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Research Article

A simple *in vitro* approach for assessing resistance and pathogenicity in the Fusarium head blight-*Triticum* spp. pathosystem

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Abstract: An effective *in vitro* approach to predict host resistance and pathogenicity of *Fusarium* species causing head blight (FHB) may improve resistance evaluation in wheat. The *in vitro* capacity of four *Fusarium* species to cause disease on young plant parts of six bread and durum wheat cultivars with known quantitative resistance was assessed using a coleoptile infection assay. Significant differences were detected in pathogenicity among *Fusarium* species and susceptibility/resistance levels among wheat cultivars. Only the resistance measured by coleoptile length (CL) was correlated with resistance criteria generated under *in vitro* (area under disease progress curve, r = -0.951), controlled (Type I and Type II, r = -0.813 and r = -0.907, respectively) and field (Type I during 2018/19 and 2019/20, r = -0.857 and r = -0.866, respectively) conditions. Moreover, the values of seed germination and CL components were significantly correlated with pathogenic indices obtained under several experimental conditions. CL predicts quantitative traits at the earliest and latest wheat development phases during disease invasion. Thus, CL may suggest a veritable potential of simple, quick, and trustworthy early screening of FHB resistance in wheat cultivars and the pathogenicity of *Fusarium* species.

Keywords: *Fusarium* species, pathogenicity assay, resistance test, wheat

Introduction

In all wheat (*Triticum* spp.)-growing areas, bread (*Triticum aestivum*) and durum (*T. durum*) wheat can be heavily damaged by pathogenic *Fusarium* species causing Fusarium head blight (FHB) (Parry *et al.*, 1995). Damage provoked by the disease may cause up to 50-70% production loss in humid and semi-humid climates (McMullen *et al.*, 2012). Furthermore, a mycotoxin produced by *Fusarium* species may contaminate and accumulate in the infected grains; consequently, infected kernels can not be used as human food or animal feed (Dahl and Wilson, 2018). The disease is caused by a complex of 17 *Fusarium* species, but just three of them are the main FHB agents, i.e., *F. graminearum*, *F. avenaceum*, and *F. culmorum*. Other FHB species causing different pathogenic levels are sampled from diseased wheat fields (Bottalico and Perrone, 2002).

It has been widely reported that the pathogenicity of *Fusarium* isolates collected from diverse regions within a country and even within species from individual fields is highly variable (Bottalico and Perrone, 2002; Xu *et al.*, 2008). Wheat resistance to FHB infection is partial and polygenic. It can be detected with

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head inoculation (Type I, resistance to initial infection) and floret inoculation (Type II, resistance to spreading within a spike) (Xu and Nicholson, 2009). Traditionally, the disease response of wheat to FHB pathogen is commonly evaluated under controlled and field conditions with a routine procedure at the mid-anthesis stage in adult plants by determining the visual scoring of head symptoms. Varying uncontrollable environmental conditions such as temperature, humidity, and the simultaneous presence of other pathogens in the field make it impossible to obtain error-free evaluations of the phenotyping of disease responses (Imathiu *et al.,* 2014). The *in vitro* approaches under controlled conditions overcome restrictions resulting from field screening, greatly simplifying the analysis of numerous pathogenic isolates and recognizing FHB disease-resistant origins in a short period (Browne and Cooke, 2005; Wu *et al.*, 2005; Browne, 2007, 2009; Purahong *et al.*, 2012; Shin *et al.*, 2014; Soresi *et al.*, 2015).

Recently, Sakr (2018a, b, c, 2019a, 2020a, b, 2021) reported that the area under the disease progress curve and latent period distinguished FHB isolates and wheat cultivars. Furthermore, these components were indicators of quantitative traits in the adult plant during disease invasion (Sakr, 2018a, b, c, 2019a, c, 2020a, b). Soresi *et al.* (2015) published an inoculation method for resistance to *F. graminearum* using durum wheat coleoptiles. This method, consisting of four components, was rapid and reliable and provided a good correlation between floret inoculation and Type II resistance in the growth chamber. Despite the significance of this approach, there are no associated reports on type I resistance, other FHB species, durum, or bread wheat. With a view to better characterizing quantitative components in the FHB-wheat association, experiments were performed, for the first time, to explore the *in vitro* coleoptile infection assay (Soresi *et al.,* 2015) for potential prediction of the head blight reaction in the whole plant measured under several experimental conditions (e.g., *in vitro* and growth chamber) for pathogenicity of four FHB species and resistance of six most cultivated wheat cultivars in Syria. Also, we assayed the relationship between coleoptile and head inoculation conducted under field conditions to check whether their ranking would be consistent.

Materials and Methods

Wheat cultivars, fungal isolates, and inoculum preparation

We utilized six durum (Acsad65, Cham7, and Cham9) and bread (Cham4, Douma4, and Bohoth10) wheat cultivars with known quantitative resistance to disease infection recognized under several experimental conditions (Sakr, 2018a, b, c, 2019c, 2020b, 2021) for assessing the quantitative components. Sixteen *Fusarium* isolates representing the four FHB species, i.e., *F. culmorum* (5 isolates), *F. solani* (6 isolates), *F. verticillioides* (4 isolates), and *F. equiseti* (one isolate) were collected from naturally FHB-infected wheat fields from Ghab Plain during the 2015 growing season. On potato-dextrose agar (PDA), single spore pure isolates were morphologically identified using the keys of Leslie and Summerell (2006). Recently, the 16 fungal isolates were molecularly analyzed by random amplified polymorphic DNA (Sakr and Shoaib 2021). For long-term storage, fungal cultures were preserved in sterile distilled water at 4 °C and frozen at-16 °C (Sakr, 2019b).

For inoculum preparation, the 16 *Fusarium* isolates were cultured at 22 °C on PDA Petri dishes for ten days in continuous dark to allow mycelial growth and sporulation. Conidia were dislodged and collected by pipetting 5 mL of sterile distilled water onto the surface of each Petri dish. 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 hemocytometer and adjusted for further experimental trials.

Quantitative component tests *in vitro*

The capacity of 16 fungal isolates of four FHB species to cause disease on juvenile plant organs *in*

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vitro was evaluated using a coleoptile infection assay, according to Soresi *et al.* (2015). Wheat seeds in Petri dishes (10 seeds per Petri dish) were imbibed for 15 minutes in 4 ml of 16 *Fusarium* culture suspensions (2×10^5 conidia/ml) or in sterile distilled water for the control treatment. Then the excess suspension was removed, and the inoculated wheat seeds were seeded on filter paper (Filtrak, Thermo Fisher Scientific Inc.) and placed in Petri dishes contatinig 0.5% agar. They were kept under incubation conditions (15 ºC with a photoperiod of 16 h light). The control treatment consisted of three non-inoculated Petri dishes per replication. Six days after inoculation, four components were quantified: seed germination, coleoptile length, coleoptile weight, and root weight. Germinated seeds were assessed in each inoculated 15-seed dish and given as a fraction of the non-inoculated dish. Roots and coleoptiles were taken from each seedling, their lengths were estimated using calipers, and weights were quantified with an analytical balance. Coleoptile and root values were recorded in each germinated individual and expressed as percentage of the non-inoculated dish mean. The experiment was repeated three times.

Quantitative component tests under field conditions

Over the two growing seasons 2018/19 and 2019/20 in the field, the 16 *Fusarium* isolates were individually inoculated on the six tested wheat cultivars to assess disease incidence (DI) for Type I resistance as indicator of the cultivar's resistance and pathogenicity of 16 FHB isolates. In brief, when the spikes reached 50% anthesis, the experimental plants were spray-inoculated with 5×10^4 spores/ml of each of the 16 FHB isolates. Control plants were sprayed with sterile distilled water. Inoculated spikes were covered with polyethylene bags for 48 h (100% relative humidity) to promote infection. FHB incidence (% of symptomatic spikes) was estimated 21 days after inoculation as the percentage of spikes in a plant with visible FHB symptoms. Disease responses measured by DI during the growing season 2018/19 were presented previously by Sakr (2020a).

Statistical analyses

Data were subjected to analysis of variance (ANOVA) using DSAASTAT add-in version 2011. Before statistical analysis, the percentages of all quantitative components were transformed using the angular transformation to stabilize variances. The differences in pathogen and cultivar responses for *in vitro* and field components were evaluated under a randomized complete block design, with blocks consisting of the replications. 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/cultivars at $P = 0.05$.

Results

Generally, inoculation of the six cultivars: Acsad65, Cham7, Cham9, Cham4, Douma4, and Bohoth10, with a set of 16 Fusarium isolates reduced the mean values of the four quantitative components of the cultivars compared to water controls. None of the six tested cultivars was immune to FHB disease. *In vitro*, the inoculated FHB seedlings were recognized by mycelia covering the wheat seed surfaces and reddish discoloration on the coleoptiles and roots, while the non-inoculated plants did not exhibit any FHB symptoms (Figure 1). Compared to the non-inoculated control, bread and durum wheat plants growing in the presence of 16 FHB tested isolates under field conditions showed typical disease symptoms, indicating a strong impact of FHB species complex on the growth of wheat plants under several experimental conditions.

p(F test)-values showed statistically significant differences in pathogenicity among the 16 *Fusarium* isolates and susceptibility/resistance levels among the six tested wheat cultivars (Table 1). In the current study, fungal isolates with lower values of the four *in vitro* components (% of control) were considered more pathogenic. For all components studied, there was a significant interaction between the two parameters: FHB isolate and wheat cultivar.

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Figure 1 *In vitro* Fusarium head blight symptoms on coleoptiles of durum wheat cultivar Cham9 inoculated with isolate F21 (*F. verticillioides*) compared with control (water) at 6 days after inoculation.

Table 1 Analyses of variance for four quantitative components detected using an *in vitro* coleoptile infection assay and disease incidence (*P*(F test)-values).

Source of variation	df	SG	CL	CW	RW	DI
Isolate (I)	15	$1.152902E^{-30}$	$9.53817E^{-23}$	$5.48434E^{-36}$	$4.74539E^{-22}$	$1.93696E^{-17}$
Cultivar (C)	5	$9.525845E^{-06}$	0.000221515	$1.82811E^{-26}$	$4.56725E^{-10}$	$4.77533E^{-23}$
I × C	75	$2.665846E^{-43}$	$1.79515E^{-36}$	$1.03682E^{-47}$	$3.64047E^{-43}$	$1.68402E^{-25}$
Error	192					
CV (%)		10.8	11.2	10.2	11.9	15.9

df – degree of freedom.
SG = seed germination; CL = coleoptile length; CW = coleoptile weight; RW = root weight; DI = disease incidence detected using head inoculation assay under field conditions over the growing season 2019/20.

In vitro assay

The number of germinated wheat seeds decreased in treatments inoculated with 16 FHB isolates. The mean values for seed germination (SG) ranged from 48.0% for the most pathogenic isolate F7 (*F. solani*), to ~ 69% for the least pathogenic isolates F30 (*F. culmorum*) and F27 (*F. verticillioides*). However, there were substantial differences in SG between wheat cultivars, with reductions ranging from 37.2% for Douma4 to ~ 42.0% for Acsad65 and Cham9 relative to non-inoculated controls (Table 2). The length of diseased coleoptiles (CL) was less than that of the healthy coleoptiles and was 10.4 mm on Acsad65, 11.6 mm on Cham4, 10.8 mm on Cham7, 10.7 mm on Douma4, 10.6 mm on Cham9 and 9.5 mm on Bohoth10 regardless of the FHB isolate. The mean values for CL varied from 48.7% for the most pathogenic isolate F7 (*F. solani*) to ~ 63.0% for the least pathogenic isolate F30 (*F. culmorum*), F15, F16, F21, and F27 (*F. verticillioides*). However, the reductions in coleoptile growth also varied widely between wheat cultivars, ranging from 38.2% for Bohoth10 to 44.6% for Acsad65 (Table 2). The weight of diseased coleoptiles (CW) was less than that of the healthy coleoptiles that amounted to 0.44 g on Acsad65, 0.38 g on Cham4, 0.53 g on Cham7, 0.63 g on Douma4, 0.84 g on Cham9 and

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0.37g on Bohoth10 irrespective of the FHB isolate. The mean values for CW ranged from ~ 53.0% for the most pathogenic isolates F2 and F30 (*F. culmorum*) and F7 (*F. solani*) to ~ 68.0% for the least pathogenic isolates F35 (*F. solani*) and F15 (*F. verticillioides*). However, intrinsic differences in CW were detected between wheat cultivars, with reductions ranging from ~ 30.0% for Cham7 and Bohoth10 to 44.7% for Acsad65 relative to non-inoculated controls (Table 2). The weight of diseased roots (RW) was less than that of the healthy roots that reached 0.58 g on Acsad65, 0.44 g on Cham4, 0.49 g on Cham7, 0.68 g on Douma4, 0.79 g on Cham9 and 0.35 g on Bohoth10 regardless of the FHB isolate. The mean values for RW ranged from 47.9% for the most pathogenic isolate F31 (*F. solani*) to ~ 69% for the least pathogenic isolates F16 and F27 (*F. verticillioides*). However, the reductions in coleoptile growth varied widely between wheat cultivars, ranging from 36.8% for Bohoth10 to 46.3% for Acsad65 (Table 2).

The reproducibility and stability of *in vitro* tests were demonstrated with the highly significant correlation coefficients for all comparisons made at p = 0.001 between the four components regarding the 16 FHB isolates measured on the six wheat cultivars from the three replications, I, II, and III (Table 3).

Quantitative traits under field conditions

The mean disease incidence (DI) scores of FHB isolates ranged ~ two-fold from 36.0% for the least pathogenic isolate F27 (*F. verticillioides*) to 54.7% for the most pathogenic isolate F20 (*F. solani*) on the tested plants as compared with 0% for the water controls (Table 2). Artificial inoculation of spikes conducted to assess Type I FHB resistance exhibited pronounced variations in the resistance of durum and bread wheat cultivars. The mean fraction of wheat plants showing disease symptoms varied from 38.2 to 53.6%. Cham4 and Bohoth10 (breads) conducted the lowest infection evaluations, with mean DI values below ~ 40.0%, whereas Acsad65 (durum) was the most affected cultivar, with a mean DI score of 53.6%. The bread wheat cultivars exhibited lower DI values than the durum ones. Pathogenic responses over the two growing seasons were relatively homogeneous. Environmental conditions did not influence wheat cultivar and FHB isolate because climatic data for the station were somewhat similar during 2018/19 and 2019/20 (Sakr, 2020a).

Combinations within and among *in vitro* and head blight assays

There were no significant correlations among the four quantitative components (SG, CL, CW, and RW) determined for 16 FHB isolates except for a significant correlation between SG and CL (r = 0.532*). No significant correlations were obtained among the four quantitative components (SG, CL, CW, and RW) detected for the six wheat cultivars except for a significant correlation between CW and RW (r = 0.966**). Correlation values of *in vitro* and field components among the six wheat cultivars showed that all possible comparisons were not significantly correlated except for six significant ones (Table 4).

Table 5 presents correlation coefficients between pathogenicity components detected using an *in vitro* coleoptile infection assay and pathogenic indices generated under several experimental conditions. The values of SG were significantly correlated with previously obtained values of DI and disease severity (DS) generated under controlled conditions. However, the values of SG were correlated with previously obtained values of *in vitro* area under the disease progress curve (AUDPC) of Petri-dish inoculation assay and DI under field conditions over the two consecutive seasons for certain wheat cultivars. The values of CL were significantly correlated with AUDPC, DI, and DS under controlled and field conditions on six wheat cultivars except for two (CL × DI in field) correlations on Cham4 and Bohoth10 over 2018/19 and one (CL × DI in the field) correlation on Bohoth10 over 2019/20. The values of CW were correlated with AUDPC and DS for certain wheat cultivars. However, the values of CW were associated with DI under controlled and field conditions. The values of RW were not correlated with all pathogenic indices except one (CW × DI in the field) correlation on Bohoth10.

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Table 2 Mean disease responses measured by four quantitative components (% of control) detected using an *in vitro* coleoptile infection assay and disease incidence for six bread and durum wheat cultivars infected with four Fusarium head blight species.

Entries	Entries	Disease responses				
		SG (% of control)	CL (% of control)	CW (% of control)	RW (% of control)	DI
Fungal isolates (identification)	F1 (*F. culmorum*)	63.1 ef	54.9 bcd	63.2 cd	67.2 gh	46.0 abcde
	F2 (*F. culmorum*)	49.3 ab	57.4 de	54.5 a	56.3 cd	48.9 abc
	F3 (*F. culmorum*)	61.0 de	51.0 ab	58.0 ab	61.9 f	47.0 abcd
	F28 (*F. culmorum*)	52.3 ab	60.3 efg	60.6 bc	61.9 f	47.0 abcd
	F30 (*F. culmorum*)	69.2 g	64.5 g	53.6 a	54.2 bc	40.5 bcde
	F7 (*F. solani*)	56.8 cd	52.1 abc	54.0 a	58.0 cdef	40.6 bcde
	F20 (*F. solani*)	48.0 a	48.7 a	68.9 e	56.6 cde	54.7 a
	F26 (*F. solani*)	58.8 de	62.7 fg	80.5 f	61.5 ef	46.0 abcde
	F29 (*F. solani*)	53.6 bc	61.0 efg	66.4 de	62.2 fg	51.2 ab
	F31 (*F. solani*)	56.9 cd	58.1 def	67.1 de	47.9 a	43.2 bcde
	F35 (*F. solani*)	51.8 ab	49.0 a	68.1 e	58.8 cdef	50.8 abc
	F15 (*F. verticillioides*)	67.1 fg	63.3 g	68.5 e	59.4 def	36.8 de
	F16 (*F. verticillioides*)	58.9 de	64.9 g	63.1 cd	69.2 h	40.5 bcde
	F21 (*F. verticillioides*)	59.2 de	63.0 g	65.4 de	50.8 ab	49.6 abc
	F27 (*F. verticillioides*)	69.0 g	63.7 g	76.0 f	69.9 h	36.0 g
	F43 (*F. equiesti*)	61.0 de	56.7 cde	60.4 bc	62.0 f	44.2 bcde
Wheat cultivars	Acsad65	57.1 c	55.4 c	55.3 d	53.7 c	53.6 a
	Cham4	57.8 bc	58.3 b	59.3 c	57.8 d	39.7 d
	Cham7	57.3 bc	57.9 bc	70.0 a	61.9 ab	49.1 b
	Douma4	62.8 a	58.6 b	67.8 ab	62.3 ab	44.2 c
	Cham9	56.5 c	57.2 bc	65.2 b	60.3 bc	46.0 bc
	Bohoth10	59.7 b	61.8 a	68.1 a	63.2 a	38.2d

Seed germination (SG), coleoptile length (CL), coleoptile weight (CW) and root weight (RW). Disease incidence (DI) was detected using a head artificial inoculation generated under field conditions during the growing seasons 2018/19. According to the Fisher's LSD test, means in a column followed by the same letters are not significantly different at P = 0.05.

Table 3 Correlation coefficients within the four quantitative components measured in the three experiments, RI, RII and RIII of an *in vitro* coleoptile infection assay after inoculation with four Fusarium head blight species on six bread and durum wheat cultivars.

Variables		Acsad65	Cham4	Cham7	Douma4	Cham9	Bohoth10
SG	RI × RII	0.992***	0.990***	0.988***	0.986***	0.989***	0.993***
	RI × RIII	0.944***	0.976***	0.966***	0.953***	0.948***	0.965***
	RII × RIII	0.968***	0.981***	0.976***	0.983***	0.962***	0.968***
CL	RI × RII	0.993***	0.997***	0.955***	0.976***	0.991***	0.976***
	RI × RIII	0.951***	0.983***	0.975***	0.968***	0.988***	0.944***
	RII × RIII	0.946***	0.987***	0.974***	0.983***	0.975***	0.954***
CW	RI × RII	0.990***	0.992***	0.982***	0.992***	0.994***	0.958***
	RI × RIII	0.983***	0.989***	0.967***	0.978***	0.992***	0.951***
	RII × RIII	0.992***	0.998***	0.987***	0.989***	0.999***	0.993***
RW	RI × RII	0.996***	0.990***	0.981***	0.988***	0.980***	0.989***
	RI × RIII	0.980***	0.984***	0.974***	0.973***	0.956***	0.985***
	RII × RIII	0.910***	0.991***	0.989***	0.985***	0.982***	0.990***

Seed germination (SG), coleoptile length (CL), coleoptile weight (CW) and root weight (RW).
***: statistically significant difference at P = 0.001.

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Table 4 Correlation coefficients of four quantitative components detected using an *in vitro* coleoptile infection assay and disease incidence detected under field conditions during the two growing seasons 2018/19 and 2019/20 among six bread and durum wheat cultivars infected four Fusarium head blight species.

Growth and disease parameters	Wheat cultivars	Acsad65	Cham4	Cham7	Douma4	Cham9	Bohoth10
Seed germination	Acsad65	1					
	Cham4	-0.337 ns	1				
	Cham7	-0.010 ns	-0.061 ns	1			
	Douma4	-0.103 ns	0.343 ns	0.239 ns	1		
	Cham9	0.313 ns	0.001 ns	0.188 ns	0.180 ns	1	
	Bohoth10	-0.072 ns	0.736**	-0.089 ns	0.166 ns	-0.164 ns	1
Coleoptile length	Acsad65	1					
	Cham4	-0.175 ns	1				
	Cham7	0.428 ns	-0.021 ns	1			
	Douma4	-0.430 ns	0.548*	0.022 ns	1		
	Cham9	-0.223 ns	-0.116 ns	0.112 ns	0.098 ns	1	
	Bohoth10	-0.234 ns	0.423 ns	-0.017 ns	0.428 ns	0.246 ns	1
Coleoptile weight	Acsad65	1					
	Cham4	-0.349 ns	1				
	Cham7	0.293 ns	0.185 ns	1			
	Douma4	0.368 ns	-0.089 ns	-0.174 ns	1		
	Cham9	0.498 ns	-0.054 ns	0.284 ns	-0.243 ns	1	
	Bohoth10	0.223 ns	0.470 ns	0.288 ns	-0.102 ns	0.298 ns	1
Root weight	Acsad65	1					
	Cham4	-0.248 ns	1				
	Cham7	-0.083 ns	0.011 ns	1			
	Douma4	0.457 ns	-0.351 ns	-0.143 ns	1		
	Cham9	-0.020 ns	0.331 ns	-0.242 ns	0.086 ns	1	
	Bohoth10	0.005 ns	0.100 ns	0.067 ns	-0.022 ns	0.397 ns	1
Disease incidence over 2018/19	Acsad65	1					
	Cham4	-0.069 ns	1				
	Cham7	-0.045 ns	0.457 ns	1			
	Douma4	-0.213 ns	0.547*	0.386 ns	1		
	Cham9	0.413 ns	0.137 ns	-0.059 ns	-0.103 ns	1	
	Bohoth10	0.452 ns	0.053 ns	-0.229 ns	-0.154 ns	0.748***	1
Disease incidence over 2019/20	Acsad65	1					
	Cham4	-0.307 ns	1				
	Cham7	-0.063 ns	0.292 ns	1			
	Douma4	-0.281 ns	0.593*	0.359 ns	1		
	Cham9	0.502*	-0.014 ns	-0.033 ns	-0.135 ns	1	
	Bohoth10	0.291 ns	0.039 ns	-0.285 ns	-0.009 ns	0.531*	1

ns = non-significant tests (P = 0.05), *, **, ***: statistically significant difference at P = 0.05, P = 0.01 and P = 0.01. Disease incidence over the growing season 2018/19 was presented previously by Sakr (2020a).

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The correlation coefficients between the resistance measured by SG, CL, CW, and RW and the resistance measured by other components conducted herein and in the past are presented in Table 6. Correlation coefficients between CL and resistance measured by AUDPC and adult FHB resistance were significant. However, the other three components showed no correlations with other components. A ranking of cultivars in terms of these five disease values (CL, AUDPC, DI, and DS in a growth chamber and DI in the field) is shown in Figure 2. The two statistically defined (Fisher's LSD test) groups of cultivars were based on CL and AUDPC with contrasting responses as termed `moderately resistant` and `susceptible,` with the remainder was listed as `susceptible to moderately susceptible. Compared to *in vitro* groups, the four statistically defined groups of cultivars were based on DI and DS under controlled and field conditions with contrasting reactions as termed `moderately resistant` and `susceptible`, with the remainder, was recorded as `moderately susceptible` and `susceptible to moderately susceptible.

Table 5 Correlation coefficients between four pathogenicity components detected using an in vitro coleoptile infection assay and several pathogenic indices generated under different experimental conditions for four Fusarium head blight species on six bread and durum wheat cultivars.

Wheat cultivars	SG × AUDPC	SG × DI	SG × DS	SG × DIFC$^{2018/19}$	SG × DIFC$^{2019/20}$
Acsad65	-0.566*	-0.653*	-0.593*	-0.671**	-0.695**
Cham4	-0.581*	-0.582*	-0.495*	-0.487 ns	-0.575*
Cham7	-0.634**	-0.630**	-0.627**	-0.407 ns	-0.463 ns
Douma4	-0.267 ns	-0.530*	-0.506*	-0.624**	-0.611*
Cham9	-0.333 ns	-0.554*	-0.513*	-0.433 ns	-0.427 ns
Bohoth10	-0.726**	-0.551*	-0.510*	-0.471 ns	-0.534*
	CL × AUDPC	CL × DI	CL × DS	CL × DIFC	SG × DIFC$^{2019/20}$
Acsad65	-0.872***	-0.525*	-0.626**	-0.686**	-0.675*
Cham4	-0.795**	-0.570*	-0.606*	-0.415 ns	-0.559*
Cham7	-0.550*	-0.663**	-0.603*	-0.579*	0.490*
Douma4	-0.660**	-0.609*	-0.505*	-0.643**	-0.741**
Cham9	-0.683**	-0.621*	-0.602*	-0.682**	-0.543*
Bohoth10	-0.737**	-0.612*	-0.612*	-0.448 ns	-0.463 ns
	CW × AUDPC	CW × DI	CW × DS	CW × DIFC	SG × DIFC$^{2019/20}$
Acsad65	-0.279 ns	-0.097 ns	-0.553*	-0.418 ns	0.234 ns
Cham4	-0.094 ns	-0.022 ns	-0.693**	-0.145 ns	0.448 ns
Cham7	-0.190 ns	0.019 ns	-0.177*	-0.490 ns	0.332 ns
Douma4	0.096 ns	0.332 ns	-0.047 ns	0.355 ns	-0.386 ns
Cham9	-0.501*	-0.068 ns	-0.626**	-0.230 ns	0.169 ns
Bohoth10	-0.421 ns	-0.005 ns	-0.510*	0.251 ns	0.335 ns
	RW × AUDPC	RW × DI	RW × DS	RW × DIFC	SG × DIFC$^{2019/20}$
Acsad65	-0.101 ns	0.235 ns	0.057 ns	0.084 ns	0.119 ns
Cham4	-0.104 ns	0.297 ns	-0.189 ns	0.133 ns	-0.030 ns
Cham7	0.047 ns	0.297 ns	0.054 ns	-0.023 ns	-0.003 ns
Douma4	-0.163 ns	-0.358 ns	-0.265 ns	0.366 ns	-0.346 ns
Cham9	-0.383 ns	-0.477 ns	0.204 ns	-0.411 ns	-0.336 ns
Bohoth10	0.436 ns	0.145 ns	0.491 ns	0.527*	0.429 ns

Seed germination (SG), coleoptile length (CL), coleoptile weight (CW), root weight (RW), standardized area under disease progress curve (AUDPC) of Petri-dish inoculation detected *in vitro*, disease incidence (DI) detected using a head artificial inoculation generated under controlled conditions, disease severity (DS) detected using a floret artificial inoculation generated under controlled conditions, disease incidence (DIFC) detected using a head artificial inoculation assay under field conditions during the two growing seasons 2018/19 and 2019/20. ns = non-significant tests (p = 0.05), *, **, ***: statistically significant difference at P = 0.05, P = 0.01 and P = 0.01. Disease incidence over the growing season 2018/19 was presented previously by Sakr (2020a).

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Table 6 Correlation coefficients between the resistance measured by four quantitative components detected using an *in vitro* coleoptile infection assay and resistance criteria generated under different experimental conditions for six bread and durum wheat cultivars infected with four Fusarium head blight species.

Variables	*In vitro* AUDPC	Type I in growth chamber	Type II in growth chamber	Type I in field during 2018/19	Type I in field during 2019/20
SG	-0.429 ns	-0.362 ns	-0.508 ns	-0.414 ns	-0.413 ns
CL	-0.951**	-0.813*	-0.907*	-0.857*	-0.866*
CW	-0.560 ns	-0.273 ns	0.588 ns	-0.317 ns	-0.368 ns
RW	0.752 ns	-0.506 ns	-0.779 ns	-0.551 ns	-0.595 ns

Seed germination (SG), coleoptile length (CL), coleoptile weight (CW), root weight (RW), standardized area under disease progress curve (AUDPC) of Petri-dish inoculation assay, spraying inoculation under controlled conditions (Type I), point inoculation under controlled conditions (Type II) and spraying inoculation under filed conditions during the two growing seasons 2018/19 and 2019/20 (Type I). ns = non-significant tests (P = 0.05), *, **, ***: statistically significant difference at p = 0.05, p = 0.01 and p = 0.01. Disease incidence over the growing season 2018/19 was presented previously by Sakr (2020a).

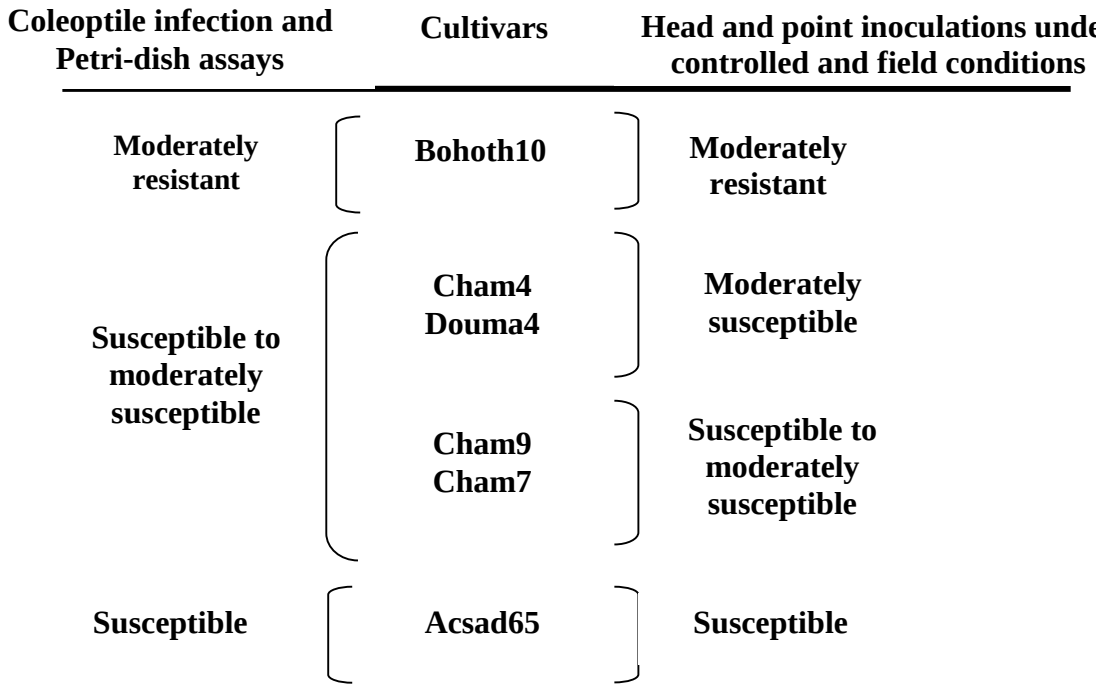

Figure 2 Ranking of six bread and durum wheat cultivars based on coleoptile length in 16 Fusarium head blight-mediated *in vitro* coleoptile infection assay and on FHB area under disease progress curve detected using a Petri-dish assay, incidence and severity following head and point inoculations, respectively, of spikes and spikelets in a growth chamber and field during the two growing seasons 2018/19 and 2019/20.

Discussion

During our research, pronounced differences in inoculated treatments were observed on individual plant organs relative to non-inoculated controls, revealing that *Fusarium* species used in this assay were found to be convenient for the differential expression of all tested quantitative components. It appears that the tested *in vitro* coleoptile infection approach carried out on *F. graminearum* to screen quantitative durum wheat resistance (Soresi *et al.,* 2015) can be used to explore pathogenicity and resistance components of four FHB species: *F. culmorum*, *F. verticillioides*, *F. solani* and *F.*

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equiseti on six widely cultivated bread and durum cultivars in Syria. The reproducibility and stability of the tested *in vitro* coleoptile infection were verified with the highly significant correlation coefficients within the four components during our investigation.

Adequate *in vitro* conditions were determined for the coleoptile infection experiment to maximize variations in disease response components among FHB species and the six wheat cultivars. The four quantitative components involved in this assay were found to be correlated, suggesting that these components are genetically identical and also reflecting into complex polygenic nature of resistance/pathogenicity in the interaction in FHB-wheat system, which is not entirely understood (Castiblanco *et al*., 2018). For all the tested quantitative components, there was a broad diversity in pathogenicity among the 16 FHB isolates as a direct result of the pathogen severity. Moreover, intrinsic differences were detected among wheat cvs., indicating differences in the ability to resist pathogen effects at early development stages.

Compared with SG and CL analyses conducted in the past in a Petri-dish inoculation methodology which exhibited no significant differences among these same *Fusarium* species and wheat cultivars (Sakr, 2018a, b, c, 2019a, 2020b), it seems that inoculum concentration, infection methods, and growth conditions had permitted to assess pronounced differences in SG and CL. In the Petri-dish inoculation method (Purahong *et al.*, 2012), a 6 ml of spore suspension at 1×10^6 spores/ml was applied, and the wheat seeds were submerged in the *Fusarium* inoculum suspension. They were then put into a Petri dish with sterile double-layer filter paper. In the *in vitro* coleoptile infection methodology (Soresi *et al.*, 2015), the wheat seeds in Petri dishes were imbibed for 15 minutes in 4 ml of 16 *Fusarium* culture suspensions (2×10^5 conidia/ml). Then the excess suspension was recovered, and the inoculated wheat seeds were seeded on filter paper and placed in Petri dishes with 0.5% agar. While infected treatments were incubated at 22 ºC in the continuous dark in the Petri-dish inoculation methodology, the infected treatments were incubated at 15 ºC with a photoperiod of 16 h light in the *in vitro* coleoptile infection method. Various infection tools could assist in recognizing the resistance/susceptibility scale in wheat cultivars in growth chamber and field (Sip *et al.*, 2011).

According to Soresi *et al.* (2015), quantitatively resistant wheat cultivars are recognized by high values (% of control) of SG, CL, CW, and RW compared with the susceptible one. The current investigation identified differences in resistance/susceptibility grades between wheat plants for the four tested components. However, not all the evaluated components were informative for FHB prediction at the earliest and latest wheat development stages. Only CL was consistently associated with the resistance as measured by AUDPC detected *in vitro* (Sakr, 2018a,b,c, 2020b) and the adult FHB resistance type I and type II under controlled and filed conditions (Sakr, 2019c). In accordance with our data, Soresi *et al.* (2015) indicated that CL was more expressive of FHB Type II adult resistance. Also, Shin *et al.* (2014) noted that decreases in SG were poorly linked with both: Type I and Type II in mature wheat plants. In contrast to our findings, Browne (2007, 2009) observed that higher SG was linked to greater FHB Type II resistance. It is widely accepted that SG and CL assays are two techniques generally used for the assessment of wheat cultivar resistance. The weight of coleoptiles and roots included in our *in vitro* blight assay did not predict the resistance/susceptibility level in FHB-wheat pathosystem at the earliest and latest wheat development stages during FHB infection as observed previously by Soresi *et al.* (2015).

Durum wheat is more susceptible to FHB infection than bread since there is a high disease resistance (Xu and Nicholson, 2009). As expected, our findings confirmed *in vitro*, growth chamber, and field data that `Acsad65, durum` was susceptible and `Bohoth10, bread` was moderately resistant (Sakr, 2018a, 2019c, 2020b, 2021). The reliability of this cultivar order was proved by the significant correlation

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between the CL and AUDPC, FHB Type I and Type II resistance under controlled and field conditions. More importantly, Bohoth10 with the highest values (% of control) for SG, CL, CW, RW and had the lowest levels of AUDPC, spike and spikelet damage, and *vice versa* for Acsad65, indicating that Bohoth10 provided broad, though incomplete, resistance to the four *Fusarium* species examined. Overall, wheat plants with the highest values for CL had the highest levels of FHB AUDPC, Type I, and Type II resistance scores. The biological clarification for a probable association between *in vitro* and adult plant reactions to FHB infection remains largely debated. Still, it can be suggested that identical genetic pathways become activated at both growth stages (Soresi *et al.*, 2015). Our data highlight that the assessment of resistance level is repeatable and constant under diverse experimental conditions.

The four analyzed FHB species were found to provide diverse and obvious symptoms on wheat coleoptiles, indicating that the *in vitro* coleoptile infection assay provides several experimental conditions for *Fusarium* species to vigorously develop, thus ensuring that their pathogenicity is stable and/or correctly distinguished or quantified. All the *Fusarium* isolates that dealt with this *in vitro* assay, fulfilled the requirement to induce FHB disease. Thus, they are pathogenic. The four FHB species applied in this test are recognized as capable of mycotoxin production. Thus, the ability of 16 *Fusarium* isolates to cause reddish discoloration on the coleoptiles and roots in changing amounts could be commonly the consequence of the phytotoxic impact of these metabolites (Xu and Nicholson, 2009).

The significant decreases noticed for values of SG, CL, CW and RW are indications of pathogenicity (Lannou, 2012). SG, CL, CW and RW did differentiate isolates within and among species. The wide range of pathogenicity variation among FHB isolates in the current study has been substantiated by other reports analyzing the pathogenicity of diverse *Fusarium* species (Bottalico and Perrone, 2002; Xu *et al.*, 2008; Sakr, 2018a, b, c, 2019c, 2020a, b). Mutation, genetic recombination or selection in the 16 *Fusarium* isolates may play a primary function in pathogenesis. Our data are in accordance with those found by Purahong *et al.* (2012); highly significant variations were reported in the pathogenicity of *F. graminearum* on the host plant as measured by CL. Brennan *et al.* (2003) reported that diminution of the CL had been linked to pathogenicity. Our results did not agree with those reported by Purahong *et al.* (2012) who showed that decreases in GS were not significant to distinguish *Fusarium* isolates. Some studies demonstrate the reduction of SG of wheat seeds caused by *F. graminearum* (Parry *et al.*, 1995; Browne and Cooke, 2005): so when the wheat seeds inoculated with the four tested FHB species in the coleoptile infection methodology are infected, their germination scores are diminished compared with the water control. Our findings also showed, for the first time, the utility of CW and RW for analyzing of pathogenicity in FHB-wheat pathosystem.

It should be pointed that a complex genotype interaction was found between FHB isolates and wheat cultivars for SG, CL, CW and RW, and disease incidence generated under field conditions over two consecutive seasons revealing by the presence of a cultivar-specific pathogenicity, suggesting that pathogenicity mechanisms and resistance genes maybe different to disease caused by individual FHB isolates. Strong arguments have been made for specific pathogenicity interactions among *Fusarium* species involved in the FHB complex and wheat plants (Parry *et al.*, 1995; Xu and Nicholson, 2009; Sakr, 2018a, b, c, 2019c, 2020a, b, 2021). Just variability may exist in pathogenicity mechanisms among different species of *Fusarium* and their corresponding defense mechanisms in wheat, indicating that the isolate-specific efficacy can lead to overcoming host resistance. However, further research is required to draw any definitive conclusions.

Correlations were obtained between the data of both: CL (with a greater extent) and SG (to a lesser extent) and AUDPC, disease incidence, and disease severity previously generated *in vitro* and in growth chamber and in the field for

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wheat plants. When considered together, these distinct researches on pathogenicity suggest the utility of CL for FHB evaluation concerning both the *Fusarium* species and wheat plants. The repeatability and the stability of the *in vitro* coleoptile infection assay over several experimental conditions were proved using different wheat cultivars by yielding correlations with the data from *in vitro*, head, and spikelet inoculations. So, in this investigation, we selected six wheat cultivars showing various FHB resistance levels (Sakr, 2019c) (one moderately resistant, two moderately susceptible, two susceptible to moderately susceptible, and one susceptible) that would be adequate to examine the stability and repeatability of coleoptile infection test *in vitro* and under controlled and field conditions. In accordance with our findings, a low and negative link ($r = -0.47$) of wheat seed germination scores obtained by *Microdochium majus* and FHB assessment generated by artificial spike inoculation of *F. graminearum* under field conditions was reported by Browne (2007). In parallel, Purahong *et al.* (2012) observed positive correlations of AUDPC scores and FHB values generated in head infection of *F. graminearum* over four durum wheat cultivars under controlled and field conditions. They found high correlations between these two parameters (Purahong *et al.*, 2012), similar to our results.

The *in vitro* coleoptile infection assay probably distinguishes the degree of disease reaction of four FHB species at the early stages of plant growth by stimulating the interaction between wheat tissues and *Fusarium* species (Soresi *et al.*, 2015). The situation in this *in vitro* assay could be identical to adult plant spike and spikelet inoculation using spraying and floret inoculation assay for disease incidence and disease severity. *Fusarium* conidia were put directly on wheat seeds (without glumes), and they could immediately penetrate and infect germinated seeds. Thus, disease development is proven via symptoms like mycelia covering the seed surface and reddish discoloration on the affected plant parts (Soresi *et al.*, 2015). Therefore, the *in vitro* component, CL, predicts quantitative traits at the earliest and latest wheat development phases during disease invasion. Since only six wheat cultivars were tested here, additional research consisting of a great number of accessible Syrian wheat cultivars is required to validate our results *in vitro*, in a growth chamber and field.

Conclusions

Based on the information generated in this study, we highlighted that CL overcomes the restrictions related to time and space dependency of field and growth chamber evaluations and allows for identifying susceptibility levels in six wheat cultivars widely cultivated in Syria. Furthermore, CL assay can be used for screening the most pathogenic isolates of different FHB species for FHB resistance breeding of wheat. The *in vitro* coleoptile infection assay has a high potential to simplify the advance of research into the wheat-FHB pathosystem since it offers a real possibility of simple, quick, and trustworthy prediction of resistance in bread and durum wheat cultivars and pathogenicity of FHB species. Further study on quantitative trait loci associated with pathogenicity in *Fusarium* species and resistance in wheat plants should be sought to better explore wheat-*Fusarium* interactions on the molecular level generated in the expression of FHB pathogenicity and resistance.

Authors' Contributions

The author's contribution is 100%.

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ارزیابی مقاومت و بیماری‌زایی فوزاریوم سنبله گندم با یک روش ساده آزمایشگاهی

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چکیده: پیش‌بینی مقاومت میزبان و بیماری‌زایی گونه‌های فوزاریوم عامل بیماری بلایت سنبله گندم (FHB) حائز اهمیت است. در این پژوهش چهار گونه فوزاریوم برای ایجاد بیماری در بخش‌های گیاه جوان شش رقم گندم با استفاده از روش آلوده‌سازی کولئوپتیل ارزیابی شد. تفاوت معنی‌داری در بیماری‌زایی در بین گونه‌های فوزاریوم و سطوح حساسیت/مقاومت در بین ارقام گندم مشاهده شد. فقط، مقاومت اندازه‌گیری شده توسط طول کلئوپتیل (CL) با معیارهای مقاومت تولید شده در شرایط آزمایشگاهی (سطح منحنی پیشرفت بیماری، $r = -0.951$)، کنترل شده (نوع I و نوع II، $r = 0.813$ و $r = -0.907$) و شرایط مزرعه‌ای (نوع I در طی سال‌های ۲۰۱۸ و ۲۰۱۹ $r = -0.857$ و $r = -0.866$) هم‌بستگی معنی‌داری داشت. علاوه براین، مقادیر جوانه‌زنی بذر و اجزای طول کلئوپتیل با شاخص‌های بیماری‌زایی به‌دست‌آمده در شرایط آزمایشی هم‌بستگی معنی‌داری داشت. طول کلئوپتیل صفات کمی را در اولین و آخرین مراحل رشد گندم در طول تهاجم بیماری پیش‌بینی می‌کند. بنابراین، طول کلئوپتیل ممکن است پتانسیل واقعی غربالگری اولیه ساده، سریع و قابل اعتماد مقاومت به FHB در ارقام گندم و بیماری‌زایی گونه‌های فوزاریوم را نشان دهد.

واژگان کلیدی: گونه فوزاریوم، سنجش بیماری‌زایی، آزمون مقاومت، گندم

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