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RESEARCH ARTICLE

In Vitro Quantitative Resistance Components in Wheat Plants to Fusarium Head Blight

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Abstract:

Background:

In vitro tools have proved to be very useful in identifying quantitative resistance in wheat to Fusarium Head Blight (FHB) infection. However, there is a need to understand how the different *in vitro* and *in planta* tests correlate to describe the level of wheat resistance to FHB infection.

Objective and Methods:

We evaluated the correlation between *in vitro* symptom assessment using nine quantitative resistance criteria and *in planta* disease severity for Type II resistance assessment using a set of 16 FHB isolates across two widely grown Syrian wheat cultivars: Cham1 (durum) and Cham6 (bread).

Results:

Cultivar differences after inoculation with fungal isolates in seedlings and adult plants relative to the controls were detected. There were significant differences in the resistance of two wheat cultivars as measured by adult FHB resistance, Latent Period (LP) of detached leaf inoculation and standardized Area Under Disease Progress Curve ($AUDPC_{standard}$) of modified Petri-dish inoculation. Correlation coefficients between FHB severity and the two *in vitro* components LP and $AUDPC_{standard}$ were significant (r=0.545 with p<0.05, and r=0.659 with p<0.01, respectively). No significant differences in the resistance of Cham6 and Cham1 were indicated for the other seven *in vitro* components: incubation period and lesion length of detached leaf inoculation, germination rate reduction and coleoptile length reduction of modified Petri-dish inoculation and lesion length of clip-dipping inoculation and percentage of infected seedlings of foliar-spraying and pin-point inoculations. Results from these seven components were not correlated with adult FHB resistance. Longer latent period and less $AUDPC_{standard}$ were related to greater FHB disease-type II resistance.

Conclusion:

LP and $AUDPC_{standard}$ are indicators of mechanisms of resistance occurring in the whole plant during FHB infection. Therefore, the idea of using *in vitro* components is based on their predictive ability of *in planta* adult FHB resistance.

Keywords: Detached leaf assay, FHB species complex, Modified petri-dish assay, Seedling assay, Wheat cultivar resistance, Lantent period.

1. INTRODUCTION

Seed infection by *Fusarium* pathogens possess a great risk for bread (*Triticum aestivum* L.) and durum (*T. durum* Desf.) wheat cultivation. *Fusarium* species are widespread pathogenic fungi, of which certain members can cause Fusarium Head Blight (FHB). FHB is one of the most widespread and damaging fungal disease affecting durum wheat and bread wheat production globally [1]. FHB infect spikes at the stage of anthesis with inter and intra-cellular colonization of floral tissues and systemically spread within the spikelets, ultimately resulting in bleaching and premature death of spikes [2]. When favorable environmental conditions enable severe epidemics, it causes great reduction in grain yield (up to 50-70%) and quality by producing mycotoxins that make wheat unfit for food and feed [3]. A complex of seventeen *Fusarium* species has been isolated from wheat heads with FHB symptoms. Globally, *F. graminearum* and *F. culmorum* are found to be the main disease causal agent. In addition, other causal agents are less frequently encountered species such as *F. equiseti*, *F. cerealis* and *F. poae* and, to a lesser extent, *F. oxysporum*, *F. solani*, and *F. verticillioides* [4, 5].

Practices to manage FHB include the breeding of resistant varieties to the fungus, disease forecasting, direct chemical

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fungicide treatment, bacterial antagonists as biocontrol agents and crop rotation [2, 6]. To date, the development of tolerant or resistant cultivars is the most practical, cost-effective and environmentally friendly disease management approach [7, 8]. Wheat exhibits two primary kinds of quantitative resistance to FHB which are termed type I (resistance to initial infection after spray inoculation) and type II (resistance to fungal spread within the head after point inoculation) resistance [9]. Resistance to FHB is a quantitative trait showing polygenic inheritance, and is modulated by significant cultivar-environment interactions. Compared to bread wheat, little information is available about Quantitative Trait Loci (QTLs) involved in FHB durum resistance, with most current durum wheat cultivars highly susceptible to FHB infection [10].

Wheat is one of the main crops in Mediterranean countries. Its domestication has occurred between 12000 and 10000 years BP in Syria. Wheat (durum and bread) is one of the most important crops occupying up to 24% of the cultivated area, with an annual production of 3.9 million tons in 2011 [11]. There are five distinct agro-climatic zones based on rainfall [12]. Bread wheat is cultivated mainly in irrigated areas (zones 1 and 2), and durum wheat in rainfed areas (zones 3, 4 and 5), *i.e.*, Cham1 (durum) is planted in rainfed and irrigated areas (zone 1) and Cham6 (bread) is adapted for rainfed agriculture and planted in both zones (1 and 2) [12]. Namely FHB species: *culmorum* was the most frequent (43.8%), followed by *F. equiseti* (23.3%), *F. moniliforme* (14.6%), *F. proliferatum* (7.1%), *F. sambucinum* (2.9%), *F. compactum* (2.1%), *F. solani* (1.7%), *F. crookwellense* (0.8%), *F. avenaceum* (0.8%), and *F. semitectum* (0.4%) were recovered during spring of three seasons (2008-2010) from wheat seeds showing FHB across 20 locations/fields of Ghab Plain (zone 1), one of the principal Syrian wheat production areas [13]. Most frequent FHB species were *F. tricinctum* (30% of all *Fusarium* isolates), *F. culmorum* (18%), *F. equiseti* (14%) and *F. graminearum* (13%) from wheat spikes with FHB symptoms across five different Syrian provinces (zones 1, 2 and 3), except for Ghab Plain [14]. Earlier studies have reported the evaluation of type I and Type II resistance in the field, growth chamber and *in vitro* assays of Syrian wheat cultivars to FHB pathogens and differential reactions on cultivars were detected [15 - 17], *i.e.*, Cham6 showed lower levels of spikelet (type II) damage than did Cham1 [15, 17].

Under controlled and field conditions, evaluation of FHB resistance of wheat at the adult plant stage is determined by evaluating disease symptoms *in planta*. However, it is laborious, time consuming, requires a large space and appropriate equipment over several months and depends whether the environmental conditions are favorable for disease development [2, 5, 7, 8]. Therefore, there has been an interest in developing *in vitro* assays to provide methods for prescreening FHB resistance. *In vitro* methodologies predicting resistance at early plant stage have been evaluated, even though results are varied [18 - 24]. Recently, Sakr [25] quantified FHB resistance in barley plants using nine components involved in three *in vitro* assays (detached leaf, modified Petri-dish and seedling tests) widely used in FHB-wheat studies [19 - 23, 26]. The quantitative components were: Incubation period, latent period and lesion length of detached leaf assay; germination rate reduction, area under disease progress curve and coleoptile length reduction of modified Petri-dish assay; and percentage of infected seedlings of foliar-spraying and pin-point inoculations and lesion length of clip-dipping inoculation of seedling assay.

There is a need to understand how the different *in vitro* and *in planta* tests correlate to describe the level of wheat resistance to FHB infection. The aim of the current study was to investigate the use of three *in vitro* assays (detached leaf, modified Petri-dish and seedling) for identification of resistance or susceptibility levels in wheat to FHB infection at early stages. To achieve this objective, we evaluated the corre-lation between *in vitro* symptom assessment using nine quanti-tative resistance criteria and *in planta* disease severity for Type II resistance assessment using a set of 16 FHB isolates across two Syrian wheat cultivars.

2. MATERIALS AND METHODS

2.1. Syrian Wheat Cultivars and Fungal Isolates

Two Syrian widely grown wheat cultivars: Cham1 (durum, released in 1983) and Cham6 (bread, released in 1991) with highest agronomic characteristics and resistance to biotic and abiotic stresses [12] were used to set up experimental trials for *in planta* and *in vitro* assays. Sixteen *Fusarium* isolates comprising four species were obtained during the 2015 growing season on wheat spikes with FHB symptoms across nine villages in Ghab Plain. These 16 isolates consisted of *F. culmorum* (isolates F1, F2, F3, F28 and F30), *F. verticillioides* (isolates F15, F16, F21 and F27), *F. solani* (isolates F7, F20, F26, F29, F31 and F35), and *F. equiseti* (isolate F43), which were used for artificial inoculation. Although *F. graminearum* is considered the major causative of FHB complex worldwide [4, 5], this species was not found in the surveyed region as observed in other studies investigating the composition of FHB complex species in Ghab Plain [13, 15]. To ensure adequate pathogenicity on the two wheat cultivars, the pathogenicity of the 16 FHB isolates was pre-screened for *in planta* and *in vitro* disease reactions [27 - 31]. Isolates were identified morpho-logically according to Nelson *et al.* [32]. For long term preservation, fungal cultures were maintained in sterile distilled water at 4°C and freezing at -16°C [33].

For inoculum preparation, fungal suspension and four to six agar plugs out of the stored isolates were plated onto PDA in 9-cm Petri dishes and incubated for 10 days at 22°C in the dark to allow mycelial growth and sporulation. Ten ml of sterile distilled water were added to each dish, and the conidia concentration was directly quantified under an optical micro-scope with a Neubauer chamber and diluted to desirable concentrations according to *in planta* and *in vitro* inoculation tests.

2.2. Evaluation of FHB Resistance *In Planta*

The FHB resistance (type II) was evaluated in a growth chamber at 20°C day/night temperature, and 16/8 h light/dark cycle. Seeds of the two wheat cultivars were surface sterilized with 5% sodium hypochlorite solution for 8 min and then washed six times in sterile distilled water. They were sown into

plastic pots (9 cm) filled with 0.9 kg of sterilized clay soil (five seeds per pot), and arranged in a complete randomized design with three replicates. The potting soil consisted of 57% clay, 39% loam and 2% sand collected from Sojji research station (located east of Damascus, Syria, 33°30′54.4″N 36°07′33.2″E) with pH 7.8, organic matter 1.25%, was air dried, sieved to pass a 2 mm screen, and pasteurized at 5 kGy of Gamma Ray (GR) with 60Co source using a gamma irradiator (ROBO, Russia). Three pots per treatment were left un-inoculated as control treatment. Following emergence, plants were thinned to three per pot (the pot with 9 cm diameter is suitable for 3 wheat plants to be grown to disease assessment) and nitrogen fertilizer (0.078 g/pot) was applied twice at two times: emergence and tillering. The plants were watered once a week until infection. At mid-anthesis stage, a single central floret of the spikelet of the main stem was point single isolate inoculated with 10 μl of a spore suspension (5×10^4 spores/ml) of FHB isolates or sterile distilled water for control treatment using a micropipette. Wheat heads were covered with clear plastic bags for 2 days to provide constant high humidity. FHB resistance (type II) was estimated as the percentage of spikelets on the inoculated spikes with visually detectable disease symptoms 21 Days After Inoculation (DAI).

2.3. Evaluation of FHB Resistance *In Vitro*

The detached-leaf resistance assay was assessed using the methodology described by Browne and Cooke [19, 20], Browne *et al.* [21] and Browne [22], the modified Petri-dish resistance assay was performed using the methodology of Purahong *et al.* [26], and the seedling resistance assay was done using the methodology of Shin *et al.* [23]. All methods are presented in detail by Sakr [24] on barley plants. In the detached-leaf resistance assay [19 - 22], the components measured were: incubation period (period in days from inoculation to first appearance on the leaf surface, a dull gray-green water-soaked lesion), latent period (period in days from inoculation to sporulation), and lesion length (measured after 7 DAI as a visible necrotic area). In the modified Petri-dish resistance assay [26], the components measured were: germination rate reduction and coleoptile length reduction determined by comparison with the water control treatment 6 DAI and standard area under disease progress curve ranged from 0 (very resistant) to 1 (not resistant) and calculated from the percentage of healthy coleoptiles as a function of time (from 2 to 6 DAI). In the seedling assay [23], the components tested were: the percentage of infected seedlings with visible necrotic lesion and/or sporulation of fungal disease symptoms measured 7 DAI and assessed using pin-point and foliar-spraying tests and lesion length with a water-soaked necrotic and/or cholortic area assessed using clip-dipping inocualtion. Each treatment was arranged in a complete randomized design with three replicates, and the experiment was repeated. Results were similar between the experiments and the data from the second experiment are presented.

2.4. Statistical Analyses

The *in planta* and *in vitro* data were performed using StatView, 4.57® Abacus Concepts, Berkley, Canada. Prior to analysis, the percentage of disease severity, germination rate reduction, coleoptile length reduction and infected seedlings were transformed using the angular transformation to stabilize variances. ANOVA incorporating the Fisher's LSD test at the 5% level of significance was used to evaluate differences in the two tested wheat cultivars [34]. The significant wheat cultivar x FHB isolate interactions were assessed for all measured quantitative resistance criteria. The sample correlation coefficients (Pearson *r*) were calculated among data of FHB resistance (Type II) and *in vitro* assays at a significant level of 5%.

3. RESULTS

3.1. FHB Resistance *In Planta*

Results showed that neither of the two tested wheat cultivars: Cham1 (durum) and Cham6 (bread) escaped from head blight disease (Table **1**). Typical FHB symptoms induced by the 16 fungal isolates were clear and easy to score in the inoculated spikelets, while no symptoms were present in the control plants (Fig. **1**). The symptoms showed after 7 DAI, and disease resistance was scored 21 DAI. Point inoculation of central spikelets conducted to asses FHB resistance revealed statistically significant differences (Probability, *p*=0.0261) in the resistance of Cham1 and Cham6, calculated as the average percentage of affected spikelets per ear at 21 DAI. The fraction of plants showing disease symptoms ranged from 32 to 49% on Cham6 and from 30 to 55% on Cham1. The fungus/host interaction for FHB severity was significant (*p*=0.0102). The statistical analysis shows that there is no significant difference between the two tested cultivars, except for F1 and F28 (*F. culmorum*).

3.2. FHB Resistance *In Vitro*

Overall, exposure of treatments for the nine quantitative resistance components on two tested wheat cultivars to 16 FHB isolates reduced mean values relative to the water controls, suggesting a strong effect of different *Fusarium* isolates on the growth of these cultivars (Tables **2-4**). Seedlings of two wheat cultivars growing in the presence of 16 FHB isolates showed typical *in vitro* FHB symptoms according to three tested assays, whereas the control plants did not show any disease symptoms (Fig. **1**).

In the detached-leaf assay Table **2**, all FHB isolates caused lesions on detached leaves of two wheat cultivars by the sixth day post inoculation. No lesions were observed on control leaf segments. Visible lesions were almost oval in shape and appeared as a dull gray-green water-soaked area. Incubation period and lesion length rates, after the inoculation of leaf segments with conidial suspensions of FHB isolates, showed no variation between Cham1 and Cham6 (*p*=0.851, *p*=0.3430, respectively). Mean incubation period on Cham1 was 2.07 days and on Cham7 was 2.17 days. Correlation between incubation period and Type II resistance was not significant (*r*=0.341 not significant, ns). Mean lesion length on Cham1 was 7.57 mm and on Cham7 was 7.45 mm. There was not a statistically significant correlation between lesion length and Type II FHB resistance (*r*=-0.114 ns). The rates of latent period varied widely between the two tested culitvars (*p*=0.0066), ranging from 4.5 to 8.6 days on Cham6 and from 2.0 to 11 days on Cham1. The interaction between fungus and host for latent period was significant (*p*=0.0041). The statistical analysis

Table 1. Fusarium head blight severity (% symptomatic spikelets/spike) for *in planta* Type II resistance under controlled conditions in two Syrian wheat cultivars (Cahm1, durum and Cham6, bread) inoculated with 16 fungal isolates of four Fusarium head blight species.

FHB Species	Fungal Isolates	Disease Severity (%)	
		Cham1	Cham6
F. culmorum	F1	55 a	32 b
	F2	42 a	40 a
	F3	45 a	40 a
	F28	52 a	35 b
	F30	30 a	39 a
F. solani	F7	55 a	49 a
	F20	55 a	40 a
	F26	50 a	42 a
	F29	40 a	42 a
	F31	44 a	45 a
	F35	47 a	44 a
F. verticillioides	F15	43 a	35 a
	F16	33 a	45 a
	F21	35 a	44 a
	F27	38 a	37 a
F. equiseti	F43	40 a	38 a
-		F isolates=1.913; P=0.0381	
-		F cultivars=5.187; P=0.0261	
-		F interactions=2.326; P=0.0102	

According to the Fisher's LSD test, means followed by the same letter are not significantly different at $p<0.05$, F tests ($p<0.05$) (F), Probability (P).

Fig. (1). *in planta* and *in vitro* inoculations techniques used to assess Fusarium head blight resistance on plant materials of Syrian durum wheat cultivar Cham1 infected with F1 (*F. culmorum*), **(a)** point inoculation assay, **(b)** detached-leaf assay, **(c)** foliar-spraying assay, **(d)** clip-dipping assay, **(e)** pin-point assay and **(f)** modified Petri-dish assay.

Table 2. Disease responses of *in vitro* detached leaf assay in two Syrian wheat cultivars (Cahm1, durum and Cham6, bread) inoculated with 16 fungal isolates of four Fusarium head blight species.

FHB Species	Fungal Isolates	Incubation Period (days)		Latent Period (days)		Lesion Length (mm)	
		Cham1	Cham6	Cham1	Cham6	Cham1	Cham6
F. culmorum	F1	2.27	2.23	5.5 a	6.3 a	7.84	7.43
	F2	2.08	2.23	4.1 a	6.1 a	7.58	7.20
	F3	1.98	2.27	4.0 a	5.3 a	7.43	7.51
	F28	2.23	2.20	4.5 a	5.3 a	7.43	8.01
	F30	2.27	1.98	6.6 a	6.3 a	7.51	6.77
F. solani	F7	2.03	2.35	7.5 a	8.6 a	7.62	7.31
	F20	2.35	1.97	11 a	7.5 b	7.12	7.31
	F26	2.04	2.35	7.5 a	7.4 a	7.12	7.84
	F29	2.03	2.35	6.5 a	7.5 a	7.31	7.12
	F31	2.03	1.98	5.1 a	5.6 a	8.02	7.12
	F35	1.95	2.10	6.6 a	6.5 a	7.43	7.43
F. verticillioides	F15	1.98	2.23	4.5 a	4.9 a	8.28	7.84
	F16	2.04	1.95	2.0 b	5.1 a	7.21	7.86
	F21	1.73	2.23	5.5 a	7.5 a	7.59	7.12
	F27	1.98	2.04	5.1 a	4.5 a	7.86	7.84
F. equiseti	F43	2.08	2.23	5.6 a	7.5 a	7.84	7.43
Mean		2.07 a	2.17 a			7.57 a	7.45 a
-		F isolates=0.158 ns; P=0.9999		F isolates=10.224; P=0.0001		F isolates=0.894 ns; P=0.5743	
-		F cultivars=0.851 ns; P=0.3599		F cultivars=7.872; P=0.0066		F cultivars=0.913 ns; P=0.3430	
-		F interactions=0.291 ns; P=0.9948		F interactions=2.604; P=0.0041		F interactions=0.909 ns; P=0.5585	

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 tests ($p<0.05$) (F), Probability (P).

Table 3. Disease reactions of *in vitro* Petri-dish assay in two Syrian wheat cultivars (Cahm1, durum and Cham6, bread) inoculated with 16 fungal isolates of four Fusarium head blight species.

FHB Species	Fungal Isolates	Germination Rate Reduction (%)		AUDPC$_{standard}$		Coleoptile Length Reduction (%)	
		Cham1	Cham6	Cham1	Cham6	Cham1	Cham6
F. culmorum	F1	22	23	0.49	0.30	58	57
	F2	22	21	0.41	0.38	58	52
	F3	23	19	0.49	0.38	57	58
	F28	22	22	0.49	0.34	52	62
	F30	17	23	0.37	0.37	58	52
F. solani	F7	23	20	0.52	0.44	59	56
	F20	20	23	0.52	0.39	55	56
	F26	23	18	0.47	0.41	55	59
	F29	23	20	0.43	0.41	56	55
	F31	18	20	0.43	0.36	48	55
	F35	18	20	0.47	0.43	59	48
F. verticillioides	F15	18	19	0.40	0.27	58	59
	F16	17	19	0.36	0.34	55	60
	F21	22	17	0.54	0.38	58	55
	F27	19	17	0.36	0.38	60	51
F. equiseti	F43	22	21	0.54	0.35	60	60
Mean		21 a	20 a	0.46 a	0.37 b	57 a	56 a
-		F isolates=0.429 ns; P=0.9647		F isolates=2.015; P=0.0276		F isolates=0.763 ns; P=0.7113	
-		F cultivars=0.250 ns; P=0.6185		F cultivars=30.421; P=0.0001		F cultivars=0.385 ns; P=0.5373	
-		F interactions=0.501 ns; P=0.9311		F interactions=1.252; P=0.2588		F interactions= 1.384 ns; P=0.1825	

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 tests ($p<0.05$) (F), Probability (p). In the current study, isolates F2, F35, F27 and F43 were reanalyzed on Cham1 and Cham6, however, disease reaction for the four isolates was analyzed previously and presented by Sakr [8].

Table 4. Disease reactions of *in vitro* seedling assay in two Syrian wheat cultivars (Cahm1, durum and Cham6, bread) inoculated with 16 fungal isolates of four Fusarium head blight species.

FHB Species	Fungal Isolates	Spraying (%)		Pin-Point (%)		Clip-Dipping (cm)	
		Cham1	Cham6	Cham1	Cham6	Cham1	Cham6
F. culmorum	F1	45	45	41	41	2.23	2.27
	F2	45	42	40	41	2.23	2.27
	F3	45	44	41	40	2.27	2.20
	F28	44	47	43	41	2.20	2.35
	F30	34	45	39	40	2.20	2.55
F. solani	F7	47	41	41	41	2.35	2.03
	F20	39	47	41	38	1.97	2.27
	F26	47	41	39	41	2.35	2.03
	F29	47	47	45	39	2.35	2.35
	F31	35	35	43	34	2.27	1.76
	F35	35	41	39	41	2.20	2.03
F. verticillioides	F15	37	51	44	43	1.83	2.20
	F16	35	41	41	39	1.73	2.03
	F21	45	39	43	41	2.23	1.93
	F27	39	41	40	42	1.93	2.20
F. equiseti	F43	45	45	43	42	2.23	2.27
Mean		41 a	43 a	41 a	40 a	2.16 a	2.17 a
-		F isolates=0.246 ns; P=0.9979		F isolates=0.684 ns; P=0.7907		F isolates=0.364 ns; P=0.9836	
-		F cultivars=0.190 ns; P=0.6641		F cultivars=2.391 ns; P=0.1269		F cultivars=0.008 ns; P=0.9281	
-		F interactions=0.481 ns; P=0.9421		F interactions=0.887 ns; P=0.5817		F interactions=0.385 ns; P=0.9784	

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 tests ($p<0.05$) (F), Probability (P).

shows that there is no significant difference between the two analyzed cultivars, except for F20 (*F. solani*) and F16 (*F. verticillioides*). Correlation coefficients between latent period and Type II FHB resistance were significant (r=0.545 with $p<0.05$).

In the modified Petri-dish assay Table **3**, all the 16 FHB isolates tested with this *in vitro* assay caused brown spots on the coleoptiles, and/or mycelium that completely covered the seeds of the two tested wheat cultivars. ANOVA detected significant differences for standard Area Under Disease Progress Curve (AUDPC$_{standard}$) (p=0.0001) and non-significant differences for germination rate reduction and coleoptile length reduction between Cham1 and Cham6 (p=0. 6185, p=0.5373, respectively). There were substantial differences in AUDPC$_{standard}$ between the two tested cultivars, with rates scored a value of 0.37 on Cham6 and 0.46 on Cham1. AUDPC$_{standard}$ of Cham6 was less 17.78% than Cham1. Correlation coefficients between AUDPC$_{standard}$ and adult FHB resistance were significant (r=0.659 with $p<0.01$). Mean germination rate reduction on Cham1 was 21% and on Cham6 was 20%. Germination rate reduction did not show a correlation with disease severity (r=0.386 ns). Diseased ~5 mm long coleoptiles were only one-half of mean lengths of healthy coleoptiles that reached 11.0 mm and 9.39 mm on Cham1 and Cham6, respectively whatever was the FHB isolate. Mean coleoptile length reduction on Cham1 was 57% and on Cham6 was 56%. A statistically significant correlation between coleoptile length reduction and FHB resistance-Type II was not detected (r = -0.153 ns).

In the seedling assay Table **4**, seedlings inoculated with

conidia from *Fusarium* species showed visible fungal disease symptoms after 3 DAI. Brown lesions developed from the inoculated sections in three-day old seedling coleoptiles and stems. Seven days after inoculation, the seedlings of the two cultivars exhibited varying degrees of visual necrosis as a consequence of the seedling resistance. Seven-day old visually necrotic area exhibited varying degrees as a consequence of the seedling resistance. No significant differences were observed between Cham1 and Cham6 for percentage of infected seedlings following foliar-spraying inoculation, percentage of infected seedlings following pin-point inoculation and lesion length following clip-dipping inoculation (41%, 41% and 2.16 cm on Cham1 and 43%, 40% and 2.17 cm on Cham6) (p=0.6641, p=0.1269, p=0.9281, respectively). Correlation between Type II resistance and these three *in vitro* components was not significant (r= 0.150 ns, r=-0.230 ns, and r=-0.037 ns, respectively).

4. DISCUSSION

Knowledge of Fusarium Head Blight (FHB) resistance in wheat gene pool could be pivotal in forecasting schemes for disease control and reducing damage inflicted by FHB [2, 7, 8]. Type I and Type II resistance detected using *in planta* spray and point inoculations (visual scoring of diseased head and spikelet symptoms) is critical for the selection of resistant or tolerant wheat cultivars. However, the head and floret inoculation techniques are costly, time consuming, laborious and can be greatly influenced by growth stage and environmental factors [2, 5, 7, 8]. More rapid and accurate methods to identify resistance to FHB infection are need for cereal breeding. Syrian wheat cultivars may constitute a valuable genetic resource,

possessing various desirable agronomic traits, including acceptable-level resistance to FHB [15 - 17]. To update our knowledge, we analyzed the relationship between *in vitro* resistance assays and *in planta* adult resistance in two widely grown Syrian durum and bread wheat cultivars.

Durum wheat is more susceptible to FHB infection than bread wheat [5]. The data show that the two cultivars differed in their FHB resistance and susceptibility behaviour, in which the spread of the pathogen within the head (type II resistance) was related to cultivar resistance [35]. *In planta* testing provides evidence that significant differences were detected between Cham6 (bread) and Cham1 (durum). Cham1 seems to exhibit more FHB disease severity than Cham6. Thus, Cham6 appears to be more resistant than Cham1. Our results are in accordance with those found by Alazem [15], in which the mean disease score for Type II resistance measured under controlled conditions of 32 fungal isolates of nine FHB species was 13.40% on Cham6 and it was considered as resistant. Moreover, Alkadri *et al.* [17] observed that Cham1 was susceptible after inoculation with Syrian and Italian *F. culmorum* strains in the growth chamber and in the field and the mean disease development score for Type II resistance was recorded 25.7%. Indeed, four durum Syrian wheat landraces had a low mean FHB rating (19–25%) compared to German durum varieties (56–60%) under German field conditions [16]. Since Alazem [15] and Alkadri *et al.* [17] conducted their studies under similar conditions that our study, the differences in the values of disease severity rating for the two wheat cultivars (from 32 to 49% on Cham6 and 30 to 55% on Cham1) are comparable with the previous data [15, 17]. One possibility could be that the isolates used are more aggressive, however, further investigation is required in order to draw any final conclusions. It appears possible that wide usage of wheat cultivars showing high levels of quantitative resistance could lead to increases in aggressiveness in FHB as observed for the pathosystem *Mycosphaerella graminicola / T. aestivum* [36]. Despite these differences in disease severity scores, the standard Area Under Disease Progress Curve (AUDPC$_{standard}$) did indicate that Cham6 was more resistant than Cham1.

Detached leaf, modified Petri-dish and seedling assays were selected on the basis of their similarity to adult plant spikelet inoculation using point inoculation assay [19, 21 - 23, 26]. These assays provide different experimental conditions by simulating the interaction between wheat tissues and fungi. FHB inocula were put directly on the wheat seeds, leaves and seedlings (without glumes) and they could directly penetrate and infect germinating seeds as well as leaves and seedlings. Thus, disease development is manifested through appearance of symptoms such as brown spots on the coleoptiles and/or by mycelium completely covering the seeds, discoloured, malformed, necrotic or chlorotic areas on the infected plant part [19, 21 - 23, 26]. Although the detached leaf and modified Petri-dish assays predicted initial infection after spray inoculation [19, 21, 22, 26], however, FHB resistance determined by seedling assay did not correlate with type I resistance in adult plants [23]. Thus, we hypothesized that fungal spread within the head after point inoculation, type II resistance, will be more appropriate to establish relationships with the three tested *in vitro* assays in one experimentation as noted for detached leaf

[19, 21, 22], modified Petri-dish [26] and seedling [23] assays.

Effects of incubation period and lesion length did not appear to be important characteristics of quantitative resistance to FHB in Syrian wheat cultivars. Incubation period and lesion length did not distinguish between Cham1 and Cham6 showing different levels of type II quantitative resistance. Moreover, results from these two assays were poorly correlated with resistance in adult plants. Our results are in contradiction with those reported for commercially grown European wheat cultivars [19] and U.S. soft red winter wheat entries [21], in which shorter or longer incubation periods were correlated with higher levels of quantitative resistance according to origin of wheat cultivars. Furthermore, the results in the present research are not in accordance with those previously obtained for lesion length; moderately resistant European cultivars and four moderately resistant U.S. commercial cultivars had short lesion lengths in the detached leaf assay [19, 21]. In contrast to our findings, Browne [22] found that shorter incubation periods and lesions have been related to an important component of whole-wheat FHB resistance measured by single point inoculation (type II). It could be that dichotomy among Syrian, European and U.S. wheat cultivars reflects the different genetic control of the highly effective FHB resistance as hypothesized by Browne and Cooke [19]. However, there were significant differences in the resistance of two wheat cultivars as measured by Latent Period (LP). LP appears to be distinctive of the resistance or susceptibility levels in wheat to FHB infection at early stages, and longer latent periods has related to type II resistance on adult spikes. Similarly, longer latent periods have been related to FHB resistance (type II) under controlled conditions [22]. Also, our results agree with those reported for resistant European and U.S. wheat cultivars [19, 21]. Also, the presence/absence of certain QTLs may explain these variations in the resistance responses of wheat cultivars following infection with FHB agents since some QTLs, *i.e.*, *Fhb1* and *Qfhs.ifa-5A*, has related to most effective wheat resistance to FHB [10]. The pattern of chlorosis formation was similar to its observed pattern in the detached leaf assay described by Browne and Cooke [20]; chlorosis of the infected area occurred when lesions first developed.

The reductions in germination rate and coleoptile length were not reliable methods to distinguish the two tested wheat cultivars. Our results are in accordance with those previously obtained *in vitro* [28, 29]. Moreover, FHB resistance determined by reductions in germination rate and coleoptile length was also poorly correlated with resistance in adult plants. 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 [18 - 22]. Soresi *et al.* [24] found that coleoptile length was related with head blight resistance. Contrary, Shin *et al.* [23] noticed that reductions in germination rate were not correlated with FHB types I and II resistance. AUDPC$_{standard}$ was of primary importance across all whole FHB disease components, accounting for the reduction of 17.78% between the two tested cultivars. AUDPC$_{standard}$ was calculated from the decreasing number of healthy wheat seedlings after fungal inoculation of the seeds [26]. The slower the reduction of the number of

healthy seedlings, the more resistant is the cultivar [26]. Our results are in accordance with previous *in vitro* analysis in which this criterion did distinguish between wheat cultivars [28, 29]. Therefore, AUDPC_{standard} seems to be an efficient criterion for screening FHB resistance at early stages and has related to type II resistance on adult spikelets. *In vitro* AUDPC_{standard} data were highly significantly correlated with artificial inoculation data in the growth chamber on durum and bread wheat cultivars (Types I and II) [26, 27, 30, 31].

The three quantitative components: percentage of infected seedlings of foliar-spraying, pin-point inoculations and lesion length of clip-dipping inoculation did not differentiate between the two tested Syrian wheat cultivars carrying different levels of quantitative resistance. Furthermore, results from these three *in vitro* components were not correlated with adult FHB resistance. However, lesion length was highly related with FHB head resistance [23]. Also, the percentage of infected seedlings (of foliar-spraying and pin-point inoculations) was poorly correlated with adult FHB resistance [23]. Thus, these three assays were not efficient methods to predict Type II resistance in adult plants. This is not surprising since the correlation between some *in vitro* assays and *in planta* indices for Type II was not stable and depended on resistance source origin; the exotic wheat germplasms which provide highly effective resistances to FHB resistance do not appear to be detected in the detached leaf [19, 20] or seed germination [18] assays.

Quantitative resistant wheat cultivars are identified by low *in planta* disease spikelet values of the fungus compared with the susceptible one [7 - 9], and long Latent Period and less AUDPC_{standard} determined *in vitro* [19 - 22, 26]. Cham1 seems to exhibit more FHB disease severity and less latent period than Cham6. AUDPC_{standard} of Cham6 was 17.78% less that that of Cham1. Thus, Cham6 appears to be more resistant than Cham1 detected using *in vitro* and floret inoculation assays. This observation suggests that in Cham6, the development of the FHB pathogens was slowed down, and might be due to resistance mechanisms expressed by differential responses conferred by QTL during FHB infection in wheat plants [10].

In this research, the two *in vitro* components, latent period (LP) and AUDPC_{standard}, partially reproduced *in planta* testing may provide a better understanding to describe the level of wheat resistance to FHB infection. Since greater FHB disease-type II resistance were related to longer latent period and less AUDPC_{standard}, these two *in vitro* components could reflect aspects of a resistance reaction by the plant rather than simply susceptibility and pathogen development. For example, LP and AUDPC_{standard} may indicate tolerance of infection by the host, and asymptomatic colonization, rather than inhibition of pathogen development [18]. Also, the measured differences in LP and AUDPC_{standard} appear to be different aspects of similar response mechanisms against FHB in the whole plant at the cellular level [18]. Thus, this work supports the view that the current model of types of resistance is an oversimplification of the interacting mechanisms underlying expression of FHB resistance [22, 25]. The two *in vitro* components, LP and AUDPC_{standard}, as indicators of mechanisms of resistance occurring in the whole plant during FHB infection, did predict

in planta Type II resistance in adult plants, and the other seven tested components did not. It appears that the criteria such as latent period and AUDPC_{standard} may be useful to measure quantitative resistance in wheat to FHB at early stages as reported for *in vitro* barley resistance [25]. However, the other measured seven *in vitro* components seem to be not distinctive of the resistance or susceptibility levels in wheat to FHB infection at subsequent stages of disease development as noted for *in vitro* FHB-barely association [25].

In wheat- and barley-FHB researches, it has been proposed that the individual *in vitro* and *in planta* components not only influence the total level of resistance but also have a variable individual influence on the disease development [23, 25]. Furthermore, this work reflects the polygenic nature of head blight resistance and the importance of *in vitro* methods to determine specific sources/mechanisms of FHB resistance, which cannot be distinguished on the basis of *in planta* whole-plant resistance alone as proposed by Browne and Cooke [19]. The biological explanation for an association between *in vitro* and *in planta* responses to FHB infection remains unknown, but it can be hypothesized that similar genetic pathways become activated at both developmental stages [24]. Since only two wheat cultivars were tested here, further research using a large sample of available Syrian wheat cultivars is needed to validate our results *in vitro*, under controlled and field conditions.

CONCLUSION

The current research provides interesting *in vitro* and *in planta* wheat quantitative resistance data. Bread wheat appears to be more resistant than durum detected using *in vitro* and *in planta* floret inoculation assays. The two *in vitro* components, LP and AUDPC_{standard}, could be of potential use in predicting *in planta* adult FHB wheat resistance. Detached leaf and modified Petri-dish also proved to be high-throughput methods and reliable pre-screening methods for FHB resistance. Using these techniques, it should be possible to screen large wheat populations during the earlier stages in breeding programs, and develop wheat cultivars with improved FHB resistance. It is important to determine QTLs associated with FHB resistance in Syrian wheat cultivars. Furthermore, it will be necessary to analyze the relationship between *in vitro*-determined quantitative resistance components and FHB resistance using *in planta* spray inoculation, termed Type I resistance to better understand the utility of *in vitro* components involved in the expression of FHB resistance.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

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