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 (AUDPCstandard) of modified Petri-dish inoculation. Correlation coefficients between FHB severity and the two in vitro components LP and AUDPCstandard 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 AUDPCstandard were related to greater FHB disease-type II resistance.

Conclusion: LP and AUDPCstandard 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, Latent 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
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 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 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 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. verticilloides* (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 morphologically 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 microscope 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...
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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 Sojii 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 × 10⁴ 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 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 chlorotic area assessed using clip-dipping inoculation. 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 assess 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 cultivars (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     |          |

According to the Fisher’s LSD test, means followed by the same letter are not significantly different at *p*<0.05. *F* isolates=1.913; *P*=0.0381; *F* cultivars=5.187; *P*=0.0261; *F* interactions=2.326; *P*=0.0102.

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  | 2.35  | 5.6 a | 7.5 a | 7.84  | 7.43  |

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 (%) | AUDP<sub>std</sub> | 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    |
| *F. verticillioides* | F35         | 18    | 20    | 0.47  | 0.43  | 59    | 48    |
|                 | 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    |

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 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 |
|                   | 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. verticillioides* | F43          | 45 | 45 | 43 | 42 | 2.23 | 2.27 |
| Mean              |                | 41 a | 43 a | 41 a | 40 a | 2.16 a | 2.17 a |

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<sub>standard</sub>) (*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<sub>standard</sub> between the two tested cultivars, with rates scored a value of 0.37 on Cham6 and 0.46 on Cham1. AUDPC<sub>standard</sub> of Cham6 was less 17.78% than Cham1. Correlation coefficients between AUDPC<sub>standard</sub> 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 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* = -0.230 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 behavior, 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\textsubscript{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, discoulored, malformed, necrotic or chlorotic areas on the infected plant part [19, 21 - 23, 26]. Although the detached leaf and modified Petridish 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\textsubscript{standard} was of primary importance across all whole FHB disease components, accounting for the reduction of 17.78% between the two tested cultivars. AUDPC\textsubscript{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_{autot} 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_{autot} 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 resistant and the susceptible one [7 - 9], and long Latent Period and less AUDPC_{autot} determined in vitro [19 - 22, 26]. Cham1 seems to exhibit more FHB disease severity and less latent period than Cham6. AUDPC_{autot} 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_{autot} 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_{autot}, 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_{autot} 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_{autot} 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_{autot} 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_{autot} 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_{autot} 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

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