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Assessment of Different Resistance Types of Syrian Durum Wheat Cultivars Towards FHB Agent

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ABSTRACT

Wheat is one of the most important crops in Syria, for both local consumption and export commodity and can be infected by *Fusarium* Head Blight (FHB) a world-widespread disease. *Fusarium* Head Blight reduces yield, grain quality and causes accumulation of deoxynivalenol (DON). The most promising and effective management strategy is to avail cultivars resistant to FHB. Ten Syrian durum wheat cultivars and “Simeto”, one of the most susceptible Italian cultivars, were artificially infected, under growth chamber and field conditions. They were inoculated with Syrian and Italian strains of *Fusarium culmorum*, one of the main agents of FHB, to screen their tolerance toward FHB infection, FHB spread, kernel infection and mycotoxin levels. Jory was the most tolerant cultivar in the growth chamber and field, while Sham 9, was the most susceptible.

Key words: *Fusarium culmorum*, wheat, syrian cultivars, tolerance, *Fusarium* head blight

INTRODUCTION

Fusarium Head Blight (FHB) or scab is a serious global cereal disease caused by several *Fusarium* species, mainly *Fusarium culmorum* (W.G. Sm.) Sacc. and *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein) Petch]. *Fusarium* Head Blight (FHB) of wheat causes great yield losses (Brennan *et al.*, 2005), bleached and shrunken kernels, decreasing baking quality due to destruction of starch, proteins and cell walls of the infected kernels (Shinha and Bhatnagar, 1998). Some *Fusarium* species, besides colonization of host tissues, held to a most serious human and animal health risk for mycotoxin accumulation in food and feed (Pestka, 2010). Mycotoxins are fungal poisonous chemical compounds and are also involved in the inhibition of host resistance reactions (Maier *et al.*, 2006). The most important mycotoxins associated with FHB are deoxynivalenol (DON) and nivalenol (NIV), trichothecenes type (A) and zearalenone (Muller *et al.*, 2012).

Control strategies are difficult and expensive. Breeding for resistance, besides different control practices (Xu and Nicholson, 2009), has been lately worldwide considered of

high priority since it seems to be the most promising (Browne and Cooke, 2005) and the most cost-effective tool to control the disease (Buerstmayr *et al.*, 2000). Up to now no wheat cultivars are immune to FHB agents, most of them are susceptible and only a few are moderately resistant (Parry *et al.*, 1995; Cai *et al.*, 2005). *Triticum durum* L. is more susceptible than *Triticum aestivum* L. (common wheat) and its grains are exposed to a higher mycotoxin concentration (Stack *et al.*, 2002).

Mesterhazy (1995) found that wheat cultivars have very similar resistance reactions against *F. graminearum* and *F. culmorum*. Resistance of wheat to FHB is a complex phenomenon; It is a quantitative character and different components of physiological resistance should be considered: (1) Resistance to initial infection, (2) Resistance to spreading, (3) Resistance to kernel infection, (4) Tolerance to infection and (5) Resistance to DON accumulation (Mesterhazy, 2002).

Wheat (durum and common) is one of the most important crops in Syria, for local consumption and export commodity (NAPC., 2006). The cultivation area is divided into five sub-regions, according to agro-meteorological conditions (FAO., 2003).

There are no reports about the presence of FHB in Syria, but *Fusarium* species are present and frequently isolated in Syrian wheat kernel samples (Alkadri *et al.*, 2013). The main species isolated were *F. culmorum* and in less quantity, *F. graminearum* (Alkadri *et al.*, 2013).

Studies on the FHB resistance of Syrian cultivars are, to our knowledge, very limited; therefore, in this research for the first time, a screening over Syrian durum wheat cultivars in a multi approach experiment (field and growth chamber) has been conducted.

The aim of the study was to investigate varietal differences in ten Syrian durum wheat cultivars, after inoculation with Syrian and Italian *F. culmorum* strains, in comparison with an Italian susceptible variety. *Fusarium* Head Blight (FHB) disease severity, kernel infection and mycotoxin accumulation, are used to define Mesterhazy's resistance types.

MATERIALS AND METHODS

Plant materials and fungal strains: Ten Syrian durum wheat cultivars, the most cultivated in different Syrian areas, were kindly offered by Arab Center for the Study of Arid zones and Dry lands (ACSAD-Syria) to set up experimental trials in the field and growth chamber. The susceptible Italian cultivar, "Simeto", was used as positive control (Table 1).

Six *F. culmorum* strains (F960, F961 and F966 from Syria and F11, F24 and F35 from Italy), isolated from durum wheat kernels, characterized in 3-ADON chemotype for the presence of gene *Tri 12* (Alkadri *et al.*, 2013; Prodi *et al.*, 2011) and for the aggressiveness (Alkadri, 2012) in the laboratory of Phytopathological Mycology-Department of Agricultural Sciences-University of Bologna (Italy), were used for the artificial inocula.

Macroconidia production: Each *F. culmorum* strain was cultured on potato dextrose agar (PDA, Difco) plates for 7 days. Two mycelium plugs were cut from each strain, were submerged into flasks with autoclaved V8 broth (Singleton *et al.*, 1992) and placed in a refrigerated horizontal type shaker at 140 rpm, 25°C under incident sun light for 2 weeks. The mixture of macroconidia with mycelium in the V8 medium was filtered through a sterile syringe with double layers of autoclaved cheesecloth. Macroconidia suspension was adjusted for each strain to 1×10^4 mL⁻¹ conidia for floret inoculations in the growth chamber and to 2×10^5 mL⁻¹ conidia for ear inoculations in the field trials (Purahong *et al.*, 2012).

Floret inoculations in growth chamber: Seeds of the eleven durum wheat cultivars were seeded in trays. After 15 days, each seedling was transplanted into a pot and placed in a growth chamber at 25/19°C day/night temperature, 14/10 h light/dark cycle. One week after transplantation, approximately 3 g of commercial fertilizer (N/P/K) were applied to each plant. The plants were watered three times a week until harvest to avoid water stress conditions. At anthesis (BBCH 63),

20 µL of suspension, at a concentration of 1×10^4 mL⁻¹ conidia, for each strain were injected into two florets (10 µL per floret) at the middle of each spike, between lemma and palea without wounding the ovary. Spikes were then covered with polyethylene bags for 48 h to ensure constant high humidity. For each isolate eight spikes were inoculated. Each pot was considered as a replication and disposed in a completely randomized design. The florets of the control spikes were injected with water. In total, for each cultivar, 56 spikes were evaluated. Diseased-Head Severity (DHS) evaluation was carried out at 7, 14 and 21 days after inoculation (DAI) and based on the percentage of infected area on individual head with a modified Parry's scale (Purahong *et al.*, 2012): 0% (no symptoms), 2, 5, 10, 25, 50, 75 and 90% (90% or more of bleached area). Diseased-Head Severity is defined as the average proportion of diseased spikelets per diseased spike (sum of the proportion of diseased spikelets per diseased spike divided by the total number of diseased spikes sampled).

The average value of DHS caused by all *F. culmorum* strains is considered, for each cultivar, a parameter to determine type I resistance. Mean severity (average of DHS values of 1st, 2nd and 3rd evaluations), terminal severity (the 3rd DHS evaluation) and disease development (the means of each evaluation; 7, 14 and 21 DAI-plotted over the estimation time), were used to determine type II resistance for each cultivar.

Koch's postulate was fulfilled by the re-isolation of *F. culmorum* from the infected spikes.

Ear inoculations in the field: The eleven durum wheat cultivars were sown in the field of the experimental farm of the University of Bologna (Cadriano, 44°33'4.15"N; 11°24'39.02"E) in autumn 2010-2011. The field was subdivided into 44 micro-plots at double rows (1 m length, 15 cm between the row and 20 cm between micro-plots). Two hundred seeds were sown in each micro-plot for each cultivar (100 per row).

Two mixtures of fungal suspensions: the first was a mixture of the Syrian *F. culmorum* strains (F960, F961 and F966) and the second of the Italian strains (F11, F24 and F35), at concentration 2×10^5 mL⁻¹ macroconidia, were prepared. At 30% anthesis (BBCH 63), 60 mL of each conidial suspension were sprayed on each micro-plot using a hand sprayer. The experimental field contained 44 micro-plots disposed in a completely randomized design; 22 inoculated with Syrian strain mixtures and 22 inoculated with Italian ones. The experiment was repeated twice on the 11 cultivars. Natural rain on the inoculation day ensured high humidity, so no additional irrigation was applied.

Five groups of 10 spikes per micro-plot were chosen randomly and marked with plastic labels for disease assessment. Diseased-Head Severity (DHS) and Disease Incidence (DI) at 14 and 21 Days After Inoculation (DAI) were evaluated. The DHS was determined as previously described for the growth chamber. The DI was calculated as

the proportion of diseased spikes (number of infected spikes divided by the total number of spikes sampled) (50 spikes per replicate). Mean FHB index was calculated as the product of DI and DHS divided by 100.

The Hectoliter Weight (HW) was measured after the harvest at BBCH 99. *Fusarium* Damage Kernels (FDK) was determined on one hundred seeds for each replicate, estimating visually the number of scabby "tombstone" infected kernels and recorded as percentage of FDK (Mesterhazy *et al.*, 1999). Koch's postulate was fulfilled by the re-isolation of *F. culmorum* from the infected kernels.

DON analysis: Wheat grains obtained from the field experiment were ground and DON was estimated by AgraQuant DON Kit (Romer Labs, Austria), an enzyme immunoassay for the quantitative analysis of DON in cereals. The analysis were set up following the condition indicated by manufacturer; starting from 20 g of each sample shaken into flasks of 300 mL containing 100 mL double distilled water in a rotary shaker (200 rpm) for 3 min. Two replicates for each cultivar inoculated with each mixture were analyzed and DON content was calculated using a microtiter plate spectrometer (OpsysMR, Dynex technologies) and a software package distributed by the manufacturer.

Statistical analysis: Data analysis was performed using SPSS (SPSS Inc. Chicago, IL, v17, 1993-2007). The correlation coefficients among different variables were determined using the Pearson product-moment correlation at a significant level of 5%. ANOVA incorporating the post hoc "Tukey" test at the 5% level of significance, was used to differentiate the means.

RESULTS

Floret inoculations in growth chamber: Typical FHB symptoms were observed in the inoculated spikelets while, no symptoms were present in the control.

The values of DHS evaluations (%) at 7-14-21 DAI for all the cultivars are shown in Fig. 1, cv Jory had the lowest DHS values: DHS-7DAI = 6.8, DHS-14DAI = 17.2, DHS-21 DAI = 28.5.

Based on the terminal severity assessment at 21 DAI, Jory and ACSAD1333 were the most tolerant cultivars (28.6 and 35%, respectively), while all the other Syrian cultivars, except Horani (50.8%), were more susceptible than Simeto (56.8%) (Table 1).

The difference among cultivars, based on the mean diseased head severity, was not significant.

Disease development, statistically significant, ranged between 10.87 for Jory and 30.8 for Sham 5 (Table 1).

Ear inoculations in the field and DON analysis: The inoculated cultivars either with Syrian or Italian inoculum showed the same behaviour (sig (2-tailed) = 0.393 > 0.05 using independent t-test).

The mean values of FHB index at 14 DAI ranged, for all the different cultivars, from 1.72-7.36% and at 21 DAI from

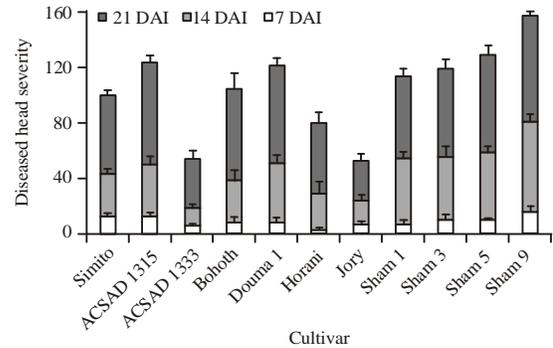


Fig. 1: Diseased-head severity evaluations (7, 14 and 21 DAI), in growth chamber for the eleven durum wheat cultivars tested, Error bars represent the standard error of mean

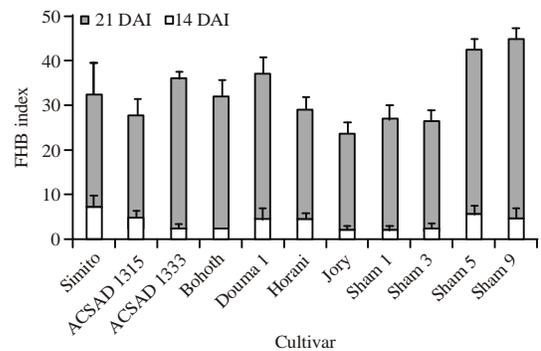


Fig. 2: FHB index evaluation (14 and 21 DAI) in %, in the field for the eleven durum wheat cultivars tested, Error bars represent the standard error of mean

Table 1: Values of Diseased-Head Severity (DHS) and disease development in growth chamber for the eleven durum wheat cultivars tested

Cultivar	Mean DHS	Terminal DHS	Disease development
Simeto	33.32 ^a	56.8 ^{bc}	21.97 ^{bc}
ACSAD 1315	41.27 ^a	73.6 ^c	30.41 ^c
ACSAD 1333	18.01 ^a	35.0 ^{ab}	14.40 ^{ab}
Bohoth1	34.73 ^a	65.8 ^c	28.74 ^c
Douma1	40.38 ^a	70.3 ^c	30.70 ^c
Horani	26.57 ^a	50.8 ^{bc}	23.75 ^{bc}
Jory	17.53 ^a	28.6 ^a	10.87 ^a
Sham 1	37.86 ^a	58.9 ^{bc}	25.73 ^c
Sham 3	39.61 ^a	63.8 ^c	26.58 ^c
Sham 5	43.03 ^a	70.9 ^c	30.83 ^c
Sham 9	52.39 ^a	76.4 ^c	30.02 ^c

*Means with the same letter within a column are not significantly different at p = 0.05 according to Tukey test

21.98-40.81 (Table 2). Jory (21.98%) and ACSAD1333 (23.50%) were the most tolerant cultivars, showing significant difference with the most susceptible cultivar Sham 9 (40.81%). The disease development (type II) among all the cultivars was different using ANOVA test; Simeto, Jory, Horani, ACSAD1333 showed a low variability (17.91, 19.91, 20.31 and 20.97, respectively), while Sham 9 had the highest value (36.16) (Table 2 and Fig. 2).

Table 2: Field trial: values of FHB index (%) in the 1st and 2nd evaluations after inoculation with Syrian and Italian *F. culmorum* strains (mix) in the eleven durum wheat cultivars tested, Disease development (%), Fusarium-Damaged Kernels (FDK) in %, Hectoliter Weight (HW) in kg hL⁻¹ and DON level in ppm

Cultivar	FHB index						Disease development type II	FHB variables		
	Syrian mix		Italian mix		2nd mean FHB index type I	FDK type III		HW	DON type V	
	1st evaluation	1st evaluation	1st mean FHB index	2nd evaluation						2nd evaluation
Simeto	3.42	11.30	7.36	13.96	36.58	25.27 ^{ab}	17.91 ^a	34 ^a	94.09 ^{bc}	1.88 ^a
ACSAD1315	2.56	6.51	4.53	23.31	23.68	23.50 ^{ab}	29.40 ^{ab}	45 ^a	91.96 ^{ab}	1.67 ^a
ACSAD1333	2.85	2.21	2.53	39.64	28.23	33.94 ^{ab}	20.97 ^a	32 ^a	92.87 ^{abc}	1.18 ^a
Bohoth1	1.63	3.15	2.39	24.05	35.75	29.90 ^{ab}	27.51 ^{ab}	45 ^a	93.44 ^{abc}	2.10 ^a
Douma1	0.79	8.77	4.78	28.82	36.57	32.70 ^{ab}	27.92 ^{ab}	41 ^a	93.58 ^{abc}	1.41 ^a
Horani	4.47	4.69	4.58	21.13	28.64	24.89 ^{ab}	20.31 ^a	30 ^a	95.26 ^{bc}	1.81 ^a
Jory	3.03	1.10	2.06	22.19	21.76	21.98 ^a	19.91 ^a	33 ^a	94.30 ^{bc}	0.33 ^a
Sham 1	2.08	1.35	1.72	29.56	21.82	25.69 ^{ab}	23.98 ^{ab}	39 ^a	94.73 ^{bc}	0.75 ^a
Sham 3	3.25	1.16	2.20	26.86	22.53	24.70 ^{ab}	22.49 ^{ab}	33 ^a	96.10 ^c	0.86 ^a
Sham 5	5.56	6.15	5.85	38.79	34.52	36.65 ^{ab}	30.80 ^{ab}	46 ^a	90.01 ^a	1.09 ^a
Sham 9	2.05	7.26	4.66	39.70	41.93	40.81 ^b	36.16 ^b	46 ^a	91.28 ^{ab}	0.63 ^a

*Means with the same letter within a column are not significantly different at p = 0.05 according to Tukey test

Table 3: Correlations among different variables; in the eleven durum wheat cultivars evaluated for resistance to FHB in the field and growth chamber

Parameters	FHB index (%)	FDK (%)	DON (ppm)	HW (kg hL ⁻¹)	Disease development in growth chamber (%)	DHS (%)
FDK	0.880**	-	-	-	-	-
DON	-	-	-	-	-	-
HW	-0.791**	-0.744**	-	-	-	-
Disease development in growth chamber	0.768**	0.734*	-	-	-	-
Disease development in field	0.956**	0.902**	-	-0.729**	0.729**	0.769**
DHS	0.820**	0.773**	-	-	0.978**	-

*, **: Significant at p < 0.05 and p < 0.01, respectively. HW: Hectoliter weight, DHS: Disease head severity, FHB: Fusarium head blight, FDK: Fusarium-damaged kernels and DON: Deoxynivalenol

There were no significant differences among all the different cultivars for FDK (type III) and DON levels (type V), however FDK ranged from 30-46% whilst DON from 0.33-2.1 ppm (Table 2).

HW showed a significant diversity among the cultivars; the highest value was for Sham 3 (96.1 kg m⁻³) while Sham 5 had the lowest (90.01 kg m⁻³) (Table 2).

Comparison between the results of growth chamber and field trials: The correlations among the different variables in the growth chamber and field are reported in Table 3.

Mean FHB index showed high positive correlation with FDK and negative correlation with HW (r = 0.88 and -0.791, p < 0.01, respectively). Furthermore, high correlation was found between DHS and FHB index (r = 0.820, p < 0.01). Disease development in the field was correlated with disease development in the growth chamber (r = 0.729, p < 0.01). Deoxynivalenol (DON) levels did not show a correlation with any variable.

DISCUSSION

The present study provides preliminary data on FHB infection in most of Syrian cultivated durum wheat cultivars tested in growth chamber and in the Italian experimental field using Syrian and Italian *F. culmorum* strains.

The data obtained show that all the cultivars differed in their behaviour within the same variable but they were similar, when compared under growth chamber and field conditions, in fact the most tolerant and the most susceptible cultivars kept

FHB scores. Furthermore, the terminal DHS in the growth chamber was higher than FHB index in the field. This might be attributed to the fact that humidity and temperature in the growth chamber were adjusted to be ideal for disease development, while the conditions in the field were not controlled. Resistance to initial infection (type I) was strongly influenced by environmental conditions, while the spread of the pathogen within the spike (type II) was related to cultivar resistance (Wisniewska *et al.*, 2004). It is necessary to combine type I and type II resistance to get FHB resistant wheat plants and furthermore, HW could be used to determine the tolerance of cultivars, since it is considered a quality parameter influenced by FHB.

The high correlation between FDK and FHB index is in accordance with Mesterhazy (2002) and Wegulo *et al.* (2011). According to our results, there was a significant difference within HW among the cultivars and a correlation between HW and FHB index. Ramirez-Marchand *et al.* (2003) also reported a correlation between FHB and HW.

The DON levels were not significantly different and were not correlated to other variables which imply that cultivars with resistance to FHB do not necessarily show low DON levels. Moreover, toxin and disease resistance are two different phenomena. This is in accord with Wisniewska *et al.* (2004) and Chrpova *et al.* (2007), who reported that some cultivars with FHB symptoms clearly expressed high resistance to DON accumulation. Bai *et al.* (2001) reported that severe visual symptoms may not always be associated with high DON levels. On the contrary, Perkowski and Chelkowski (1993) and Lemmens *et al.* (1997) observed a significant correlation

between resistance to FHB and DON accumulation in seeds after natural infection. Karlovsky (2011) reported that an increase in resistance against FHB was moderate in wheat expressing DON acetylation activity.

These contradictions could be interpreted as the mechanisms of DON accumulation are rather complicated and depend on ecological conditions other than to host and fungal genotypes (Mesterhazy *et al.*, 1999).

Preliminary results indicate that the majority of Syrian cultivars assayed, grown in growth chamber and under Italian field conditions, are susceptible to FHB. Nevertheless, “Jory” showed better tolerance than “Simeto”, the most cultivated cultivar in southern Italy, where the climatic conditions are quite similar to some Syrian wheat growing areas. Environmental changes might induce FHB spread in Syrian disease-free areas, being *F. culmorum* already present in Syrian wheat kernels (Alkadri *et al.*, 2013). Evaluation of a wider range of durum wheat cultivars grown in Syrian regions towards the tolerance/resistance to FHB and DON accumulation would provide more choices and increased benefits to producers and to the food processing industries.

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