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AKADÉMIAI KIADÓ

In vitro variation in pathogenicity of *Fusarium* species causing head blight in relation to their isolation from barley head

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RESEARCH ARTICLE

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ABSTRACT

Till now, no published study is available on the variation in pathogenicity of Fusarium head blight (FHB) pathogens in relation to their isolation origin in barley head. To end this, two barley cultivars of contrasting quantitative resistance were artificially infected by four FHB species under field conditions over two consecutive growing seasons. Then, pathogenicity tests were conducted under *in vitro* conditions on single-spore cultures originated from both kernels and glumes in the heads. Different pathogenicity was detected among *Fusarium* species originated from both kernels and glumes, indicating that the same isolate from glumes and kernels differs in pathogenicity on leaves/seedlings. Isolates of *Fusarium culmorum* and *Fusarium verticillioides* originated from infected kernels had shorter latent periods and higher area under disease progress curves compared to isolates originated from glumes, and the reverse was observed for the *Fusarium equiseti* isolate. In the case of *Fusarium solani*, isolates originated from kernels or from glumes were equally pathogenic. Primarily findings in this first in-depth study have implications for breeding programs relied principally on actual quantification of pathogenicity in *Fusarium* species present in a given environment. The sampling of fungi should take into account the presence of *Fusarium* species of interest on kernels or glumes.

KEYWORDS

area under disease progress curve, FHB causative agents, glumes, kernel, latent period

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INTRODUCTION

Fusarium head blight (FHB) has been extensively reported to be a destructive fungal disease that causes enormous economic impacts to barley (*Hordeum vulgare* L.) and other small-grain cereals (i.e. wheat, oat, rye and triticale) cultivation (Parry et al., 1995). From crop production and food safety points of view, while yield losses can occur under epidemic conditions, the greatest effect of FHB disease occurs through the negative effect on grain quality due to the existence of mycotoxins. Very low mycotoxin levels are often enough to render grains unfit in the food and feed chains (Fernando et al., 2021). During flowering and under favorable conditions (warm, humid and wet), FHB species penetrate directly glumes, palea and rachilla by forming invasive mycelia which spread internally throughout vascular bundles in the spikelet and cause bleaching of the florets. With time, it results in sterility or production of discolored kernels scattered throughout the head (Xue et al., 2004). In barley, FHB disease is attributable to a number of fungi of the *Fusarium* and *Microdochium* genera. *Fusarium graminearum* is known to be a principal FHB causative agent damaging barley worldwide (Parry et al., 1995; Bottalico and Perrone, 2002).

Fusarium species causing FHB are pathogens of high concern for the barley production due to their high pathogenicity and ability to cause severe disease under epidemic conditions (Xue et al., 2004, 2006, 2019). Studies have shown that species of *Fusarium* widely differ in pathogenicity. *F. graminearum*, *Fusarium culmorum* and *Fusarium crookwellense* are the most pathogenic species, *Fusarium equiseti*, *Fusarium acuminatum*, *Fusarium sporotrichioides* and *Fusarium poae* are weakly pathogenic, and *Fusarium avenaceum* has a moderate pathogenicity (Xue et al., 2004, 2006; Browne and Cooke, 2005; Fernandez and Chen, 2005). In the barley-FHB literature, it is known that that species of *Fusarium* do not have vertical races and the resistance is race-non-specific. Another important feature is that the resistance is also species-non-specific, meaning that the same quantitative traits locus gives protection against all the *Fusarium* species tested (Fernando et al., 2021). Nevertheless, previous research has shown that host resistance seems to be species-specific as expressed by significant cultivar × species interactions (Xue et al., 2006); indicating that barley cultivars may each possess different genes for resistance to the respective *Fusarium* species. Studies have generally concluded that there are distinct host range differences. *M. majus* isolates were more pathogenic in detached leaves in wheat than *Microdochium nivale* isolates (Diamond and Cooke, 1999; Browne and Cooke, 2004; Brennan et al., 2005). The opposite situation was found in rye, isolates of *M. nivale* were more competitive on rye seedlings than isolates of *M. majus* (Simpson et al., 2000), suggesting that the preferential species specialization exists towards plant organs/tissues.

Till now, no published studies are available on the variation in pathogenicity of FHB pathogens in relation to their isolation origin in barley head. Therefore, it is of interest to compare the susceptibility of different barley head parts to *Fusarium* species. *In vitro* methodologies are equally effective but less time consuming and potentially less resource demanding compared to traditional phenotyping in growth chamber and field domains (Browne and Cooke, 2004; Purahong et al., 2012). The availability of these *in vitro* tools makes it now possible to detect whether they differ in pathogenicity. Latent period (LP) (time from inoculation to sporulation) and area under disease progress curve (AUDPC) have been regarded as the most important *in vitro* components for assessing pathogenicity in the barley–FHB association (Sakr, 2019). As long as LP and AUDPC are *in vitro* indicators of mechanisms of pathogenicity occurring in the adult barley plants during FHB infection (Sakr, 2019), the measured ratings of LP and AUDPC can be considered to be largely the same as pathogenic responses in barley

plants grown in the field. Given this evidence, the objective of this present work to determine whether *F. culmorum*, *Fusarium solani*, *Fusarium verticillioides* and *F. equiseti* originated from glumes and kernels in the head of two barley cultivars differ in their pathogenicity determined using latent period and area under disease progress curves under *in vitro* conditions.

MATERIAL AND METHODS

In each of two growing seasons, two barley cultivars were artificially and individually infected with 16 FHB isolates under field conditions. When barley spikes were at the soft dough stage, the 16 fungal isolates were collected from both kernels and glumes in the infected barley head. Then, pathogenicity tests were conducted under *in vitro* conditions on these 16 isolates originated from both kernels and glumes.

Overview of field experiments

Field experiments were carried out over the two consecutive growing seasons 2018/19 and 2019/20 (Sakr, 2020a) on two morphologically, physiologically and genetically different barley cultivars (Ceccarelli et al., 1987) of contrasting susceptibility to FHB, including in decreasing order of susceptibility cv. Arabi Abiad (AB, moderately susceptible) and cv. Arabi Aswad (AS, moderately resistant) as ranked from previous *in vitro*, growth chamber and field observations (Sakr, 2018a, 2018b, 2019, 2020a, 2020b; Sakr and Shoaib, 2021). Sixteen single-spore derived cultures of four FHB species, i.e., *F. culmorum* (F1, F2, F3, F28 and F30), *F. solani* (F7, F20, F26, F29, F31 and F35), *F. verticillioides* (synonym *Fusarium moniliforme*) (F15, F16, F21 and F27) and *F. equiseti* (F43) were selected for their contrasting pathogenicity (based on previously several experimental observations (Sakr, 2018a, 2018b, 2019, 2020a, 2020b; Sakr and Shoaib, 2021). The above-cited barley cultivars were individually and artificially inoculated to assess disease incidence for Type I resistance, resistance to initial penetration of the pathogen, as indicator of the cultivar's resistance and pathogenicity of 16 FHB isolates. These fungal cultures, referred herein as original isolates, were collected during the 2015 growing season from naturally infected wheat kernels exhibiting FHB symptoms from Ghab Plain with a history of head blight epidemics, one of the principal Syrian wheat production areas. Recently, the 16 *Fusarium* species causing FHB isolates were molecularly analyzed by random amplified polymorphic DNA (Sakr and Shoaib, 2021). Field findings at the Deir Al-Hajar Agricultural Experiment Station, located south east of the countryside of Damascus, Syria (33°20′ N, 36°26′ E, altitude 600 m) during 2018/19 and 2019/20 confirmed pathogenic and resistance data generated under *in vitro* and growth chamber conditions (Sakr, 2020a; Sakr and Shoaib, 2021). In brief, the spikes of AB and AS were sprayed during anthesis (Zadok stage 69) with a spore suspension at 5×10^4 spores/ml of 16 *Fusarium* isolates. Barley spikes were sealed with *transparent* plastic bags for 48 h to supply stationary high humidity to enhance primary infection. The experiment layout was arranged in a completely randomized block design with 3 replicates for each isolate and cultivar.

Re-isolation of fungal isolates from the field and inoculum preparation

When barley plants were at the soft dough stage 21 days post inoculation (dpi), fungal isolates were obtained from 5 kernels and 5 glumes randomly selected per each experimental unit, from

the field over the two consecutive growing seasons 2018/19 and 2019/20. Plant tissue was surface-sterilized in a 0.5% NaOCl solution for 1 min, rinsed in sterile distilled water, and plated onto 9-cm Petri plates containing on potato dextrose agar (PDA, HiMedia Laboratories) with 13 mg L^{-1} kanamycin sulphate added after autoclaving. Plates were placed in an incubator (JSPC, JS Research Inc) for 10 days at 23 ± 1 °C in the dark to allow mycelial growth and sporulation. Fungal cultures were identified to species level based on their cultural (pigmentation and growth rate) and morphological (size of macroconidia, presence of microconidia and chlamydospores) characteristics by using the keys of Leslie and Summerell (2006) and transferred to fresh media, and a single-spore culture was obtained. Koch's postulate was fulfilled by the re-isolation of 16 fungal isolates from the infected kernels and glumes. *Fusarium* cultures were stored in sterile distilled water at 4 °C or at −16 °C until needed (Sakr, 2020c).

FHB inocula were prepared by independently growing each of the 16 fungal cultures obtained from different barley head parts on PDA in Petri plates for 10 days at 23 ± 1 °C in the dark. Following incubation, conidia were dislodged and harvested by flooding the cultures with 10 mL of sterile distilled water and the suspensions of the FHB isolates were filtered through two layers of sterile cheesecloth to remove mycelia. The resulting spore suspensions were directly quantified under an optical microscope with a hemacytometer and adjusted to 5×10^4 spores mL^{-1}.

Establishment of in vitro assay and pathogenicity comparison

In vitro pathogenicity analyses for original isolates and isolates obtained from kernels and isolates obtained from glumes were carried out on AB and AS for latent period (LP) detected in a detached leaf and area under disease progress curve (AUDPC) detected in a Petri-dish experiments.

Methods for LP assay were conducted as described previously by Browne and Cooke (2004) to assess *in vitro* quantitative resistance components and used recently by Sakr (2019) to quantify pathogenicity in the barley–FHB association. Assessments of symptom manifestation and sporulation were analyzed once a day under a stereo microscope (magnification X40), and the pathogenicity of an isolate (LP) was quantified daily from inoculation to sporulation. Five replicates of each isolate based on observations on 120 detached leaves, each replicate consisted of 24 detached leaves, were set up in which the plates were arranged in a randomized block design, and the experiment was repeated twice.

Methods for AUDPC assay were carried out as characterized previously by Purahong et al. (2012) to assess *in vitro* aggressiveness components and utilized recently by Sakr (2018a, 2019) to assess pathogenicity in the system of barley and FHB pathogens. The pathogenicity component of an isolate (AUDPC) was measured as disease progress over 6 dpi and its value was ranged from 0 (not pathogenic) to 1 (very pathogenic). Five replicates of each isolate were set up in which the plates were arranged in a randomized block design, and the experiment was repeated twice.

The change of pathogenicity of isolates originated from kernels and glumes was measured by comparing LP and AUDPC of the isolates obtained from kernels to the isolates obtained from glumes.

Statistical analyses

The experimental data were subjected to analysis of variance (ANOVA) using [DSAASTAT (2015)], version 1.514, Department of Agriculture and Environmental Science, University of

Perugia, Italy. A combined analysis of data during the two growing seasons was conducted to verify if pathogenicity may have a significant and stable effect over years. Environments being years (the two growing seasons 2018/19 and 2019/20) were classed as random effects, while treatment (fungi) was classed as fixed effects. The differences between isolates obtained from both: kernels and glumes and their original isolates were compared using Fisher's least significant difference test at the 5% level of significance.

RESULTS

Since no significant interaction year × isolate was observed, $P > 0.05$ for LP and AUDPC trials (climatic data for the station were quasi-similar over the two growing seasons), data are shown as the averages of the two growing seasons on the two quantitatively different barley cultivars (Tables 1 and 2). Tables 1 and 2 presents mean pathogenicity values over the two growing seasons determined using *in vitro* detached leaf assay and Petri-dish assay of FHB isolates on AS

Table 1. Mean values for pathogenicity component, latent period (days) detected in an *in vitro* detached leaf assay in a set of 16 fungal isolates of four Fusarium head blight species. Original isolates (Ori.) used to artificially inoculate barley spikes under field conditions, isolates obtained from kernels (K.) and isolates obtained from glumes (G.) measured on two barley cultivars over the two growing seasons 2018/19 and 2019/20

Fungal isolates (identification)	Arabi Abiad			Arabi Aswad		
	Ori.	K.	G.	Ori.	K.	G.
F1 (*Fc*)	8.2b	8.1b (18%)*	9.9a	7.7b	7.8b (20%)	9.8a
F2 (*Fc*)	3.7b	3.5b (22%)	4.5a	5.8b	5.7b (23%)	7.4a
F3 (*Fc*)	4.8b	4.9b (16%)	5.9a	4.5b	4.6b (18%)	5.6a
F28 (*Fc*)	6.3b	6.0b (23%)	7.8b	5.9b	6.0b (20%)	7.4a
F30 (*Fc*)	8.4b	8.6b (16%)	10.2a	7.6b	7.6b (16%)	9.0a
F7 (*Fs*)	9.4a	9.2a	9.3a	9.1a	9.2a	9.0a
F20 (*Fs*)	5.6a	5.8a	5.6a	8.0a	8.1a	8.1a
F26 (*Fs*)	5.7a	5.7a	5.6a	7.9a	8.0a	8.0a
F29 (*Fs*)	8.5a	8.4a	8.6a	7.5a	7.4a	7.6a
F31(*Fs*)	4.4a	4.2a	4.3a	6.6a	6.7a	6.4a
F35 (*Fs*)	5.3a	5.5a	5.3a	7.7a	7.90a	7.8a
F15 (*Fv*)	3.5b	3.4b (17%)	5.1a	4.5b	4.4b (14%)	5.1a
F16 (*Fv*)	3.0b	3.0b (18%)	3.7a	5.0b	5.1b (17%)	6.1a
F21 (*Fv*)	5.3b	5.5b (17%)	6.7a	7.1b	7.2b (15%)	8.5a
F27 (*Fv*)	5.9b	5.7b (23%)	7.4a	6.3b	6.2b (22%)	7.9a
F43 (*Fe*)	4.8a	4.7a	3.9b (17%)	8.1a	8.1a	6.8b (16%)

Abbreviations: Fc: *Fusarium culmorum*, Fs: *F. solani*, Fv: *F. verticillioides*, Fe: *F. equiseti*.
According to the Fisher's LSD test, means followed by the same letter within a linage are not significantly different at $P=0.05$. Original isolates were collected during the 2015 growing season from naturally infected wheat kernels exhibiting FHB symptoms. *: The change of pathogenicity (%) of isolates obtained from kernels and glumes was measured by comparing LP of the 16 *Fusarium* isolates obtained from kernels to the 16 *Fusarium* isolates obtained from glumes.

Table 2. Mean values for pathogenicity component, area under disease progress curve detected in an *in vitro* Petri-dish assay in a set of 16 fungal isolates of four Fusarium head blight species. Original isolates (Ori.) used to artificially inoculate barley spikes under field conditions, isolates obtained from kernels (K.) and isolates obtained from glumes (G.) measured on two barley cultivars over the two growing seasons 2018/19 and 2019/20

Fungal isolates (identification)	Arabi Abiad			Arabi Aswad		
	Ori.	K.	G.	Ori.	K.	G.
F1 (*Fc*)	0.35a	0.34a (19%)*	0.28b	0.22a	0.22a (19%)	0.17b
F2 (*Fc*)	0.25a	0.26a (22%)	0.21b	0.29a	0.30a (21%)	0.23b
F3 (*Fc*)	0.58a	0.59a (19%)	0.47b	0.39a	0.39a (18%)	0.33b
F28 (*Fc*)	0.44a	0.46a (19%)	0.36b	0.29a	0.28a (20%)	0.23b
F30 (*Fc*)	0.70a	0.71a (18%)	0.56b	0.34a	0.35a (15%)	0.28b
F7 (*Fs*)	0.68a	0.66a	0.67a	0.45a	0.46a	0.44a
F20 (*Fs*)	0.42a	0.40a	0.41a	0.40a	0.41a	0.39a
F26 (*Fs*)	0.47a	0.49a	0.47a	0.52a	0.50a	0.51a
F29 (*Fs*)	0.39a	0.40a	0.42a	0.39a	0.38a	0.39a
F31(*Fs*)	0.30a	0.29a	0.31a	0.33a	0.35a	0.34a
F35 (*Fs*)	0.39a	0.38a	0.40a	0.37a	0.38a	0.39a
F15 (*Fv*)	0.26a	0.25a (20%)	0.20b	0.22a	0.23a (23%)	0.19b
F16 (*Fv*)	0.41a	0.42a (23%)	0.32b	0.31a	0.32a (23%)	0.26b
F21 (*Fv*)	0.38a	0.39a (19%)	0.32b	0.35a	0.36a (18%)	0.28b
F27 (*Fv*)	0.22a	0.21a (24%)	0.16b	0.26a	0.25a (21%)	0.19b
F43 (*Fe*)	0.34b	0.33b	0.39a (18%)	0.41b	0.39b	0.46a (17%)

Abbreviations: Fc: *Fusarium culmorum*, Fs: *F. solani*, Fv: *F. verticillioides*, Fe: *F. equiseti*.
According to the Fisher's LSD test, means followed by the same letter within a linage are not significantly different at $P = 0.05$. Original isolates were collected during the 2015 growing season from naturally infected wheat kernels exhibiting FHB symptoms. *: The change of pathogenicity (%) of isolates obtained from kernels and glumes was measured by comparing AUDPC of the 16 *Fusarium* isolates obtained from kernels to the 16 *Fusarium* isolates obtained from glumes.

and AB, respectively. Generally, all *Fusarium* isolates obtained from both kernels and glumes were pathogenic. *Fusarium* infected seedlings were identified by discolored, malformed, necrotic or chlorotic areas in the detached leaves for LP assay and by brown spots on the coleoptiles and/or by mycelium completely covering the seeds for AUDPC experiment.

Overall, on AB and AS, isolates of *F. culmorum* and *F. verticillioides* originated from infected kernels were more pathogenic than isolates originated from glumes, and the opposite was found for the *F. equiseti* isolate. However, in the case of *F. solani*, isolates originated from kernels or from glumes were equally pathogenic. Isolates of *F. culmorum* and *F. verticillioides* originated from infected kernels had shorter LPs and higher AUDPCs (19%) compared to isolates originated from glumes. There were no significant differences among original isolates used in the artificial inoculation of barley spikes under field conditions during the two consecutive growing seasons 2018/19 and 2019/20 and isolates obtained from kernels. The reverse was observed for the *F. equiseti* isolate; F43 obtained from infected glumes had shorter LPs (17%) and higher AUDPCs (18%) compared to isolate obtained from kernels; there were no significant differences among original isolate and isolate obtained from kernels. However, in the case of *F. solani*

isolates obtained from kernels or from glumes were equally pathogenic; there were no significant differences among original isolates and isolates obtained from both kernels and glumes.

DISCUSSION

In cereals-FHB pathosystem, previous researches have reported that there is a biologically significant preference in pathogen causative agents into organs/tissues (Diamond and Cooke, 1999; Simpson et al., 2000; Browne and Cooke, 2004; Brennan et al., 2005). It appears likely that precise and correct quantification of pathogenicity of several pathogens might differ in linkage to their isolation from infected head parts. As far as we know, no published work till now has clarified this crucial biological issue in the barley-FHB association. Therefore, we have aimed to fill this gap by providing new findings on comparing the pathogenicity of four *Fusarium* species originated from glumes and kernels in the head with two different bio-experiments (latent period (LP) detected in a detached leaf and area under disease progress curve (AUDPC) detected in a Petri-dish) under *in vitro* conditions. Because of the correlation between both pathogenicity components, LP and AUDPC and disease development in the field (Sakr, 2019, 2020a), knowledge of diversity of LP and AUDPC for FHB populations on barley cultivars should be helpful in predicting disease reactions in the field.

The current work has confirmed that there are biological differences among *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* originated from glumes and kernels in the heads artificially sprayed with the tested isolates herein under field conditions over the two consecutive growing seasons 2018/19 and 2019/20 (Sakr, 2020a). In our study, the *Fusarium* isolates obtained from a given head part had shorter LPs they had also greater AUDPCs, suggesting the high correlation between both pathogenicity components: LP and AUDPC as reported earlier (Sakr, 2019). Shorter LP and higher AUDPC might lead to better performance of FHB pathogens in the field (Purahong et al., 2012; Sakr, 2020a). Like recent studies analyzing *in vitro* pathogenicity in the barley-FHB system (Sakr, 2018a, 2018b, 2019, 2020a), FHB isolates having shorter LPs and greater AUDPCs are considered to be more pathogenic on barley tissue than isolates having longer LPs and lesser AUDPCs.

Pathogenicity of *Fusarium* species causing FHB is probably the consequence of timely expression of many genes, governing production of mycotoxins, cell-wall-degrading enzymes, hormones, and specific metabolites that alter the host's resistance reaction (Fernando et al., 2021). Different pathogenicity was detected among *Fusarium* species originated from both kernels and glumes in AB and AS, indicating that the same isolate from glumes and kernels differs in pathogenicity on leaves/seedlings. Earlier works have reported biological differences for *M. nivale* var. *nivale* and var. *majus* (Diamond and Cooke, 1999; Simpson et al., 2000) and for *F. graminearum* (Carter et al., 2002); this may also occur for the other *Fusarium* species analyzed herein. The explanation for this phenomenon regarding the four tested species herein: *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* would be some kind of physiological adaptation, physiological specialization, or something similar. The adaptive potential of plant pathogen species highly depends on the forces shaping the pathogen evolution; among these forces, the selection by the host-plant resistances is of particular importance (McDonald and Linde, 2002). Such host specialization evolves when host species differ in natural properties (e.g. resistance) as a consequence of inherent plant host–pathogen interactions (Kniskern et al.,

2011). Pathogenicity of the four *Fusarium* species tested in the present work on barley head parts may be affected by the degree of kernels/glumes resistance to mycotoxin production. As toxins may act as a pathogenicity factor, the level of disease is also expected to rise under infection by pathogens with elevated toxigenic potential (Fernando et al., 2021). Compounds present in anther extracts have been reported to promote initial hyphal growth of some *Fusarium* species (*F. graminearum* and *F. culmorum*) but have no effect on others (*M. nivale*) (Strange et al., 1974), possibly explaining why the tested FHB species had a distinct difference in the kernels/glumes range.

CONCLUSION

This first in-depth study shows conclusively that there is a physiological adaptation or physiological specialization among *F. culmorum*, *F. solani*, *F. verticillioides* and *F. equiseti* in which the same isolate from glumes and kernels differs in pathogenicity on leaves/seedlings. While the use of quantitatively barley resistant cultivars is an essential component of a sustainable management strategy of FHB, our findings have implications for barley breeding programs relied principally on actual quantification of pathogenicity in *Fusarium* species present in a given environment. The sampling of FHB fungi should take into account the presence of *Fusarium* species of interest on kernels or glumes.

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REFERENCES

Bottalico, A. and Perrone, G. (2002). Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. *European Journal of Plant Pathology*, 108(7): 611–624.

Brennan, J. M., Egan, D., Cooke, B. M., and Doohan, F. M. (2005). Effect of temperature on head blight of wheat caused by *Fusarium culmorum* and *F. graminearum*. *Plant Pathology*, 54(2): 156–160.

Browne, R. A. and Cooke, B. M. (2004). Development and evaluation of an *in vitro* detached leaf assay for pre-screening resistance to Fusarium head blight in wheat. *European Journal of Plant Pathology*, 110(1): 91–102.

Browne, R. A. and Cooke, B. M. (2005). A comparative assessment of potential components of partial disease resistance to Fusarium head blight using a detached leaf assay of wheat, barley and oats. *European Journal of Plant Pathology*, 112(3): 247–258.

Carter, J. P., Rezanoor, H. N., Holden, D., Desjardins, A. E., Plattner, R. D., and Nicholson, P. (2002). Variation in pathogenicity associated with the genetic diversity of *Fusarium graminearum*. *European Journal of Plant Pathology*, 108(7): 573–583.

Ceccarelli, S., Grando, S., and Van Leur, J. Ag. (1987). Genetic diversity in barley landraces from Syria and Jordan. *Euphytica*, 36(2): 389–405.

Diamond, H. and Cooke, B. M. (1999). Towards the development of a novel *in vitro* strategy for early screening of Fusarium ear blight resistance in adult winter wheat plants. *European Journal of Plant Pathology*, 105(4): 363–372.

DSAASTAT (2015). An introduction to DSAASTAT statistics software by Andrea Onofri. Available at: https://vdocuments.mx/dsaastat-by-andrea-onofri.html (Accessed: October 21, 2015).

Fernandez, M. R. and Chen, Y. (2005): Pathogenicity of *Fusarium* species on different plant parts of spring wheat under controlled conditions. *Plant Disease*, 89(2): 164–169.

Fernando, W. G. D., Oghenekaro, A. O., Tucker, J. R., and Badea, A. (2021). Building on a foundation: advances in epidemiology, resistance breeding, and forecasting research for reducing the impact of Fusarium head blight in wheat and barley. *Canadian Journal of Plant Pathology*, 43(4): 495–526.

Kniskern, J. M., Barrett, L. G., and Bergelson, J. (2011). Maladaptation in wild population of the generalist plant pathogen *Pseudomonas syringae*. *Evolution*, 65(3): 818–830.

Leslie, J. F. and Summerell, A. B. (2006). *The Fusarium laboratory manual*. Blackwell Publishing Professional, Ames, pp. 1–388.

McDonald, B. A. and Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology*, 40: 349–379.

Parry, D. W., Jekinson, P., and Mcleod, L. (1995). Fusarium ear blight (scab) in small grain cereals – a review. *Plant Pathology*, 44(2): 207–238.

Purahong, W., Alkadri, D., Nipoti, P., Pisi, A., Lemmens, M., and Prodi, A. (2012). Validation of a modified petri-dish test to quantify aggressiveness of *Fusarium graminearum* in durum wheat. *European Journal of Plant Pathology*, 132(3): 381–391.

Sakr, N. (2018a). Aggressiveness variation among and within Fusarium head blight species on barley *in vitro*. *Acta Phytopathologica et Entomologica Hungarica*, 53(1): 11–18.

Sakr, N. (2018b). Components of quantitative resistance in barley plants to Fusarium head blight infection determined using three *in vitro* assays. *Journal of Plant Protection Research*, 58(3): 176–183.

Sakr, N. (2019). Variation in aggressiveness of Fusarium head blight species towards barley plants determined using three *in vitro* assays. *Pakistan Journal of Phytopathology*, 31(1): 19–33.

Sakr, N. (2020a). Aggressiveness of *Fusarium* species causing head blight in barley landraces grown under Fertile Crescent conditions. *Pakistan Journal of Phytopathology*, 32(1): 41–52.

Sakr, N. (2020b). An efficient in vitro assay to predict resistance and pathogenicity in the Fusarium head blight – *Hordeum vulgare* pathosystem. *Open Agriculture Journal*, 13: 9–18.

Sakr, N. (2020c). Conservation of cereal fungi following different methods of preservation for long terms. *Pakistan Journal of Phytopathology*, 32(2): 159–168.

Sakr, N. and Shoaib, A. (2021). Pathogenic and molecular variation of *Fusarium* species causing head blight on barley landraces. *Acta Phytopathologica et Entomologica Hungarica*, 56(1): 5–23.

Simpson, D. R., Rezanoor, H. N., Parry, D. W., and Nicholson, P. (2000). Evidence for differential host preference in *Microdochium nivale* var. *majus* and *Microdochium nivale* var. *nivale*. *Plant Pathology*, 49(2): 261–268.

Strange, R.N., Majer, J.R., and Smith, H. (1974). The isolation and identification of choline and betaine as the two major components in anthers and wheat germ that stimulate *Fusarium graminearum in vitro*. *Physiological Plant Pathology*, 4(2): 277–290.

Xue, A. G., Armstrong, K. C., Voldeng, H. D., Fedak, G., and Babcock, C. (2004). Comparative aggressiveness of isolates of *Fusarium* species causing head blight on wheat in Canada. *Canadian Journal of Plant Pathology*, 26(1): 81–88.

Xue, A. G., Chen, Y., Seifert, K., Guo, W., Blackwell, B. A., Harris, L. J., and Overy, D. P. (2019). Prevalence of *Fusarium* species causing head blight of spring wheat, barley and oat in Ontario during 2001–2017. *Canadian Journal of Plant Pathology*, 41(3): 392–402.

Xue, A. G., Ho, K. M., Butler, G., Vigier, B. J., and Babcock, C. (2006). Pathogenicity of *Fusarium* species causing head blight in barley. *Phytoprotection*, 87(1): 55–61.