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ORIGINAL ARTICLE

# Evaluation of germination and vigor indices associated with *Fusarium*-infected seeds in pre-basic seeds wheat fields

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**Abstract**

Seed-borne diseases of wheat such as Fusarium head blight (FHB), a fungal disease caused by several species of *Fusarium*, results in reduced yield and seed quality. The aim of this study was to identify the *Fusarium* species, the effect of *Fusarium*-infected seeds on germination and vigor indices and to determine the location of *Fusarium* spp. in seeds, as well as to investigate the pathogenicity and variability of aggressiveness of the isolates obtained from pre-basic seeds wheat fields in Iran. According to morphological and molecular characters, the species *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* were identified. Among the isolates, *F. graminearum* was the predominant species with the highest frequency and relative density of 92.9% and 70.9%, respectively. We observed that germination and vigor indices were decreased due to increased *Fusarium*-infected seeds. Results indicated significant differences among cultivars and seed-borne *Fusarium* levels. While a higher infection level of *Fusarium* spp. most commonly occurred in the seed coat, only *F. graminearum* was observed in embryos. Our study about pathogenicity showed that 77.3% of the *Fusarium* spp. isolates were not pathogenic and 22.7% isolates of *Fusarium* spp. were pathogenic or weakly pathogenic. Our results indicated that variability in aggressiveness among isolates of a species and positive correlation may be determined by pathogenicity tests. This is the first time the location of *Fusarium* spp. in seeds has been identified. It is also the first time that *Fusarium*-infected seeds in pre-basic seeds wheat fields of Iran have been evaluated.

**Keywords:** Fusarium head blight, *Fusarium* spp., location, seed-borne, wheat

## Introduction

Wheat (*Triticum aestivum* L.) is one of the most important small grain cereals used for animal feed and human consumption worldwide. In 2014, world production of wheat was 726 million tons, making it the third most-produced cereal after maize and rice (FAOSTAT 2015). Major wheat producers include: China, India, Russia, United States of America, France, Canada, Germany, Pakistan, Australia, Ukraine, Turkey, United Kingdom, Kazakhstan, Poland and Iran contributing 79.4% of world production. In Iran, it is the most important food grain and is cultivated on an area of about 7.3 million hectares with a total production of 10.6 million tons. The seed production of wheat follows a generation system that includes breeder, pre-basic, foundation and certified seeds, based on nomenclature of the Organization for Economic Cooperation and Development (OECD) (Anonymous 2015).

Seed-borne diseases of wheat such as Fusarium head blight (FHB) are considered to be a constraint in wheat seed production, because they reduce yield and

seed quality (Agarwal *et al*. 2008; Kumar *et al*. 2008). FHB-affected seed may become contaminated with mycotoxins, such as trichothecenes, which can cause several health problems for humans and livestock as well as reduce seed germination and embryo growth (Pestka 2010).

The disease is caused by different species of *Fusarium*, with different life styles especially *F. graminearum* (Schwabe) [teleomorph *Gibberella zeae* (Schw.) Petch], *F. culmorum* (W.G. Smith) Sacc. (teleomorph unknown), and *F. avenaceum* (Fries) Sacc (teleomorph *Gibberella avenacea* R.J. Cooke) (Boutigny *et al*. 2014).

Fusarium head blight causes losses in yield of wheat from 30 to 70% (Parry *et al*. 1995). In Iran, FHB occurs frequently in Golestan, Mazandaran, and Ardabil provinces. In recent years, the wheat losses in Iran due to FHB have increased significantly (Haratian *et al*. 2008; Davari *et al*. 2013; Khaledi *et al*. 2017). Fusarium head blight may cause yield losses in some fields of up to 70% (Davari *et al*. 2006). Although previous studies have identified losses in yield of wheat, so far, there has been no report from seed production fields of wheat. Numerous of studies have examined the effects of FHB on seed germination (Browne and Cooke 2005; Tekle *et al*. 2013; Shin *et al*. 2014). If FHB-affected seed is used for planting, seedling blight on roots and coleoptiles, reduced germination and vigor (Browne and Cooke 2005) and pre- and post-emergence death of seedlings (Asran and Eraky Amal 2011), and variation in plant morphology may occur (Rajput *et al*. 2005).

Aggressiveness is an important factor for determining the potential ability of *Fusarium* isolates to cause yield losses and FHB epidemics in wheat. More detailed knowledge about the extensive variability of aggressiveness is essential for understanding the interaction between wheat and *Fusarium* species causing FHB (Wu *et al*. 2005; Khaledi *et al*. 2017). *Fusarium graminearum* caused the greatest reduction in germination of wheat seeds followed by *F. avenaceum* and *F. culmorum*, and was the least for *F. poae* (Browne and Cooke 2005). Several strategies have been used to manage FHB disease, including crop rotation, planting resistant or tolerant cultivars, and chemical and biological control (Wegulo *et al*. 2015).

Different wheat genotypes express various levels of resistance against *Fusarium* spp. causing FHB (Mesterházy *et al*. 2005). Resistance to FHB is a complex trait, with polygenic inheritance, and its expression is highly influenced by the environment (Ruan *et al*. 2012; Buerstmayr and Buerstmayr 2015). To date, two main types of resistance to FHB are widely accepted: type I – resistance to initial infection; and type II – resistance to fungal spread within the spike. Additionally, three other types of resistance were reported by Mesterházy *et al*. (1999): type III – resistance to deoxynivalenol accumulation; type IV – resistance to kernel infection; type V – tolerance.

Despite the economic importance of FHB in wheat seed production, understanding the percentage of infected seeds, the frequency and identification of seed-borne *Fusarium* species in pre-basic seeds wheat fields is limited. Therefore, the main objectives of this study were to identify and determine: (i) the frequency of *Fusarium* spp. isolated from wheat seeds in Iran, (ii) the effect of *Fusarium* spp. on germination and vigor indices, (iii) the location of the *Fusarium* spp. in the seed, and (iv) the pathogenicity and variability of aggressiveness of *Fusarium* spp. and to identify possible correlation.

## Materials and Methods

### Sample collection

A total of 85 seed samples of pre-basic seeds of various wheat cultivars with different levels of FHB resistance were collected from various wheat fields, located in Golestan, Mazandaran and Ardebil provinces in Iran after harvest in 2016–2017 (Fig. 1). Sampling was done according to International Seed Testing Association guidelines (ISTA 1986). The seed samples were packed in paper envelopes and stored in a freezer at the Seed and Plant Certification and Registration Institute (SPCRI) in Iran, until used for subsequent studies.

### Isolation and identification of *Fusarium* species

For isolation of *Fusarium* spp., 400 seeds of each sample were surface sterilized by immersion in 1% sodium hypochlorite for 3 min and rinsed three times in sterile distilled water. The sterilized samples were placed in water agar as a general medium (Burgess *et al*. 1994) and a semi-selective medium for *Fusarium*, i.e., peptone-pentachloronitrobenzene agar (PPA), and incubated at 25 ± 1°C in a 12h light/dark cycle for 10 days. The resulting *Fusarium* colonies were single-spored and transferred to potato dextrose agar (PDA), carnation leaf agar (CLA) and spezieller nährstoffarmer agar (SNA) plates for morphological identification (Leslie and Summerell 2006). Morphological identification was based on Starkey *et al*. (2007) and Leslie and Summerell (2006). After identification, the isolates were deposited in the fungal culture collection of the Seed Health Laboratory of Seed and Plant Certification and Registration Institute (SPCRI) in Iran. All fungi were maintained on PDA medium at 4°C and sub-cultured monthly. The isolation frequency (*Fr*) and relative density (*RD*) of fungi were recorded and calculated (Hajihasani *et al*. 2012; Tsedaley 2016; Nayyar *et al*. 2018) as follow:


**Fig. 1.** Map of Iran showing sampling regions and data related to *Fusarium* isolated from different locations in pre-basic seeds wheat fields. Gray areas show where sampling was performed

$$Fr = \frac{ns}{N} \times 100 \ [\%],$$

$$RD = \frac{ni}{Ni} \times 100 \ [\%],$$

where: *ns* = the number of samples in which a fungus occurred, *N* = the total number of samples, *ni* = the number of isolates of a given fungal species, *Ni* = the total number of fungal isolates obtained.

## DNA extraction

Mycelial plugs (0.5 $cm^2$) were removed from PDA plates, transferred into 250 ml Erlenmeyer flasks containing 100 ml potato dextrose broth (PDB) medium, and incubated at 25°C for 10 days. Mycelial mats were dried between sterile filter papers and ground to a fine powder with liquid nitrogen. Total genomic DNA was extracted with a commercially available DNA extraction kit (Genomic DNA isolation kit IV; DENA Zist Asia, Iran) according to the manufacturer's instructions. DNA concentration was quantified with a NanoDrop spectrophotometer and the quality was verified by 1% agarose gel electrophoresis. The DNA samples were diluted using sterilized distilled water to a final concentration of 50 ng · $\mu l^{-1}$ and stored at –20°C until use.

## *Fusarium* species identification by PCR

To confirm the morphological identification of species, conventional polymerase chain reaction (PCR) was performed using specific primers (Table 1) for molecular identification of four *Fusarium* species, which may potentially infect wheat seeds in the investigated area. The PCR reaction was performed in a 25 µl volume, each reaction contained 7.5 µl of sterile water, 12.5 µl of PCR Master Mix (Pars Tous, Iran), 1 µl of 10pMeach forward and reverse primers and 3 µl of template DNA. The PCR cycle consisted of an initial denaturation step at 94°C for 2 min followed by 35 cycles of denaturation (95°C for 35 s), annealing (times and temperatures for each primer pair are listed in Table 1), extension (72°C for 30 s) and final extension at 72°C for 7 min. All primers used in this study were purchased from

**Table 1.** Primer sequences, product sizes and annealing temperatures used for PCR identification of *Fusarium* species

| Species | Primer | Sequences (5′–3′) | Product size [bp] | PCR conditions (anneal/ extend) | Reference |
|---|---|---|---|---|---|
| *F. graminearum* | Fg16F<br>Fg16R | CTCCGGATATGTTGCGTCAA<br>GGTAGGTATCCGACATGGCAA | 400–500 | 60°C/60 s | Nicholson *et al*. (1998) |
| *F. culmorum* | OPT18F<br>OPT18R | GATGCCAGACCAAGACGAAG<br>GATGCCAGACGCACTAAGAT | 472 | 59°C/30 s | Schilling *et al*. (1996) |
| *F. avenaceum* | FA-F<br>FA-R | GCTAATTCTTAACTTACTAGGGGCC<br>CTGTAATAGGTTATTTACATGGGCG | 272 | 60°C/30 s | Schilling *et al*. (1996) |
| *F. poae* | Fp82-F<br>Fp82-R | CAAGCAAACAGGCTCTTCACC<br>TGTTCCACCTCAGTGACAGGTT | 220 | 61°C/30 s | Parry and Nicholson (1996) |

Macrogen (South Korea). Amplification products were separated by electrophoresis (90 V, 60 mA, 100 W, 30 min) in 1% agarose gels in 1 TAE (Tris-acetate-EDTA) buffer and visualized by SYBR Green staining on a UV gel documentation system.

## Standard germination test

The standard germination test of wheat seeds was assessed using the rolled paper towel method (Warham 1990). This method was employed to determine the effect of seed-borne pathogen inoculum on seed quality parameters of wheat i.e. to carry out germination and vigor tests. Briefly, 400 seeds, randomly taken from each sample, were selected and placed between a pair of moist paper towels, with 25 seeds per row. Then, these seeds were covered with another moist paper and rolled carefully to avoid any excess pressure on seeds. Paper towels were placed individually in plastic bags and the bags were sealed with an elastic band. These towels were incubated at 20 ± 1°C in 16/8 h light/dark photoperiod with 70% moisture for 7 days. Seeds were visually assessed according to the ISTA rules (ISTA 2013). After a 7 day incubation period, the germination percentage, normal and abnormal seedlings, shoot and root lengths, fresh and dry weights were determined by placing them in an oven for 24 h at 75°C). Also, seedling length vigor index (*SLVI*) and seedling weight vigor index (*SWVI*) relations were determined by the following equations (Nautiyal *et al*. 2009):

$$SLVI = (\text{mean of shoot length} + \text{mean of root length}) \times \times \text{percentage of seed germination},$$

$$SWVI = \text{dry weight seedling} \times \times \text{percentage of seed germination}.$$

## Location of *Fusarium* spp. in wheat seed

The location of *Fusarium* spp. in wheat seed was studied by employing the component plating technique (Maden *et al*. 1975). Naturally infected wheat seed samples were used for the study. Twenty seeds were washed four times with tap water, surface sterilized in 1% sodium hypochlorite for 3 min. These seeds were again washed with sterile water and soaked in water for 60 min and then the seeds were dissected aseptically using a sterile needle and forceps. The separated seed parts viz., seed coat and embryo were plated immediately before drying on PDA plates. The plates were incubated at 25 ± 1°C for 7 days. Then, they were examined under a stereo-binocular microscope for the presence of *Fusarium* spp. in different seed parts. The infection level of each part was evaluated according to the following formula (Mahmoud *et al*. 2013):

$$\text{Infection level} = \left( \frac{\text{Total number of infected seed parts}}{\text{Total number of seed parts}} \right) \times 100\,[\%].$$

## Plant materials

Spring wheat cultivar (cv.) Falat, which is known as being susceptible to FHB (Soltanloo *et al*. 2011), obtained from the Seed and Plant Improvement Institute of Karaj, Iran, was used for pathogenicity tests. The seeds were surface sterilized with 1% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water and incubated for 5 days on wet sterile filter paper in Petri dishes at 25°C. Germinated seeds were sown in each of the 15 cm-diameter plastic pots filled with sterile potting soil, which had been autoclaved at 121°C for a minimum of 30 min at 100 kPa (15 psi) on 2 successive days and grown in a greenhouse (28 ± 4°C; 16/8 h light/dark photoperiod). The soil used in this experiment was a combination of peat moss, vermiculite and perlite at the ratio of 2 : 1 : 1 (v/v/v).

## Inoculum preparation

Fungal inocula were produced on Mung Bean Broth (MBB) and Synthetic Nutrient Agar (SNA) media as

described by Zhang *et al.* (2013) and Koch *et al.* (2013), respectively. Conidial suspensions were diluted to a final concentration of $1 \times 10^5$ conidia · $ml^{-1}$ containing 0.05 % (v/v) Tween 20.

### Pathogenicity test

In the greenhouse experiments, the pathogenicity test of *Fusarium* isolates on wheat spikes was investigated using the method described by Yoshida *et al.* (2007). At the flowering stage (ZGS 64 to 65), 10 ml of spore suspension ($1 \times 10^5$ conidia · $ml^{-1}$) amended with Tween 20 was sprayed onto the spikes of each plant. The inoculated plants were incubated overnight in a greenhouse at 18–25°C, with 100% humidity. Then, the plants were placed in a plastic bag for 3 days to maintain high relative humidity. Control plants were treated only with sterile distilled water. Inoculated wheat heads were evaluated after 10 days and the FHB disease severity was estimated. Disease severity was measured as the percentage of infected spikelet(s) within the spike using a 0 to 5 scale (0 = no disease, 1 = to 20%, 2 = to 40%, 3 = to 60%, 4 = to 80% and 5 = more than 80% disease severity) (Wan *et al.* 1997) and the FHB index was calculated as described previously (Amarasinghe *et al.* 2013). Each test had ten replicates arranged in a completely randomized design, and the experiment was repeated three times.

In the detached leaf bioassay, 4-cm long segments from the mid-section were prepared from the apical leaves of 4-week old wheat plants. Each leaf segment was placed adaxial surface uppermost on the surface of 0.5% water agar as described by Browne and Cooke (2004). Leaf segments were inoculated at the center of the adaxial surface with 5 µl inoculum suspension of $1 \times 10^5$ conidia · $ml^{-1}$. Control leaf segments were inoculated using a drop of sterile distilled water without the fungus. Petri dishes were incubated at 25°C with a 12/12 h light/dark cycle. After 5 days, the length of necrotic lesions was measured. The test included four replicates for each isolate and the experiment was repeated three times.

### Assessment of aggressiveness

The aggressiveness of each isolate of *Fusarium* spp. on seedlings and detached leaves was determined using the method described by Khaledi *et al.* (2017). Analysis of aggressiveness was based on determining hours post inoculation (hpi) for disease symptom appearance.

### Statistical analysis

All experiments were set up in a completely randomized design. The data were analyzed by one-way analysis of variance (ANOVA) and comparison of means was carried out using the least significant difference (LSD) at the level of $p < 0.05$. Statistical analysis was performed using PROC GLM in SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA).

## Results

### Morphological and molecular identification of *Fusarium* isolates

In this study, a total of 453 *Fusarium* isolates were recovered from different locations of pre-basic seeds wheat fields after harvest in 2016–2017. All isolates were classified into four species based on morphological criteria using the keys for *Fusarium* species identification of Starkey *et al.* (2007) and Leslie and Summerell (2006). Based on morphological characters of conidia, chlamydospores and conidiophores, *Fusarium* isolates were identified which belonged to four species (Table 2, Fig. 2). *Fusarium graminearum* Schwabe was the most common species, recovered from 92.9% of the seeds tested, and represented 70.9% of the seed-borne *Fusarium* pathogen population. *Fusarium culmorum* (W.G. Smith) Sacc., *F. avenaceum* (Corda: Fr.) Sacc., and *F. poae* (Peck) Wollenw. were less common and were recovered from 50.6, 25.9, and 5.8% of the seeds and represented 21.2, 5.9 and 1.9% of the pathogen population, respectively. Morphological observations showed that the length of macroconidia ranged from 24 to 72 µm for *F. graminearum* (Figs 2A–B), 37 to 55 µm for *F. culmorum* (Fig. 2C), 44 to 70 µm for *F. avenaceum* (Figs 2D–E), and 36 to 47 µm for *F. poae* (Fig. 2F).

Molecular identification of the selected isolates was further confirmed using species-specific primers. Species-specific PCR assay was performed on the genomic DNA of the selected isolate. The list of PCR primers used to identify *Fusarium* species is presented in Table 1. The results of PCR confirmed morphological identification of *Fusarium* isolates and proved that the isolates were properly identified as *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*.

### Distribution, frequency and density of isolates

*Fusarium*-infected seeds were observed in 79 of 85 sampling seeds grown in different regions of pre-basic seed fields. Among different wheat samples of various cultivars in the investigated provinces, Ardebil farms showed the highest distribution of *Fusarium* isolates. *Fusarium graminearum* was detected in all sampling regions except WHSH499, WHSH501, WHSH502, WHSH503, WHSH520 and WHSH556 samples. *Fusarium graminearum*, *F. culmorum* and *F. avenaceum*

**Table 2.** Morphological characters of *Fusarium* spp. isolated from different locations in pre-basic seeds wheat (*Triticum aestivum* L.) fields

| Name of the species | Pigmentation on PDA | Growth rate | Chlamydospore | Sporodochium | Microconidia | Macroconidia | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | number of septa | apical cell | basal cell | size [µm] |
| *Fusarium graminearum* | vary from white to pale pink to red | R | + | pale orange | – | 5–7 | tapered | Fs | 24–72 × 4–7 |
| *F. culmorum* | red | R | + | orange to brown | – | 3–4 | rounded and blunt | Nfs | 37–55 × 4–7 |
| *F. avenaceum* | yellow | S | – | tan to orange | + | 3–4 | tapered to pointed | Nfs | 44–70 × 3–4 |
| *F. poae* | red | S | + | tan to pale orange | + | 3 | curved | Fs | 36–47 × 3–5 |

R = rapid, S = slow, "+" = presence, "–" = absence, Fs = foot shape, Nfs = notched and without a distinct foot shape

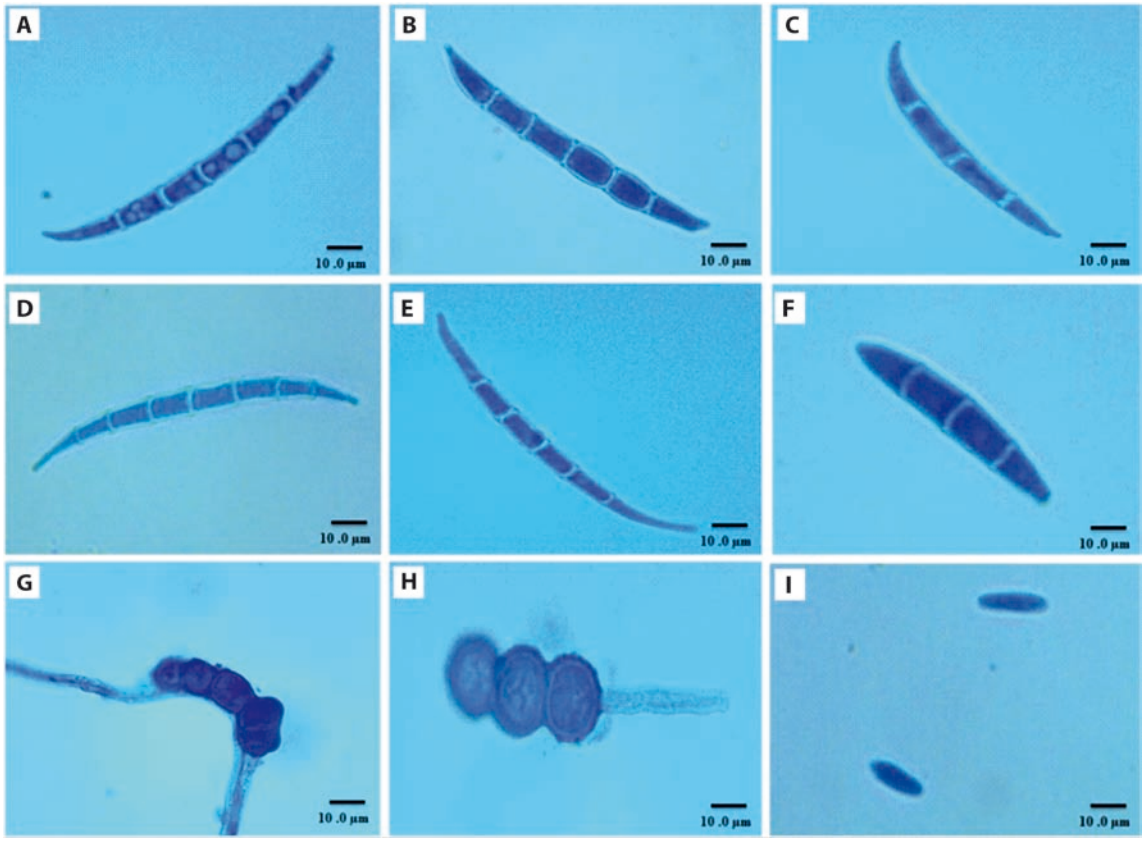


**Fig. 2.** Morphological characters of *Fusarium* species: macroconidium isolate FHB1 of *F. graminearum* (A), macroconidium isolate FHB84 of *F. graminearum* (B), macroconidium isolate FHB22 of *F. culmorum* (C), macroconidium isolate FHB29 of *F. avenaceum* (D), macroconidium isolate FHB100 of *F. avenaceum* (E), macroconidium isolate FHB66 of *F. poae* (F), chlamydospores isolate FHB76 of *F. graminearum* (G), chlamydospores isolate FHB80 of *F. culmorum* (H), microconidia FHB94 of *F. poae* (I) (scale bars = 10 µm). Photographs were taken under a microscope (Olympus BX51) at 400× magnification

were detected from different fields of Golestan, Ardebil, Mazandaran provinces, while *F. poae* was detected only from Golestan province, Iran. In our study, the highest and lowest incidences of *Fusarium* spp. were observed in samples from Ardebil and Mazandaran provinces, respectively. Approximately 5.32% of the analysed seeds were contaminated by four *Fusarium* spp. Among the isolates, *F. graminearum* was the predominant species with the highest incidence, frequency and relative density of 3.8, 92.9 and 70.9%, respectively (Fig. 1).

### Germination and vigor indices of wheat seeds

The germination percentages, normal and abnormal seedling, seedling vigor index, shoot and root lengths, fresh weight and dry weight as affected by natural infection are shown in Table 3. The seed germination percentage varied from 87.75 to 100% in all samples. Standard germination results showed that the percentage of normal seedlings was greater than 93.75%; however, the percent of abnormal seedlings was less than 6.25% for all samples. The SLVI and SWVI of the seedlings varied from 3,400 to 1,601.4 and from 28.85 to 12.1, respectively. Samples WHM0129 (belonging to Golestan province) showed the lowest vigor index.

Among cultivars, there were significant differences in the shoot and root lengths, fresh weights and dry weights (Table 3). The shoot and root lengths of the seedlings varied from 16.75 to 9 cm and from 18 to 9.25 cm, respectively. The fresh and dry weights of the seedlings varied from 2.9 to 1.8 g and from 0.28 to 0.14 g, respectively. The lowest seed germination, shoot and root lengths, fresh and dry weights were observed on samples WHM0129 (belonging to Golestan province).

### Location of *Fusarium* spp. in wheat seed

The incubation of seeds without coats allowed the internal development of *Fusarium* spp. The location of *Fusarium* spp. in the seed was studied by employing the component plating technique and the results are presented in Table 4. A high percentage of *F. graminearum* was noticed in seed coats and a lower degree of infection in embryos. *Fusarium avenaceum* and *F. poae* were located in seed coats. Only *F. graminearum* was recorded in embryos of seeds at low frequency (Table 4).

### Pathogenicity and aggressiveness assays

Comparison of the data obtained from inoculation of *Fusarium* isolates on wheat seedlings, wheat spikes and leaf segments revealed significant differences in the pathogenicity of the different *Fusarium* isolates (Table 5). The pathogenicity test revealed that 103 *Fusarium* isolates were found to be pathogenic or weakly pathogenic and the others were not pathogenic. Significant differences in Fusarium head blight index (FHB index) and leaf lesion length were observed among isolates tested.

The pathogenicity test on wheat spikes showed that the FHB index of pathogenic and weakly pathogenic isolates ranged from 19.5 ± 0.28 to 1.5 ± 0.28. The highest FHB index was observed for isolate FHB1 of *F. graminearum* with an average DI of 32.75 ± 0.25. Isolates FHB35 of *F. graminearum*, FHB37 of *F. graminearum* and FHB46 of *F. graminearum* were weakly pathogenic and showed the lowest disease progress among all *Fusarium* isolates.

Leaf assay revealed that the average lesion length of pathogenic and weakly pathogenic isolates ranged from 19.5 ± 0.28 to 1.5 ± 0.28 mm. The longest lesion length was produced by the FHB1 isolate of *F. graminearum* and the shortest lesion length was produced by FHB35 isolates of *F. graminearum* and FHB46 of *F. graminearum*, respectively. The results of the aggressiveness test on wheat spikes and detached-leaves showed earlier development of disease symptoms by isolate FHB1 of *F. graminearum* than other isolates tested (Table 5, Fig. 3).

## Discussion

In this study, a total of 453 *Fusarium* spp. isolates obtained from pre-basic seeds wheat fields were identified using molecular and morphological characters. Morphological identification was confirmed using a set of species-specific primers. This is the first detailed identification of seed-borne *Fusarium* species and their frequency in pre-basic seeds wheat fields in Iran. Furthermore, the location of *Fusarium* spp. in seeds was determined. Also, we evaluated the effect of *Fusarium* spp. on germination and vigor indices of wheat seeds.

Based on morphological observations and molecular analyses, all isolates belonging to four *Fusarium* species were isolated from wheat seed samples. Three hundred and 21 isolates were identified as *F. graminearum*, 96 isolates as *F. culmorum*, 27 isolates as *F. avenaceum* and 9 isolates as *F. poae*. The results of morphological and molecular identification of *Fusarium* species were in accordance with the reports of Abedi-Tizaki and Sabbagh (2012) and Khaledi *et al.* (2017).

The *Fusarium* species causing FHB were observed in different regions of sampling. Only 7% of the samples were found to be unaffected. Golestan had the highest percentage of infected samples. It is possible that cultures of the sensitive varieties, climate conditions and crop rotation with maize and rapeseed cause the prevalence of FHB in regions. The main species associated with FHB in wheat in Iran is *F. graminearum* as suggested by previous studies (Abedi-Tizaki and Sabbagh 2012; Davari *et al.* 2013; Khaledi *et al.* 2017). Mobasser *et al.* (2012) reported that 42.89% of the isolates recovered from wheat seeds in Iran belong to *F. graminearum*, which is in accordance with our data. Similar results were obtained in Pakistan (Bhatti and Bhutta 2002), India (Pathak and Zaidi 2012) and Serbia (Lević *et al.* 2012).

The study revealed that *F. graminearum* and *F. culmorum* were the most frequent species (70.9 and 21.2%,

**Table 3.** Means comparison of germination and vigor indices as affected by natural *Fusarium* infection in wheat seeds

| Sample code | Sample name | Cultivar/ line | Sample site | *GP* | *AS* | *SL* | *RL* | *FW* | *DW* | *SLVI* | *SWVI* | *NFI* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | WHM0144 | N-91-9 | Golestan | 95.0 | 3.2 | 13.0 | 14.7 | 2.5 | 0.24 | 2636.2 | 23.3 | 5 |
| 2 | WHM0145 | N-91-8 | Golestan | 92.2 | 5.2 | 10.6 | 12.7 | 2.4 | 0.23 | 2156.3 | 21.5 | 9 |
| 3 | WHM0135 | N-91-17 | Golestan | 95.2 | 3.0 | 13.1 | 15.4 | 2.6 | 0.24 | 2714.6 | 23.3 | 5 |
| 4 | WHM0136 | Qaboos | Golestan | 100.0 | 0.5 | 14.6 | 16.8 | 2.8 | 0.27 | 3150.0 | 28.1 | 2 |
| 5 | WHM0137 | Aftab | Golestan | 100.0 | 0.0 | 15.4 | 17.6 | 2.8 | 0.27 | 3300.0 | 26.6 | 1 |
| 6 | WHM0138 | Kohdasht | Golestan | 91.7 | 5.2 | 10.6 | 10.7 | 2.1 | 0.20 | 1961.2 | 18.2 | 10 |
| 7 | WHM0139 | Karim | Golestan | 94.2 | 4.7 | 12.2 | 14.2 | 2.6 | 0.24 | 2497.6 | 21.9 | 9 |
| 8 | WHM0140 | Line 17 | Golestan | 100.0 | 0.5 | 14.5 | 17.2 | 2.8 | 0.27 | 3175.0 | 26.4 | 2 |
| 9 | WHM0141 | Morvarid | Golestan | 98.5 | 2.7 | 13.6 | 15.9 | 2.8 | 0.27 | 2905.7 | 26.2 | 4 |
| 10 | WHM0142 | N-87-20 | Golestan | 95.0 | 3.5 | 12.9 | 15.0 | 2.6 | 0.24 | 2648.1 | 22.4 | 5 |
| 11 | WHM0143 | N-91-17 | Golestan | 95.0 | 3.2 | 13.3 | 15.2 | 2.6 | 0.24 | 2695.6 | 22.8 | 5 |
| 12 | WHM0127 | Kohdasht | Golestan | 89.7 | 5.7 | 10.2 | 10.2 | 1.8 | 0.16 | 1828.7 | 13.8 | 14 |
| 13 | WHM0128 | Line 17 | Golestan | 92.0 | 5.0 | 10.6 | 11.9 | 2.1 | 0.21 | 2070.0 | 20.1 | 11 |
| 14 | WHM0129 | Karim | Golestan | 87.7 | 6.2 | 9.0 | 9.25 | 1.8 | 0.14 | 1601.4 | 12.1 | 19 |
| 15 | WHM0130 | Qaboos | Golestan | 90.7 | 5.0 | 10.5 | 10.5 | 1.9 | 0.16 | 1905.7 | 14.85 | 12 |
| 16 | WHM0131 | N-87-20 | Golestan | 99.5 | 2.0 | 14.1 | 16.5 | 2.8 | 0.27 | 3047.2 | 26.5 | 3 |
| 17 | WHM0132 | Morvarid | Golestan | 95.2 | 3.2 | 13.2 | 15.5 | 2.6 | 0.26 | 2738.4 | 23.7 | 7 |
| 18 | WHM0133 | N-91-8 | Golestan | 94.5 | 4.5 | 12.8 | 14.5 | 2.6 | 0.23 | 2586.9 | 22.1 | 8 |
| 19 | WHM0134 | N-91-9 | Golestan | 94.0 | 5.0 | 12.2 | 14.1 | 2.66 | 0.24 | 2479.2 | 22.1 | 10 |
| 20 | WHM013 | N-92-9 | Mazandaran | 94.2 | 4.5 | 13.0 | 14.7 | 2.6 | 0.24 | 2615.4 | 23.4 | 8 |
| 21 | WHM03 | N-92-9 | Mazandaran | 94.0 | 5.0 | 12.2 | 14.2 | 2.6 | 0.23 | 2491.0 | 22.2 | 9 |
| 22 | WHM010 | N-91-8 | Mazandaran | 94.5 | 4.0 | 13.2 | 15.0 | 2.6 | 0.24 | 2669.6 | 23.8 | 8 |
| 23 | WHM05 | N-92-9 | Mazandaran | 99.2 | 1.5 | 14.2 | 16.6 | 2.8 | 0.27 | 3064.3 | 27.3 | 3 |
| 24 | WHM06 | N-92-19 | Mazandaran | 95.2 | 2.5 | 13.6 | 15.6 | 2.7 | 0.26 | 2786.1 | 24.8 | 4 |
| 25 | WHM01 | Gonbad | Mazandaran | 98.5 | 2.0 | 14.0 | 16.1 | 2.7 | 0.27 | 2967.3 | 25.8 | 4 |
| 26 | WHM04 | N-87-20 | Mazandaran | 95.0 | 3.0 | 13.2 | 15.5 | 2.6 | 0.26 | 2731.2 | 24.2 | 5 |
| 27 | WHM07 | N-91-17 | Mazandaran | 92.5 | 5.0 | 12.2 | 14.2 | 2.5 | 0.23 | 2451.2 | 21.4 | 9 |
| 28 | WHM011 | Gonbad | Mazandaran | 99.0 | 1.5 | 14.2 | 16.5 | 2.8 | 0.27 | 3044.2 | 26.5 | 5 |
| 29 | WHM015 | N-91-9 | Mazandaran | 99.7 | 1.0 | 15.2 | 16.8 | 2.8 | 0.28 | 3204.4 | 27.1 | 3 |
| 30 | WHM02 | Morvarid | Mazandaran | 93.5 | 5.0 | 12.2 | 14.25 | 2.6 | 0.23 | 2477.7 | 21.8 | 10 |
| 31 | WHM014 | N-87-20 | Mazandaran | 100.0 | 1.0 | 15.3 | 16.8 | 2.8 | 0.28 | 3225.0 | 27.5 | 2 |
| 32 | WHM016 | N-92-19 | Mazandaran | 94.5 | 4.5 | 13.0 | 14.7 | 2.6 | 0.24 | 2622.4 | 23.8 | 7 |
| 33 | WHM012 | Morvarid | Mazandaran | 99.2 | 1.5 | 14.2 | 16.6 | 2.8 | 0.27 | 3064.3 | 27.2 | 6 |
| 34 | WHSH497 | Homa | Ardebil | 99.7 | 0.5 | 15.7 | 16.2 | 2.8 | 0.28 | 3192.0 | 25.7 | 3 |
| 35 | WHSH498 | Homa | Ardebil | 97.7 | 1.0 | 14.2 | 15.5 | 2.7 | 0.26 | 2908.1 | 28.6 | 5 |
| 36 | WHSH499 | Azar 2 | Ardebil | 100.0 | 0.0 | 16.0 | 18.0 | 2.9 | 0.28 | 3400.0 | 28.1 | 0 |
| 37 | WHSH500 | Azar 2 | Ardebil | 100.0 | 0.0 | 16.0 | 17.7 | 2.8 | 0.28 | 3375.0 | 28.2 | 1 |
| 38 | WHSH501 | Baran | Ardebil | 100.0 | 0.0 | 16.0 | 18.0 | 2.9 | 0.29 | 3400.0 | 25.5 | 0 |
| 39 | WHSH502 | Baran | Ardebil | 100.0 | 0.0 | 16.0 | 18.0 | 2.8 | 0.28 | 3400.0 | 28.7 | 0 |
| 40 | WHSH503 | Saein | Ardebil | 100.0 | 0.0 | 16.0 | 18.0 | 2.8 | 0.28 | 3400.0 | 28.5 | 0 |
| 41 | WHSH504 | Saein | Ardebil | 100.0 | 0.5 | 15.9 | 16.6 | 2.8 | 0.27 | 3250.0 | 28.5 | 3 |
| 42 | WHSH505 | Rasad | Ardebil | 98.0 | 0.7 | 14.0 | 15.8 | 2.7 | 0.26 | 2927.8 | 28.8 | 6 |
| 43 | WHSH506 | Rasad | Ardebil | 98.5 | 0.7 | 14.0 | 16.0 | 2.7 | 0.26 | 2955.0 | 28.5 | 5 |
| 44 | WHSH507 | Azar 2 | Ardebil | 100.0 | 0.0 | 16.0 | 17.8 | 2.8 | 0.29 | 3387.5 | 28.4 | 1 |

**Table 3.** *Continuation*

| Sample code | Sample name | Cultivar/ line | Sample site | *GP* | *AS* | *SL* | *RL* | *FW* | *DW* | *SLVI* | *SWVI* | *NFI* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 45 | WHSH508 | Azar 2 | Ardebil | 100.0 | 0.5 | 15.8 | 17.5 | 2.8 | 0.27 | 3337.5 | 28.0 | 1 |
| 46 | WHSH515 | Orum | Ardebil | 98.5 | 0.7 | 14.0 | 15.8 | 2.7 | 0.26 | 2942.7 | 28.5 | 4 |
| 47 | WHSH516 | Mihan | Ardebil | 97.7 | 1.2 | 14.0 | 15.8 | 2.7 | 0.26 | 2920.3 | 28.8 | 5 |
| 48 | WHSH517 | Heydari | Ardebil | 97.0 | 2.7 | 13.1 | 15.5 | 2.6 | 0.25 | 2776.6 | 27.8 | 7 |
| 49 | WHSH518 | Gaskogen | Ardebil | 99.2 | 0.7 | 15.4 | 16.1 | 2.8 | 0.27 | 3126.4 | 28.0 | 6 |
| 50 | WHSH519 | Pishgam | Ardebil | 95.5 | 3.0 | 13.2 | 15.1 | 2.6 | 0.24 | 2709.8 | 28.3 | 7 |
| 51 | WHSH520 | Saysoniz | Ardebil | 100.0 | 0.0 | 16.0 | 18.0 | 2.8 | 0.28 | 3400,0 | 28.6 | 0 |
| 52 | WHSH528 | Zagros | Ardebil | 96.5 | 2.7 | 13.5 | 15.3 | 2.6 | 0.25 | 2786.4 | 28.8 | 6 |
| 53 | WHSH529 | Gaskogen | Ardebil | 100.0 | 0.2 | 15.9 | 17.5 | 2.8 | 0.27 | 3337.5 | 28.8 | 1 |
| 54 | WHSH530 | Shiroudi | Ardebil | 94.2 | 4.5 | 13.0 | 14.6 | 2.6 | 0.24 | 2603.6 | 28.4 | 9 |
| 55 | WHSH531 | Karim | Ardebil | 97.5 | 1.2 | 14.1 | 15.5 | 2.71 | 0.27 | 2888.4 | 28.1 | 6 |
| 56 | WHSH532 | N-91-17 | Ardebil | 96.2 | 2.7 | 13.4 | 15.2 | 2.6 | 0.24 | 2755.1 | 28.6 | 6 |
| 57 | WHSH533 | Kohdasht | Ardebil | 97.5 | 1.5 | 13.9 | 15.5 | 2.7 | 0.26 | 2864.0 | 25.3 | 7 |
| 58 | WHSH534 | Gonbad | Ardebil | 95.7 | 2.7 | 13.2 | 15.1 | 2.6 | 0.24 | 2716.9 | 25.5 | 6 |
| 59 | WHSH535 | Dehdasht | Ardebil | 94.7 | 3.7 | 13.1 | 14.9 | 2.6 | 0.24 | 2653.0 | 23.8 | 9 |
| 60 | WHSH536 | N-87-20 | Ardebil | 99.7 | 0.5 | 15.6 | 16.25 | 2.8 | 0.27 | 3179.5 | 28.9 | 5 |
| 61 | WHSH537 | Qaboos | Ardebil | 99.0 | 0.5 | 15.4 | 16.1 | 2.8 | 0.27 | 3118.5 | 23.6 | 5 |
| 62 | WHSH538 | Zagros | Ardebil | 91.7 | 5.0 | 10.5 | 11.7 | 2.1 | 0.20 | 2041.4 | 25.6 | 8 |
| 63 | WHSH539 | Qaboos | Ardebil | 96.7 | 3.0 | 12.8 | 15.4 | 2.5 | 0.25 | 2733.2 | 25.8 | 7 |
| 64 | WHSH540 | Saysoniz | Ardebil | 98.7 | 0.7 | 14.0 | 16.0 | 2.7 | 0.27 | 2962.5 | 28.6 | 3 |
| 65 | WHSH541 | Morvarid | Ardebil | 100.0 | 0.2 | 15.8 | 17.4 | 2.8 | 0.28 | 3325.0 | 28.7 | 2 |
| 66 | WHSH542 | Morvarid | Ardebil | 100.0 | 0.0 | 16.0 | 18.0 | 2.8 | 0.28 | 3400.0 | 28.5 | 1 |
| 67 | WHSH543 | N-87-20 | Ardebil | 100.0 | 0.5 | 15.8 | 17.5 | 2.8 | 0.27 | 3337.5 | 28.0 | 2 |
| 68 | WHSH544 | N-91-8 | Ardebil | 95.5 | 2.7 | 13.2 | 15.0 | 2.6 | 0.24 | 2697.8 | 28.9 | 6 |
| 69 | WHSH545 | N-91-9 | Ardebil | 97.2 | 1.5 | 13.8 | 15.5 | 2.7 | 0.26 | 2856.7 | 27.9 | 5 |
| 70 | WHSH546 | Zagros | Ardebil | 97.2 | 1.5 | 13.8 | 15.5 | 2.7 | 0.26 | 2856.7 | 28.9 | 6 |
| 71 | WHSH547 | N-91-9 | Ardebil | 98.7 | 0.7 | 14.0 | 16.0 | 2.7 | 0.27 | 2962.5 | 28.9 | 4 |
| 72 | WHSH548 | Aftab | Ardebil | 99.7 | 0.5 | 15.6 | 16.2 | 2.8 | 0.28 | 3179.5 | 28.7 | 3 |
| 73 | WHSH549 | Kohdasht | Ardebil | 95.5 | 2.7 | 13.2 | 15.0 | 2.6 | 0.24 | 2697.9 | 28.9 | 6 |
| 74 | WHSH550 | Gonbad | Ardebil | 92.0 | 5.0 | 10.6 | 11.8 | 2.1 | 0.21 | 2070.0 | 28.6 | 9 |
| 75 | WHSH551 | Morvarid | Ardebil | 97.7 | 1.2 | 14.0 | 15.8 | 2.7 | 0.27 | 2920.3 | 28.6 | 6 |
| 76 | WHSH552 | N-91-17 | Ardebil | 94.7 | 4.0 | 13.0 | 14.8 | 2.6 | 0.24 | 2641.1 | 27.5 | 8 |
| 77 | WHSH553 | N-91-8 | Ardebil | 95.5 | 2.7 | 13.2 | 15.0 | 2.6 | 0.24 | 2697.8 | 27.8 | 9 |
| 78 | WHSH554 | Karim | Ardebil | 97.7 | 1.25 | 14.0 | 16.0 | 2.7 | 0.26 | 2932.5 | 27.5 | 4 |
| 79 | WHSH555 | Kohdasht | Ardebil | 99.7 | 0.5 | 15.3 | 16.2 | 2.84 | 0.27 | 3154.5 | 27.8 | 3 |
| 80 | WHSH556 | Chamran | Ardebil | 100.0 | 0.0 | 16.0 | 18.0 | 2.8 | 0.28 | 3400.0 | 28.6 | 0 |
| 81 | WHSH557 | N-91-8 | Ardebil | 96.7 | 3.0 | 12.85 | 15.3 | 2.5 | 0.24 | 2733.2 | 28.7 | 7 |
| 82 | WHSH558 | Dehdasht | Ardebil | 100.0 | 0.5 | 15.1 | 17.0 | 2.8 | 0.27 | 3212.5 | 25.8 | 3 |
| 83 | WHSH559 | N-87-20 | Ardebil | 100.0 | 0.5 | 15.8 | 17.3 | 2.8 | 0.27 | 3325.0 | 27.5 | 2 |
| 84 | WHSH560 | N-91-9 | Ardebil | 99.7 | 0.7 | 15.8 | 16.2 | 2.8 | 0.27 | 3204.4 | 27.8 | 4 |
| 85 | WHSH561 | Mihan | Ardebil | 96.5 | 3.0 | 13.3 | 15.2 | 2.6 | 0.24 | 2762.3 | 23.9 | 7 |
| LSD (0.05) | | | | 0.7 | 0.5 | 0.3 | 0.4 | 0.03 | 0 | 64.8 | 0.7 | |

*GP* = germination percent, *AS* = abnormal seedling, *SL* = shoot length, *RL* = root length, *FW* = fresh weight, *DW* = dry weight, *SLVI* = seedling length vigor index, *SWVI* = seedling weight vigor index and *NFI* = number of *Fusarium* isolates. Each experiment was repeated two times with similar results

**Table 4.** Location of *Fusarium* species in the different seed parts of wheat

| *Fusarium* species | Seed component | |
|---|---|---|
| | seed coat* | embryo* |
| *F. graminearum* | 70.0 a | 1.7 a |
| *F. culmorum* | 18.3 b | 0 b |
| *F. avenaceum* | 3.3 c | 0 b |
| *F. poae* | 1.7 d | 0 b |

*infection level [%]
Different letters indicate significant differences according to LSD analysis using SAS software ($p$ = 0.05). Each experiment was repeated two times with similar results

respectively), with high (3.8%) and moderate incidence (1.1%), and with relative densities of 92.9 and 50.6%, respectively, on wheat seeds. Our data were in accordance with observations of Zare *et al.* (2006), Hajihasani *et al.* (2012) and Mobasser *et al.* (2012). Khazaei *et al.* (2014) reported that the average infection level of *F. graminearum*, *F. culmorum*, *F. avenaceaum*, *F. poa* was 46.54% from wheat seed loads in Iran.

In this study, 5.3% incidences of *Fusarium* spp. infection were recorded in pre-basic seeds wheat fields of all provinces. Overall, the highest incidence of *Fusarium* spp. was observed in Ardebil province, followed by Golestan and Mazandaran provinces. It was shown that the frequency and relative density of *Fusarium* species on all cultivars highly varied from region to region and within the region, from variety to variety. These findings were in accordance with the results obtained by Haratian *et al.* (2008) and Davari *et al.* (2013).

Research has shown that several biotic factors, intrinsic to the seed and/or interactions with other organisms, pests, pathogens and abiotic factors can delay, reduce or prevent germination (Baskin and Baskin 1998; Souza *et al.* 2015). Environmental factors such as pH, constant temperature, osmotic stress, salt stress, dry storage, light, and soil moisture are known to affect seed germination (Koger *et al.* 2004; Zhou *et al.* 2005). Biotic stresses such as seed-borne and soil-

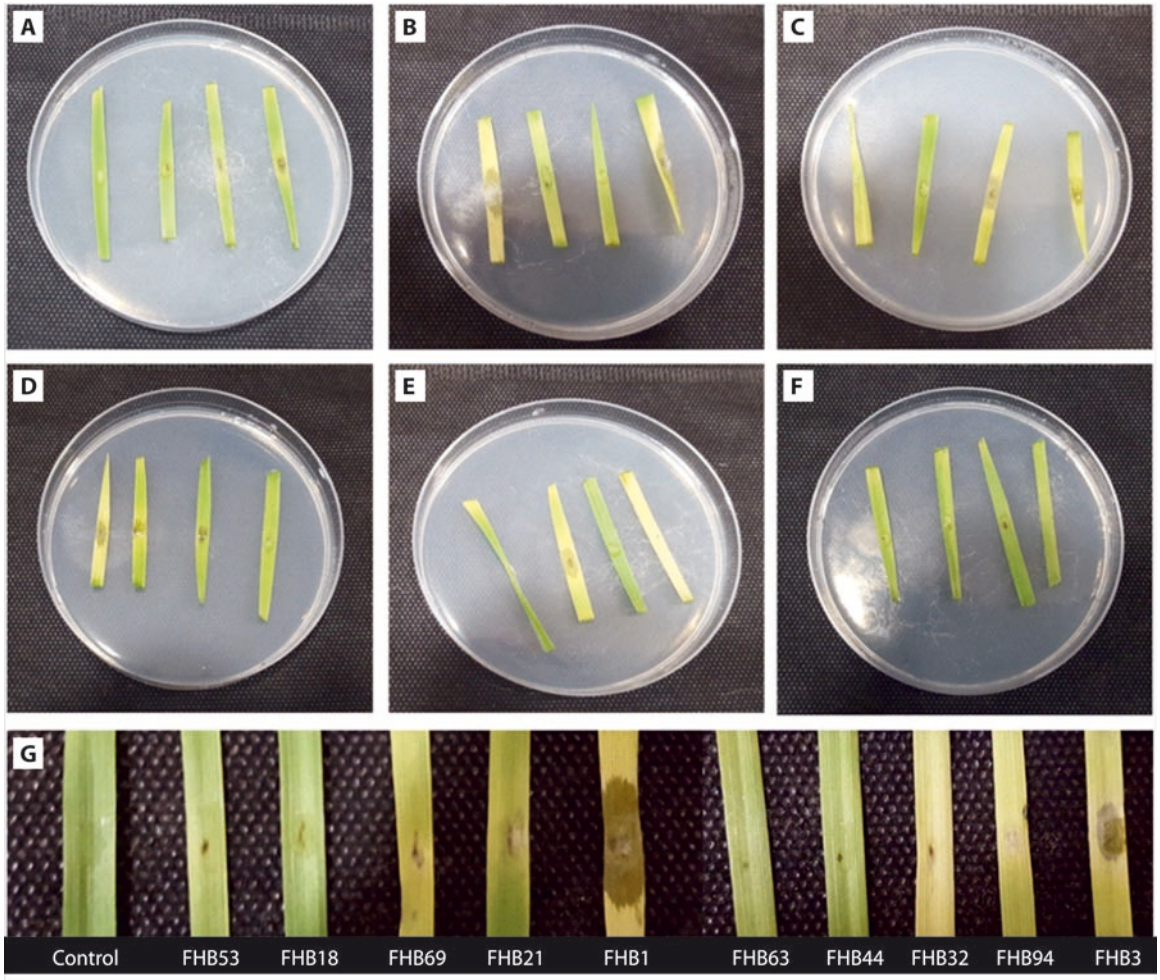


**Fig. 3.** Disease symptoms on leaf segments by isolate FHB16 of *Fusarium culmorum* (A), isolate FHB1 of *F. graminearum* (B), isolate FHB2 of *F. graminearum* (C), isolate FHB99 of *F. graminearum* (D), isolate FHB84 of *F. graminearum* (E), isolate FHB25 of *F. graminearum* (F), and control, isolates FHB53, FHB18, FHB30, FHB1, FHB44, FHB32 and FHB3 of *F. graminearum,* isolate FHB63 of *F. culmorum*, isolate FHB69 of *F. avenaceum* and isolate FHB94 of *F. poae* (G)

**Table 5.** Pathogenicity and aggressiveness of *Fusarium* isolates on inoculated wheat spikes and leaf segments

| Isolate code | Sample name | Species-specific PCR | Pathogenicity and aggressiveness analysis | | | |
|---|---|---|---|---|---|---|
| | | | leaf | | spike | |
| | | | pathogenicity [LL, mm] | aggressiveness [hpi] | pathogenicity [FHB index] | aggressiveness [hpi] |
| FHB1 | WHM0138 | *F. graminearum* | 19.5 ± 0.28 a | 71 | 32.75 ± 0.25 a | 119 |
| FHB2 | WHM0142 | *F. graminearum* | 17 ± 0 b | 74 | 28 ± 0 c | 123 |
| FHB3 | WHM0131 | *F. graminearum* | 15 ± 0 d | 78 | 26.75 ± 0.25 d | 124 |
| FHB4 | WHM03 | *F. graminearum* | 14 ± 0 e | 79 | 24.75 ± 0.25 f | 125 |
| FHB5 | WHM07 | *F. graminearum* | 14.75 ± 0.25 d | 78 | 24.75 ± 0.25 f | 124 |
| FHB6 | WHSH532 | *F. graminearum* | 14 ± 0 e | 79 | 24.25 ± 0.25 g | 125 |
| FHB7 | WHSH538 | *F. graminearum* | 14.75 ± 0.25 d | 78 | 24.75 ± 0.25 f | 124 |
| FHB8 | WHSH553 | *F. graminearum* | 15 ± 0 d | 78 | 25 ± 0 f | 124 |
| FHB9 | WHM0145 | *F. graminearum* | 14.75 ± 0.25 d | 79 | 24.75 ± 0.25 f | 124 |
| FHB10 | WHSH561 | *F. graminearum* | 14 ± 0 e | 79 | 24.25 ± 0.25 g | 125 |
| FHB11 | WHM0139 | *F. avenaceum* | 8 ± 0 l | 100 | 19 ± 0 lm | 167 |
| FHB12 | WHSH504 | *F. graminearum* | 9 ± 0 k | 99 | 20 ± 0 k | 127 |
| FHB13 | WHM015 | *F. graminearum* | 10 ± 0 j | 97 | 21 ± 0 j | 127 |
| FHB14 | WHM03 | *F. culmorum* | 13 ± 0 f | 96 | 23 ± 0 h | 126 |
| FHB15 | WHM0130 | *F. graminearum* | 11 ± 0 i | 97 | 22.25 ± 0.25 i | 128 |
| FHB16 | WHM02 | *F. culmorum* | 11 ± 0 i | 97 | 22.25 ± 0.25 i | 128 |
| FHB17 | WHM0143 | *F. graminearum* | 13 ± 0 f | 96 | 24.75 ± 0.25 f | 126 |
| FHB18 | WHM0135 | *F. graminearum* | 10 ± 0 j | 97 | 21 ± 0 j | 128 |
| FHB19 | WHM0128 | *F. avenaceum* | 9 ± 0 k | 99 | 19 ± 0 lm | 167 |
| FHB20 | WHSH559 | *F. graminearum* | 14 ± 0 e | 79 | 24.75 ± 0.25 f | 125 |
| FHB21 | WHSH544 | *F. graminearum* | 13 ± 0 f | 96 | 23 ± 0 h | 126 |
| FHB22 | WHSH539 | *F. culmorum* | 13 ± 0 f | 96 | 23 ± 0 h | 126 |
| FHB23 | WHSH540 | *F. graminearum* | 9 ± 0 k | 99 | 18.75 ± 0.25 m | 168 |
| FHB24 | WHM012 | *F. graminearum* | 12 ± 0 g | 96 | 23 ± 0 h | 128 |
| FHB25 | WHM02 | *F. graminearum* | 9 ± 0 k | 99 | 19 ± 0 lm | 167 |
| FHB26 | WHSH550 | *F. culmorum* | 7 ± 0 m | 101 | 17.25 ± 0.25 no | 168 |
| FHB27 | WHSH536 | *F. graminearum* | 10 ± 0 j | 97 | 21 ± 0 j | 128 |
| FHB28 | WHM04 | *F. graminearum* | 11.5 ± 0.28 h | 96 | 22.25 ± 0.25 i | 126 |
| FHB29 | WHSH553 | *F. avenaceum* | 13 ± 0 f | 96 | 24 ± 0 g | 126 |
| FHB30 | WHSH535 | *F. graminearum* | 14.75 ± 0.25 d | 78 | 25 ± 0 f | 124 |
| FHB31 | WHSH551 | *F. graminearum* | 7 ± 0 m | 101 | 17.75 ± 0.25 n | 168 |
| FHB32 | WHSH541 | *F. graminearum* | 4 ± 0 p | 103 | 14 ± 0 q | 191 |
| FHB33 | WHM0130 | *F. culmorum* | 7 ± 0 m | 101 | 17 ± 0 o | 169 |
| FHB34 | WHSH560 | *F. graminearum* | 3 ± 0 q | 104 | 12 ± 0 s | 192 |
| FHB35 | WHSH547 | *F. graminearum* | 1.5 ± 0.28 r | 120 | 8 ± 0 w | 195 |
| FHB36 | WHM010 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 9.75 ± 0.25 uv | 193 |
| FHB37 | WHM0128 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 8 ± 0 w | 194 |
| FHB38 | WHSH529 | *F. graminearum* | 3 ± 0 q | 104 | 13 ± 0 r | 191 |
| FHB39 | WHSH528 | *F. graminearum* | 4.75 ± 0.25 o | 101 | 15 ± 0 p | 191 |
| FHB40 | WHSH531 | *F. avenaceum* | 6 ± 0 n | 102 | 17.25 ± 0.25 o | 169 |
| FHB41 | WHSH533 | *F. graminearum* | 7 ± 0 m | 101 | 17.25 ± 0.25 o | 168 |
| FHB42 | WHSH561 | *F. culmorum* | 8 ± 0 l | 99 | 18 ± 0 n | 167 |
| FHB43 | WHSH497 | *F. graminearum* | 5 ± 0 o | 102 | 14.75 ± 0.25 p | 191 |
| FHB44 | WHSH544 | *F. graminearum* | 3 ± 0 q | 104 | 12 ± 0 s | 191 |

**Table 5.** Pathogenicity and aggressiveness of *Fusarium* isolates on inoculated wheat spikes and leaf segments – *continuation*

| Isolate code | Sample name | Species-specific PCR | Pathogenicity and aggressiveness analysis | | | |
|---|---|---|---|---|---|---|
| | | | leaf | | spike | |
| | | | pathogenicity [LL, mm] | aggressiveness [hpi] | pathogenicity [FHB index] | aggressiveness [hpi] |
| FHB45 | WHSH542 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 10.75 ± 0.25 t | 193 |
| FHB46 | WHM05 | *F. graminearum* | 1.5 ± 0.28 r | 120 | 8 ± 0 w | 194 |
| FHB47 | WHSH505 | *F. graminearum* | 4 ± 0 p | 103 | 13 ± 0 r | 191 |
| FHB48 | WHM014 | *F. graminearum* | 4.75 ± 0.25 o | 101 | 14.75 ± 0.25 p | 191 |
| FHB49 | WHSH500 | *F. graminearum* | 3 ± 0 q | 104 | 13 ± 0 r | 192 |
| FHB50 | WHSH534 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 12 ± 0 s | 192 |
| FHB51 | WHM0136 | *F. graminearum* | 6 ± 0 n | 102 | 17 ± 0 o | 168 |
| FHB52 | WHM011 | *F. graminearum* | 4.75 ± 0.25 o | 102 | 15 ± 0 p | 191 |
| FHB53 | WHM016 | *F. graminearum* | 7 ± 0 m | 101 | 17.75 ±0.25 n | 167 |
| FHB54 | WHSH546 | *F. avenaceum* | 9 ± 0 k | 99 | 19 ± 0 lm | 168 |
| FHB55 | WHM01 | *F. graminearum* | 13 ± 0 f | 96 | 23 ± 0 h | 126 |
| FHB56 | WHSH543 | *F. graminearum* | 15 ± 0 d | 78 | 25 ± 0 f | 124 |
| FHB57 | WHSH552 | *F. graminearum* | 14 ± 0 e | 79 | 24.25 ± 0.25 g | 125 |
| FHB58 | WHM0129 | *F. culmorum* | 6.75 ± 0.25 m | 101 | 17.25 ± 0.25 o | 167 |
| FHB59 | WHSH557 | *F. graminearum* | 4.75 ± 0.25 o | 102 | 14.75 ± 0.25 p | 191 |
| FHB60 | WHM0140 | *F. graminearum* | 3.25 ± 0.25 q | 103 | 14 ± 0 q | 191 |
| FHB61 | WHSH498 | *F. graminearum* | 3 ± 0 q | 104 | 9.75 ± 0.25 uv | 193 |
| FHB62 | WHSH534 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 9.75 ± 0.25 uv | 193 |
| FHB63 | WHSH506 | *F. culmorum* | 3 ± 0 q | 104 | 9.75 ± 0.25 uv | 193 |
| FHB64 | WHM0141 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 9.5 ± 0.28 v | 193 |
| FHB65 | WHSH548 | *F. graminearum* | 3 ± 0 q | 104 | 14 ± 0 q | 191 |
| FHB66 | WHM0127 | *F. poae* | 6 ± 0 n | 102 | 15 ± 0 p | 191 |
| FHB67 | WHSH554 | *F. graminearum* | 8 ± 0 l | 99 | 19.25 ± 0.25 l | 167 |
| FHB68 | WHM07 | *F. graminearum* | 10 ± 0 j | 97 | 21.25 ± 0.25 j | 126 |
| FHB69 | WHSH552 | *F. avenaceum* | 12 ± 0 g | 96 | 23 ± 0 h | 126 |
| FHB70 | WHM0131 | *F. graminearum* | 14 ± 0 e | 79 | 24.75 ± 0.25 f | 124 |
| FHB71 | WHSH545 | *F. graminearum* | 8 ± 0 l | 99 | 18 ± 0 n | 167 |
| FHB72 | WHSH517 | *F. culmorum* | 10 ± 0 j | 97 | 20 ± 0 k | 127 |
| FHB73 | WHM0134 | *F. graminearum* | 6.75 ± 0.25 m | 101 | 17 ± 0 o | 168 |
| FHB74 | WHSH519 | *F. graminearum* | 4 ± 0 p | 103 | 14 ± 0 q | 191 |
| FHB75 | WHSH515 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 9.75 ± 0.25 uv | 193 |
| FHB76 | WHSH498 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 9.5 ± 0.28 v | 193 |
| FHB77 | WHM0130 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 9.75 ± 0.25 uv | 193 |
| FHB78 | WHSH519 | *F. graminearum* | 3 ± 0 q | 104 | 14.75 ± 0.25 p | 176 |
| FHB79 | WHSH528 | *F. graminearum* | 5 ± 0 o | 102 | 14.75 ± 0.25 p | 175 |
| FHB80 | WHM013 | *F. culmorum* | 5 ± 0 o | 102 | 14.75 ± 0.25 p | 175 |
| FHB81 | WHM0137 | *F. graminearum* | 8 ± 0 l | 99 | 17.75 ± 0.25 n | 167 |
| FHB82 | WHSH549 | *F. graminearum* | 10.75 ± 0.25 i | 96 | 21 ± 0 j | 127 |
| FHB83 | WHM0134 | *F. graminearum* | 15 ± 0 d | 78 | 25 ± 0 f | 124 |
| FHB84 | WHSH518 | *F. graminearum* | 17.25 ± 0.25 b | 74 | 28.5 ± 0.28 b | 121 |
| FHB85 | WHSH555 | *F. graminearum* | 16 ± 0 c | 76 | 26 ± 0 e | 123 |
| FHB86 | WHSH505 | *F. graminearum* | 14 ± 0 e | 79 | 24.75 ± 0.25 f | 125 |
| FHB87 | WHM0138 | *F. graminearum* | 14.75 ± 0.25 d | 78 | 24.75 ± 0.25 f | 124 |
| FHB88 | WHM0132 | *F. graminearum* | 3 ± 0 q | 104 | 14.75 ± 0.25 p | 177 |

**Table 5.** *Continuation*

| Isolate code | Sample name | Species-specific PCR | Pathogenicity and aggressiveness analysis | | | |
|---|---|---|---|---|---|---|
| | | | leaf | | spike | |
| | | | pathogenicity [LL, mm] | aggressiveness [hpi] | pathogenicity [FHB index] | aggressiveness [hpi] |
| FHB89 | WHSH530 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 10 ± 0 u | 193 |
| FHB90 | WHSH516 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 9.5 ± 0.28 v | 193 |
| FHB91 | WHSH532 | *F. graminearum* | 2.75 ± 0.25 q | 104 | 10 ± 0 u | 193 |
| FHB92 | WHM0133 | *F. graminearum* | 4 ± 0 p | 103 | 15 ± 0 p | 176 |
| FHB93 | WHSH557 | *F. graminearum* | 5 ± 0 o | 102 | 15 ± 0 p | 175 |
| FHB94 | WHM0129 | *F. poae* | 6 ± 0 n | 102 | 17.25 ± 0.25 o | 169 |
| FHB95 | WHSH537 | *F. graminearum* | 8.25 ± 0.25 l | 98 | 17.75 ± 0.25 n | 169 |
| FHB96 | WHM0145 | *F. culmorum* | 10.75 ± 0.25 i | 97 | 21 ± 0 j | 127 |
| FHB97 | WHSH508 | *F. graminearum* | 13 ± 0 f | 96 | 24.25 ± 0.25 g | 127 |
| FHB98 | WHM06 | *F. graminearum* | 14.5 ± 0.28 d | 78 | 25 ± 0 f | 124 |
| FHB99 | WHM0129 | *F. graminearum* | 17 ± 0 b | 74 | 28.25 ± 0.25 bc | 121 |
| FHB100 | WHM0144 | *F. avenaceum* | 11.5 ± 0.28 h | 96 | 22 ± 0 i | 126 |
| FHB101 | WHSH558 | *F. graminearum* | 11 ± 0 i | 96 | 21 ± 0 j | 127 |
| FHB102 | WHSH530 | *F. culmorum* | 6.25 ± 0.25 n | 101 | 17.75 ± 0.25 n | 168 |
| FHB103 | WHSH507 | *F. graminearum* | 4.75 ± 0.25 o | 102 | 15 ± 0 p | 175 |
| LSD (0.05) | | | 0.4 | | 0.5 | |

hpi = hours post inoculation, LL = lesion length, FHB index = Fusarium head blight index; average ± standard error; different letters indicate significant differences according to LSD analysis using SAS software ($p = 0.05$); each experiment was repeated two times with similar results

borne pathogens are known to affect seed germination (Lamichhane *et al*. 2018).

The germination ability of wheat seeds is one of the most important indicators of quality, viability and germination of wheat seed. In our study there was a significant negative impact of seed-borne *Fusarium* on germination and vigor indices of wheat seeds. As in previous *in vitro* assays of wheat (Browne and Cooke 2005; Browne 2007), a significant association between germination and *Fusarium* infection was observed in our study. According to the national seeds standard the minimal percentage of germination in pre-basic seeds wheat fields was reported to be 90%, which is less than the percentage of germination in our data, except WHM0127 and WHM0129 samples (Osroush 2010). *Fusarium* spp. infection in wheat seed causes decreased germination percentage. Similar results were obtained by Hare *et al*. (1999), who observed that reduced germination of seedlings of wheat was associated with infection by *F. culmorum*. Browne and Cooke (2005) reported that *F. graminearum* caused the greatest reduction with a mean seed germination of 61.7% relative to the controls followed by *F. avenaceum* (65.5%), *F. culmorum* (76.6%), and was least for *F. poae* (92.5%), which is in accordance with our observations.

The number of abnormal seedlings increased significantly in samples which contained more infected seeds. The standard germination in all the samples was 93.75% normal and 6.25% abnormal seedlings. We found that shoot and root lengths decreased with increased natural infection by *Fusarium* spp., however, the fresh and dry weights as well as the length of the seedlings tended to decrease compared to more infected seeds. The respiration of seeds and *Fusarium* spp. results in a loss in dry weight as well as the production of heat and moisture which contribute to further disease spread and postharvest losses. Numerous reports have indicated that germination and vigor indices were decreased due to increased *Fusarium*-infected seeds (Browne 2007; Franke *et al*. 2014), which is supported by our results. Browne (2007) reported that seed-borne *Fusarium* had a significantly negative relationship with seed weight and germination.

Some studies revealed the location activities of some fungal pathogens in seed coats, cotyledons, endosperms and embryonic axes (Dubey and Singh 2005; Thippeswamy *et al*. 2006; Nagaraja and Krishnappa 2016). The results showed that a higher infection level of *F. graminearum* most commonly occurred in the seed coat followed by the embryo (1.7%). Most *Fusarium* spp. were observed in the seed coat and cotyledon. Similar results were obtained by Mahmoud *et al*. (2013), who reported that *Fusarium* sp. were more active in the seed coat than in the embryo. Among the species of *Fusarium* detected in wheat seeds, only *F. graminearum* was observed in the embryo of seeds.

This is in accordance with the observations of Geleta *et al*. (2005) who detected *F. graminearum* in the embryo of seeds.

The genus *Fusarium* has no specialized structures for penetration into plant cells and enters the host via natural openings or penetrates the epidermal cell walls directly with short infection-hyphae (Pritsch *et al*. 2000; Wanyoike *et al*. 2002). *Fusarium* spp. are able to penetrate and invade a host with the help of secreted cell wall degrading enzymes. Production of cell wall degrading enzymes also enables the pathogen to penetrate, grow and infect the plant tissue (Kikot *et al*. 2009). Aggressiveness and pathogenicity of *Fusarium* spp. involve different mechanisms such as production of extracellular enzymes and mycotoxins (Ortega *et al*. 2013; Khaledi *et al*. 2017). Various cell wall degrading enzymes such as cellulase, xylanase, pectinase and lipase could be produced by *Fusarium* spp. during infection of wheat spikes (Ortega *et al*. 2013). Mycotoxins such as trichothecenes, zearalenone and fumonisins have a significant role in determining the pathogenicity of *Fusarium* isolates (Purahong *et al*. 2013; Hernandez-Nopsa *et al*. 2014).

*Fusarium* isolates without the ability of producing trichothecenes were unable to cause severe disease in wheat tissues (Bai *et al*. 2002; Khaledi *et al*. 2017). According to other investigators, a strong association has been found between the severity of FHB and mycotoxin concentration (Wegulo 2012; Hernandez-Nopsa *et al*. 2014; Panthi *et al*. 2014). Trichothecenes are considered to be virulence factors during plant infection and are also associated with pathogen aggressiveness (Bai *et al*. 2002; Foroud and Eudes 2009; Pasquali and Migheli 2014). *Fusarium* isolates without the ability of producing trichothecenes were unable to cause severe disease in wheat tissues (Bai *et al*. 2002; Khaledi *et al*. 2017).

The results of our study about pathogenicity of *Fusarium* isolates on wheat spikes and leaf segments showed that isolates of *Fusarium* spp. were pathogenic or weakly pathogenic on wheat and differences in aggressiveness and pathogenicity were found. We found a correlation between aggressiveness and pathogenicity of *Fusarium* isolates. Our data are in accordance with observations of Khaledi *et al*. (2017), who found a correlation between virulence factors, aggressiveness and pathogenicity of *Fusarium* isolates on seedlings, spikes and leaves of wheat plants.

In our study about pathogenicity of *Fusarium* isolates on wheat spikes and leaf segments we saw differences in pathogenicity. One hundred and three (about 22.7%) of 453 isolates were pathogenic or weakly pathogenic in wheat (cv. Falat). The *Fusarium* isolates recovered from wheat seeds revealed differences in their pathogenicity in wheat cultivars. A detached leaf assay was used to pre-screen *Fusarium* isolates pathogenicity causing FHB in wheat cultivars. Results showed that 77.3% isolates of *Fusarium* spp. were found not to be pathogenic. Partial disease resistance components detected in the detached leaf assay have been correlated to FHB in wheat (Browne 2007; Shin *et al*. 2014). Wu *et al*. (2005) reported that leaf lesion length by *Fusarium* isolates was significantly correlated with FHB index of the same *Fusarium* isolates under field conditions. Overall, isolate FHB1 of *F. graminearum* caused the highest disease progress with an average FHB index of 32.75 ± 0.25 and leaf lesion length of 19.5 ± 0.28. In our investigations, the aggressiveness of the *Fusarium* isolates varied as much within a species as among species. This is in accordance with the results obtained by Sakr (2017), Purahong *et al*. (2012) and Wu *et al*. (2005). Significant differences in pathogenicity were observed among the *Fusarium* spp. isolates, which is evidence of differences in aggressiveness. The highest variation in aggressiveness was observed among the *F. graminearum* isolates. Alvarez *et al*. (2010) reported high variation in aggressiveness among *F. graminearum* isolates from Argentina, which is in accordance with our data.

In summary, this study indicates that germination and vigor indices of wheat were significantly influenced with different seed contamination rates. Seed-borne *Fusarium* caused reduction in quality and germination of seed and, also, loss in seed viability. The data on the frequency and relative density of the fungal species on wheat is important for the application of strategy, which will reduce their incidence on wheat seeds. Certified and healthy seeds of wheat can contribute to food security in the world (Rahman *et al*. 2018), since they provide significant input for crop production and consequently avoid the reduction of yield and quality caused by seed-borne *Fusarium*. Knowledge about seed-borne *Fusarium* species associated with wheat and pathogenicity could be useful for management strategies to reduce destructive effects of FHB disease in seeds and increase the seed production of wheat.

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