

ORIGINAL ARTICLE

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

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Vol. 59, No. 1: 69–85, 2019

DOI: 10.24425/jppr.2019.126037

Received: August 3, 2018

Accepted: February 21, 2019

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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  $25 \pm 1^\circ\text{C}$  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  $4^\circ\text{C}$  and sub-cultured monthly. The isolation frequency (*Fr*) and relative density (*RD*) of fungi were recorded and calculated (Hajjhasani *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<sup>2</sup>) 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 · μl<sup>-1</sup> 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
<i>F. graminearum</i>	Fg16F Fg16R	CTCCGGATATGTTGCGTCAA GGTAGGTATCCGACATGGCAA	400–500	60°C/60 s	Nicholson <i>et al.</i> (1998)
<i>F. culmorum</i>	OPT18F OPT18R	GATGCCAGACCAAGACGAAG GATGCCAGACGCACTAAGAT	472	59°C/30 s	Schilling <i>et al.</i> (1996)
<i>F. avenaceum</i>	FA-F FA-R	GCTAATTCTTAACCTACTAGGGGCC CTGTAATAGGTTATTACATGGGCG	272	60°C/30 s	Schilling <i>et al.</i> (1996)
<i>F. poae</i>	Fp82-F Fp82-R	CAAGCAAACAGGCTCTTCACC 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  $20 \pm 1^\circ\text{C}$  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  $75^\circ\text{C}$ . Also, seedling length vigor index (SLVI) and seedling weight vigor index (SWVI) relations were determined by the following equations (Nautiyal *et al.* 2009):

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

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

### 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  $25 \pm 1^\circ\text{C}$  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  $25^\circ\text{C}$ . Germinated seeds were sown in each of the 15 cm-diameter plastic pots filled with sterile potting soil, which had been autoclaved at  $121^\circ\text{C}$  for a minimum of 30 min at 100 kPa (15 psi) on 2 successive days and grown in a greenhouse ( $28 \pm 4^\circ\text{C}$ ; 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  $\cdot$  ml<sup>-1</sup> 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  $\cdot$  ml<sup>-1</sup>) 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  $\mu$ l inoculum suspension of  $1 \times 10^5$  conidia  $\cdot$  ml<sup>-1</sup>. 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  $\mu$ m for *F. graminearum* (Figs 2A–B), 37 to 55  $\mu$ m for *F. culmorum* (Fig. 2C), 44 to 70  $\mu$ m for *F. avenaceum* (Figs 2D–E), and 36 to 47  $\mu$ m 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*.

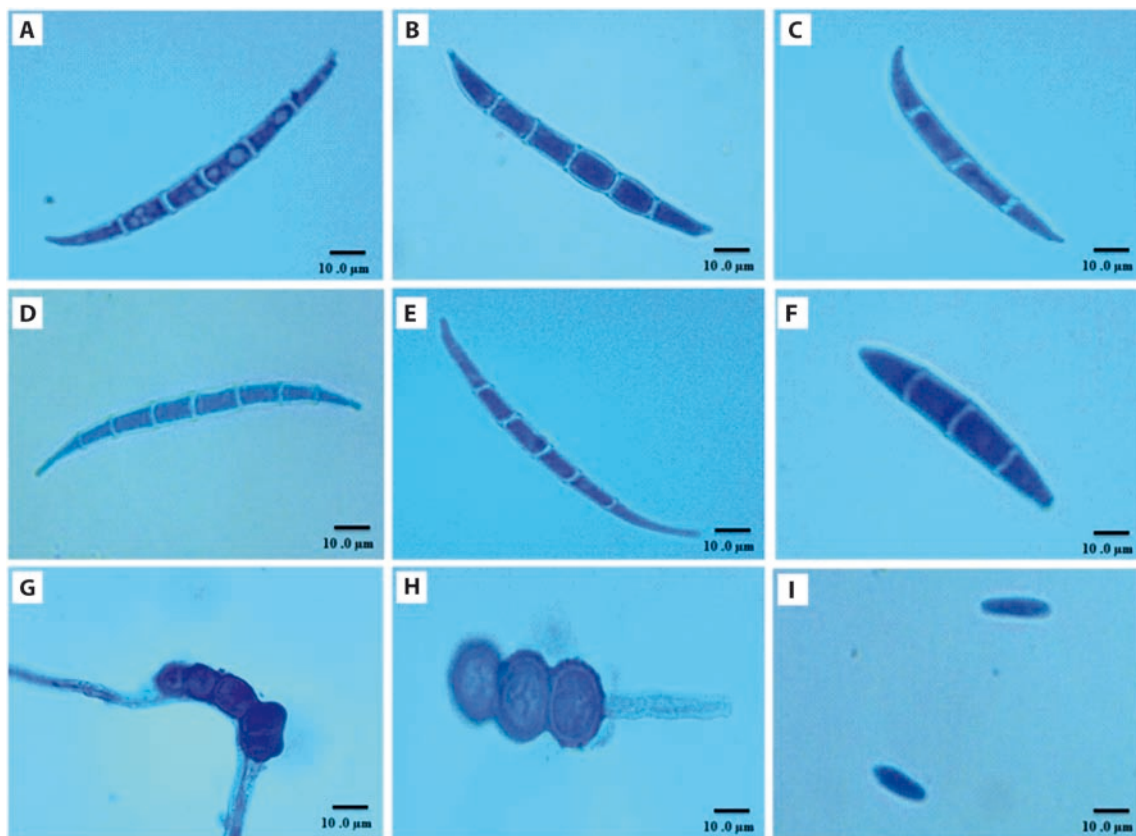
### Distribution, frequency and density of isolates

*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 WSH499, WSH501, WSH502, WSH503, WSH520 and WSH556 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 [ $\mu\text{m}$ ]
<i>Fusarium graminearum</i>	vary from white to pale pink to red	R	+	pale orange	-	5-7	tapered	Fs	24-72 $\times$ 4-7
<i>F. culmorum</i>	red	R	+	orange to brown	-	3-4	rounded and blunt	Nfs	37-55 $\times$ 4-7
<i>F. avenaceum</i>	yellow	S	-	tan to orange	+	3-4	tapered to pointed	Nfs	44-70 $\times$ 3-4
<i>F. poae</i>	red	S	+	tan to pale orange	+	3	curved	Fs	36-47 $\times$ 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  $\mu\text{m}$ ). Photographs were taken under a microscope (Olympus BX51) at 400 $\times$  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  $19.5 \pm 0.28$  to  $1.5 \pm 0.28$ . The highest FHB index was observed for isolate FHB1 of *F. graminearum* with an average DI of  $32.75 \pm 0.25$ . 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  $19.5 \pm 0.28$  to  $1.5 \pm 0.28$  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	WHS497	Homa	Ardebil	99.7	0.5	15.7	16.2	2.8	0.28	3192.0	25.7	3
35	WHS498	Homa	Ardebil	97.7	1.0	14.2	15.5	2.7	0.26	2908.1	28.6	5
36	WHS499	Azar 2	Ardebil	100.0	0.0	16.0	18.0	2.9	0.28	3400.0	28.1	0
37	WHS500	Azar 2	Ardebil	100.0	0.0	16.0	17.7	2.8	0.28	3375.0	28.2	1
38	WHS501	Baran	Ardebil	100.0	0.0	16.0	18.0	2.9	0.29	3400.0	25.5	0
39	WHS502	Baran	Ardebil	100.0	0.0	16.0	18.0	2.8	0.28	3400.0	28.7	0
40	WHS503	Saein	Ardebil	100.0	0.0	16.0	18.0	2.8	0.28	3400.0	28.5	0
41	WHS504	Saein	Ardebil	100.0	0.5	15.9	16.6	2.8	0.27	3250.0	28.5	3
42	WHS505	Rasad	Ardebil	98.0	0.7	14.0	15.8	2.7	0.26	2927.8	28.8	6
43	WHS506	Rasad	Ardebil	98.5	0.7	14.0	16.0	2.7	0.26	2955.0	28.5	5
44	WHS507	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	WHS508	Azar 2	Ardebil	100.0	0.5	15.8	17.5	2.8	0.27	3337.5	28.0	1
46	WHS515	Orum	Ardebil	98.5	0.7	14.0	15.8	2.7	0.26	2942.7	28.5	4
47	WHS516	Mihan	Ardebil	97.7	1.2	14.0	15.8	2.7	0.26	2920.3	28.8	5
48	WHS517	Heydari	Ardebil	97.0	2.7	13.1	15.5	2.6	0.25	2776.6	27.8	7
49	WHS518	Gaskogen	Ardebil	99.2	0.7	15.4	16.1	2.8	0.27	3126.4	28.0	6
50	WHS519	Pishgam	Ardebil	95.5	3.0	13.2	15.1	2.6	0.24	2709.8	28.3	7
51	WHS520	Saysoniz	Ardebil	100.0	0.0	16.0	18.0	2.8	0.28	3400.0	28.6	0
52	WHS528	Zagros	Ardebil	96.5	2.7	13.5	15.3	2.6	0.25	2786.4	28.8	6
53	WHS529	Gaskogen	Ardebil	100.0	0.2	15.9	17.5	2.8	0.27	3337.5	28.8	1
54	WHS530	Shiroudi	Ardebil	94.2	4.5	13.0	14.6	2.6	0.24	2603.6	28.4	9
55	WHS531	Karim	Ardebil	97.5	1.2	14.1	15.5	2.71	0.27	2888.4	28.1	6
56	WHS532	N-91-17	Ardebil	96.2	2.7	13.4	15.2	2.6	0.24	2755.1	28.6	6
57	WHS533	Kohdasht	Ardebil	97.5	1.5	13.9	15.5	2.7	0.26	2864.0	25.3	7
58	WHS534	Gonbad	Ardebil	95.7	2.7	13.2	15.1	2.6	0.24	2716.9	25.5	6
59	WHS535	Dehdasht	Ardebil	94.7	3.7	13.1	14.9	2.6	0.24	2653.0	23.8	9
60	WHS536	N-87-20	Ardebil	99.7	0.5	15.6	16.25	2.8	0.27	3179.5	28.9	5
61	WHS537	Qaboos	Ardebil	99.0	0.5	15.4	16.1	2.8	0.27	3118.5	23.6	5
62	WHS538	Zagros	Ardebil	91.7	5.0	10.5	11.7	2.1	0.20	2041.4	25.6	8
63	WHS539	Qaboos	Ardebil	96.7	3.0	12.8	15.4	2.5	0.25	2733.2	25.8	7
64	WHS540	Saysoniz	Ardebil	98.7	0.7	14.0	16.0	2.7	0.27	2962.5	28.6	3
65	WHS541	Morvarid	Ardebil	100.0	0.2	15.8	17.4	2.8	0.28	3325.0	28.7	2
66	WHS542	Morvarid	Ardebil	100.0	0.0	16.0	18.0	2.8	0.28	3400.0	28.5	1
67	WHS543	N-87-20	Ardebil	100.0	0.5	15.8	17.5	2.8	0.27	3337.5	28.0	2
68	WHS544	N-91-8	Ardebil	95.5	2.7	13.2	15.0	2.6	0.24	2697.8	28.9	6
69	WHS545	N-91-9	Ardebil	97.2	1.5	13.8	15.5	2.7	0.26	2856.7	27.9	5
70	WHS546	Zagros	Ardebil	97.2	1.5	13.8	15.5	2.7	0.26	2856.7	28.9	6
71	WHS547	N-91-9	Ardebil	98.7	0.7	14.0	16.0	2.7	0.27	2962.5	28.9	4
72	WHS548	Aftab	Ardebil	99.7	0.5	15.6	16.2	2.8	0.28	3179.5	28.7	3
73	WHS549	Kohdasht	Ardebil	95.5	2.7	13.2	15.0	2.6	0.24	2697.9	28.9	6
74	WHS550	Gonbad	Ardebil	92.0	5.0	10.6	11.8	2.1	0.21	2070.0	28.6	9
75	WHS551	Morvarid	Ardebil	97.7	1.2	14.0	15.8	2.7	0.27	2920.3	28.6	6
76	WHS552	N-91-17	Ardebil	94.7	4.0	13.0	14.8	2.6	0.24	2641.1	27.5	8
77	WHS553	N-91-8	Ardebil	95.5	2.7	13.2	15.0	2.6	0.24	2697.8	27.8	9
78	WHS554	Karim	Ardebil	97.7	1.25	14.0	16.0	2.7	0.26	2932.5	27.5	4
79	WHS555	Kohdasht	Ardebil	99.7	0.5	15.3	16.2	2.84	0.27	3154.5	27.8	3
80	WHS556	Chamran	Ardebil	100.0	0.0	16.0	18.0	2.8	0.28	3400.0	28.6	0
81	WHS557	N-91-8	Ardebil	96.7	3.0	12.85	15.3	2.5	0.24	2733.2	28.7	7
82	WHS558	Dehdasht	Ardebil	100.0	0.5	15.1	17.0	2.8	0.27	3212.5	25.8	3
83	WHS559	N-87-20	Ardebil	100.0	0.5	15.8	17.3	2.8	0.27	3325.0	27.5	2
84	WHS560	N-91-9	Ardebil	99.7	0.7	15.8	16.2	2.8	0.27	3204.4	27.8	4
85	WHS561	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

<i>Fusarium</i> species	Seed component	
	seed coat*	embryo*
<i>F. graminearum</i>	70.0 a	1.7 a
<i>F. culmorum</i>	18.3 b	0 b
<i>F. avenaceum</i>	3.3 c	0 b
<i>F. poae</i>	1.7 d	0 b

\*infection level [%]

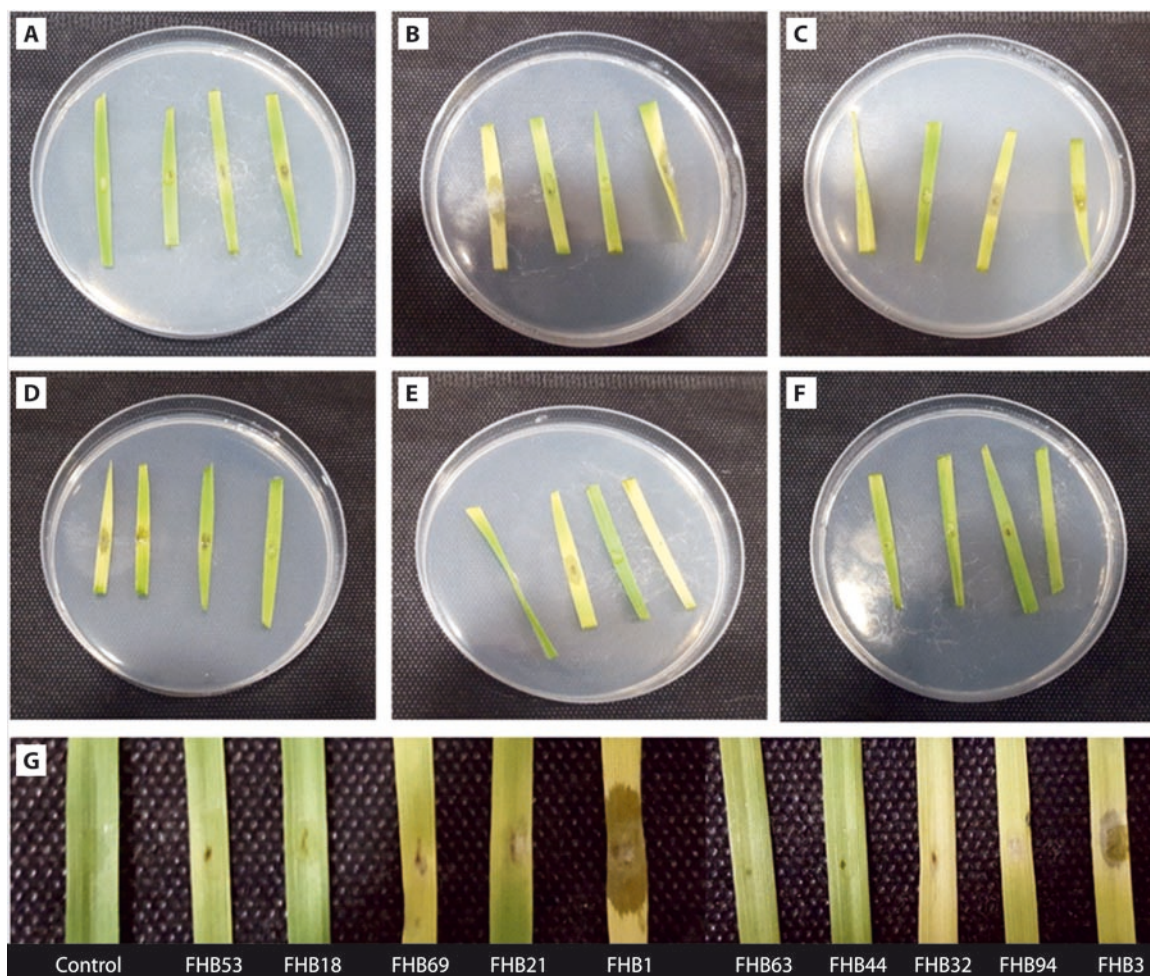
Different letters indicate significant differences according to LSD analysis using SAS software ( $p = 0.05$ ). Each experiment was repeated two times with similar results

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), Hajjhasani *et al.* (2012) and Mobasser *et al.* (2012). Khazaei *et al.* (2014) reported that the average infection level of

*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae* 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 FHB94 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	<i>F. graminearum</i>	19.5 ± 0.28 a	71	32.75 ± 0.25 a	119
FHB2	WHM0142	<i>F. graminearum</i>	17 ± 0 b	74	28 ± 0 c	123
FHB3	WHM0131	<i>F. graminearum</i>	15 ± 0 d	78	26.75 ± 0.25 d	124
FHB4	WHM03	<i>F. graminearum</i>	14 ± 0 e	79	24.75 ± 0.25 f	125
FHB5	WHM07	<i>F. graminearum</i>	14.75 ± 0.25 d	78	24.75 ± 0.25 f	124
FHB6	WHS532	<i>F. graminearum</i>	14 ± 0 e	79	24.25 ± 0.25 g	125
FHB7	WHS538	<i>F. graminearum</i>	14.75 ± 0.25 d	78	24.75 ± 0.25 f	124
FHB8	WHS553	<i>F. graminearum</i>	15 ± 0 d	78	25 ± 0 f	124
FHB9	WHM0145	<i>F. graminearum</i>	14.75 ± 0.25 d	79	24.75 ± 0.25 f	124
FHB10	WHS561	<i>F. graminearum</i>	14 ± 0 e	79	24.25 ± 0.25 g	125
FHB11	WHM0139	<i>F. avenaceum</i>	8 ± 0 l	100	19 ± 0 lm	167
FHB12	WHS504	<i>F. graminearum</i>	9 ± 0 k	99	20 ± 0 k	127
FHB13	WHM015	<i>F. graminearum</i>	10 ± 0 j	97	21 ± 0 j	127
FHB14	WHM03	<i>F. culmorum</i>	13 ± 0 f	96	23 ± 0 h	126
FHB15	WHM0130	<i>F. graminearum</i>	11 ± 0 i	97	22.25 ± 0.25 i	128
FHB16	WHM02	<i>F. culmorum</i>	11 ± 0 i	97	22.25 ± 0.25 i	128
FHB17	WHM0143	<i>F. graminearum</i>	13 ± 0 f	96	24.75 ± 0.25 f	126
FHB18	WHM0135	<i>F. graminearum</i>	10 ± 0 j	97	21 ± 0 j	128
FHB19	WHM0128	<i>F. avenaceum</i>	9 ± 0 k	99	19 ± 0 lm	167
FHB20	WHS559	<i>F. graminearum</i>	14 ± 0 e	79	24.75 ± 0.25 f	125
FHB21	WHS544	<i>F. graminearum</i>	13 ± 0 f	96	23 ± 0 h	126
FHB22	WHS539	<i>F. culmorum</i>	13 ± 0 f	96	23 ± 0 h	126
FHB23	WHS540	<i>F. graminearum</i>	9 ± 0 k	99	18.75 ± 0.25 m	168
FHB24	WHM012	<i>F. graminearum</i>	12 ± 0 g	96	23 ± 0 h	128
FHB25	WHM02	<i>F. graminearum</i>	9 ± 0 k	99	19 ± 0 lm	167
FHB26	WHS550	<i>F. culmorum</i>	7 ± 0 m	101	17.25 ± 0.25 no	168
FHB27	WHS536	<i>F. graminearum</i>	10 ± 0 j	97	21 ± 0 j	128
FHB28	WHM04	<i>F. graminearum</i>	11.5 ± 0.28 h	96	22.25 ± 0.25 i	126
FHB29	WHS553	<i>F. avenaceum</i>	13 ± 0 f	96	24 ± 0 g	126
FHB30	WHS535	<i>F. graminearum</i>	14.75 ± 0.25 d	78	25 ± 0 f	124
FHB31	WHS551	<i>F. graminearum</i>	7 ± 0 m	101	17.75 ± 0.25 n	168
FHB32	WHS541	<i>F. graminearum</i>	4 ± 0 p	103	14 ± 0 q	191
FHB33	WHM0130	<i>F. culmorum</i>	7 ± 0 m	101	17 ± 0 o	169
FHB34	WHS560	<i>F. graminearum</i>	3 ± 0 q	104	12 ± 0 s	192
FHB35	WHS547	<i>F. graminearum</i>	1.5 ± 0.28 r	120	8 ± 0 w	195
FHB36	WHM010	<i>F. graminearum</i>	2.75 ± 0.25 q	104	9.75 ± 0.25 uv	193
FHB37	WHM0128	<i>F. graminearum</i>	2.75 ± 0.25 q	104	8 ± 0 w	194
FHB38	WHS529	<i>F. graminearum</i>	3 ± 0 q	104	13 ± 0 r	191
FHB39	WHS528	<i>F. graminearum</i>	4.75 ± 0.25 o	101	15 ± 0 p	191
FHB40	WHS531	<i>F. avenaceum</i>	6 ± 0 n	102	17.25 ± 0.25 o	169
FHB41	WHS533	<i>F. graminearum</i>	7 ± 0 m	101	17.25 ± 0.25 o	168
FHB42	WHS561	<i>F. culmorum</i>	8 ± 0 l	99	18 ± 0 n	167
FHB43	WHS497	<i>F. graminearum</i>	5 ± 0 o	102	14.75 ± 0.25 p	191
FHB44	WHS544	<i>F. graminearum</i>	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	WHS542	<i>F. graminearum</i>	2.75 ± 0.25 q	104	10.75 ± 0.25 t	193
FHB46	WHM05	<i>F. graminearum</i>	1.5 ± 0.28 r	120	8 ± 0 w	194
FHB47	WHS505	<i>F. graminearum</i>	4 ± 0 p	103	13 ± 0 r	191
FHB48	WHM014	<i>F. graminearum</i>	4.75 ± 0.25 o	101	14.75 ± 0.25 p	191
FHB49	WHS500	<i>F. graminearum</i>	3 ± 0 q	104	13 ± 0 r	192
FHB50	WHS534	<i>F. graminearum</i>	2.75 ± 0.25 q	104	12 ± 0 s	192
FHB51	WHM0136	<i>F. graminearum</i>	6 ± 0 n	102	17 ± 0 o	168
FHB52	WHM011	<i>F. graminearum</i>	4.75 ± 0.25 o	102	15 ± 0 p	191
FHB53	WHM016	<i>F. graminearum</i>	7 ± 0 m	101	17.75 ± 0.25 n	167
FHB54	WHS546	<i>F. avenaceum</i>	9 ± 0 k	99	19 ± 0 lm	168
FHB55	WHM01	<i>F. graminearum</i>	13 ± 0 f	96	23 ± 0 h	126
FHB56	WHS543	<i>F. graminearum</i>	15 ± 0 d	78	25 ± 0 f	124
FHB57	WHS552	<i>F. graminearum</i>	14 ± 0 e	79	24.25 ± 0.25 g	125
FHB58	WHM0129	<i>F. culmorum</i>	6.75 ± 0.25 m	101	17.25 ± 0.25 o	167
FHB59	WHS557	<i>F. graminearum</i>	4.75 ± 0.25 o	102	14.75 ± 0.25 p	191
FHB60	WHM0140	<i>F. graminearum</i>	3.25 ± 0.25 q	103	14 ± 0 q	191
FHB61	WHS498	<i>F. graminearum</i>	3 ± 0 q	104	9.75 ± 0.25 uv	193
FHB62	WHS534	<i>F. graminearum</i>	2.75 ± 0.25 q	104	9.75 ± 0.25 uv	193
FHB63	WHS506	<i>F. culmorum</i>	3 ± 0 q	104	9.75 ± 0.25 uv	193
FHB64	WHM0141	<i>F. graminearum</i>	2.75 ± 0.25 q	104	9.5 ± 0.28 v	193
FHB65	WHS548	<i>F. graminearum</i>	3 ± 0 q	104	14 ± 0 q	191
FHB66	WHM0127	<i>F. poae</i>	6 ± 0 n	102	15 ± 0 p	191
FHB67	WHS554	<i>F. graminearum</i>	8 ± 0 l	99	19.25 ± 0.25 l	167
FHB68	WHM07	<i>F. graminearum</i>	10 ± 0 j	97	21.25 ± 0.25 j	126
FHB69	WHS552	<i>F. avenaceum</i>	12 ± 0 g	96	23 ± 0 h	126
FHB70	WHM0131	<i>F. graminearum</i>	14 ± 0 e	79	24.75 ± 0.25 f	124
FHB71	WHS545	<i>F. graminearum</i>	8 ± 0 l	99	18 ± 0 n	167
FHB72	WHS517	<i>F. culmorum</i>	10 ± 0 j	97	20 ± 0 k	127
FHB73	WHM0134	<i>F. graminearum</i>	6.75 ± 0.25 m	101	17 ± 0 o	168
FHB74	WHS519	<i>F. graminearum</i>	4 ± 0 p	103	14 ± 0 q	191
FHB75	WHS515	<i>F. graminearum</i>	2.75 ± 0.25 q	104	9.75 ± 0.25 uv	193
FHB76	WHS498	<i>F. graminearum</i>	2.75 ± 0.25 q	104	9.5 ± 0.28 v	193
FHB77	WHM0130	<i>F. graminearum</i>	2.75 ± 0.25 q	104	9.75 ± 0.25 uv	193
FHB78	WHS519	<i>F. graminearum</i>	3 ± 0 q	104	14.75 ± 0.25 p	176
FHB79	WHS528	<i>F. graminearum</i>	5 ± 0 o	102	14.75 ± 0.25 p	175
FHB80	WHM013	<i>F. culmorum</i>	5 ± 0 o	102	14.75 ± 0.25 p	175
FHB81	WHM0137	<i>F. graminearum</i>	8 ± 0 l	99	17.75 ± 0.25 n	167
FHB82	WHS549	<i>F. graminearum</i>	10.75 ± 0.25 i	96	21 ± 0 j	127
FHB83	WHM0134	<i>F. graminearum</i>	15 ± 0 d	78	25 ± 0 f	124
FHB84	WHS518	<i>F. graminearum</i>	17.25 ± 0.25 b	74	28.5 ± 0.28 b	121
FHB85	WHS555	<i>F. graminearum</i>	16 ± 0 c	76	26 ± 0 e	123
FHB86	WHS505	<i>F. graminearum</i>	14 ± 0 e	79	24.75 ± 0.25 f	125
FHB87	WHM0138	<i>F. graminearum</i>	14.75 ± 0.25 d	78	24.75 ± 0.25 f	124
FHB88	WHM0132	<i>F. graminearum</i>	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	WHS530	<i>F. graminearum</i>	2.75 ± 0.25 q	104	10 ± 0 u	193
FHB90	WHS516	<i>F. graminearum</i>	2.75 ± 0.25 q	104	9.5 ± 0.28 v	193
FHB91	WHS532	<i>F. graminearum</i>	2.75 ± 0.25 q	104	10 ± 0 u	193
FHB92	WHM0133	<i>F. graminearum</i>	4 ± 0 p	103	15 ± 0 p	176
FHB93	WHS557	<i>F. graminearum</i>	5 ± 0 o	102	15 ± 0 p	175
FHB94	WHM0129	<i>F. poae</i>	6 ± 0 n	102	17.25 ± 0.25 o	169
FHB95	WHS537	<i>F. graminearum</i>	8.25 ± 0.25 l	98	17.75 ± 0.25 n	169
FHB96	WHM0145	<i>F. culmorum</i>	10.75 ± 0.25 i	97	21 ± 0 j	127
FHB97	WHS508	<i>F. graminearum</i>	13 ± 0 f	96	24.25 ± 0.25 g	127
FHB98	WHM06	<i>F. graminearum</i>	14.5 ± 0.28 d	78	25 ± 0 f	124
FHB99	WHM0129	<i>F. graminearum</i>	17 ± 0 b	74	28.25 ± 0.25 bc	121
FHB100	WHM0144	<i>F. avenaceum</i>	11.5 ± 0.28 h	96	22 ± 0 i	126
FHB101	WHS558	<i>F. graminearum</i>	11 ± 0 i	96	21 ± 0 j	127
FHB102	WHS530	<i>F. culmorum</i>	6.25 ± 0.25 n	101	17.75 ± 0.25 n	168
FHB103	WHS507	<i>F. graminearum</i>	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-Noposa *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-Noposa *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  $32.75 \pm 0.25$  and leaf lesion length of  $19.5 \pm 0.28$ . 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.

## Acknowledgements

The authors would like to acknowledge the financial support of the Seed and Plant Certification and Registration Institute (SPCRI), Iran.

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