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Identification of *Fusarium* species associated with Fusarium head blight of wheat in the North of Iran and phylogenetic analysis of the dominant species

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Abstract

In order to determine the causal agents of Fusarium head blight, during 2015–16, wheat heads with disease symptom were collected from 10 and six main wheat production areas of Golestan and Mazandaran provinces (N Iran), respectively. A total of 431 *Fusarium* isolates were obtained belonging to nine *Fusarium* species based on their morphological characteristics. *Fusarium graminearum* species complex had the highest frequency among the species in both provinces, Golestan (52.0%) and Mazandaran (55.8%). *Fusarium culmorum*, *F. equiseti*, *F. Acuminatum*, and *F. compactum* had the highest mean of frequency in both provinces with 13.5, 9.7, 6.0 and 3.7%, respectively after *F. graminearum*. Also, *F. cerealis*, *F. Avenaceum*, and *F. proliferatum* and some unidentified isolates a total of 14% of the isolates were calculated. The lowest frequency was related to *F. subglutinans* (1%) that was isolated only from Golestan province. In order to determine the phylogeny of *F. graminearum* species complex in the North Iran, 53 out of 229 isolates were selected based on their distribution in the sampled areas. Partial genes of translation elongation factor 1-alpha (*TEF*) and putative reductase (*RED*) were amplified using specific primers. A commercial sequencing facility was used to generate fungal sequences. Almost all strains of *F. graminearum* species complex belonged to *F. graminearum* sensu stricto. The results indicated a homogeniety within *F. graminearum* species complex, however, there was a minor morphologically differences between some strains.

Keywords: *Fusarium graminearum*, Golestan, Mazandaran, *RED*, *TEF*, wheat scab

شناسایی گونه‌های فوزاریوم مرتبط با بلایت خوشه گندم و تجزیه فیلوژنتیکی گونه غالب در شمال ایران*

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خلاصه

به منظور تعیین عوامل بیماری بلایت فوزاریومی، خوشه‌های گندم با علایم بیماری از ۱۰ و شش منطقه مهم کشت گندم به ترتیب از استان‌های گلستان و مازندران در سال زراعی ۹۵-۱۳۹۴ نمونه‌برداری شد. براساس خصوصیات ریخت‌شناسی، ۴۳۱ جدایه منسوب به فوزاریوم تشخیص داده شد که به نه گونه مختلف تعلق داشتند. گونه مرکب *Fusarium graminearum* بیشترین فراوانی را در استان‌های گلستان (۵۲٪) و مازندران (۵۵/۸٪) داشت. قارچ‌های *F. culmorum*، *F. equiseti*، *F. acuminatum* و *F. compactum* پس از *F. graminearum* به ترتیب با میانگین‌های ۱۳/۵، ۹/۷، ۶ و ۳/۷٪ در دو استان مذکور بیشترین فراوانی را داشتند. میانگین فراوانی *F. cerealis*، *F. avenaceum* و *F. proliferatum* و جدایه‌های شناسایی نشده در مجموع ۱۴٪ در شمال ایران برآورد شد. کمترین فراوانی (۱٪) مربوط به قارچ *F. subglutinans* بود که فقط از نمونه‌های استان گلستان جدا شد. برای تعیین خصوصیات ریخت‌شناسی و فیلوژنتیکی گونه مرکب *F. graminearum*، ۵۳ جدایه از بین ۲۲۹ جدایه به دست آمده از استان‌های مذکور براساس مناطق انتشار انتخاب شد. بخشی از ژن‌های translation elongation factor 1-alpha (*TEF*) و reductase (*RED*) با استفاده از آغازگرهای اختصاصی تکثیر و تعیین توالی شد. همه سویه‌های گونه مرکب *F. graminearum* متعلق به *F. graminearum* sensu stricto بودند. داده‌های فیلوژنتیکی نشان‌دهنده وجود همگنی ژنتیکی در بین جمعیت مربوط به گونه مرکب *F. graminearum* بود، اگرچه بین بعضی از سویه‌های این گونه اختلافات کوچک ریخت‌شناسی وجود داشت.

واژه‌های کلیدی: اسکب گندم، گلستان، مازندران، *Fusarium graminearum*، *RED*، *TEF*

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Introduction

Fusarium head blight (FHB) or Fusarium ear blight, which is also called wheat head scab, is an economically important disease of wheat (*Triticum aestivum* L., and *T. durum* Desf.), barley (*Hordeum vulgare* L.) and other small grain cereals worldwide (Parry *et al*. 1995). The disease causes yield and quality loss of grains. *Fusarium graminearum* has resulted in more than $3 billion in cereal losses in the United States of America during the past two decades (Prussin *et al*. 2014). Contamination of the harvested grain with mycotoxins especially deoxynivalenol is the most serious threat of FHB in cereals (Astoreca *et al*. 2013). Common wheat and durum wheat are major crops cultivated in Golestan and Mazandaran provinces in the North of Iran. The disease is one of the most important wheat diseases that has caused epidemics in Ardabil (Dasht-e Moghan), Golestan, Mazandaran, Khuzestan provinces of Iran (Zamanizadeh & Khorsandi 1995).

The most prevalent species involved in FHB are *Fusarium graminearum* Schwabe species complex [teleomorph *Gibberella zeae* (Schwein.) Petch], *F. culmorum* (W.G. Sm.) Sacc., *F. poae* (Peck) Wollenw., and *F. avenaceum* (Fr.) Sacc. (teleomorph *Gibberella avenacea* R.J. Cook) (Parry *et al*. 1995). In the North of Iran, more frequently occurring species are *F. graminearum* species complex (FGSC), and *F. culmorum* (Zamanizadeh & Khorsandi 1995, Golzar *et al.* 1998) where FGSC is more aggressive producing mycotoxins (Mirabolfathy & Karami-Osboo 2013). Mycotoxin contaminated grains are serious threat to global food safety as these toxins have been linked to toxic secondary metabolites of humans and livestock (Peraica *et al.* 1999). Trichothecenes inhibit eukaryotic protein synthesis and immune function in humans and animals (Ueno *et al.* 1973, Pestka & Smolinski 2005). Deoxynivalenol (DON) is a member of the trichothecenes group, which is produced by *Fusarium* species (Sobrova *et al*. 2010). FGSC isolates are major agents that produce deoxynivalenol, nivalenol (NIV) in wheat, barley and corn (Kimura *et al*. 2003). During vegetation period of cereals, DON is produced by FGSC (Desjardins & Proctor 2001). In addition, some phytotoxic trichothecenes function as virulence factors on sensitive cereal hosts (Jansen *et al.* 2005).

In the last two decades, many deficiencies and problems of morphological taxonomy have been solved using molecular techniques (Money 2013). Due to the overlap observed in the morphological systematic studies within the genus, molecular techniques based on multilocus DNA sequencing analysis have enabled researchers to solve these problems (Geiser *et al*. 2013, O'Donnell *et al*. 2012). In 2000s, more accurate evaluation of morphological characters of *F. graminearum* s.l. was carried out and combined with results of phylogenetic analysis based on sequencing of 13 genes. The results indicated that the population diversity of *F. graminearum* s.l. has been derived from the combination of this species. Therefore, it was divided into nine phylogenetic species: *F. vorosii* B. Toth *et al*., *F. gerlachii* T. Aoki *et al*., *F. graminearum* Schwabe s.s., *F. acaciae-mearnsii* O'Donnell *et al*., *F. meridionale* T. Aoki *et al*., *F. boothii* O'Donnell *et al*., *F. mesoamericanum* T. Aoki *et al*., *F. asiaticum* O'Donnell *et al*., and *F. cortaderiae* O'Donnell *et al*. (O'Donnell *et al*. 2000, Starkey *et al*. 2007, Ward *et al*. 2002). Recently, several studies have been done on FGSC and results indicated that, this group consists of 15 phylogenetic species, in addition to above mentioned species, six species were identified as new phylogenetic species including: *F. austroamericanum* T. Aoki *et al*., *F. aethiopicum* O'Donnell *et al*., *F. ussurianum* T. Aoki *et al*., *F. louisianense* Gale *et al*., *F. nepalense* T. Aoki *et al*., and *F. brasilicum* T. Aoki *et al*. (Sarver *et al.* 2011, Aoki *et al.* 2012).

Since cereal fields of Golestan and Mazandaran provinces (N Iran) are located in the high risk areas for FHB disease occurrence, this study was conducted to determine *Fusarium* species associated with FHB on wheat and phylogenetic analysis of the major agent of the disease.

Materials and Methods

- Sampling and fungal isolation

Wheat head samples with FHB disease symptom from major wheat producing areas of Golestan (Aliabad, Azadshahr, Bandar Gaz, Galikesh, Gonbad Kavus, Gorgan,

Minoodasht, Kalaleh, Kordkuy and Ramian) and Mazandaran (Babolsar, Behshahr, Ghaemshahr, Juybar, Neka and Sari) provinces were collected during 2015–16 (Table 1). For the isolation of causal agents of FHB disease, three spikes were selected from each farm and two spikelets from each spike were surface-sterilized in 1% (v/v) sodium hypochlorite for 3 min, rinsed with sterile water and dried on sterile filter paper. The spikelets were cultured on potato dextrose agar (PDA, Merck, Germany) with 0.2 $g.L^{-1}$ streptomycin sulfate (Sigma-Aldrich, Germany) and Fusarium-selective Nash & Snyder medium (Dhingra & Sinclair 1995, Leslie & Summerell 2007). Plates were stored at room temperature for 4 days. Colonies were transferred to PDA medium for the next steps.

Table 1. Identification code and geographic origin of *Fusarium graminearum* species complex in Iran (isolates obtained from wheat head used in this study for morphological and phylogenetic analysis)

Isolate code	Geographic origin
IRFHBN1	Seyyed Miran, Gorgan, Golestan
IRFHBN2	Nowdeh Khanduz, Azadshahr, Golestan
IRFHBN3	Khanduz-e-Sadat, Azadshahr, Golestan
IRFHBN4	Hajikord, Aliabad, Golestan
IRFHBN5	Miandarreh, Kordkuy, Golestan
IRFHBN6	Kordabad, Aliabad, Golestan
IRFHBN7	Derab Kola, Sari, Mazandaran
IRFHBN8	Tushan, Gorgan, Golestan
IRFHBN9	Balajaddeh, Kordkuy, Golestan
IRFHBN10	Khanbebin, Ramian, Golestan
IRFHBN11	Qareqach, Ramian, Golestan
IRFHBN12	Bahnamir, Babolsar, Mazandaran
IRFHBN13	Badeleh, Sari, Mazandaran
IRFHBN14	Dasht-e Naz, Sari, Mazandaran
IRFHBN15	Paeen Zarandin, Neka, Mazandaran
IRFHBN16	Kiakola, Ghaemshahr, Mazandaran
IRFHBN17	Qarehtappeh, Behshahr, Mazandaran
IRFHBN18	Seyed Mahalleh, Sari, Mazandaran
IRFHBN19	Talesh Mahalleh, Juybar, Mazandaran
IRFHBN20	Derka Sar, Juybar, Mazandaran
IRFHBN21	Sarv Kola, Juybar, Mazandaran
IRFHBN22	Gharakheil, Ghaemshahr, Mazandaran
IRFHBN23	Gharakheil, Ghaemshahr, Mazandaran
IRFHBN24	Divdasht, Ghaemshahr, Mazandaran
IRFHBN25	Divdasht, Ghaemshahr, Mazandaran
IRFHBN27	Nowmal, Gorgan, Golestan
IRFHBN28	Nowmal, Gorgan, Golestan
IRFHBN29	Nowmal, Gorgan, Golestan
IRFHBN30	Sali Kandeh, Kordkuy, Golestan
IRFHBN31	Abbasabad, Aliabad Golestan
IRFHBN32	Fazel Abad, Aliabad, Golestan
IRFHBN33	Kafshgiri, Gorgan, Golestan
IRFHBN34	Kafshgiri, Gorgan, Golestan
IRFHBN35	Kafshgiri, Gorgan, Golestan
IRFHBN36	Kafshgiri, Gorgan, Golestan
IRFHBN37	Kafshgiri, Gorgan, Golestan
IRFHBN38	Kafshgiri, Gorgan, Golestan
IRFHBN40	Ahangar Mahalleh, Gorgan, Golestan
IRFHBN41	Gharn Abad, Gorgan, Golestan
IRFHBN42	Gharn Abad, Gorgan, Golestan
IRFHBN43	Valesh Abad, Gorgan, Golestan
IRFHBN44	Valesh Abad, Gorgan, Golestan
IRFHBN45	Valesh Abad, Gorgan, Golestan
IRFHBN46	Valesh Abad, Gorgan, Golestan
IRFHBN47	Valesh Abad, Gorgan, Golestan
IRFHBN48	Valesh Abad, Gorgan, Golestan
IRFHBN49	Valesh Abad, Gorgan, Golestan
IRFHBN50	Baylar, Gonbad Kavus, Golestan
IRFHBN51	Ghalami, Minudasht, Golestan
IRFHBN52	Kazem Khvajeh, Kalaleh, Golestan
IRFHBN53	Salehabad-e Chaqorli, Kalaleh, Golestan
IRFHBN54	Darabad, Galikesh, Golestan
IRFHBN55	Estunabad, Bandar Gaz, Golestan

- Fungal purification and morphological characterization

Fusarium-like isolates were recovered from wheat spikelets and purified by single-spore isolation. To examine colony morphology, strains were grown on potato dextrose agar at 25° C and in the dark for 10 days. To examine microscopic characters, all isolates were grown on synthetic nutrient-poor agar (SNA) (O'Donnell 1996) and carnation-leaf agar (CLA) (Leslie & Summerell 2007) for 10 days at 25° C under continuous black light (Aoki & O'Donnell 1999). Colony growth rate was determined using PDA at 10, 15, 20, 25 and 30° C in the dark in triplicates. Periodically, mycelial growth was recorded by measuring colony diameter. The length and width of 20, 5-septate conidia of each isolate was measured (Aoki & O'Donnell 1999, Leslie & Summerell 2007). The daily colony growth rate and length and width of macroconidia were analyzed using SAS 9.1 software (Anova test $P \leq 0.05$) (SAS Institute, Inc., Cary NC, 2002). *Fusarium* species were identified using their morphological characters: types of phialides, presence or absence of chlamydospores and microconidia, macroconidia, pigmentation and colony growth rate (Gerlach & Nirenberg 1982, Nelson *et al.* 1983, Leslie & Summerell 2007).

- Genomic DNA extraction

Fifty-three isolates belong to *F. graminearum* species complex were selected for phylogenetic study. These isolates were grown in 100 ml Erlenmeyer flasks containing 50 ml of liquid yeast medium (YM) broth culture (2% D-glucose, 0.3% malt extract, 0.3% yeast extract, 0.5% peptone) at 25° C for seven days (O'Donnell 1996). Mycelium was harvested using sterile gauze and rinsed with sterile distilled water. Dried mycelium was crushed into fine powder in liquid nitrogen by mortar. Total genomic DNA was isolated using a modified CTAB (cetyltrimethyl ammonium bromide) technique (Huang *et al.* 2000). Approximately 100 mg mycelium powder was suspended in 900 µL of extraction buffer (100 mM Tris-HCl, 100 mM EDTA, 250 mM NaCl) then 100 µL of 10% *N*-Lauroylsarcosine (Sigma-Aldrich, Germany) was added. The suspension was incubated at 60° C for 60 min and centrifuged for 10 min at 13000 × g in a Beckman microfuge (Beckman, USA). Upper phase suspension was transferred to a new microtube. 100 ml of solution of NaCl 5 mM and 200 ml CTAB 5% were added to each tube, respectively and the tubes were placed at 65° C for 10 min. According to the volume of material contained in each tube, chloroform/isoamyl alcohol (24:1) was added and after mixing, the tubes were centrifuged for 10 min at 13000 × g. Upper phase of suspension was transferred to a new 1.5 ml tube and the equal volume of isopropanol was added where the DNA was precipitated at –20° C. To precipitate DNA, tubes were centrifuged for 10 min at 13000 × g then the supernatant was discarded gently. The sediment was washed with about 100 ml of 75% ethanol and once the DNA was precipitated. The pellet was diluted in 100 ml of deionized double-distilled water.

- DNA amplification and sequencing

Partial sequences of two genes, putative reductase (*RED*) and translation elongation factor *1-α* (*TEF*), were amplified by PCR using gene specific primers (Table 2). The PCR amplification was carried out in a final volume of 30 µ1 containing: 3 µl 10× PCR buffer, 1.8 mmol l^{-1} $MgCl_2$, 0.6 mmol l^{-1} dNTPs, 0.38 µl *Taq* DNA polymerase (5 U/µl) (all from SinaClon Bioscience, Iran), 2 µ1 of DNA template (corresponding to approximately 15–20 ng) and 0.8 µmol l^{-1} of primers (O'Donnell *et al*. 1998). PCR program were conducted in a MJ Mini Personal thermal cycler (BIO-RAD, Santa Clara, USA) for amplification of putative reductase gene: 94° C for 2 min, followed by 25 cycles of 94° C for 1 min, at 58° C for 1 min, and 72° C for 2 min and then incubated at 72° C for 10 min (Suga *et al*. 2008), and for translation elongation factor *1-α*: initial heating for 6 min at 95° C, followed by 40 cycles of (30s at 95° C, 20s at 56° C and 2 min at 72° C), and 5 min at 72° C (Kristensen *et al*. 2007). Finally, quantity and quality of PCR products were evaluated and visualized on 1.5% agarose gel. The purified PCR products were sequenced for *RED* and *TEF* genes by Macrogen Co., Korea.

Table 2. List of primers used for *TEF* and *RED* genes amplification and sequencing in this study

Locus	Gene product	Length of sequence[a]	Name	Sequence (5'-3')[b]	Use			Reference
					F/R[C]	PCR	Sequence	
EF-1α	Translation elongation factor 1α	645	EF1	ATGGGTAAGGARGACAAGAC	F	*		O'Donnell *et al.* (2000)
			EF2	GGARGTACCAGTSATCATG	R	*		
			EF3-N	GTAAGGAGGASAAGACTCAC	F		*	
			EF22T	AGGAACCCTTACCGAGCTC	R		*	
RED	Putative reductase	991	RED1d	TCTCAGAAAGACGCATATATG	F	*	*	O'Donnell *et al.* (2000)
			RED1F	CAGAAAGACGCATATATGTTC	R	*	*	

[a] base pair (bp)
[b] R= A/G; S= C/G
[C] F/R= forward/reverse

Phylogenetic analysis

Additional sequence data of strains that belong to *F. graminearum* species complex (Table 3) were obtained from GenBank and used for comparison with the sequences generated in this study. All sequences were aligned using MEGA 6 (Tamura *et al.* 2013). The translation elongation factor *1-α* (*TEF*) and reductase (*RED*) genes sequences were combined and used for phylogenetic analysis. Maximum Parsimony (MP) searches were conducted using the heuristic search option and tree bisection reconnection (TBR) branch swapping method. Clade stability was assessed by 500 bootstrap replications and values greater than 60 were reported on the phylogenetic tree.

Table 3. Identification code, geographic origin and host of *Fusarium* spp. strains used in this study as reference strains in the phylogenetic analyses

Isolate code	Species	Geographic origin	Host	Reference
NRRL34207	*F. acaciae-mearnsii*	Australia	soil	Sarver *et al.* (2011)
NRRL26752	*F. acaciae-mearnsii*	South Africa	*Acacia mearnsii*	Starkey *et al.* (2007)
NRRL46718	*F. aethiopicum*	Gugsa womberma, Ethiopia	Wheat	O'Donnell *et al.* (2008)
NRRL46726	*F. aethiopicum*	Bure, Ethiopia	Wheat	O'Donnell *et al.* (2008)
NRRL13818	*F. asiaticum*	Japan	Barley	Ward *et al.* (2002)
NRRL6101	*F. asiaticum*	Japan	Barley	Ward *et al.* (2002)
NRRL2903	*F. austroamericanum*	Brazil	Polypore	O'Donnell *et al.* (2000)
NRRL36957	*F. austroamericanum*	Paysandu, Uruguay	Wheat	Ward *et al.* (2008)
NRRL26916	*F. boothii*	South Africa	Corn	Ward *et al.* (2002)
NRRL29105	*F. boothii*	Kaski, Nepal	Corn	Ward *et al.* (2002)
NRRL31238	*F. brasilicum*	Brazil	Corn	Sarver *et al.* (2011)
NRRL31281	*F. brasilicum*	Brazil	Oats	Sarver *et al.* (2011)
NRRL25805[a]	*F. cerealis*	Columbia	Soil	Ward *et al.* (2002)
NRRL31171	*F. cortaderiae*	Brazil	Barley	Sarver *et al.* (2011)
NRRL34577	*F. cortaderiae*	Unknown	Unknown	Ward *et al.* (2008)
NRRL25475[a]	*F. culmorum*	Denmark	Barley	Ward *et al.* (2002)
NRRL29298[a]	*F. dactylidis*	Oceania, New Zealand	Cocksfoot	Aoki *et al.* (2015)
NRRL29380[a]	*F. dactylidis*	Oregon, USA	Grass	Aoki *et al.* (2015)
NRRL38380	*F. gerlachii*	Unknown	Unknown	Starkey *et al.* (2007)
NRRL38405	*F. gerlachii*	Unknown	Unknown	Starkey *et al.* (2007)
NRRL38369	*F. graminearum*	Louisiana, USA	Wheat head	Starkey *et al.* (2007)
NRRL13383	*F. graminearum*	Iran	Corn	Ward *et al.* (2002)
NRRL28063	*F. graminearum*	Michigan, USA	Corn	Ward *et al.* (2002)
NRRL28439	*F. graminearum*	Rotterdam, Netherlands	Leather leaf	Starkey *et al.* (2007)
NRRL29169	*F. graminearum*	Kansas, USA	Wheat	Ward *et al.* (2002)
NRRL6394	*F. graminearum*	Hungary	Millet	Sarver *et al.* (2011)
NRRL54196	*F. louisianense*	Louisiana, USA	Wheat	Sarver *et al.* (2011)
NRRL54197	*F. louisianense*	Louisiana, USA	Wheat	Sarver *et al.* (2011)
NRRL28723	*F. meridionale*	Lalitpur, Nepal	Corn	Ward *et al.* (2002)
NRRL29010	*F. meridionale*	Transkei, South Africa	Soil	Ward *et al.* (2002)
NRRL25797	*F. mesoamericanum*	Honduras	Banana	Ward *et al.* (2002)
NRRL29148	*F. mesoamericanum*	USA	Grape	Ward *et al.* (2002)
NRRL54220	*F. nepalense*	Lamjung, Nepal	Rice	Sarver *et al.* (2011)
NRRL54222	*F. nepalense*	Nepal	Rice	Sarver *et al.* (2011)
NRRL28062[a]	*F. pseudograminearum*	Darling Downs, Australia	Barely	Ward *et al.* (2002)
NRRL45665	*F. ussurianum*	Jewish Autonomous, Russia	Wheat	Yli-Mattila *et al.* (2009)
NRRL45795	*F. ussurianum*	Kamen-Rybolov, Russia	Wheat	Yli-Mattila *et al.* (2009)
NRRL37605	*F. vorosii*	Ipolydamasd, Hungary	Wheat head	Starkey *et al.* (2007)
NRRL45790	*F. vorosii*	Ussuriysk, Russia	Wheat	Starkey *et al.* (2007)

[a] Strains of species used as outgroups

Results

- Morphological characterization

A total of 431 *Fusarium* isolates were obtained from 174 spikes of wheat that collected from 58 wheat farms in Golestan and Mazandaran provinces. According to morphological characters, isolates belonged to nine *Fusarium* species, namely *F. acuminatum* Ellis & Everh., *F. avenaceum* (Fr.) Sacc., *F. compactum* (Wollenw.) Gordon, *F. cerealis* (Cooke) Sacc., *F. culmorum*, *F. equiseti* (Corda) Sacc., *F. graminearum* species complex (FGSC), *F. proliferatum* (Matsush.) Nirenberg, and *F. subglutinans* (Wollenw. & Reinking) P.E. Nelson. FGSC had the highest frequency in both Golestan (52.0%) and Mazandaran (55.8%) provinces. Followed by *F. culmorum*, *F. equiseti*, *F. Acuminatum*, and *F. compactum* with frequency of 14.2, 10.3, 6.3 and 4.0 percentages in Golestan province, and 11.6, 8.5, 5.4, 3.1 in Mazandaran province, respectively. *Fusarium cerealis*, *F. Avenaceum*, and *F. proliferatum* formed 8.0% of the isolates while 5.8% of isolates were unidentified. The lowest frequency was related to *F. subglutinans* (1%) that was isolated only from Golestan province (Table 4).

Table 4. The frequency (%) of *Fusarium* species, isolated from contaminated wheat heads in different zones of Golestan and Mazandaran provinces (N Iran)

Geographic origin (Golestan/Mazandaran)	Number of farms sampled	*F. acuminatum*	*F. avenaceum*	*F. compactum*	*F. cerealis*	*F. culmorum*	*F. equiseti*	*F. graminearum*	*F. proliferatum*	*F. subglutinans*	Others
Aliabad, Golestan	4	9.4	0.0	3.1	3.1	12.5	6.3	56.3	3.1	3.1	3.1
Azadshahr, Golestan	2	8.3	0.0	0.0	0.0	16.7	8.3	66.7	0.0	0.0	0.0
Bandar Gaz, Golestan	1	0.0	0.0	0.0	0.0	14.3	14.3	57.1	0.0	0.0	14.3
Galikesh, Golestan	2	6.3	6.3	6.3	6.3	6.3	12.5	56.3	0.0	0.0	0.0
Gonbad Kavus, Golestan	2	5.6	0.0	11.1	5.6	22.2	22.2	16.7	5.6	0.0	11.1
Gorgan, Golestan	23	6.3	3.1	3.8	2.5	14.5	10.1	50.9	1.9	1.3	5.7
Minoodasht, Golestan	1	11.1	11.1	11.1	0.0	22.2	11.1	22.2	0.0	0.0	11.1
Kalaleh, Golestan	2	6.7	0.0	0.0	0.0	6.7	6.7	73.3	6.7	0.0	0.0
Kordkuy, Golestan	3	0.0	0.0	4.8	4.8	19.0	14.3	52.4	0.0	0.0	4.8
Ramian, Golestan	2	7.7	0.0	0.0	0.0	7.7	0.0	76.9	0.0	0.0	7.7
Total	**42**	**6.3**	**2.3**	**4.0**	**2.6**	**14.2**	**10.3**	**52.0**	**2.0**	**1.0**	**5.3**
Babolsar, Mazandaran	1	0.0	0.0	0.0	0.0	0.0	12.5	75.0	12.5	0.0	0.0
Behshahr, Mazandaran	2	6.7	0.0	6.7	0.0	20.0	13.3	33.3	6.7	0.0	13.3
Ghaemshahr, Mazandaran	5	4.3	4.3	4.3	4.3	8.7	8.7	52.2	4.3	0.0	8.7
Juybar, Mazandaran	3	5.0	5.0	0.0	0.0	15.0	0.0	75.0	0.0	0.0	0.0
Neka, Mazandaran	1	11.1	0.0	0.0	0.0	22.2	22.2	33.3	0.0	0.0	11.1
Sari, Mazandaran	4	6.5	3.2	3.2	0.0	9.7	6.5	61.3	3.2	0.0	6.5
Total	**16**	**5.4**	**3.1**	**3.1**	**1.6**	**11.6**	**8.5**	**55.8**	**3.9**	**0.0**	**7.0**
All Total	**58**	**6.0**	**2.6**	**3.7**	**2.3**	**13.5**	**9.7**	**53.1**	**2.6**	**0.7**	**5.8**

Out of 229 isolates of FGSC, 53 were selected for phylogenetic analysis based on their geographical distribution (Table 1). Regarding the daily colony growth rate, the optimum growth temperature was 25° C, and isolates IRFHBN1, IRFHBN2, IRFHBN12, IRFHBN13, IRFHBN16, and IRFHBN18 with 30.0 mm/day had the highest growth rate and the isolate IRFHBN42 with 17.0 mm/day had the slowest growth rate. At 10° C, isolates IRFHBN16 (5.2 mm/day) and IRFHBN15 (2.3 mm/day) had the highest and lowest growth rate, respectively. Thirteen isolates with 18.0 mm/day and one with 11.8 mm/day at 15° C had the highest and lowest growth

rates, respectively. At 30° C, eighteen isolates with 10.0 mm/day were grouped in the highest growth rate group and one isolate with 7.7 mm/day was placed in the lowest growth rate group (Table 5). Generally, isolates had dissimilar growth rate at different temperatures, so that the averages of growth rates at 10, 15, 20, 25 and 30° C were 3.4, 13.5, 18.5, 23.0 and 8.9 mm/day, respectively. There was no significant correlation between their geographical location and growth rate at different temperatures. Colonies of the isolates of FGSC were red, dull-red to brownish-yellow in color. Among the isolates of FGSC, aerial mycelia were also equally abundant, floccose, white and reddish-white to brownish-yellow. No significant differences were observed in morphological characters of colonies of the different isolates of FGSC on PDA at 25° C in the dark. Length of 5-septate conidia of FGSC isolates was varied within and between isolates. The longest length of macroconidia belonged to IRFHBN50 (62.2±2.48 µm), and the shortest (44.7±2.48 µm) IRFHBN11 (Table 5). Width of 5-septate conidia was in the range of 4.5–5 µm.

Table 5. Mean of colony growth (mm/d) strains of FGSC at each temperature in PDA medium (each isolate by temperature combination was replicated 3 times and length of macroconidia of strains isolated from N Iran)

Isolate code	*Growth at 10° C (mm/day)	*Growth at 15° C (mm/day)	*Growth at 20° C (mm/day)	*Growth at 25° C (mm/day)	*Growth at 30° C (mm/day)	Length of macroconidia in µm	
						Mean	Min/Max
IRFHBN1	3.8±0.11^{d}	18.0±0.00^{a}	18.0±0.00^{cd}	30.0±0.00^{a}	10.0±0.00^{a}	52.2±2.75^{efgh}	47.5–57.5
IRFHBN2	3.6±0.00^{e}	15.0±0.00^{b}	18.0±0.00^{cd}	30.0±0.00^{a}	8.2±0.00^{e}	52.2±4.63^{efgh}	50.0–65.0
IRFHBN3	2.4±0.06^{m}	15.0±0.00^{b}	15.0±0.00^{e}	22.5±0.00^{cd}	8.3±0.41^{d}	53.0±3.07^{efg}	50.0–57.5
IRFHBN4	4.1±0.00^{c}	18.0±0.00^{a}	22.5±0.00^{a}	27.5±0.43^{ab}	9.7±0.57^{ab}	51.5±2.93^{hijk}	47.5–57.5
IRFHBN5	3.4±0.11^{g}	11.3±0.00^{e}	15.0±0.00^{e}	22.5±0.00^{cd}	8.3±0.43^{d}	49.5±1.97^{mnop}	47.5–52.5
IRFHBN6	3.5±0.00^{f}	12.9±0.00^{c}	21.0±0.22^{ab}	22.5±0.00^{cd}	5.6±0.00^{j}	48.5±2.4^{nop}	45.5–52.5
IRFHBN7	3.3±0.00^{gh}	12.9±0.00^{c}	15.0±0.00^{e}	22.5±0.00^{cd}	10.0±0.00^{a}	47.5±2.35^{op}	45.5–50.5
IRFHBN8	3.1±0.00^{j}	12.9±0.00^{c}	18.0±0.00^{cd}	22.5±0.00^{cd}	6.9±0.00^{i}	47.5±2.35^{op}	45.5–52.5
IRFHBN9	3.5±0.00^{f}	18.0±0.00^{a}	22.5±0.00^{a}	27.5±0.43^{ab}	8.3±0.46^{d}	60.2±2.75^{b}	55.0–62.5
IRFHBN10	2.4±0.06^{m}	7.3±0.34^{gh}	11.8±0.00^{f}	21.0±0.26^{de}	8.3±0.46^{d}	48.7±2.96^{nop}	45.0–52.5
IRFHBN11	3.5±0.00^{f}	12.9±0.00^{c}	22.5±0.00^{a}	22.5±0.00^{cd}	7.3±0.00^{gh}	44.7±2.48^{p}	43.0–50.0
IRFHBN12	3.5±0.00^{f}	15.0±0.00^{b}	22.5±0.00^{a}	30.0±0.00^{a}	8.3±0.00^{d}	49.7±3.98^{mnop}	47.0–55.0
IRFHBN13	4.1±0.00^{c}	11.3±0.00^{e}	22.5±0.00^{a}	30.0±0.00^{a}	10.0±0.00^{a}	48.5±3.94^{nop}	43.0–55.0
IRFHBN14	3.1±0.00^{j}	18.0±0.00^{a}	21.0±2.59^{ab}	22.5±0.00^{cd}	10.0±0.00^{a}	48.7±2.70^{nop}	45.0–52.5
IRFHBN15	2.3±0.00^{n}	7.5±0.00^{g}	15.0±0.00^{e}	21.0±2.59^{de}	9.7±0.57^{ab}	52.0±6.10^{fghi}	45.5–62.5
IRFHBN16	5.2±0.17^{a}	18.0±0.00^{a}	22.5±0.00^{a}	30.0±0.00^{a}	10.0±0.00^{a}	51.0±2.68^{ijkl}	47.5–55.0
IRFHBN17	3.2±0.00^{i}	18.0±0.00^{a}	22.5±0.00^{a}	22.5±0.00^{cd}	10.0±0.00^{a}	49.5±1.97^{mnop}	47.5–52.0
IRFHBN18	3.8±0.00^{d}	18.0±0.00^{a}	22.5±0.00^{a}	30.0±0.00^{a}	10.0±0.00^{a}	49.0±2.68^{mnop}	45.0–52.5
IRFHBN19	4.1±0.00^{c}	11.3±0.00^{e}	21.0±0.25^{ab}	22.5±0.00^{cd}	7.5±0.00^{g}	54.0±3.57^{de}	50.0–60.0
IRFHBN20	3.6±0.00^{e}	12.9±0.00^{c}	18.0±0.00^{cd}	22.5±0.00^{cd}	10.0±0.00^{a}	52.2±4.32^{efgh}	47.5–57.5
IRFHBN21	3.5±0.00^{f}	12.9±0.00^{c}	17.0±0.00^{d}	19.5±0.25^{def}	7.7±0.00^{f}	50.2±1.42^{lmn}	47.5–52.5
IRFHBN22	4.3±0.00^{b}	11.3±0.00^{e}	19.5±1.73^{bc}	22.5±0.00^{cd}	10.0±0.00^{a}	51.7±2.37^{ghij}	47.5–55.0
IRFHBN23	4.1±0.00^{c}	11.3±0.00^{e}	22.5±2.59^{a}	25.0±0.43^{bc}	7.5±0.00^{g}	52.7±3.80^{efgh}	47.5–60.0
IRFHBN24	3.5±0.00^{f}	15.0±0.00^{b}	18.0±0.00^{cd}	22.5±0.00^{cd}	8.3±0.00^{d}	50.75±1.68^{klm}	47.5–52.5
IRFHBN25	4.3±0.00^{b}	15.0±0.00^{b}	18.0±0.00^{cd}	22.5±0.00^{cd}	8.3±0.00^{d}	51.2±2.13^{hijk}	47.5–52.5
IRFHBN27	3.1±0.06^{j}	10.0±0.00^{f}	18.0±0.00^{cd}	22.5±0.00^{cd}	10.0±0.00^{a}	49.2±3.54^{mnop}	45.0–52.5
IRFHBN28	2.8±0.00^{l}	7.1±0.34^{h}	11.8±0.92^{f}	18.0±0.00^{ef}	7.1±0.00^{h}	49.5±2.83^{mnop}	47.5–55.0
IRFHBN29	3.2±0.00^{i}	10.0±0.00^{f}	18.0±0.00^{cd}	22.5±0.00^{cd}	10.0±0.46^{a}	51.0±3.76^{ijkl}	47.5–57.5
IRFHBN30	2.4±0.00^{m}	11.3±0.00^{e}	11.3±0.00^{fg}	17.0±0.00^{f}	7.5±0.00^{g}	53.2±1.68^{def}	52.5–55.0
IRFHBN31	3.5±0.00^{f}	11.3±0.00^{e}	18.0±0.00^{cd}	21.0±1.73^{de}	9.3±0.57^{bc}	51.7±4.80^{ghij}	47.5–60.0
IRFHBN32	3.6±0.00^{e}	15.0±0.00^{b}	18.0±0.00^{cd}	22.5±0.00^{cd}	8.3±0.00^{d}	50.2±1.41^{lmn}	47.5–52.5
IRFHBN33	4.1±0.00^{c}	18.0±0.00^{a}	21.0±2.59^{ab}	25.0±0.43^{bc}	10.0±0.46^{a}	50.0±2.88^{lmno}	47.5–55.0
IRFHBN34	2.9±0.00^{k}	10.0±0.00^{f}	15.0±0.00^{e}	27.5±0.43^{ab}	10.0±0.46^{a}	52.0±3.68^{fghi}	47.5–55.0
IRFHBN35	4.3±0.00^{b}	18.0±0.00^{a}	18.0±0.00^{cd}	22.5±0.00^{cd}	10.0±0.00^{a}	53.0±3.68^{efg}	47.5–60.0
IRFHBN36	4.3±0.11^{b}	18.0±0.00^{a}	21.0±0.25^{ab}	25.0±0.00^{bc}	10.0±0.00^{a}	51.5±1.74^{hijk}	50.0–55.0
IRFHBN37	4.3±0.00^{b}	18.0±0.00^{a}	15.0±0.00^{e}	21.0±0.00^{de}	6.9±0.00^{i}	50.2±4.47^{lmn}	42.5–55.0
IRFHBN38	3.5±0.00^{f}	18.0±0.00^{a}	15.0±0.00^{e}	21.0±0.00^{de}	9.7±0.00^{ab}	57.5±3.53^{c}	52.5–62.5
IRFHBN40	3.8±0.00^{d}	12.9±0.00^{c}	18.0±0.00^{cd}	22.5±0.00^{cd}	8.3±0.00^{d}	52.5±2.04^{efgh}	50.0–55.0
IRFHBN41	3.8±0.00^{d}	18.0±0.00^{a}	21.0±0.25^{ab}	22.5±0.00^{cd}	7.7±0.00^{f}	51.0±3.16^{ijkl}	47.5–57.5
IRFHBN42	2.9±0.00^{k}	7.3±0.34^{gh}	9.7±0.00^{g}	13.6±0.00^{g}	6.9±0.00^{i}	50.0±2.04l^{mno}	47.5–52.5
IRFHBN43	3.6±0.00^{e}	11.8±0.92^{d}	19.5±0.00^{bc}	22.5±0.00^{cd}	9.7±0.57^{ab}	53.0±1.97^{efg}	50.0–55.0

Table 5 (contd.)

IRFHBN44	2.4±0.00^{m}	12.9±0.00^{c}	15.0±0.00^{e}	22.5±0.00^{cd}	8.3±0.46^{d}	53.0±2.58^{efg}	50.0–55.0
IRFHBN45	3.2±0.06^{i}	18.0±0.00^{a}	22.5±0.00^{a}	22.5±0.00^{cd}	10.0±0.00^{a}	54.0±4.74^{de}	47.5–60.0
IRFHBN46	3.2±0.00^{i}	11.3±0.00^{e}	21.0±2.59^{ab}	21.0±2.59^{de}	9.3±0.57^{bc}	55.0±5.00^{cd}	50.0–62.5
IRFHBN47	3.8±0.00^{d}	12.9±0.00^{c}	22.5±0.00^{a}	22.5±0.00^{cd}	8.3±0.46^{d}	52.7±3.42^{efgh}	47.5–57.5
IRFHBN48	3.2±0.00^{i}	10.0±0.00^{f}	19.5±0.00^{bc}	22.5±0.00^{cd}	10.0±0.00^{a}	52.0±5.86^{fghi}	47.5–65.0
IRFHBN49	2.8±0.06^{l}	11.3±0.00^{e}	15.0±0.00^{e}	18.0±0.00^{ef}	10.0±0.00^{a}	49.5±3.49^{mnop}	45.5–57.5
IRFHBN50	3.3±0.00^{gh}	12.9±0.00^{c}	18.0±0.00^{cd}	21.0±0.00^{de}	9.0±0.00^{c}	62.2±2.48^{a}	57.5–65.0
IRFHBN51	3.8±0.00^{d}	12.9±0.00^{c}	22.5±0.00^{a}	22.5±0.00^{cd}	9.7±0.57^{ab}	51.0±2.68^{ijkl}	47.5–55.5
IRFHBN52	2.9±0.00^{k}	15.0±0.00^{b}	15.0±0.00^{e}	21.0±0.00^{de}	8.3±0.00^{d}	51.2±3.95^{hijk}	45.0–57.5
IRFHBN53	3.8±0.00^{d}	11.8±0.92^{d}	22.5±0.00^{a}	22.5±0.00^{cd}	9.7±0.34^{ab}	54.0±3.16^{de}	52.5–60.0
IRFHBN54	2.4±0.06^{m}	15.0±0.00^{b}	18.0±0.00^{cd}	21.0±0.00^{de}	8.3±0.00^{d}	51.7±5.00^{ghij}	45.0–60.0
IRFHBN55	2.4±0.00^{m}	10.0±0.00^{f}	15.0±0.00^{e}	21.0±0.25^{de}	7.3±0.00^{gh}	50.75±2.37^{klm}	47.5–55.0

* Value followed by different letter(s) is significantly different from one another at 5% probability level, according to LSD test

- PCR products and phylogenetic analysis of FGSC

Fifty-three isolates of FGSC, obtained from wheat heads were selected for DNA sequence analyses by two genes (Table 1). After multiple alignments, the lengths of sequences were 644 bp for *TEF* and 991 bp for *RED* genes. Sequences of *TEF* and *RED* genes of 36 strains were included as references and two strains of *F. Pseudograminearum*, and *F. dactylidis* as outgroups in the phylogenetic analysis (Table 3). Tree was constructed for each region of a total of 92 sequences. All strains, isolated from Golestan and Mazandaran provinces belonged to *F. graminearum* s.s. and grouped with reference strains NRRL6394, NRRL13383, NRRL29169, NRRL28439, NRRL38369 and NRRL28063 with a high bootstrap value (94%). The sequence similarity was found to be higher than 99.5% between the observed genotypes and other reported sequences, including reference strains *F. graminearum* s.s. (Fig. 1). In clade *F. graminearum* s.s., two subclades with 94 and 86% bootstrap were observed. The isolates differed in a single nucleotide compared with other *F. graminearum* s.s. isolates (Fig. 1).

Discussion

Since Fusarium head blight (FHB) is caused by a complex of *Fusarium* species, detection and determination of the main species involved in disease is necessary for disease management strategies. In this study, 431 isolates were obtained from 174 heads that were sampled from wheat fields in Northern provinces of Iran including Golestan and Mazandaran. The isolates were characterized based on their morphological characteristics and the sequences of *TEF* and *RED* genes. The result of morphological studies showed that nine *Fusarium* species are involved as causal agent of FHB. In Northern provinces, the *F. graminearum* species complex (FGSC) was dominant species with 53.1% average frequency and average frequency of *F. culmorum*, *F. equiseti*, *F. acuminatum*, *F. compactum*, *F. avenaceum*, *F. proliferatum*, and *F. cerealis* was 13.5, 9.7, 6.0, 3.7, 2.6, 2.6 and 2.3%, respectively. *Fusarium subglutinans* was isolated only from Golestan province and had the lowest frequency (1.0%).

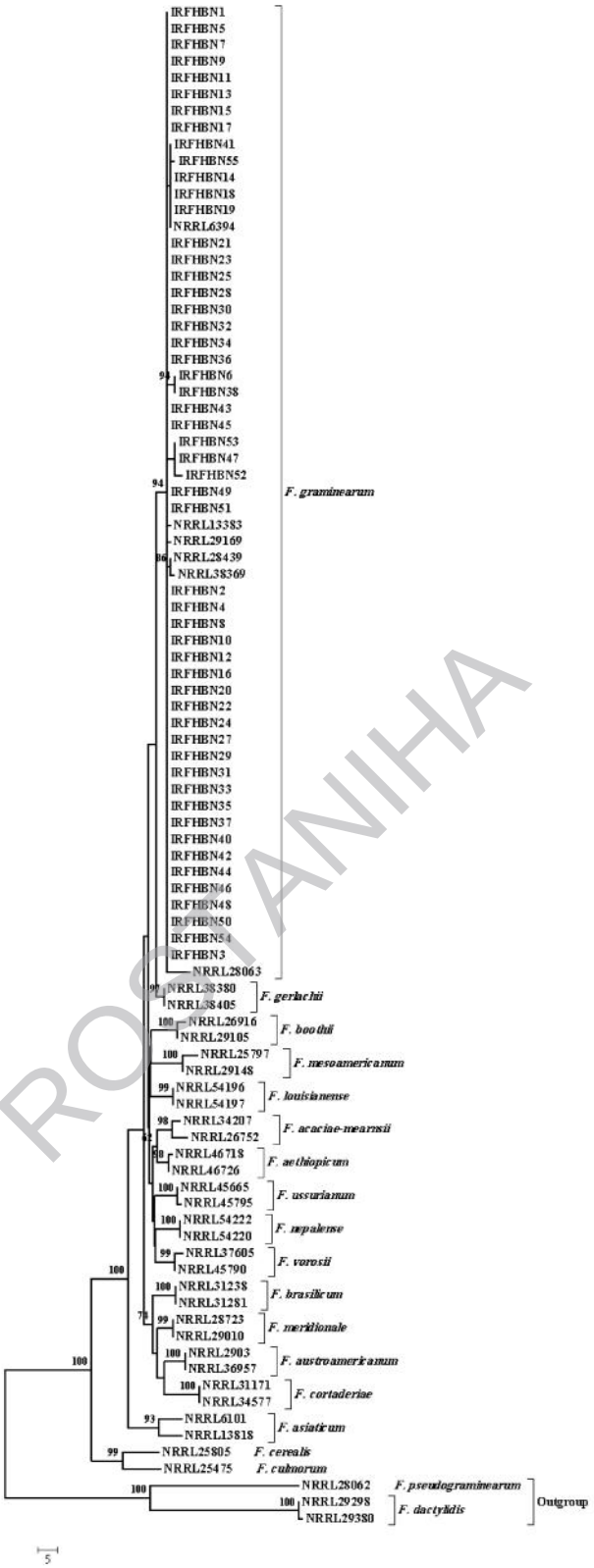

Fig. 1. The parsimonious phylogram inferred from the combined dataset of portions of two *TEF* and *RED* genes rooted with sequences of *F. pseudograminearum* NRRL 28062 and *F. ductylidis* NRRL 29380 and 29298. Branches having more than 60% Maximum Parsimony bootstrap values are indicated above branches based on 500 replicates of the data.

The results suggest that *F. graminearum* species complex is the major causal agent of wheat FHB in Northern Iran, and this is consistent with results from previous surveys conducted in Iran by other investigators (Zare & Ershad 1997, Abedi-Tizaki & Sabbagh 2012, Mirabolfathy & Karami-Osboo 2013). Consistent with previous reports (Zare & Ershad 1997), eight *Fusarium* species were reported as FHB causal agents from Golestan province in wheat and barley fields. In this study, *F. graminearum*, *F. culmorum*, *F. equiseti* and *F. proliferatum* had the most frequency and *F. acuminatum*, *F. croockwellens*, *F. chlamydosporum*, *F. Lateritium*, and *F. compactum* were identified to be less frequent species. Abedi-Tizaki & Sabbagh (2012) reported that, *F. graminearum* (48.2%), *F. culmorum* (17.1%), *F. acuminatum* (10.1%), *F. equiseti* (8.1%), *F. croockwellens* (3.7%), *F. poae* (5.5%), *F. lateritium* (2.9%), *F. proliferatum* (2.6%), and *F. subglutinans* (1.4%) were FHB causal agents in Golestan province. Comparison of these results with previous studies shows an increase in the frequency of *F. equiseti*, and *F. compactum*. This can be attributed to climate change and new wheat varieties grown in this region. Studies indicate that the spread of *F. compactum* is in hot areas while *F. acuminatum and F. equiseti* are mostly present in temperate regions (Backhouse and Burgess 1995). Both diversity and abundance of *Fusarium* species were revealed differences between the wheat varieties such that not all fungal species have colonized all varieties (Pusz *et al.* 2016, Semaskiene *et al.* 2005).

Variation of weather condition and diversity of causal agents makes controlling of FHB disease more complex and difficult. The results showed that several *Fusarium* species were isolated from the same spikelet of wheat heads simultaneously. For example, we have seen in some cases FGSC with *F. equiseti*, *F. culmorum* or other *Fusarium* species were isolated from a spikelet. Interactions of isolates of different *Fusarium* species are competitive at the individual spikes (Siou *et al*. 2015), in contrast, some results indicate there are significant positive interactions among *Fusarium* species of the FHB causal agents (Xu *et al.* 2008).

Colony growth rate measurements of FGSC isolates were revealed that 25° C (among 10, 15, 20, 25 and 30° C) was the optimum temperature for the colony growth of FGSC isolates. Suitable temperature for wheat infection to FHB by *F. graminearum* is above 25° C combined with moist periods of 24 h or more (Scala *et al*. 2016). The fastest growth rate of *F. graminearum* isolates were belonged to IRFHBN1 and IRFHBN2 isolates from Golestan, and IRFHBN12, IRFHBN13, IRFHBN16 and IRFHBN18 from Mazandaran with 30.0 mm daily increment on PDA at 25° C. The fastest growth rate of *F. graminearum* colonies on synthetic medium was reported 13.5 mm per day at 25° C (Neagu & Borda 2013). In this study, maximum and minimum colony growth rate were obtained 5.2±0.17 mm and 2.3±0.00 mm at 10° C, 18±0.00 mm and 7.1±0.34 mm at 15° C, 22.5±0.00 mm and 11.8±0.62 mm at 20° C and 10.0±0.00 mm and 7.7±0.00 mm at 30° C, respectively. Thus, the optimum growth temperature of these isolates were determined between 20–25° C. Studies have shown that, optimum temperature for infection of wheat spikes with FGSC is between 23–28° C, however in high humidity and drizzling conditions, infection can occur at lower temperatures (Brennan *et al*. 2005, Rossi *et al.* 2001). Neagu & Borda (2013) modeled using non-linear the growth rate of *F. graminearum* to determine the effects of water activity, temperature and their interactions on fungal growth on grains. They showed that growth rate increased with the raise of the water activity and maximum growth rate measured at 25° C and 0.995 a*w* value.

Phylogenetic tree resulting from the combination of sequences of two genes (*TEF* and *RED*) showed that all isolates from North of Iran belonged to *F. graminearum* s.s. and grouped with reference strains with high bootstrap values (94%). It is shown that, *RED* and *TEF-1α* sequences were able to separate most species of FGSC and used at the initial stage to discover novel species (O'Donnell *et al.* 2004, Starkey *et al.* 2007). It also indicates that *F. graminearum* s.s. is the dominant species and is the main causal agent of wheat FHB disease in these regions. Malihipour *et al.* (2012)

investigated the phylogenetic relationships among strains that were collected from Canada, Mexico, and Iran and characterized them using the *Tri101* gene sequences. The results are consistent with our study, that all Canadian and Iranian isolates are clustered in one group and were identified as *F. graminearum* s.s. Based on chemotypes and population diversity of *F. graminearum* species complex showed that, *F. graminearum* s.s. is prevalent species in the North-West and in the Northern provinces of Iran. *Fusarium graminearum* strains collected from North-West region produced abundant 15-acetyldeoxynivalenol, while strains from North of Iran produced nivalenol mycotoxins (Davari *et al*. 2013). According to recent studies, species causing FHB disease in Iran proved to be *F. graminearum* s.s., which is also the most prevalent FHB species elsewhere in the world (Aoki *et al.* 2012, O´Donnell *et al.*, 2004, Starkey *et al.* 2007, Ward *et al.* 2008), although in eastern Asia *F. asiaticum* is the prevalent species (Yang *et al.* 2008, Zhang *et al.* 2012). The *F. graminearum* s.l. was diagnosed by Gerlach and Nirenberg (1982), after 1980 according to morphological and phylogenetic analysis it was separated to *F. cerealis* (Cooke) Sacc. (=*F. crookewellense* W. Burgess *et al.*), and *F. pseudograminearum* O'Donnell et T. Aoki (Aoki *et al*. 2012). Furthermore, in recent decades, FGSC was classified into 14 novel species based on genealogical exclusivity (Aoki *et al*. 2012, O'Donnell *et al.* 2000, O'Donnell *et al*. 2008). Among identified species, *F. graminearum* s.s. has the greatest expansion and is paramount important pathogen related with cereal FHB disease. It is reported as the predominant species in North (Burlakoti *et al*. 2008, O'Donnell *et al*. 2004) and South America (Ramirez *et al*. 2007), Europe (Xu *et al*. 2005), South Africa (Boutigny *et al*. 2011), Australia (Tan *et al.* 2012), New Zealand (Cromey *et al*. 2001, Aoki *et al.* 2015), and Asia (Lee *et al*. 2009, Suga *et al.* 2008, Yli-Mattila *et al.* 2009). It has been shown that, *F. asiaticum* is dominant in areas where the mean annual temperature is above 15° C, while *F. graminearum* s.s. is dominant in regions with lower temperature (Suga *et al.* 2008).

According to this study, four *Fusarium* species including *F. graminearum* s.s., *F. culmorum*, *F. equiseti*, and *F. acuminatum* are major causal agents of FHB disease. *F. graminearum* is the predominant species with genetic homogeneity in the North of Iran. In conclusion, our study provides a detailed report on most important pathogens causing FHB disease. In order to provide FHB disease management program and contamination prevention of mycotoxins in wheat, it is important to identify and characterize the main species involve in FHB disease. The result can help to develop breeding strategies for FHB resistance in the North of Iran by characterizing major causal agents of the disease.

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