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Genetic analysis of deoxynivalenol and nivalenol chemotypes of *Fusarium graminearum* on wheat in Iran

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Abstract

Fusarium graminearum as the main cause of Fusarium head blight of wheat not only decreases the yield, but also causes mycotoxicosis in human and livestock because of producing dioxynivalenol and nivalenol. Sixty isolates of *F. graminearum* obtained from infected wheat farms of Iran were tested for the ability of deoxynivalenol, nivalenol and acetylen derivations production using specific primers of Tri13P1/Tri13P2 and Tri13F/Tri13DONR. A 583bp fragment responsible for the production of 15-Acetyl deoxynivalenol amplified in 36 isolates out of 60 tested ones, also in 24 isolates, an 859bp band responsible to product nivalenol were reproduced. None of the isolates produced 644bp fragment which is responsible for 3-Acetyl deoxynivalenol production. The data related to the distribution of *Tri13* haplotypes by the PCR method showed that the majority of *F. graminearum* isolates were DON and 15-AcDON producer. Potential of trichothecenes B production of isolates possessing Trichothecenes-generating genes were evaluated using HPLC-SPE method. 77% of isolates produced 15-AcDON and 46% produced NIV. Also none of the DON-producing isolates was able to produce NIV, whereas the low levels of DON (10- 90 ppb) were evaluated in *F. graminearum* isolates producing high levels of NIV. Distribution of DON derivatives and NIV- producing *F. graminarum* isolates in different provinces showed that the most NIV- producing isolates were obtained from Golestan and Mazandaran provinces whereas DON chemotypes isolates were more than NIV-producing ones in Fars, Kerman, Hormozgan and Ardabil provinces.

Keywords: Fusarium Head Blight, HPLC, Trichothecenes, *Tri13* genes.

بررسی ژنتیکی جدایه‌های *Fusarium graminearum* مولد دی اکسی نیوالنول و نیوالنول گندم در ایران

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چکیده

عامل اصلی بیماری فوزاریوز سنبله گندم در ایران *Fusarium graminearum* است که نه تنها باعث کاهش محصول می‌گردد، که با تولید د اکسی نیوالنول (DON) و نیوالنول (NIV) سبب بیماری‌های ناشی از زهرابه‌های قارچی در انسان و حیوانات اهلی می‌گردد. در این مطالعه تعداد ۶۰ سویه *F. graminearum* جدا شده از مزارع آلوده‌ی گندم در ایران، از نظر ژن‌های مولد د اکسی نیوالنول، نیوالنول و مشتقات استیله‌ی آن‌ها با استفاده از آغازگرهای اختصاصی Tri13P1/Tri13P2 و Tri13F/Tri13DONR آزمایش شد. در ۳۶ جدایه از ۶۰ سویه آزمایش شده، یک قطعه ۵۸۳ جفت بازی مسئول تولید ۱۵–استیل د اکسی نیوالنول و در ۲۴ جدایه قطعه ۸۵۹ جفت بازی مسئول تولید نیوالنول تکثیر گردید. در هیچکدام از جدایه‌ها قطعه ۶۴۴ جفت بازی مسئول تولید ۳–استیل دی اکسی نیوالنول تکثیر نگردید. توزیع هاپلوتیپ‌های *Tri13* نشان داد که اکثر جدایه‌ها، مولد DON و 15-AcDON هستند. توانایی تولید تریکوتسن‌ها در بین جدایه‌های واجد ژن‌های مولد تریکوتسن با استفاده از روش HPLC-SPE ارزیابی شد. ٪۷۷ جدایه‌های مورد بررسی، مولد 15-AcDON و ٪۴۶ تولیدکننده‌ی NIV بودند. هیچکدام از جدایه‌های مولد DON قادر به تولید NIV نبودند. در حالی که مقادیر کم DON (۱۰–۹۰ppb) در جدایه های مولد NIV ردیابی گردید. توزیع جدایه های *F. graminearum* مولد NIV و مشتقات DON در استان‌های مختلف نشان داد که اکثر جدایه‌های مولد NIV متعلق به استان‌های گلستان و مازندران بودند، در حالی در استان‌های فارس، کرمان، هرمزگان و اردبیل جدایه‌های مولد DON بیشتر از جدایه‌های مولد NIV بودند.

واژه‌های کلیدی: بلایت فوزاریومی سنبله (FHB)، تریکوتسن‌ها، HPLC، *Tri13*.

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Introduction

Fusarium graminearum is one of the most important species responsible for FHB or Scab in wheat and other grains (Windels 2000) and causes (30-70%) quantitative and qualitative damages to wheat all over the world (Parry *et al*., 1995; McMullen *et al*., 1997). Fusarium head blight (FHB) has been considered as the major wheat disease in the north regions of Iran since two last decades (Golzar, 1993; Forootan *et al*., 1993; Babadoost, 1995; Mirabolfathy and Karami-Osboo, 2012) and the main casual agent of FHB in Iran is *Fusarium graminearum* Schwabe with the sexual form of *Gibberella zeae* (Schwein) Petch (Ji *et al*., 2007). Regarding to high DON contamination of wheat crop production at Ardabil province in year 2011, population analysis of the *Fusarium graminearum* species complex isolated from wheat crop of this area was considered and 321 *F. graminearum* isolates were identified morphologically and molecularly using Nicholson's specific *F. graminearum* primers, also for resolving the different members of the *F. graminearum* clade, the species specific primers for partial sequences of the ammonia ligase 2 (CTPS2) gene were used, The results of PCR products showed that 90% of *F. graminearum* isolates (287 out of 321isolates) were *F. asiaticum* while in 10 % of isolates were not *F. asiaticum* but they were in the clade of *F. graminearum* (Mianabi *et al*., 2014). This fungus has periodically caused epidemics, in addition to production of mycotoxins which threatened human health and livestock (Jennings *et al*., 2004; Yang *et al*., 2008).

The genus *Fusarium* is one of the most important group of filamentous fungi and difficult to classify fungal genera (Bluhm *et al*., 2002). *Fusarium* species are typically identified by morphological features such as growth form, non-sexual and sexual forms and other features which are often arbitrary and also time-consuming and non-specific (Leslie and Summerell 2006). Studies based on mycotoxin chemotype production are very useful to characterize the Fusarium isolates (Moss and Thrane 2004). Nevertheless, the methodology for carrying out the morphological and physiological characterization of toxigenic fungi is generally time consuming. Therefore, a rapid and reliable assay for the routine identification of toxigenic *Fusarium* spp would benefit the food and feed industry. PCR-based methods are already having a significant impact on mycological research. These methods may be used in fields such as mycotoxicology (Jime´nez *et al*., 2000), plant pathology (Mirete *et al*., 2003), or fungal systematics (O'Donnell *et al*., 1998). By using Fg16F/Fg16R primers designed based on the sequence of ITS area, five different products can pair various pieces from 400 to 500bp (Nicholson *et al*., 1998) which provides the possibility of accurate and faster *Fusarium* spp diagnosis. Each geographic region has its own *F. graminearum* populations (Carter *et al*., 2002).

The important toxins produced by *F. graminearum* on the small grains include 8-ketotrichothecens like deoxynivalenol (DON) and nivalenol (NIV) and their acetylation derivations such as 15-AcDON, 3-AcDON and 4-AcNIV (Kimura *et al*., 2007). Various studies have been indicated that the most prevalent mycotoxins are DON and NIV and their production is different in various geographical regions (Ward *et al*., 2002). The DON chemotype produces DON and 3-acetyldeoxynivalenol (3-AcDON), while the NIV chemotype produces NIV and 4-acetylnivalenol (4-AcNIV). DON and NIV chemotypes are frequently found in cereals harvested in Korea and Japan (Lee *et al*., 2001; Lee *et al*., 2002; Li *et al*., 2005). NIV is present at higher levels than DON in cereals from these countries (Lee *et al*., 2001; Chandler *et al*., 2003; Sugiura *et al*., 1990). Significant infection to DON, 3-AcDON and ZON have been reported in wheat grain samples collected from the northern areas of Iran (Zamani-Zadeh and Khorsandi, 1995).

The common methods for determination of *F. graminearum* mycotoxins are HPLC or GC/MS which are time-consuming and laborious. PCR-based methods have been used to determine chemotype based on the sequences of the genes responsible for trichothecene biosynthesis (Chandler *et al*., 2003; Jennings *et al*., 2004; Lee *et al*., 2001). *Tri13* gene is responsible for converting DON to NIV in Fusarium and *Tri7* gene is responsible for acetylating NIV and producing 4-AcNIV. The sequences omitted on *Tri13* gene are associated with the acetylation area in DON-producing species and with converting DON to NIV (Wang *et al*., 2008).

The aim of present study was to determine the

frequency of *F. graminaerum* chemotypes from wheat producing area of Iran using molecular and analytical assays.

Materials and Methods

Molecular identification of *F. graminearum* : Sixty *F. graminearum* isolates from Golestan, Mazandaran, Hormozgan, Ardebil, Fars, Kerman and Khorasan provinces which had been isolated from Fusarium head blight infected wheat farms in the previous studies (Mirabolfathy and Karami-Osboo, 2012) were selected from the collection of Mycotoxin Laboratory at Iranian Plant Protection Research Institute. After culturing the isolates on PDA medium for 3 to 5 days, three 0.4mm pieces of medium containing fungus were transferred to flask containing 150ml of potato dextrose broth and maintained on the shaker incubator at 120rpm under normal laboratory conditions for 14 days. The mycelia were freeze-dried and ground to a fine powder. DNA was extracted using CTAB method (Nicholson *et al*., 1997).

PCR reaction was performed by specific kit and Thermal cycler (Techne Co.; England); each assay had a positive (genomic DNA of a known isolate) and a negative control (without genomic DNA). Thermal cycler program included a 5min step at 94°C to initiate the reaction, 30 cycles (94°C for 1min; 54°C for 1min; 72°C for 2min) and a final extension step (72°C for 5min) using Fg16F/Fg16R primers (Table 1). PCR products were separated by electrophoresis through 1.2% agarose gels which were stained using 1µg/ml ethidium bromide and photographed under UV light.

Determination of *F. graminearum* chemotypes: All *F. graminearum* isolates were studied for gene responsible to produce DON, derivatives of DON and NIV by PCR method. Tri13F/Tri13DONR specific primers (Chandler *et al*., 2003) were used to identify DON-producing isolates (Table 1). The cycling protocol for Tri13F and Tri13DONR included a 5min at 94°C to start, 35 cycles (94°C for 60s; 58°C for 45s; 72°C for 60s) and a final extension stage (72°C for 5min).

To identify the NIV-generating isolates and DON acetylation derivatives, Tri13P1/Tri13P2 primers (Wang *et al*., 2008) which produced specific species with different sizes related to *Tri13* gene were used (Table 1). The thermal cycler condition included a 5min at 94°C for starting, 35 cycles (94°C for 60 s; 58°C for 40s; 72°C for 60s) and a final extension step (72°C for 5min).

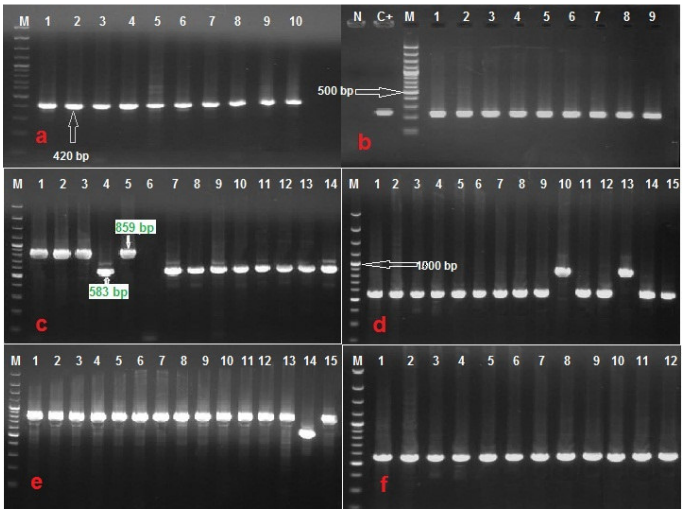

Fig. 1. (a) PCR products amplified from genomic DNA of *F. graminearum* isolates using Fg16F/Fg16R primers (expected product 420bp), M: 100bp DNA marker **(b)** Amplification products of gene encoding DON in *F. graminearum* isolates. N: Negative control, C+: Positive control (Fg170, DON producing isolate of *F. graminearum*), M: 100bp DNA marker **(c)** PCR amplification of DON- and NIV-chemotypes of *F. graminearum* isolates. M: 100bp DNA marker; Lane 1-3: NIV chemptype isolates (FgM119-FgM121); Lane 4: Positive control (15-AcDON-chemotype isolate); Lane 5: Positive control (NIV-chemotype); Lane 6: Negative control (omitting template DNA); Lane 7-14: FgH125, FgH126, FgH128, FgH129, FgK130, FgK131, FgF132, FgG133 **(d)** PCR amplification of DON- and NIV-chemotypes of *F. graminearum* isolates. M: 100bp DNA marker; Lane 1-9: FgF135, Fg136, FgF137, FgG139, FgF140, FgF142, FgG143, FgF144, FgM145, lane 10: FgM146 , Lane 11-12: FgM147, FgF148, Lane 13: FgM149, Lane 14-15: FgF150, FgG151 **(e)** M: 100bp DNA marker; Lane 1-13: FgG101, FgG102, FgG103, FgG104, FgG105, FgG108, FgG109, FgG110, FgG113, FgG118, FgM106, FgM107, FgG111, Lane 14: FgM115, lane 15: FgM112 **(f)** M: 100bp DNA marker; Lane 1-12: FgF138, FgH122, FgH124, FgA153, FgA155, FgA159, FgK156, FgK157, FgK158, FgKh160, FgKh161, FgKh162.

Evaluation of trichothecenes B productions using HPLC: Based on PCR assays, 13 *F. graminearum* representative isolates were selected and their mycotoxin-producing potential was investigated using analytical method. Mycotoxin analysis was performed using a modified method of Cea and Cammarota, (2011). Rice culture (5g) from each sample was finely ground, mixed well and placed in 25ml of acetonitrile/water (84:16, v/v) for 45 min and then filtered through No. 1 filter paper (Whatman International Ltd; UK). Three milliliters of the solution was taken for trichothecenes B analysis and passed through a charcoal–alumina–Celite SPE cartridge (Faroogh Lab.; Iran) packed with a disc of No. 1 filter paper (Whatman International Ltd, UK). The sample was allowed to seep by gravity feed through the cartridge and the column was then washed with 5 ml of a solvent mixture comprising of acetonitrile /water (84:16, v/v) at about 1 ml /min. The cleaned-up extract was collected in dark vial and evaporated to dryness, dissolved in 1 ml of HPLC mobile phase and 100 µL was injected to the HPLC (Fig. 2, Fig. 3).

Reagents and Standard: High-performance liquid chromatography (HPLC) grade water, acetonitrile, methanol, and all analytical grade extraction solvents were purchased from Merck (Germany). All standards were purchased from Sigma Aldrich (St. Louis, MO, USA). Stock solution of DON, NIV, 3-AcDON and 15-AcDON (25µg/ml) was prepared in acetonitrile and stored in dark glass vials at −20°C.

Instrumentation: A high performance liquid chromatography system equipped with auto sampler (Waters 717), binary HPLC pump (Waters 1525) and a dual λ absorbance UV detector (Waters 2487), was used for the analysis. The reverse phase column was a Waters Nova-pak® C-18, 3.9mm×250mm, 4mm particle size (Waters Milford, MA; USA) at 40°C. Separation was achieved using an isocratic mobile phase of water: acetonitrile: methanol (88:6:6; V/V) at 1.0 ml/min. Elutes (100µl) were detected using an ultraviolet detector set at 218nm (Mirabolfathy and Karami-Osboo 2012).

Accuracy of the method: To calculate the recovery and ensure the accuracy of the method, a blank sample was spiked at 1000 ng/g of each trichothecenes and according to the above described method was extracted and cleaned up. The calibration curves were calibrated between 100-1000 ng/mL for DON, 50-10000 ng/ml for NIV and 15-AcDON and 500-10000ng/ml for 3-AcDON. The quantification of trichothecenes was estimated by measuring the area under the curve at their retention time compared with the relevant calibration curve obtained from the same experiment.

Statistical analysis: The relationship between the distribution of DON, NIV and 15-AcDON chemotypes in *F. graminearum* isolates was statistically analyzed by the Fisher's exact test and logistics regression using SAS software (PROC GLM, SAS Institute).

Result and Discussion

Identification of *F. graminearum* isolates: Morphological identification of all sixty isolates (Mirabolfathy & Karami-Osboo, 2012) were confirmed as *F. graminearum* genetically using Fg16F/Fg16R primers and created the expected band with the size of 420bp on agarose gel (Fig. 1a).

Determination of *F. graminearum* chemotypes: Overall, both DON and NIV chemotypes were present in *F. graminearum* populations with different frequencies depending on geographical regions (table 3 and Fig 4). DON producing isolates were predominant in Fars, Kerman, Hormozgan and Ardebil provinces with a frequency more than 60%. On the other hand, NIV chemotypes isolates found in the Golestan and Mazandaran provinces in a higher frequency compared to the DON chemotype.

Tri13F/Tri13DONR primer amplified a 282bp fragment in 36 isolates (60%) whilst in the 24 other isolates (40%), the Tri13P1/Tri13P2 primers, related to the NIV chemotype, amplified an 859bp fragment (Fig. 1b, 1c, 1d, 1e and 1f). These results showed that 40% of sixty *F. graminearum* wheat isolates produced NIV and 60% of them produced 15-AcDON. None of the isolates produced 3-AcDON (Table 3).

Evaluation of trichothecenes B production using HPLC: All isolates possessed the 15-AcDON-producing gene produced the toxin. Furthermore, all isolates possessed the Nivalenol-generating gene produced the toxin in the range of 580-31410 µg/Kg with the mean of 10828±3904 µg/kg (Table 2).

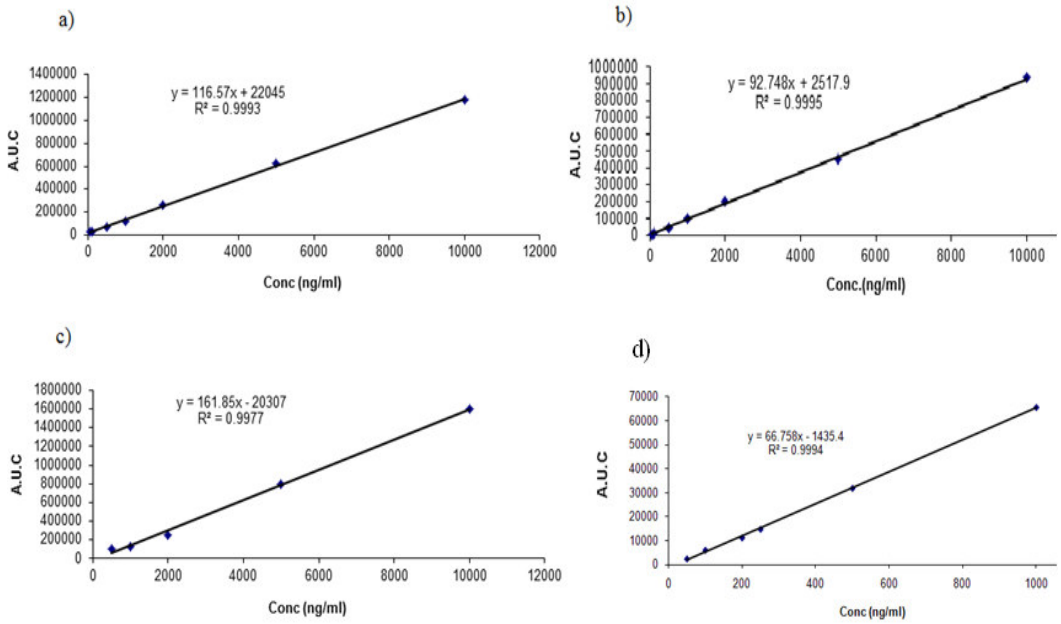

Fig. 2. Calibration curves of (a) 15- Ac DON, (b) nivalenol, (c) 3- Ac DON, and (d) DON

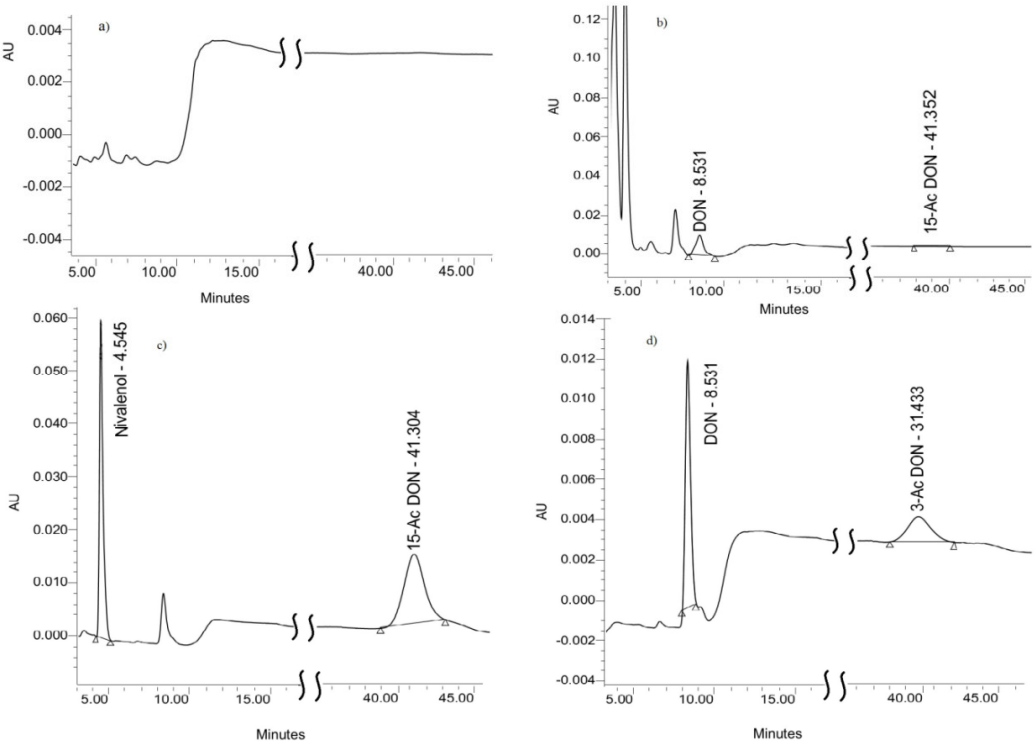

Fig. 3. HPLC chromatograms of (a) a blank sample , (b) a contaminated sample to DON and 15-Ac deoxynivalenol, (c) chromatogram of nivalenol and 15- Ac DON standards (5000 ng/mL), (d) chromatogram of DON (500 ng/ml)and 3- Ac DON standards (5000 ng/ml).

Isolate FgM111 which was geographically related to Golestan produced the maximum amount of NIV (31410 ppb) followed by FgG104 (11790 ppb) and FgG110 (10540 ppb) which were geographically belonged to Golestan province which is located in wet and marginal sea regions. Isolate FgF134 which was collected from Fars province produced the minimum amount of NIV. FgH125 isolate which was collected from Hormozgan province, produced maximum level of 15-AcDON (18746ppb). Some of NIV-producing isolates (such as FgG104) were also produce small amounts of DON (FgG104 could produce 11790 ppb of NIV and 9.96 ppb of DON as well).

The mycotoxin analysis of the 13 tested isolates using HPLC showed a perfect correlation between the geographical region and NIV potential production ($p<0.001$) which seems that is the effect of environmental impact on the distribution of NIV chemotype ($p=0.002$). There was also a correlation between geographical region and DON ($p=0.005$) and 15-AcDON ($p=0.006$) producing isolates which express the role of environmental factors on distributing DON ($p=0.004$) and 15-AcDON ($p=0.002$) producing isolates.

Up to now, various molecular methods have been introduced to diagnose pathogenic *Fusarium* species including *F. culmorum*, *F. graminearum* (Nicholson *et al*., 1998) and *F. poae* (Parry *et al*., 1995). The results of this study were consistent with others such as Nicholson *et al.* (1998) and indicated that this method is capable to identify *F. graminearum* from its closest species i.e. *F. culmorum* (Nicholson *et al*., 1998; Carter *et al*., 2002).

Table 1. Oligonucleotide primer sequences and sizes of PCR products

Primer name	Sequence	Sizes (bp)	Reference
Fg16F	5'-CTCCGGATATGTTGCGTCAA-3'	420	Nicholson *et al*., 1998
Fg16R	5'-GGTAGGTATCCGACATGGCAA-3'		
Tri13F	5'-CATCATGAGACTTGTKCRAGTTTGGG-3'	282	Chandler *et al*., 2003
Tri13DONR	5'-GCTAGATCGATTGTTGCATTGAG-3'		
Tri13P1	5'-CTCSACCGCATCGAAGASTCTC-3'	583, 644, 859	Wang *et al*., 2008
Tri13P2	5'-GAASGTCGCARGACCTTGTTTC-3'		

Table 2. The ability of DON, DON derivations and NIV productions of the representatives isolates of *Fusarium graminearum* using HPLC method

Isolate code	Geographical location	Mycotoxin concentration (ppb)			
		DON	NIV	3-AcDON	15-AcDON
FgG104	Golestan	9.96	11790	–	–
FgG110	Golestan	–	10540	–	–
FgG139	Fars	640	–	–	LOD/2*
FgM106	Mazandaran	56.3	2982	–	–
FgG111	Golestan	89.4	31410	–	–
FgM147	Mazandaran	1930.6	–	–	LOD/2
FgF134	Fars	38.5	5828	–	–
FgF136	Fars	1119.2	–	–	LOD/2
FgH125	Hormozgan	-	–	–	18746
FgA154	Ardebil, Moghan	50.3	7678	–	–
FgA155	Ardebil, Moghan	242.9	–	–	LOD/2
FgK130	Kerman	180	–	–	LOD/2
FgKh161	Khorasan	–	–	–	LOD/2

*LOD/2= below than LOD

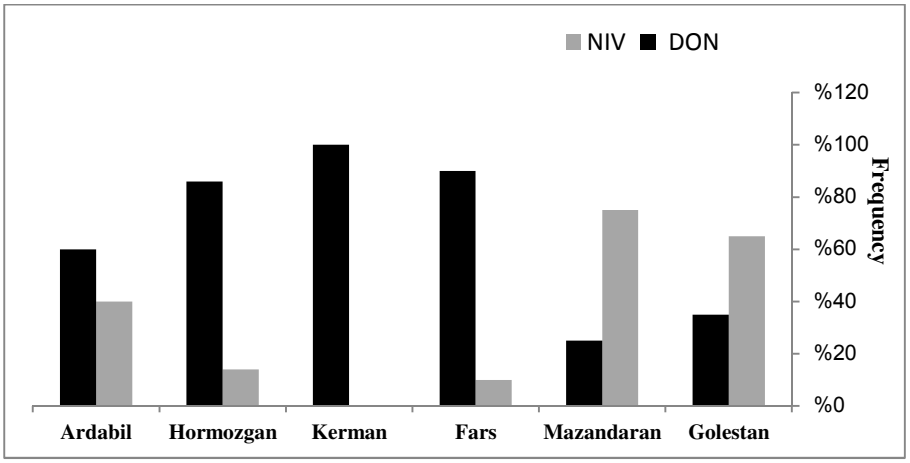

Fig. 4. Distribution (%) of DON derivatives and NIV-producing *F. graminarum* isolates in different provinces

F. graminearum not only causes disease and reduction of yield in wheat, but also can contaminate the grains by producing various types of trichothecenes (Bottalico and Perrone 2002). Various studies have been conducted using *Tri13* gene to identify trichothecenes- producing isolates of *F. graminearum* (Jennings *et al.*, 2004; Ji *et al*., 2007). The results of the present study indicated that a primer pair of Tri13P2 and Tri13P1 can differentiate NIV, 15-AcDON and 3-AcDON-producing isolates from each other.

Deoxynivalenol-producing isolates can produce certain and specific components which indicate that it has a protected structure in the sequence of *Tri13* gene (Brown *et al.*, 2002). The results of PCR reactions using Tri13F/Tri13DONR primers in this study were in consistent with the results of the previous studies (Carter *et al*., 2002; Qu *et al*., 2008).

Our results were different in percentage of trichothecenes producing isolates compared with those of Prodi *et al*. (2009) who studied 74 isolates of *F. graminearum* from the infected regions of Italy; among them, 87.2% produced 15-AcDON, 2.7% produced NIV and 8.1% produced 3-AcDON, compare with our results which showed among representative tested isolates 77% produced 15-AcDON and 46% produced NIV. Also the majority of *F. graminearum* isolates were DON and 15-AcDON producer that were consistent with the results obtained by the PCR method. Studies show that DON-producing species are not able to produce NIV or 4-AcNIV because they have a non-functional *Tri13* gene or removed *Tri13* gene (Lee *et al.*, 2002). The results revealed that none of the DON-producing isolates is able to produce NIV. On the simultaneous production of DON and NIV *F. graminearum* isolates, Sugiora *et al*. (1990) found that a number of *G. zeae* isolates being NIV-producing species can produce small amounts of DON whereas DON-producing isolates are not able to produce NIV. In similar studies, the low levels of DON were observed in *F. graminearum* species producing high levels of NIV (O'Donnell *et al*., 2000; Jurgenson *et al*., 2002). Also simultaneous producing of NIV and DON by a single isolate of *F. graminearum* were reported by previous studies (Sugiura *et al.*, 1990; O'Donnell *et al.*, 2000; Jurgenson *et al*., 2002). The results of our research indicated that FgG104, FgM106, FgF134 and FgA154 isolates which were major NIV producer could produce small amounts (10-90ppb) of DON. In contrast all major DON producer including FgF136, FgG139, FgM147and FgA155 isolates could not produce NIV at all (Table 3).

It is believed that *F. graminearum* isolates have different mechanisms in the biosynthesis of DON isomers in terms of geographical origin (Miller *et al*., 1991). In general, it seems that 3-AcDON and 15-AcDON are the analogs of two main toxins, DON and NIV, respectively. Prodi *et al.* (2009) showed that environmental factors affect the rate of production and the chemotype of trichothecenes. Determining the chemotype of three species of Fusarium, Miller *et al.* (1991) reported that identification of mycotoxins produced by the various species of *Fusarium* may help to verify the accuracy of classification or changes in the ability of toxin-producing species.

Table 3. *Fusarium graminearum* isolates, geographical location and *F. graminearum* chemotypes using different primers

Isolate Code	Geographical location	*Fusarium graminearum* Specific PCR primers (Fg16F/Fg16R)	*F. graminearum* chemotype		
			Tri13F/ Tri13DONR primers	Tri13P1/Tri13P2 primers	
				NIV	15-AcDON
FgG101	Golestan	+	−	+	−
FgG102	Golestan	+	−	+	−
FgG103	Golestan	+	−	+	−
FgG104	Golestan	+	−	+	−
FgG105	Golestan	+	−	+	−
FgG108	Golestan	+	−	+	−
FgG109	Golestan	+	−	+	−
FgG110	Golestan	+	−	+	−
FgG113	Golestan	+	−	+	−
FgG118	Golestan	+	−	+	−
FgG133	Golestan	+	+	−	+
FgG139	Golestan	+	+	−	+
FgG143	Golestan	+	+	−	+
FgG144	Golestan	+	+	−	+
FgG145	Golestan	+	+	−	+
FgG151	Golestan	+	+	−	+
FgM106	Mazandaran	+	−	+	−
FgM107	Mazandaran	+	−	+	−
FgG111	Golestann	+	−	+	−
FgM112	Mazandaran	+	−	+	−
FgM114	Mazandaran	+	−	+	−
FgM115	Mazandaran	+	+	−	+
FgM119	Mazandaran	+	−	+	−
FgM120	Mazandaran	+	−	+	−
FgM121	Mazandaran	+	−	+	−
FgM146	Mazandaran	+	−	+	−
FgM147	Mazandaran	+	+	−	+
FgM149	Mazandaran	+	−	+	−
FgF132	Fars	+	+	−	+
FgF134	Fars	+	−	+	−
FgF135	Fars	+	+	−	+
FgF136	Fars	+	+	−	+
FgF137	Fars	+	+	−	+
FgF138	Fars	+	+	−	+
FgF140	Fars	+	+	−	+
FgF142	Fars	+	+	−	+
FgF148	Fars	+	+	−	+
FgF150	Fars	+	+	−	+
FgH122	Hormozgan	+	+	−	+
FgH123	Hormozgan	+	−	+	−
FgH124	Hormozgan	+	+	−	+
FgH125	Hormozgan	+	+	−	+
FgH126	Hormozgan	+	+	−	+
FgH128	Hormozgan	+	+	−	+
FgH129	Hormozgan	+	+	−	+
FgA152	Ardebil, Moghan	+	−	+	−
FgA153	Ardebil, Moghan	+	+	−	+
FgA154	Ardebil, Moghan	+	−	+	−
FgA155	Ardebil, Moghan	+	+	−	+
FgA159	Ardebil, Moghan	+	+	−	+
FgK130	Kerman	+	+	−	+
FgK131	Kerman	+	+	−	+
FgK156	Kerman	+	+	−	+
FgK157	Kerman	+	+	−	+
FgK158	Kerman	+	+	−	+
FgKh160	Khorasan	+	+	−	+
FgKh161	Khorasan	+	+	−	+
FgKh162	Khorasan	+	+	−	+
FgKh163	Khorasan	+	+	−	+
FgKh164	Khorasan	+	+	−	+

Positive and negative sign (+, -) respectively indicated to produce expected fragments of DNA using the related primers

Distribution of DON derivatives and NIV- producing *F. graminarum* isolates in different provinces showed that the most NIV- producing isolates were obtained from Golestan and Mazandaran provinces (Fig. 4), whereas DON derivatives- producing *F. graminearum* isolates were more than NIV chemotypes in Kerman, Fars, Hormozgan and Ardabil provinces with 100%, 90%, 86%, and 60%, respectively. This result may be related to environmental factors and the phylogenetic members of *F. graminarum* complex species in different geographical areas, to prove this hypothesis a lot of isolates from all wheat fields throughout Iran must be studied.

Few studies in Iran have so far been conducted to find the relationship between production of trichothecenes and presence of the related genes. Although the activity of *Tri6* and *Tri5* (Haratian *et al*., 2006) and *Tri13* (Haratian *et al*., 2008) genes and the possibility of trichothecenes production were studied in Iran but more studies about the effective genes in production of trichothecenes provides extensive information such as the relationship between these genes and the other genes in the cluster, its relationship with the genetic variation of isolates and the possibility of mutation occurrence.

Assessments performed in the current study revealed that species-specific primers can be used for rapid detection *F. graminearum* in infected tissue. This method can even be applied to identify pathogens species in the early stages that the disease symptoms are not still quite recognizable. Moreover, based on PCR assays, the capability of producing trichothecenes in *F. graminearum* isolates can be checked. In addition, information on the distribution of *Tri13* gene haplotypes in different provinces may provide information for epidemiological studies

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