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RESEARCH PAPERS

Pathogenicity and mycotoxin chemotypes of Iranian *Fusarium culmorum* isolates on durum wheat, and comparisons with Italian and Syrian isolates

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Summary. *Fusarium* isolates obtained from Iran and Italy were identified by morphological characters and confirmed by using species-specific PCR assays. The genetic chemotyping for each strain, as a preliminary assessment for trichothecene production, was defined using PCR. Subsequently, artificial infection on durum wheat (cv. Normanno) was carried out in the greenhouse to study the pathogenicity and aggressiveness of Iranian, Italian and Syrian *F. culmorum* strains, the causal agent of crown and root rot of wheat. All *F. culmorum* strains from Iran and Italy belonged to the 3-acetyl-deoxynivalenol (3Ac-DON) chemotype, while *F. culmorum* strains from Syria, previously characterized, belonged to the 3Ac-DON and nivalenol (NIV) chemotypes. All of the strains were pathogenic and caused typical Fusarium crown rot (FCR) symptoms. Italian and Iranian strains showed similar mean aggressiveness levels (19.1 and 18.7% respectively), while the mean aggressiveness level for Syrian strains was 11.8%. There were statistically significant differences among the strains. Two Italian strains, F383 and F1126, had the greatest level of aggressiveness while FC9 (Italian) and F961 (Syrian) were the least aggressive strains. No significant differences relating to agro-ecological origin were detected among Iranian, Italian and Syrian strains. This is the first genetic chemotyping characterization and comparison of *F. culmorum* strains, isolated from different agro-ecological countries, Iran, Syria (Middle East) and Italy (Europe), which has estimated their potential for producing mycotoxins, and the aggressiveness levels of *F. culmorum* for development of FCR. These results support the increasing concerns about the risk of FCR in many wheat producing countries, particularly in Iran and Syria.

Key words: *Fusarium culmorum*, Fusarium crown rot, chemotype, aggressiveness.

Introduction

Wheat, the most strategically important crop worldwide, is widely grown in many countries, including Iran, Italy and Syria. Based on international wheat production statistics (FAOSTAT database), the total production of wheat in 2012 was 13.8 MT in Iran, 7.7 MT in Italy and 3.6 MT in Syria (http://faostat.fao.org).

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Fusarium species, ubiquitous soil saprophytes, have been isolated from debris, roots, stems and seeds of a wide variety of plants. Two distinct diseases, Fusarium crown rot (FCR) and Fusarium head blight (FHB), occur on small-grain cereals, in particular on wheat and barley (Leslie and Summerell, 2006; Scherm *et al*., 2013).

FCR is an important disease of wheat, affecting plants in the early stages of growth causing yield losses, stand reductions and rotting of root, crown and lower stem tissues (Fernandez and Jefferson, 2004). FHB affects host plants in the flowering stage

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leading to reductions in quantity and quality of the grains. The colonization of wheat with *Fusarium* species can cause grain contamination with toxic fungal secondary metabolites, mycotoxins, which are health hazards both for humans and farm animals (Desjardins, 2006).

Crown rot caused by *Fusarium* spp. is of economic importance in Australia, Europe, North America, South America, West Asia, North and South Africa (Chakraborty *et al.*, 2006). This disease causes economical yield losses in most wheat growing areas of Iran, in particular in the northwest regions (Saremi *et al.*, 2007; Pouzeshimiab *et al.*, 2014). The key factors for development are previous crops, residue management, nitrogen fertilization, plant density and environmental conditions (Scherm *et al.*, 2013). FCR is a disease complex induced by different pathogens, including *Fusarium culmorum* (W.G. Sm.) Sacc., along with *F. pseudograminearum* O'Donnell and Aoki (group I) (= *Gibberella coronicola*) and *F. graminearum* Schwabe (group II) (= *G. zeae* (Schwein.) Petch) (Pettit *et al.*, 2003). Hollaway *et al.* (2013) reported that *F. culmorum* and *F. pseudograminearum* DNA concentrations in soil prior to planting were positively related to crown rot expression and negatively related to grain yield of durum wheat, bread wheat and barley, and that the losses due to FCR were greater in durum wheat than bread wheat and much less in barley.

The severity of FCR, caused by *F. culmorum,* is greater in dry soils and in areas with high temperatures. These conditions are present in the South of Italy, where durum wheat is commonly grown (Balmas *et al.*, 2006), and in the Middle East countries such as Turkey (Tunalı *et al.*, 2006), Iran (Eslahi, 2012) and Syria (El-Khalifeh *et al.*, 2009). In Iran Eslahi (2012) reported that *F. pseudograminearum* and *F. culmorum* were the main fungi associated with FCR disease in the Khuzestan region. In the North West of Iran, however, the predominant pathogens implicated in causing root and crown rot diseases were *F*. *pseudograminearum*, *Rhizoctonia solani* and *F. culmorum* (Saremi *et al.*, 2007). FCR is known to occur in Syria, and the species found in the infected wheat plants include *F. graminearum, F. culmorum, F. avenaceum* and *F. equiseti* (El-Khalifeh *et al.*, 2009), but no data about prevalent causal agents are available.

FCR is also involved in the production and spread of conidia inocula for subsequent spike infections leading to FHB, the predominant agents of which are the trichothecene producers *F. graminearum* and *F. culmorum*. Trichothecenes are a large family of chemically related mycotoxins. The ability of aggressive *Fusarium* strains to infect wheat is related to their ability to produce large amounts of trichothecenes in culture or in infected tissues (Hestbjerg *et al.*, 2002; Scherm *et al.*, 2011), although the correlation is not always direct (Gang *et al.*, 1998). The trichothecene mycotoxins produced by *F. culmorum* are responsible of the spread of the disease by inhibiting the defence mechanisms activated by host plants (Wagacha and Muthomi, 2007). Two trichothecene chemotypes are present in *F. culmorum*: the deoxynivalenol (DON) chemotype and/or its acetylated derivatives (3Ac-DON, 15Ac-DON), and the nivalenol (NIV) chemotype and/or fusarenone-X (FUS). NIV is reported to be ten times more toxic than DON (Minervini *et al.*, 2004). Chemotype studies worldwide have increased greatly over the last 10 years, but given the lower general importance of *F. culmorum* as primary cause of FHB, less work has been dedicated to chemotype determination in this species (Pasquali and Migheli, 2014).

Chemotype identification may provide insight into the toxigenic potential of *F. culmorum* strains. For instance, the accumulation of NIV in wheat grains, harvested in Luxembourg during the years 2007 and 2008 was linked to the presence of *F. culmorum* NIV chemotype (Pasquali *et al.*, 2010). Furthermore, Covarelli *et al.* (2012) demonstrated that there was a translocation of DON to wheat heads after inoculation of stem bases of soft wheat seedlings with *F. culmorum,* even although the fungus was unable to grow systemically beyond the third node of inoculated plants.

In Middle Eastern countries, the investigations on *F. culmorum* chemotypes have been less frequent than those focusing on *F. graminearum*. However, it is possible to trace the distribution of the fungi in some geographical areas. For example, in Turkey, 100% of *F. culmorum* strains belong to the 3Ac-DON chemotype (Yörük and Albayrak, 2012), while in Syria 55% of the strains were 3Ac-DON and 45% were NIV chemotypes (Alkadri *et al.*, 2013). To our knowledge, no data about genetic chemotyping characterization of Iranian strains are available. In Italy, all the *F. culmorum* strains from wheat belonged to 3Ac-DON (Quarta *et al.*, 2005; Covarelli *et al.*, 2014) except for two NIV strains found in two Italian regions, Tuscany and Emilia-Romagna (Prodi *et al.*, 2010). To date, no 15Ac-DON chemotype has been reported in *F. culmorum*.

The research reported in this paper focussed on: 1) molecular identification and characterization of *F. culmorum* strains from Iran and Italy into genetic chemotyping, comparing the results obtained with those from Syria; and 2) evaluation of the aggressiveness of *F. culmorum* strains isolated from wheat from Italy, Iran and Syria on durum wheat plants.

Since little information on FCR agents is available for the Middle East countries, this research is important because it will allow development of agricultural policy to reduce the risk of *F. culmorum* presence in wheat and to prevent mycotoxin contamination in the production wheat grain chain.

Materials and methods

Fungal strains

During the years 2012–2014, *F. culmorum* strains were isolated from different wheat fields in three agro-ecological countries, Iran (from three regions of the north, including Golestan, Mazandran and Ardebil), Italy and Syria (Figure 1, Tables 1 and 2). These isolates have been stored in the laboratory of Phytopathological Mycology, Department of Agricultural Sciences, University of Bologna, Italy, and some isolates are also in the fungal collection of the Seed and Plant Improvement Institute, Karaj, Iran.

DNA extraction

DNA of Iranian and Italian strains was extracted using the cetyl-trimethyl-ammonium bromide (CTAB) method from *Fusarium* mycelium, harvested from 6-d-old single-spore cultures grown on potato dextrose agar (PDA; Prodi *et al.*, 2011). Syrian strains were previously identified and characterized for chemoptype (Alkadri *et al.*, 2013).

Species-specific PCR

Morphological identification of *F. culmorum* strains was verified using the species-specific primer Fc01F/Fc01R (Nicholson *et al.*, 1998). The reaction mixtures were prepared in a total volume of 25 μL. For each reaction 0.6 U of Ampli Taq polymerase (Applied Biosystems), 15 pmol of each primer and approximately 25 ng of fungal template DNA were used. Amplification was done in a T3 thermocycler (Biometra) using a touchdown PCR protocol with the annealing temperature at 66°C for the first five cycles, and 64°C for the next five cycles, followed by eight cycles at 62°C and 11 at 58°C. The temperature cycle used consisted of denaturation (95°C) for 30 s, annealing (as described above) for 20 s and extension (72°C) for 45 s. The amplification products were resolved on 1% agarose gels stained with ethidium bromide (0.4 μg mL^{-1}) and visualized under UV light, alongside a 100 bp DNA ladder (Promega). Control tubes without DNA template were included in each experiment.

Chemotype assays

Fusarium culmorum strains were characterized by multiplex PCR assays to distinguish their chemotypes regarding trichothecene synthesis. Primers, amplifying parts of the *Tri3* and *Tri7* genes, were used to classify 3Ac-DON, 15Ac-DON and NIV chemotypes (Quarta *et al.*, 2005). The primer set *Tri3*F1325/*Tri3*R1679 identified 3Ac-DON and the set *Tri3*F971/*Tri3*R1679 identified the 15Ac-DON chemotypes, while the primer set *Tri7*F340/*Tri7*R965 identified the NIV chemotype. To confirm chemotyping identifications, negative (water blank) and positive controls (isolates F966 and F967; Alkadri *et al.*, 2013) were used.

Greenhouse experiment

The durum wheat cultivar 'Normanno' was used. This cultivar is susceptible to *Fusarium* diseases and is among the most commonly cultivated in areas of North-Center Italy. Plants were grown in controlled environmental conditions in a greenhouse. A total of 107 pots were filled with sterile soil and ten seeds, at approximately 2 cm below the soil surface, were sown for each pot. The soil was watered every 2 d.

Three mycelium plugs (0.6 × 0.6 cm) from each of the 34 *F. culmorum* strains were seeded into flasks containing autoclaved V8 broth (Singleton *et al.*, 1992) and set in a refrigerated horizontal shaker at 120 rpm, 25°C under incident light for 2 weeks to produce the inoculum. The mixtures of macroconidia with mycelium and V8 medium were filtered through a sterile syringe filled with double layers of autoclaved cheesecloth. Spore concentration was determined using a haemocytometer and the suspension adjusted to 2×10^5 macroconidia mL^{-1}.

Figure 1. Map of Iran with provinces where *Fusarium culmorum* isolates were collected (▲).

Pathogenicity assay

A pathogenicity assay was carried out for 34 *F. culmorum* strains from the three different countries. Three pots for each strain plus five for uninoculated controls were used. Ten surface disinfected wheat seeds were planted in each pot (diam. 15 cm; height 10 cm) containing sterilized wheat growing soil (autoclaved twice at 121°C for 1 h, at 24 h interval). At 2 weeks after sowing (the wheat seedlings had two leaves: Zadoks' GS 12), the wheat seedlings were inoculated with 3 mL of *F. culmorum* suspension, or water for the control plants. The soil around the seedlings was removed and the inoculum was applied with a pipette along the stems, 1–2 cm above the soil, to allow the suspension to reach the stem bases. The soil was then replaced around the plants.

The treatments were arranged in a completely randomized design, replicated three times. Plants were grown in a greenhouse (23–24ºC; 45–60% relative humidity). Three weeks after inoculation (Zadoks' GS 20), each plant was carefully removed from the soil and washed. Disease symptoms included brown and necrotic discoloration in the crown tissues. For FCR evaluation, five disease classes were used: class 0 = healthy stem; 1 = mild browning on the stem; 2 = browning on one-half of the stem; 3 = complete browning of the stem; and 4 = plant death. The disease severity (DS) of each treatment was calculated using McKinney (1923) index, which expresses the percentage of the maximum severity of the disease (i.e., 100), according to the formula

$$DS = [\sum(c \times f)/n \times N] \times 100$$

where c = disease class, f = frequency, n = number of observations, and N = the greatest value of the empirical scale adopted (class 4).

Table 1. Origins of 27 *Fusarium culmorum* isolates from three Provinces of Iran, obtained from infected wheat crown tissues, and isolates from Italy. Their chemotypes (determined by PCR assays) are also indicated.

Isolate	Geographical origin	Province (Location)	Year	Chemotype
F1	Iran	Golestan (Gorgan)	2013	3Ac-DON
F2	Iran	Golestan (Gorgan)	2013	3Ac-DON
F3	Iran	Golestan (Gorgan)	2013	3Ac-DON
F4	Iran	Golestan (Gorgan)	2013	3Ac-DON
F5	Iran	Golestan (Gorgan)	2013	3Ac-DON
F6	Iran	Golestan (Gorgan)	2013	3Ac-DON
F7	Iran	Golestan (Gorgan)	2013	3Ac-DON
F8	Iran	Golestan (Gorgan)	2013	3Ac-DON
F9	Iran	Mazandaran (Sari)	2013	3Ac-DON
F10	Iran	Mazandaran (Sari)	2013	3Ac-DON
F11	Iran	Mazandaran (Sari)	2013	3Ac-DON
F12	Iran	Mazandaran (Sari)	2013	3Ac- DON
F13	Iran	Mazandaran (Sari)	2013	3Ac-DON
F14	Iran	Mazandaran (Sari)	2013	3Ac-DON
F15	Iran	Mazandaran (Sari)	2013	3Ac-DON
F16	Iran	Mazandaran (Sari)	2013	3Ac-DON
F17	Iran	Mazandaran (Sari)	2013	3Ac-DON
F18	Iran	Mazandaran (Sari)	2013	3Ac-DON
F19	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
F20	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
F21	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
F22	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
F23	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
F24	Iran	Ardebil (Dashte moghan)	2013	3Ac- DON
F25	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
F26	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
F27	Iran	Ardebil (Dashte moghan)	2013	3Ac-DON
FC9	Italy	Emilia-Romagna	2008	3Ac-DON
F383*	Italy	Basilicata	ITEM 354	3Ac-DON
F385*	Italy	Marche	ITEM 4695	3Ac-DON
F451	Italy	Emilia-Romagna	2006	3Ac-DON
F597	Italy	Tuscany	2007	3Ac-DON
F1047	Italy	Tuscany	2009	3Ac-DON
F1048	Italy	Tuscany	2009	3Ac-DON
F1049	Italy	Tuscany	2009	3Ac-DON
F1059	Italy	Tuscany	2009	3Ac-DON
F1060	Italy	Tuscany	2009	3Ac-DON
F1061	Italy	Tuscany	2009	3Ac-DON
F1062	Italy	Tuscany	2009	3Ac-DON
F1063	Italy	Tuscany	2009	3Ac-DON
F1072	Italy	Umbria	2009	3Ac-DON
F1126	Italy	Sicily	2011	3Ac-DON

Table 2. Number, origins and chemotypes of *Fusarium culmorum* strains, and disease severity (DS; determined in a pathogenicity assay), in durum wheat cv. Normanno. Mean disease severities accompanied by the same letter are not significantly different ($P \geq 0.05$).

Strain	Geographical origin	Chemotype	Disease severity
F1	Iran	3Ac-DON	6.3[abc]
F2	Iran	3Ac- DON	13.0[bcde]
F3	Iran	3Ac- DON	12.9[bcde]
F4	Iran	3Ac- DON	23.0[ef]
F5	Iran	3Ac- DON	25.9[ef]
F6	Iran	3Ac- DON	24.2[ef]
F7	Iran	3Ac- DON	24.4[ef]
F8	Iran	3Ac- DON	20.0[ef]
F960	Syria	3Ac- DON	6.6[abc]
F961	Syria	3Ac- DON	3.5[a]
F962	Syria	3Ac- DON	14.4[cdef]
F963	Syria	NIV	6.4[ab]
F964	Syria	NIV	14.5[cdef]
F965	Syria	NIV	16.2[cdef]
F966	Syria	3Ac- DON	13.1[bcde]
F967	Syria	NIV	17.9[def]
F968	Syria	3Ac- DON	7.4[abcd]
F969	Syria	3Ac- DON	13.8[cde]
F970	Syria	NIV	16.2[cdef]
FC9	Italy	3Ac- DON	2.9[a]
F383*	Italy	3Ac- DON	27.5[f]
F385*	Italy	3Ac- DON	21.3[ef]
F451	Italy	3Ac- DON	16.3[cdef]
F597	Italy	3Ac- DON	14.1[bcde]
F1047	Italy	3Ac- DON	19.8[ef]
F1048	Italy	3Ac- DON	17.6[def]
F1049	Italy	3Ac- DON	18.9[ef]
F1059	Italy	3Ac- DON	24.4[ef]
F1060	Italy	3Ac- DON	14.4[cdef]
F1061	Italy	3Ac- DON	14.0[cdef]
F1062	Italy	3Ac- DON	23.8[ef]
F1063	Italy	3Ac- DON	22.3[ef]
F1072	Italy	3Ac- DON	21.1[ef]
F1126	Italy	3Ac- DON	27.7[f]

* Reference strains obtained from ITEM Bank (F383 = ITEM 354; F385 = ITEM 4695).

Re-isolation of *F. culmorum* from infected crown and root tissues was done to verify Koch's postulates. The tissues were disinfected in sodium hypochlorite solution (2% available chlorine) for 2 min, rinsed with sterile water, dried on sterile filter paper, placed in Petri dishes containing PDA supplemented with neomycin (100 mg L^{-1}) and streptomycin sulfate (200 mg L^{-1}) and incubated at 22°C in darkness. After 7 d, presence of *F. culmorum* was determined.

Statistical analyses

Data analyses (standard ANOVA) were performed at using the SPSS software (SPSS version 21). A probability level of 5% ($P \leq 0.05$) was used to differentiate the means.

Results

Molecular identification and chemotyping

The products of DNA amplification of the 42 *Fusarium* strains (Iranian and Italian), morphologically identified as *F. culmorum*, were about 570 bp. This size corresponds to published values for species-specific PCR products for *F. culmorum*, confirming the morphological identifications.

Table 1 shows genetic chemotyping results for each strain, as a preliminary assessment of trichothecene production. An amplification product of about 350 bp, as expected for 3Ac-DON chemotypes, was obtained from all Italian and Iranian *F. culmorum* strains (100%). A 700-bp fragment, specific for 15Ac-DON chemotypes, and a 625 bp fragment, expected for NIV producers, were not found in any of the tested Italian or Iranian strains.

Pathogenicity assay

All the 34 tested *F. culmorum* strains were pathogenic and induced stem browning, typical FCR symptoms, in the inoculated durum cv. Normanno wheat plants. The values (%) of DS evaluations for all the strains, at 21 d after inoculation, are shown in Table 2.

The aggressiveness levels of the strains assayed had significant differences: for instance within the eight Iranian isolates, the lowest DS value (6.3%) occurred for F1 whereas the most aggressive strains, with values greater than 20%, were F4 (23.0%), F5

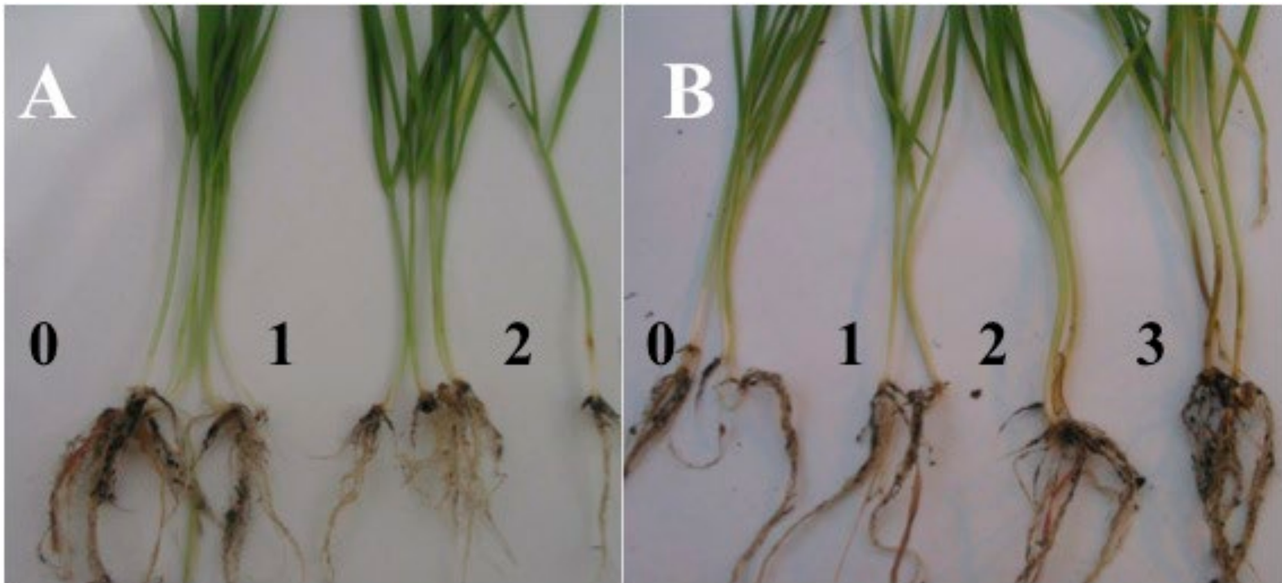

Figure 2. Different symptom severities observed in crowns and roots of durum wheat cv. Normanno 21 days after inoculation with *Fusarium culmorum* (A: strain F961; B: strain F1126).

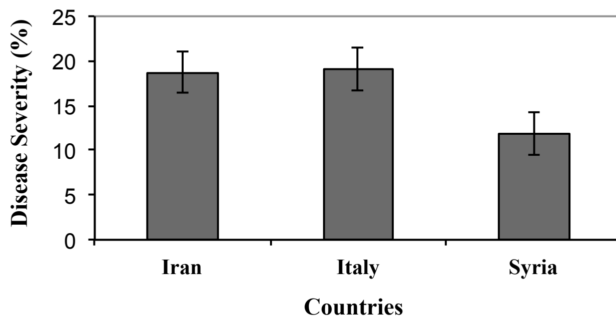

Figure 3. Mean disease severity of eight Iranian, 15 Italian and 11 Syrian *Fusarium culmorum* strains on durum wheat cv. Normanno in a greenhouse assay, 21 d after inoculation. Bars represent the standard errors of means.

(25.9%), F6 (24.2%), F7 (24.4%) and F8 (20.0%). Within the 15 Italian strains, the least DS value was for FC9 (2.9%), and the most aggressive strains were F383 (27.5%) and F1126 (27.7%). Within the 11 Syrian strains, F961 gave the least DS (3.5%), while F962 (14.4%), F964 (14.5%), F965 (16.2%), F966 (13.1%), F967 (17.9%), F969 (13.8%) and F970 (16.2%) were significantly more aggressive. There were significant differences in the variability of aggressiveness among the strains from the three countries: F383 and F1126, the two Italian strains, showed the greatest aggressiveness, while FC9 (Italian) and F961 (Syrian) were the least aggressive strains (Figure 2). However, it was not possible to cluster the strains based on their geographical origins. The results showed that the mean DS for the Iranian strains was 18.7%, the Italian strains were the most aggressive (DS = 19.1%), and the Syrian strains were less aggressive (DS = 11.8%: Figure 3). All the tested isolates caused discoloration on the seedling stems whereas the uninoculated control plants did not show any symptoms (DS = 0%).

Discussion

Iran is a large agricultural country, being cultivated with a large number of economically important crops. Extensive areas have been dedicated to monocultures for a long time, giving opportunities for emergence of plant diseases caused by *Fusarium*, and these are problems in wheat growing areas (Chehri, 2011). *Fusarium culmorum* in wheat is more aggressive in warm areas (Balmas *et al.*, 2006; El-Khalifeh *et al.*, 2009; Eslahi, 2012), where water stress and drought conditions increase the susceptibility of plants rather than the virulence of the fungi (Scherm *et al.*, 2013). In Australia yield losses due to FCR

were greater when rainfall during September and October (crop maturation) was below the long-term average (Hollaway *et al.*, 2013). Rainfall decrease and drought risk are serious problems affecting many countries of the world. Especially in Iran and Syria, the climatic conditions, warm weather and drought, could increase the risk of FCR. The climatic conditions in Iran and Syria are quite similar, which could enhance the possibility of spread of these pathogens among Middle East countries.

All Iranian and Italian *F. culmorum* strains assayed in the present study belonged to the 3Ac-DON chemotype. To our knowledge this is the first report of Iranian *F. culmorum* chemotype strains. This result is similar to that reported by Yörük and Albayrak (2012) in Turkey, where all 21 *F. culmorum* strains tested were 3Ac-DON producers. Studies conducted in several European countries, including Norway, Denmark, Germany, Netherlands and Poland (Langseth *et al*., 2001; Tòth *et al*., 2004; Quarta *et al*., 2005), also showed that the 3Ac-DON chemotype was prevalent in *F. culmorum* populations. For Syrian strains, 3Ac-DON (54.5%) and NIV (45.5%) producers are evenly distributed (Alkadri *et al*., 2013), and this is similar to surveys made in Luxemburg, where 53.2% of all *F. culmorum* isolates were 3Ac-DON chemotypes and 46.8% were NIV chemotypes (Pasquali *et al*., 2010).

In the present study, the pathogenicity assay revealed that the most of *F. culmorum* strains had a low variability; F383 and F 1126, both isolated from Italian fields, showed the greatest levels of aggressiveness on durum wheat, and FC9 and F961 were the least aggressive. No correlation based on geographical origin was found.

This is the first report on aggressiveness levels of Iranian and Syrian *F. culmorum* strains responsible of FCR. El-Khalifeh *et al.* (2009) in Syria, and Eslahi (2012) and Hajieghrari (2009) in Iran, only identified the fungi associated with foot and root rot of wheat. Furthermore, Iranian researchers tested only the pathogenicity of the strains.

Based on our results on aggressiveness levels, we conclude that Iranian and Syrian strains showed similarities to Italian *F. culmorum* isolates. In Italy FCR is mainly controlled by adopting preventive measures, such as crop rotation, use of tolerant cultivars, management of crop residues, reduced use of nitrogen fertilization and, the most important, seed coating with fungicides (Balmas *et al.*, 2006). In the Middle East countries the risk of FCR is progressively increasing but most of these preventive measures are not adopted.

The risk of a progressive spread of *F. culmorum* through different countries should increase the development of appropriate agricultural policies to gain knowledge on *F. culmorum* distribution on a larger scale. The data obtained in this work, concerning *F. culmorum* genetic chemotyping and aggressiveness, are important as a basis for the development of agricultural practices that will target to the prevention of mycotoxin contamination in wheat production chain. More studies on *F. culmorum* populations, genetic diversity and large scale distribution are required among different agro-ecological Middle East and European countries.

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