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Occurrence of *Fusarium* species associated with economically important agricultural crops in Iran

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Plant diseases caused by *Fusarium* spp. will results in yield losses and are becoming more significant in Iran. In this study, infected plant samples suspected to *Fusarium* infection, plant debris and rhizosphere soil were collected from the most important crops that is, wheat, rice, corn, barley, potato, cucurbits from different provinces in Iran during 2002 to 2009. A total of 2,500 *Fusarium* isolates were obtained and classified into 30 species based on morphological characters. *Fusarium sporotrichioides, F. chlamydosporum, F. graminearum* and *F. pseudograminearum* were obtained only from tissue samples whereas *F. scirpi, F. longipes* and *F. eumartii* were from soils. Among the isolates, 41% were recovered from plant tissue, 36% from soil and 23% from plant debris samples. In cereals tissues, *F. graminearum, F. pseudograminearum, F. sambucinum, F. culmorum, F. crookwellense, Fusarium proliferatum, F. verticillioides, F. fujikuroi* and *F. nygamai* were predominant species. *Fusarium* spp. recovered from potato tissues were *F. sambucinum, F. culmorum, F. crookwellense, F. trichothecioides, F. proliferatum, F. verticillioides, F. subglutinans* and *F. anthophilum* whereas *F. solani* and *F. oxysporum* were predominant in cucurbits, sorghum, tomato, sugar beet and bean. To our knowledge this is the first comprehensive report on the identification of large number of *Fusarium* spp. in different crops from Iran.

Key words: Fusarium spp., occurrence, distribution, agricultural crops, Iran.

INTRODUCTION

The genus *Fusarium* is one of the most important fungi that include many pathogenic species, causing a wide range of plant diseases. The genus Fusarium is a ubiquitous soil saprophyte and has been isolated from debris and roots, stems and seeds of a wide variety of plants (Leslie and Summerell, 2006). The widespread distribution of Fusarium species may be attributed to the ability of these fungi to grow on a wide range of substrates and their efficient mechanisms for dispersal (Burgess, 1981). A number of Fusarium species produces mycotoxins that can contaminate a wide variety of crop plants; therefore, mycotoxins potentially could occur in a wide variety of feeds and foods (Nelson et al., 1994). Contamination of agricultural products is a serious concern for animal and human health. Another interesting role of Fusarium is the interaction with higher plants as endophytes (Pitt and Hocking, 1999; Munkvold and

Desiardins, 1997).

Iran is a large agricultural country being cultivated with a number of economically important agricultural crops especially in the last two decades for self sustainability. Monocultures in a large area for a very long time have given ample opportunity for emergence of a number of plant diseases caused by Fusarium. Among the most important diseases by Fusarium are ear and stalk rot of wheat (Zamanizadeh and Khorsandi, 1995), corn (Bujari and Ershad, 1994), barley (Nejatsalari and Ershad, (1994), rice (Naeimi et al., 2002) and other economically important crops. The correct identification of the species is necessary in order to formulate quick actions to control the pathogenic and toxigenic Fusarium species (Salleh, 1996) and also certain strains of Fusarium species have been used in industry as biological control agents to control wide range of weeds, plant pests and diseases

(Salleh, 2007). Therefore, the aim of this study was to observe the diversity of *Fusarium* species from soils, plant debris and plant tissues obtained from different agricultural fields in Iran.

MATERIALS AND METHODS

Sample collection

All samples including rhizosphere soils, plant debris and plant tissues suspected of *Fusarium* infections were collected during 2002 to 2009 from Azarbaeigan, Esfahan, Fars, Gilan, Hamadan, Kermanshah, Khorasan, Khuzaestan, Lorestan and Mazandaran provinces in Iran (Table 1). From each of the provinces, 10 soil samples were collected. Each and every composite soil sample and the tissue samples were kept in a paper envelope and brought to the laboratory for isolation.

Isolation of Fusarium spp.

The soil samples were immediately air-dried for 24 h ground and sieved with 20 mesh gauze. Materials retained on the gauze were termed as 'debris' and materials that passed through the gauze was the 'soil'. Fusarium species from soil were isolated using serial dilution technique and the debris were directly plated onto peptonpentachloronitrobenzene agar (PPA) plates (Nash and Snyder, 1962). For isolation of Fusarium spp. from roots and stems, the samples were thoroughly washed under running tap water. The samples were cut into small pieces and surface sterilized with 70% ethanol, soaked in 1% sodium hypochlorite for 3 min and rinsed in several changes of sterile distilled water. All the sterilized samples were placed onto water agar (WA) and PPA plates. The plates were incubated under standard incubation conditions (Salleh and Sulaiman, 1984) for 24 h. The resulting single-spored Fusarium colonies were transferred onto fresh potato dextrose agar (PDA) plates.

Identification of Fusarium species

Fusarium species were identified on the basis of macroscopic characteristics such as pigmentations and growth rates on PDA plates, as well as their microscopic features including size of macroconidia, presence of microconidia and its production in chains or false heads, type of conidiogenous cells (polyphialides and monophialides) and also absence or presence of chlamydospores (Leslie and Summerell, 2006). To study the growth rate and pigmentations, monoconidial Fusarium isolates were sub-cultured (5 mm disc) onto PDA plates and incubated at 25±1°C for 3 days. Ten replications were made for each strain. For microscopic observations, all the strains of Fusarium spp. were transferred (5 mm disc) onto carnation leaf agar (CLA) (Fisher et al., 1982) and potassium chloride agar plates (Fisher et al., 1983). Species delimitation was carried out based on species description of Gerlach and Nirenberg (1982) and Leslie and Summerell (2006).

RESULTS

Isolation, identification and morphological characters of *Fusarium* species

In this study, Fusarium strains were recovered in almost

all samples collected from different locations in Iran (Table 1). A total of 2,500 Fusarium isolates were obtained from soils, plant debris and diseased plant tissues. Among the samples, 41% of Fusarium isolates were obtained from diseased plant tissues, 36% from soils and 23% from plant debris. From diseased plant tissues, 48% of the isolates were from cereals (wheat, rice, corn and barley), 26% from cucurbit plants (watermelon, cantaloupe, cucumber, squash, and pumpkin), 14% from potato and 12% from other crops such as sorghum, tomato, sugar beet and beans. All isolates were identified into species level by using morphological characters. Based on the identification manuals, the strains were identified as F. avenaceum, F. acuminatum, F. aywerte, F. anthophilum, F. chlamydosporum, F. culmorum, F. crookwellense, F. dlamini, F. eumartii, F. equiseti, F. fujikuroi, F. graminearum, F. longipes, F. lateritium, F. nygamai, F. oxysporum, F. proliferatum, F. pseudograminearum, F. pseudonygamai, F. poae, F. F. solani. F. semitectum. sambucinum. sporotrichioides, F. subglutinans, F. scirpi, F. sacchari, F. trichothecioides, F. tricinctum and F. verticillioides (Table 2).

Distribution and occurrence of *Fusarium* species in different crops

Tissues from diseased cereal plants (Table 1) were colonized with different members of the Discolor section namely, F. graminearum (20%), F. sambucinum (9%), F. culmorum (8%), F. crookwellense (6%), pseudograminearum (6%), F. trichothecioides (3%) and Liseola section (F. proliferatum (14%), F. verticilloides (12%), F. fujikuroi (10%), F. subglutinans (7%)} and Sporotrichiella other sections such as chlamydosporum (1%), F. sporotrichioides (1%), F. tricinctum (1%), F. poae (1%)} and Roseum (F. avenaceum). Among the tissue samples, the most common species found in wheat tissues were F. crookwellense (100%), F. pseudograminearum (100%), followed by F. culmorum (90%), F. sambucinum (88%), F. graminearum (78%), F.chlamydosporum (75%), F. sporotrichioides (72%), F. avenaceum (68%), tricinctum, F. poae (64%), F. proliferatum (28%), F. nygamai (19%), F. verticillioides (18%), rice tissues with F. fujikuroi (83%), F. subglutinans (74%), F. verticillioides (48%), F. nygamai (46%) and F. proliferatum (43%). Distribution of Fusarium spp. in corn tissue was similar to that found in rice and in barley similar to that found in wheat. Tissues of diseased potatoes were colonized with different members of Fusarium species such as from the Martiella section (F. solani), Elegans section (F. oxysporum), Discolor section (F. sambucinum, F. culmorum, F. crookwellense and F. trichothecioides) and Liseola section (F. proliferatum, F. verticillioides, F. subglutinans and F. anthophilum).

Table 1. Distribution of *Fusarium* spp. in different host samples collected from different places in Iran.

Location of sample collection	Host	Tissue	Debris	Soil		
Azerbaijan	Wheat, Cucurbit	F.pr, F.gr, F.ch, F.ve, F.so, F.ox, F.sp	F.so, F.ox, F.se, F.eq, F.sa	F.pr, F.ve, F.so, F.ox, F.se, F.eq, F.ac, F.cu, F.trich, F.po		
Esfahan	Cucurbit, Potato	F.pr, F.gr, F.ch, F.av, F.ve, F.so, F.ox	F.se, F.eq, F.pr, F.ve, F.so, F.ox, F.av, F.ac	F.se, F.eq, F.pr, F.ve, F.so, F.ox, F.av, F.ac		
Fars	Wheat	F.po, F.gr, F.ch, F.ve, F.av, F.ac	F.so, F.ox, F.pro, F.ve, F.av, F.ac	F.se, F.eq, F.pro, F.ve, F.so, F.ox, F.av, F.ac		
Gilan	Rice, Wheat, Corn	F.pr, F.gr, F. ch, F.ve, F. ox, F.av, F.ac, F.cu, F. sp, F.fu, F. sac, F. su, F.ny	F.pro, F. ve, F.ox, F.av, F.ac, F. sa, F.cu, F.trici, F.po, F.trich, F. cr, F. F.an, F.fu, F. sac, F.su, F.ny	F.lo, F.sc, F. ay, F. la, F.dl, F. eu, F. pro, F.ve, F.se, F.eq, F.so, F.sa, F.cu, F.trici, F. po, F.trich, F.cr, F.an, F. F.fu, F.sac, F.su, F. ny		
Hamadan	Wheat, Potato, Barley, Corn, Tomato	F.pr, F.gr, F. ch, F.ve, F.so, F.ox, F.sp, F. psg, F.av, F.ac, F.sa, F. cu, F.trich, F. cr	F.pro, F. ve, F. so, F.ox, F.av, F. fu, F.ac, F. sa, F. cu, F.trici, F.po, F.sac, F.trich, F. cr, F.an	F.lo, F. ay, F.eu, F. eq, F.ac, F. se, F.psn		
Kermanshah	Rice, Wheat, Barley, Corn, Tomato	F.pr, F.gr, F. ch, F.ve, F.so, F.ox, F.av, F.trici, F. cr, F.ps, F. sp, F.an, F.fu, F. trich, F. psg, F.psn	F.pro, F.ve, F.so, F.ox, F. sa, F. cu, F.trich, F. po, F.trici, F.cr, F. an, F.fu, F. sac, F.su, F. ny	F.lo, F. sc, F.ay, F. la, F.dl, F.eu, F.pro, F.ve, F.se, F.eq, F. so, F.ox, F.av, F.ac, F.sa, F.cu, F.trich, F.po, F.trici, F.cr, F.an, F.fu, F. psn		
Khorasan	Wheat, Cucurbit	F.pr, F.gr, F.ch, F.ve, F.so, F.ox, F.ac, F.cr	F.se, F. eq, F. pro, F.ve, F.so, F.ox, F.av, F.ac	F.se, F. eq, F.pro, F. ve, F.so, F. ox, F. av, F.ac		
Khuzestan	Wheat, Corn	F.gr, F.ch, F.ve, F.so, F. ox, F.ac, F.cr	F.an, F. fu, F. sac, F.sc, F.su, F.ny, F.la	F.lo, F. ay, F.la, F.dl, F.eu, F.se, F.eq, F.an, F.fu, F.eu		
Lorestan	Wheat, Barley	F.pr, F.gr, F. ch, F.ve, F.av, F.sp, F.psg	F.se, F.eq, F. cr, F.trich, F.trici, F. cu, F.sa, F.ac	F.so, F.ox, F. se, F. eq, F.an, F. cr, F.trich, F.trici, F.cu, F.sa, F. F.ac, F.eu		
Mazandaran	Rice, Wheat, Corn	F.pr, F. gr, F.ch, F.ve, F.po, F. cr, F.psg, F.sp, F.an, F.fu, F. sac, F.su, F.ny	F.pro, F. ve, F. se, F.eq, F.so, F. ox, F.av, F.ac, F.sa, F.cu, F. po, F. an, F.fu, F. sac, F. ny	F.lo, F.sc, F.ay, F.la, F.dl, F.pro, F. ve, F.se, F.eq, F.so, F.sa, F.cu, F.po, F.cr, F.an, F. fu, F.sac, F. su, F. ny		

F.av=F. avenaceum, F.ac=F. acuminatum, F. ay= F. aywerte, F. an= F. anthophilum, F. ch= F. chlamydosporum, F. cu= F. culmorum, F. cr= F. crookwellense, F. dl= F. dlamini, F. eu= F. eumartii, F. eq= F. equiseti, F. fu= F. fujikuroi, F. gr= F. graminearum, F. lo= F. longipes, F. la= F. lateritium, F. ny= F. nygamai, F. ox= F. oxysporum, F. pr= F. proliferatum, F. ps= F. pseudograminearum, F. so= F. semitectum, F. sp= F. sporotrichioides, F. su= F. subglutinans, F. sc= F. scirpi, F. sac= F. sacchari, F. trich= F. trichothecioides, F. trici= F. tricinctum and F. ve= F. verticillioides

DISCUSSION

Occurrence of Fusarium species in sections

Discolor, Liseola, Martiella, Elegans and Gibbosum was observed in all the soil samples, debris and diseased plant tissues. In this survey,

the association was observed between the types of crop, the number and different species of *Fusarium* recovered from different fields. For

Table 2. Morphological characters of Fusarium spp. isolated from agricultural plants and soils in Iran

Name of the Species	Chlamydospores	Pigmentation on PDA	Number of septa	Microconidia	Types of conidigenious cells		General morphology		Macroconidia
					Poly	Mono	Apical cell	Basal cell	size (µm)
F. avenaceum	-	yellow	3-5	+	-	+	tapered to pointed	Nfs	42-70 × 3.0-4.0
F. anthophilum	-	violet	3-4	+	+	+	curved	Pdfs	31-66 × 2.7-4.5
F. aywerte	-	pale rose	3-9	-	-	+	hooked	Fs	44-133 × 3.8-5
F. acuminatum	-	red	3-5	-	-	+	tapered to pointed	Fs	35-65 × 3.2-5.4
F. crookwellense	+	red	5	-	-	+	tapered to pointed	Fs	31-71 × 4.0-6.8
F. culmorum	+	red	3-4	-	-	+	rounded	Notch	28-56 × 4.0-7.0
F. chlamydosporum	+	red	3-5	+	+	+	curved and pointed	Nfs	30-42 × 3.0-4.5
F. compactum	+	red	5	-	-	+	tapered, elongated	fs	28-68 × 3.0-6.3
F. dlamini	+	purple	3-5	+	-	+	curved	Fs	30-55 × 3.0-5.0
F. equiseti	+	brown	5-7	-	-	+	tapered, elongated	Fs	42-82 × 3.2-5.6
F. fujikuroi	-	violet	3-5	+	+	+	tapered	Pdfs	21-55 × 3.0-5.0
F. graminearum	+	red	5-7	-	-	+	tapered	Fs	39-70 × 4.0-6.5
F. lateritium	+	beige	4-7	+	-	+	hook or break	Nfs	38-75 × 3.6-6.0
F. longipes	+	red	5-7	-	-	+	tapered and whip-like	LFs	32-98 × 3.8-5.5
F. nygamai	+	violet	3-5	+	+	+	short and tapered	Nfs	25-56 × 2.1-5.0
F. oxysporum	+	violet	3	+	-	+	curved	Fs	34-58 × 3.0-5.8
F. proliferatum	-	violet	3-5	+	+	+	curved	Pdfs	24-60 × 3.0-5.0
F. poae	+	red	3-5	+	-	+	curved	Fs	35-48 × 3.0-5.4
F. pseudograminearum	+	red	3-9	-	-	+	curved	Fs	39-84 × 3.0-4
F. pseudonygamai	-	violet	3-5	+	+	+	capered	Pdfs	24-64 × 2.0-4.2
F. solani	+	cream	3-5	+	-	+	counded	Nfs	32-68 × 3.6-6.0
F. semitectum	+	brown	3-5	-	+	+	curved and tapered	Fs	37-58 × 3.0-5.0
F. sambucinum	+	red	3-5	-	-	+	pointed	Fs	32-56 × 3.0-5.1
F. sporotrichioides	+	red	3-5	+	+	+	curved and tapered	Nfs	24-55 × 3.0-5.5
F. scirpi	+	violet	6-7	+	+	+	tapered	Fs	40-76 × 3.9-6.0
F. subglutinans	-	dark purple	3	+	+	+	curved	Pdfs	26-68 × 3.0-5.0
F. sacchari	-	violet	3	+	+	+	curved	Pdfs	23-49 × 3.1-4.0
F. trichothecioides	+	red	3-5	-	-	+	tapered to pointed	Fs	35-53 × 3.2-5.4
F. tricinctum	+	red	3-5	+	-	+	curved	Fs	24-51 × 3.0-5.0
F. verticillioides	-	violet	3-5	+	-	+	curved	Nfs	32-61 × 2.4-4.3

^{+ =} presence, - = absence, Poly= polyphialidic, Mono= monophialidic, Pdfs= poorly developed foot shape, Nfs= Notch or foot shape, Fs= foot shape, Lfs= Long foot shape.

example, tissues from wheat and barley were colonized with different members of the Discolor and Liseola, whereas rice and corn were colonized with members of the Liseola section. These results were in agreement with previous reports of Nur Ain Izzati and Salleh (2009), Golzar (1994), Zamanizadeh and Frootan (1992), Zamanizadeh and Khorsandi (1995), Bujari and Ershad (1994), Nejatsalari and Ershad (1994), Naeimi et al. (2002). In rice and corn tissues, F. fujikuroi, F. subglutinans, F. verticillioides and F. proliferatum were predominant species. These particular species can be seed borne internally in symptomless, apparently healthy corn kernels (Nur Ain Izzati et al., 2009; Yli-Mattila, 2010). This particular species have potential to produce potent mycotoxins associated with serious animal and human diseases (Yli-Mattila, 2010).

In potato, *F. solani*, *F. oxysporum* and *F. sambucinum* were the predominant species These three species are plant pathogenic fungi, toxin producers and causing storage rots (Gerlach and Nirenberg, 1982). *F. proliferatum* and *F. solani* were frequently isolated from patients suffering from eye keratitis (inflammation of the cornea) (Nelson et al., 1994). The widespread occurrence of this *Fusarium* species indicates the potential for toxicological problems from agricul-tural crops such as wheat, barley, rice, corn and sorghum in Iran. Thus identification of the different species of *Fusarium*, including saprobe, patho-genic and toxigenic species is important.

In this study, F. scirpi, F. longipes and F. eumartii were only found in soils. The distribution of these species in this survey confirmed that part of the native soil colonization with Fusarium species in the soil niches (Sangalang et al., 1995). In this survey Fusarium spp. were associated with soils, debris, asymptomatic and symptomatic crop plants. In asymptomatic crop plants, this correlation makes it complicated to confirm if the fungus is primary disease causal agent, a second-dary pathogen, or an endophyte. The information on association is most important for management of the true causal agent of the disease. For example, several species of Fusarium have a long-term association with stalk rot of corn (Warn and Kommedahl, 1973; Kommedahl and Windels, 1977). From the data obtained in this survey, it is apparent that a study on Fusarium population is incomplete without studying the population on both healthy and diseased plants. This study demonstrates that Fusarium species are diverse in many agricultural crops in Iran. Fusarium species were recovered from wheat, barley, rice, corn, potato, cucurbit plants and other crops in the different regions of Iran. We believe that this study will serve as a foundation for further studies on Fusarium species particularly their mycotoxin profiles in cereals and other crops from Iran and to develop proper management strategies to control Fusarium diseases and reduce the risks of mycotoxin contamination.

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