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Fusarium species associated with root, crown, stem, and leaf sheath of rice in Iran

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Abstract: Rice is the most important food for a significant portion of the world's population, especially in Asia. The infection of rice plants with *Fusarium* species is a major global problem. For the morphological and phylogenetic identification of *Fusarium* species associated with rice, a widespread sampling was conducted from the root, crown, leaf sheath, and stem of the rice plants in various rice-cultivating areas across Fars, Golestan, Guilan, Isfahan, Khuzestan and Mazandaran provinces. Species-specific primers were used to identify three closely related species: *F. fujikuroi*, *F. proliferatum,* and *F. verticillioides*, which could not be distinguished based on morphological criteria alone. By combining morphological characteristics and sequence data of *TEF1-α* and *β-tubulin* genomic regions, nine species including *F. fujikuroi*, *F. verticillioides*, *F. proliferatum*, *F. globosum*, *F. andiyazi*, *F. anthophilum*, *F. incarnatum*, *F. culmorum* and *F. oxysporum* were identified among 242 isolates examined. This study reports the first occurrence of *Fusarium andiyazi* on rice in Iran. In addition to the known causal agents of bakanae disease, the pathogenicity test revealed that two additional species *F. culmorum* and *F. anthophilum* can also be considered agents of bakanae disease on rice.

Keywords: Bakanae disease, Non-pathogen, *Oryza sativa*, Pathogen, Phylogeny.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most extensively cultivated cereal crop in the world after wheat, constituting the staple diet of more than 50% of the global population in terms of cultivation and consumption (Makun et al. 2007, FAO 2012). It is grown on over 150 million hectares across more than one hundred countries, with over 90 percent of the world's rice produced and consumed in Asia (Faostat 2013, Hossain 2014).

According to FAO statistics released in 2013, Iran produced 2.4 million tons of rice, making it a significant producer within Asia (Faostat 2013). Mazandaran province leads in rice cultivation area with 37%, followed by Guilan at 28.2%. Khuzestan, Golestan, and Fars rank third to fifth, respectively. Collectively, these five provinces account for approximately 93.9% of Iran's total rice cultivation land. (Agricultural Statistics, Ministry of Agriculture Jihad 2015).

Rice is susceptible to damage from pathogens, pests, weeds, and various climatic factors, resulting in significant annual crop losses (Musanezhad 2002). Rice diseases are one of the main limiting factors affecting rice cultivation and production. Bakanae disease is a significant complication associated with rice crops. This disease was known to exist in Japan since 1828 (Ito & Kimura 1931), but was first described by Hori (1898). Bakanae disease is known to exist in almost all rice-growing areas of the world (Kazempour & Elahinia 2007). Initially, the pathogen responsible for the bakanae disease of rice was identified as *F. moniliforme* Sheldon (Snyder & Hansen 1945, Booth 1971, Nirenberg 1976). This pathogen was later reclassified as *Fusarium fujikuroi* Nirenberg, the anamorph of *Gibberella fujikuroi* Sawada (Nirenberg 1976). Recent findings have revealed conflicting results and suggested that other species of *Fusarium* in the section Liseola may also be involved in bakanae disease infection (Amoah et al. 1996; Desjardins et al. 2000, 2003).

The *F. fujikuroi* species complex (FFSC) represents an important component within the genus *Fusarium* and consists of more than 50 phylogenetically distinct species (O'Donnell et al. 2015). Among these members, *F. verticillioides*, *F. fujikuroi*, and *F. proliferatum* are well known for their potential to cause devastating cereal diseases, such as rice bakanae, maize ear rot, and soybean root rot, leading

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to considerable reductions in crop yields and economic income (Qui et al. 2020).

So far, *F. fujikuroi* and *F. proliferatum*, both from the *G. fujikuroi* species complex have been known as causal agents for Bakanae disease, and *F. verticillioides*, another species of this species complex has also been reported in some studies. Bakanae is one of the oldest diseases that infects rice in Asia. In Iran, it was first reported by Ebrahim Nesbat (1964) from Fooman region within Guilan province. Depending on the circumstances, the fungi may secrete fusaric acid (causing dwarfism) or gibberellins (causing elongation) (Sharifnabi 2016). While significant information exists on *Fusarium* species causing bakanae and foot rot of rice, the role of other *Fusarium* species in causing these and other diseases on rice remains largely unknown. From this point of view, it is important to know *Fusarium* species associated with rice plants.

Given that morphological traits can be unreliable due to sensitivity towards environmental changes and misinterpretations arising therefrom (Geiser et al. 2004), introducing molecular phylogenetic concepts greatly enhances our understanding through precise classifications utilizing current technological advancements, the taxonomy of *Fusarium* has entered a new phase with the advent of molecular phylogenetic approaches (Taylor et al. 2000).

In this study, after collecting samples from different rice-growing regions of Iran, *Fusarium* species associated with different parts of rice plants have been identified by morphological and molecular characterization. A pathogenicity test has been performed for each identified species to determine their role in causing disease and reducing crop yield. Therefore, this study aimed to identify *Fusarium* species associated with different organs of rice in different rice cultivating areas of Iran.

MATERIALS AND METHODS

Sampling and isolation of fungi

During the summer seasons of 2014 and 2015, extensive sampling was conducted from the root, crown, leaf sheath, and stem of rice plants exhibiting symptoms such as weakness or discoloration in the aerial organs, in rice-cultivating areas of Fars, Golestan, Guilan, Isfahan, Khuzestan and Mazandaran provinces. After surface disinfection in 5% sodium hypochlorite (NaOCl) solution, the samples were transferred to PPA, a selective medium for *Fusarium* species (Leslie & Summerell 2006), at 25 °C in darkness.

Morphological characterization

Morphological identification of *Fusarium* species was conducted using macroscopic criteria such as color, growth rate on PDA medium after incubation for 1-2 weeks at 25 °C in darkness, and microscopic criteria such as presence or absence, shape, and production (chains or false heads) of microconidia, along with the type of phialides after one week growing on SNA medium at 20-22 °C in darkness. The size and shape of macroconidia and the presence of sporodochium were considered on CLA medium at 20 °C under fluorescent light with a 12-hour photoperiod after 10-14 days (Booth 1971, Nelson 1983, Leslie & Summerell 2006).

DNA extraction and PCR amplification

The isolates were cultured on potato dextrose agar (PDA) for 7 to 10 days at 25 °C in darkness. Total DNA was extracted from fungal mycelia using Zhong & Steffenson (2001) method. The *TEF1-α* and *β-tubulin* regions were amplified using primers EF1T/EF2T (5′-ATGGGT AAGGAGGACAAGAC/ GGAAGTACCAGTGATCATGTT-3′) and T1/T2 (5′-AACATGCGTGAGATTGTAAGT/TAGTGACC CTTGGCCCAGTTG-3′), respectively (Geiser et al. 2004, O'Donnell & Cigelnik 1998). A 25-µl polymerase chain reaction (PCR) mixture was prepared, which contained 12.5 µl Master Mix Red (CinnaGen, Iran), 1.5 mM $MgCl_2$, 0.5 µl of each primer (10 pmol/µL) and 10-20 ng of DNA extract. The PCR conditions for *TEF1-α* were initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 50 s, annealing at 56 °C for 50 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 7 min. For *β-tubulin*, the PCR conditions were initial denaturation at 95 °C for 3 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 45 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 10 min.

In order to confirm morphological identification of *Fusarium* isolates, species-specific primers were used for identification of three species *F. fujikuroi* (Fuji1F TEF/TEF1R:5′-ACGTGTCAAACTAAACATTCG A-3′/ 5′-GCGACAACATACCAATGACG-3′), *F. proliferatum* (Proli1F TEF/ TEF1R: (5′-GT CACGTGTCAAGCAGCGA-3′/5′-GCGACAAC AT ACCAATGACG-3′) and *F. verticilioides* (VER1/ VER2:5′-CTTCCTGCGATGTTTCTCC-3′/5′-AATT GGCCATTGGTATTATATATCTA-3′) (Amatulli et al. 2012; Mule` et al. 2004). A 12.5-µl polymerase chain reaction (PCR) mixture was prepared, which contained 6.25 µl Master Mix Red (CinnaGen, Iran), 1.5 mM MgCl2, 0.25 µl of each primer and 10 ng of DNA extract.

In this study, species-specific primers were used to identify three species *F. fujikuroi*, *F. proliferatum* and *F. verticillioides*, which could not be separated in terms of morphological features. For *F. fujikuroi* and

F. proliferatum species-specific primers, the PCR conditions were initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at 67 °C and 69 °C for 40 s, and extension at 72 °C for 40 s; and final extension at 72 °C for 5 min. For *F. verticillioides* species-specific primers, the PCR conditions were initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94 °C for 50 s, annealing at 65 °C for 50 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 7 min.

DNA sequencing and phylogenetic analysis

The PCR products were purified using the GeneAll Purification Kit (combo GP, 50 p). Purified PCR products were sequenced in the forward direction by Macrogen Company (South Korea). After receiving the files for sequenced DNA fragments, chromatograms of the sequence were edited by using softwares ChromasPro Version 1.7.6 (https://technelysium.com.au/wp/chromaspro/) and Editseq version 5.01 f the DNAStar package (DNASTAR. Madison, WI., https://www.dnastar.com/software). The Basic Local Alignment Search Tool (BLAST) was utilized to compare the similarities of nucleotide sequences with GenBank database seeds were leached multiple times and soaked in distilled water for 24 hours. Then a drop of dishwashing liquid was added to a Falcon 50 ml tube containing distilled water and rice seeds were placed in it for 15-10 minutes. Seeds were washed and transferred to 50% alcohol. Seeds were washed again and immersed in a 1% sodium hypochlorite solution for 15-10 minutes and washed, then placed on wet filter paper (humid chamber) to germinate. Germinated seeds were planted under greenhouse conditions. Pathogenicity tests were performed by injecting a suspension containing 10^6 spores per ml from seven to 10-day-old cultures of the target fungus on a PDA medium, onto the crowns of rice seedlings. The symptoms were evaluated after 15 days.

RESULTS

Morphological identification

A total of 285 infected plant samples were collected including 102 from Mazandaran, 43 from Guilan, 52 from Golestan, and 27, 38, 23 from Esfahan, Fars and Khuzestan provinces, respectively. In total, 242 *Fusarium* isolates were obtained, which included 83, 30, 47, 36, 19 and 27 isolates from Mazandaran, Guilan, Golestan, Fars, Khuzestan and Esfahan, respectively. Among these isolates, 86 isolates were collected from the stem, as well as 62, 51 and 43 isolates from the sheath, crown, and root, respectively. The morphologically identified *Fusarium* species included *Fusarium fujikuroi*, *F. verticillioides*, *F. oxysporum*, *F. incarnatum*, *F. culmorum*, *F. globosum*, *F. anthophilum* and *F. andiyazi*.

Fusarium fujikuroi was the predominant species (with a prevalence of 43%) for the Mazandaran collection, while for Khuzestan, *F. incarnatum* was the most common species with a prevalence of 57%. To confirm these morphological identifications, representative isolates of each morphologically identified species were selected for molecular characterization.

Species identification using species-specific primers

PCR was successfully performed using the Fuji1F TEF, Proli1F TEF and VER primer sets designed to differentiate between *F. fujikuroi*, *F. proliferatum* and *F. verticillioides*. Of the 172 isolates evaluated, 160 isolates were positive for *F. fujikuroi* and an amplicon fragment about 179 bp was produced by the Fuji1F TEF/ TEF1R primers. PCR with the Proli1F TEF/ TEF1R primers resulted in fragments about 188 bp for two isolates and confirmed the presence of *F. proliferatum*. Seven isolates were positive for *F. verticillioides* when the VER1 / VER2 primers were used and an amplicon about 578 bp was produced in reaction. However, three of the

(http://www.ncbi.nlm.nih.gov/blast) and FUSARIUM ID. Sequences generated from materials in this study and retrieved from GenBank were aligned using the MEGA7 software (Kumar et al. 2016) and multiple alignments option (Kumar et al. 2016). *Microdochium nivale* was selected as a suitable outgroup. Maximum Likelihood method, based on the Tamura-Nei model, was estimated using Nearest-Neighbor-Interchange (NNI) and employing the default pattern settings in MEGA7 software (Kumar et al. 2016). To assess the relative stability of the branches, bootstrap analysis was performed with 1,000 replications. The sequences used in this analysis were deposited in GenBank.

Pathogenicity tests

To confirm the pathogenicity of species isolated from the roots, crowns, stems and leaf sheath of rice, pathogenicity tests were conducted on Binam (a traditional tolerant cultivar) and Khazar cultivar (an improved susceptible cultivar) (Padasht 1993). The experiments took place in the greenhouse at Sari University of Agriculture and Natural Resources. Rice seeds were provided by the Rice Research Institute of Iran, Deputy of Mazandaran, Amol, Iran. For seed disinfection and seedling production, rice

172 isolates presumed to belong to the three mentioned species failed to amplify with any of the primers used, and therefore, their identification remained ambiguous until a PCR was conducted with universal primers that amplify *TEF1-α* and *β-tubulin* gene regions.

Phylogenetic analysis

PCR amplification of the *TEF1-α* region for 13 selected isolates, representatives of morphologically identified species generated fragments ranging from 407 to 646 bp, and alignment of the sequences resulted in a dataset of 557 characters. The *β-tubulin* (*TUB2*) gene generated fragments ranging from 451 to 595 bp, and comprised 448 characters after alignment. Phylogenetic trees constructed from single gene analyses and combined datasets of partial *TEF1-α* and β-tubulin exhibited essentially similar topologies and species groupings (the phylogram based on the combined dataset is shown) (Fig. 1). The phylogram analysis revealed that all 13 isolates were clustered into three main clades (I, II and III) and five subclades labeled A to E, alongside sequences obtained from GenBank (Table. 1). Subclades A and C include isolates from the *Fusarium fujikuroi* complex. The species *Fusarium fujikuroi*, *F. globosum*, *F. proliferatum*, *F. anthophilum* and *F. verticillioides* are clustered into four distinct clades with high bootstrap support (>85%). Subclade B contained isolates of the *F. oxysporum* species and is distinct from other species with a support value of 85%. Subclade D effectively separated the *F. culmorum* species with strong support (99%). Lastly, subclade E was associated with isolates from the *F. incarnatum* complex. These associations also received high bootstrap support (95%) and were positioned alongside isolates obtained from GenBank.

Taxonomy

Based on data produced in this study, nine species were identified namely *F. andiyazi*, *F. anthophilum*, *F. culmorum*, *F. fujikuroi*, *F. globosum*, *F. incarnatum*, *F. oxysporum*, *F. proliferatum*, and *F. verticillioides*. *Fusarium andiyazi* has previously been reported from maize and sugarcane (Chehri et al. 2010, Heidarian et al. 2013); However, this is the first time it has been documented on rice in Iran and described and illustrated here.

Table. 1. List of *Fusarium* strains used for phylogenetic analysis in this study.

Species	Collection No.	Origin	Accession No. *TEF1-α*	Accession No. *β-tubulin*
Fusarium fujikuroi	NRRL13566	*Oryza sativa* (Taiwan)	AF160279.1	U34415.1
Fusarium glubosum	CBS:428.97	Zea mays seed (South Africa)	MT011022.1	MN534124.1
Fusarium proliferatum	PT5	Grape (China)	KP313249.1	KP313250.1
Fusarium anthophilum	NRRL13602	Yellow-eyed grass (South America)	AF160292.1	U61541.1
Fusarium oxysporum	FJ-2-1	Banana (China)	KC889014.1	KC869323.1
Fusarium verticillioides	NRRL22172	Mango (Mexico)	AF160262.1	U34413.1
Fusarium andiyazi	PB07	Sugarcane (India)	PQ421509.1	PQ421504.1
Fusarium andiyazi	CBS 119856	Sorghum grain (Ethiopia)	MN533989.1	KU603868.1
Fusarium culmorum	FRC R-09618	Hordeum (Denmark)	GQ915506.1	GQ915440.1
Fusarium incarnatum	DE15	Mangrove (Malaysia)	KF993967.1	KJ020862.1
Microdochium nivale	10106	*Triticum* sp. (Italy)	JX280540.1	JX280575.1
Fusarium glubosum	UTFC-F7	*Oryza sativa* (Iran)	PQ849660	PQ849673
Fusarium oxysporum	UTFC-F19	*Oryza sativa* (Iran)	PQ849661	PQ849674
Fusarium verticillioides	UTFC-F42	*Oryza sativa* (Iran)	PQ849662	PQ849675
Fusarium anthophilum	UTFC-F111	*Oryza sativa* (Iran)	PQ849663	PQ849676
Fusarium andiyazi	UTFC-F114	*Oryza sativa* (Iran)	PQ849664	PQ849677
Fusarium anthophilum	UTFC-F120	*Oryza sativa* (Iran)	PQ849665	PQ849678
Fusarium proliferatum	UTFC-F136	*Oryza sativa* (Iran)	PQ849666	PQ849679
Fusarium culmorum	UTFC-F205	*Oryza sativa* (Iran)	PQ849667	PQ849680
Fusarium fujikuroi	UTFC-F236	*Oryza sativa* (Iran)	PQ849668	PQ849681
Fusarium incarnatum	UTFC-F270	*Oryza sativa* (Iran)	PQ849669	PQ849682
Fusarium incarnatum	UTFC-F159	*Oryza sativa* (Iran)	PQ849670	PQ849683
Fusarium incarnatum	UTFC-F116	*Oryza sativa* (Iran)	PQ849671	PQ849684
Fusarium incarnatum	UTFC-F103	*Oryza sativa* (Iran)	PQ84967	PQ849685

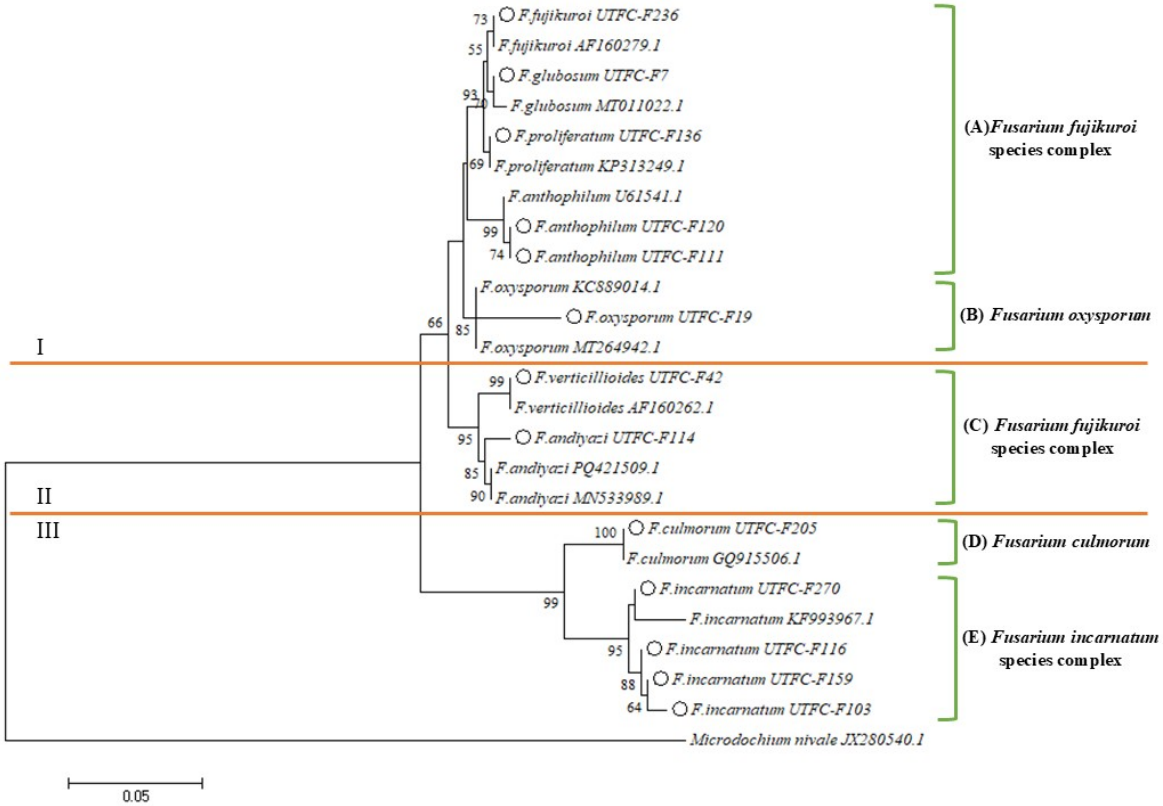

Fig.1. Maximum Likelihood phylogenetic tree inferred from a combined analysis of *TEF1-α* and *β-tubulin* sequence data, showing phylogenetic relationships of *Fusarium* species isolated from rice and selected sequences of *Fusarium* species. The numbers above the branches represent branch support using 1000 bootstrap replications. *Microdochium nivale* isolate 10106 is used as an outgroup.

***Fusarium andiyazi* Marasas, Rheeder, Lampr., K.A. Zeller & J.F. Leslie, Mycologia 93: 1205 (2001). Fig. 2**

Colonies on PDA exhibit floccose to powdery mycelium, that are initially white but may become violet with age. Colony diameter on PDA was approximately 6.3–7 cm after seven days at 25 °C and darkness. Orange sporodochia on CLA usually begin to form after 20 days. Macroconidia are hyaline and straight to slightly curved. The apical cell is slightly curved and the basal cell is pedicellate. Macroconidia typically have three septate, and also six septate macroconidia were rarely observed in some strains. Macroconidia were measured 23- 58×3- 3.5 µm. Microconidia are clavate to ovoid with a flattened base. Usually 0-septate. Microconidia were measured 5-15×1.5-3 µm. The conidiogenous cells are elongated and take the form of stretched monophialides. Aerial mycelium forms false heads, long chains that often collapse to short chains as the colony ages. Chlamydospores or sclerotia were not observed. *Fusarium andiyazi* is very similar to *F. verticillioides*. The difference between the two species is that *F. verticillioides*, unlike *F. andiyazi*, does not produce pseudo-chlamydospores (Fig. 2).

Specimen examined: Iran, Guilan Prov., Astane City, on stem of *Oryza sativa* L., Aug. 2015, H. Vardasbi, (UTFC-F114).

Pathogenicity

The symptoms were evaluated on rice seedlings after 15 days of inoculation. The severity of the symptoms was assessed by measuring the length of spots on the seedlings. Validation of pathogenicity for all species was successfully conducted following Koch's postulates. According to the results, the length of spots caused by three species *F. globosum*, *F. andiyazi,* and *F. incarnatum* was smaller than that caused by *F. fujikuroi*, *F. proliferatum*, *F. verticillioides*, *F. anthophilum* and *F. culmorum*. Additionally, spots caused by *F. oxysporum* were less than that observed in the aforementioned three species. Among the tested species, *F. proliferatum*, *F. anthophilum* and *F. culmorum* caused the most severe symptoms characterized by large and elongated spots on both rice cultivars (Khazar and Binam). In contrast, the remaining species produced only small spots on the tolerant Binam cultivar and did not cause significant symptoms on either cultivar (Fig. 3).

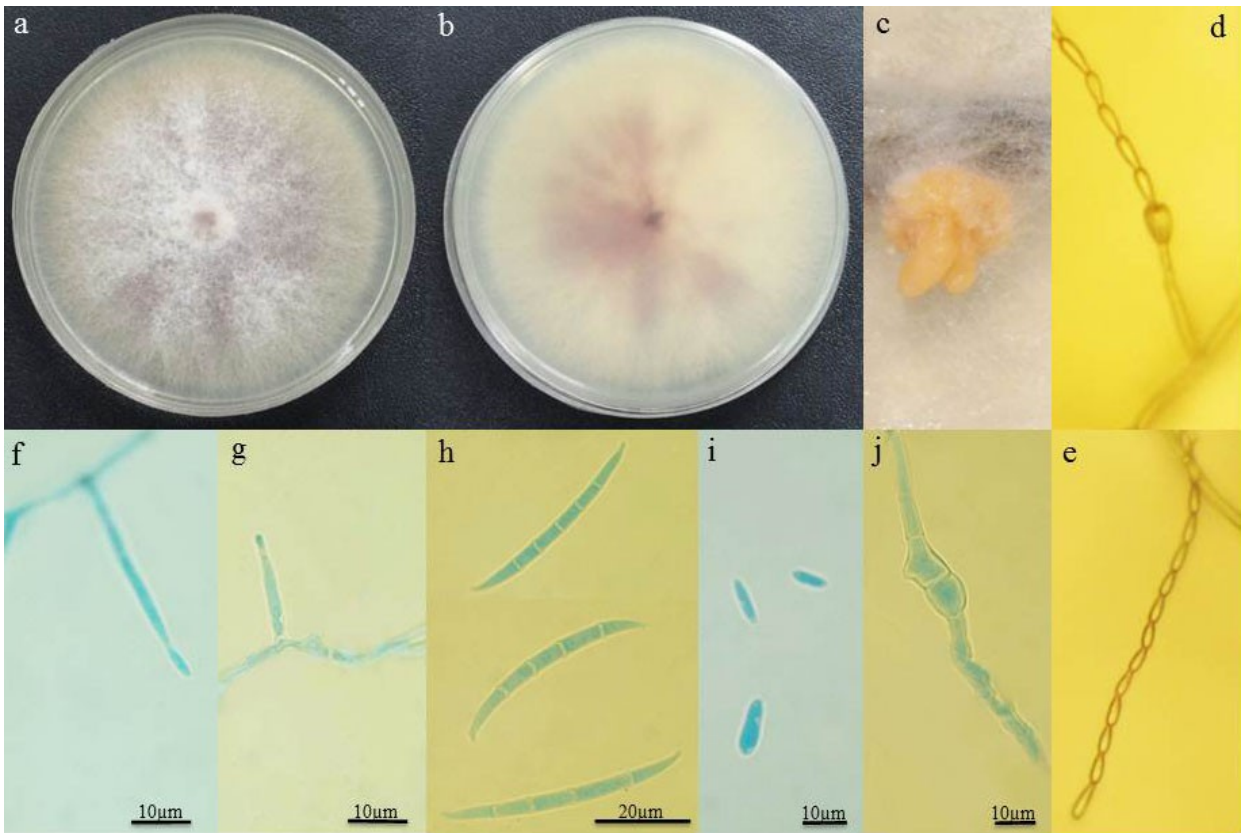

Fig. 2. *Fusarium andiyazi*. a. Upper and b. Reverse of colony on PDA; c. sporodochium; d, e. false head and chains; f, g. Monophialide; h. Macroconidia; i. Micriconidia and j. pseudoclamydospore. — Scale bars = 10 µm.

Phylogenetic classification using the *TEF1-α* and *β-tubulin* genes revealed that the *TEF1-α* gene sequence was most effective for species identification and successfully distinguished members of the *F. fujikuroi* species complex from other species with higher bootstrap support value.

In this study, the isolates of *Fusarium andiyazi* were positioned within the *Fusarium fujikuroi* species complex clade, showing a close relation with an isolate retrieved from GenBank. However, Kvas et al. (2009) indicated an uncertain phylogenetic position for *F. andiyazi* within this complex. Furthermore, research focused on Fusarium species linked to bakanae disease concluded that *F. andiyazi* is separated from closely related species such as *F. verticillioides* only by morphological characteristics. So, in their study, they separated these two species based on the type of mycotoxin that was produced by each species. (Wulff et al. 2010).

In this study, there was no clear taxonomic position for isolates of *F. globosum* in the phylogenetic tree and these isolates were ambiguously placed near the isolates of *F. proliferatum*. According to Kvas et al. (2009), *F. proliferatum* and *F. globosum* are morphologically similar. Phylogenetic analysis further supported this observation, placing these two species in a well-supported subclade.

Morphological identification and morphometric studies have shown that because of the high similarity of the morphological traits of *Fusarium* spp., separating members of this genus is not possible by using only morphological traits. The information obtained from morphological identification must be accompanied by molecular biology techniques. These results correspond with the findings of Aoki & O'Donnell (1999) and Taylor et al. (2000).

Pathogenicity tests revealed that *F. anthophilum* and *F. culmorum* can also cause bakanae disease on rice. In addition to previously identified causal agents, *F. fujikuroi*, *F. proliferatum* and *F. verticillioides*, two species *F. culmorum* and *F. anthophilum* also caused severe symptoms in Binam cultivar. Our findings regarding the pathogenicity of *F. anthophilum* are align with previous research by Hossain (2014), but this study introduces *F. culmorum*, for the first time as a cause of the bakanae disease.

Fig. 3. Symptoms of bakanae disease on rice, 15 days after inoculation in the greenhouse, a, b. Spots created by *Fusarium culmorum* on Khazar and Binam cultivars, respectively. c, d. Spots created by *F. anthophilum* on Khazar and Binam cultivars, respectively. e, f. Spots created by *F. fujikuroi* on Khazar and Binam cultivars, respectively. g, h. Spots created by *F. proliferatum* on Khazar and Binam cultivars, respectively.

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REFERENCES

Amatulli, M.T., Spadaro, D., Gullino, M.L. and Garibaldi, A. 2012. Conventional and real-time PCR for the identification of *Fusarium fujikuroi* and *Fusarium proliferatum* from diseased rice tissues and seeds. European Journal of Plant Pathology 134: 401-408.

Amoah, K., Macdonald, M.V., Rezanoor, N. and Nicholson, P. 1996. The use of random amplified polymorphic DNA technique to identify mating groups in the *Fusarium* section Liseola. Plant Pathology 45: 115-125.

Aoki, T. and O'Donnell, K. 1999. Morphological and molecular characterization of *Fusarium pseudograminearum* sp. nov., formerly recognized as the Group 1 population of *F. graminearum*. Mycologia 91: 597-609.

Booth, C. 1971. The genus *Fusarium* Commonwealth Mycological Institute, Kew Survey. England.

Chehri, K., Darvishnia, M. and Zafari, D. 2010. Six new *Fusarium* species isolated from maize in Iran. Rostaniha 11(1): 69-81.

Desjardins, A., Manandhar, H., Plattner, R., Manandhar, G., Poling, S. and Maragos, C. 2000. *Fusarium* species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. Applied and Environmental Microbiology 66: 1020-1025.

Desjardins, A.E. 2003. *Gibberella* from A (venaceae) to Z (eae) 1 2. Annual Review of Phytopathology 41: 177-198.

Ebrahim Nesbat, F. 1964. Rice foot rot. Journal of Applied Entomology and Phytopathology 1(1): 11.

FAO [Food and Agriculture Organization of the United Nations]. 2012. Rice market monitor. Trade and Markets Division 15: 33-35.

FAOSTAT, 2013. Food and Agriculture Organization of the United Nations. FAO

Statistical Databases. http://www.faostat.fao.org.

Geiser, D.M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T.J. and O'Donnell, K. 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying fusarium molecular diversity and PCR-detection of toxigenic *Fusarium* species and ochratoxigenic fungi. European Journal of Plant Pathology 110: 473–479.

Heidarian, Z., Javan-Nikkhah, M., Fotouhifar, K.B. and Ahmadpour, A. 2013. Morphological and phylogenetic investigation on selected *Fusarium* species belong to *Gibberella fujikuroi* species complex in Iran. Rostaniha 14(2): 163-174.

Hori, S. 1898. Some observations on bakanae disease of the rice plant. Mem Agric Res Sta (Tokyo) 12:110–119.

Hossain, M.T. 2014. Studies of the impact of mycoflora associated with *Oryza sativa* (rice) in South Africa. PhD thesis, University of South Africa, Pretoria.

Ito, S. and Kimura, J. 1931. Studies on the 'bakanae' disease of the rice plant. Report of the Hokkaido National Agricultural Experiment Station 27:1–95.

Kazempour, M.N. and Elahinia, S.A. 2007. Biological Control of *Fusarium fujikuroi* the causal agent of bakanae disease by rice-associated antagonistic bacteria. Bulgarian Journal of Agricultural Science 13:393-408.

Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870-1874.

Kvas, M., Marasas, W.F.O., Wingfield, B. D., Wingfield, M.J. and Steenkamp, E.T. 2009. Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex. Fungal Diversity 34: 1-21.

Leslie, J. and Summerell, B. 2006. The *Fusarium* laboratory manual. Blackwell Publishing Professional. Ames, IA, USA.

Makun, H.A., Gbodi, T.A., Akanya, O.H., Ezekiel, A.S. and Ogbadu, G.H. 2007. Fungi and some mycotoxins contaminating rice (*Oryza sativa*) in Niger State, Nigeria. African Journal of Biotechnology 6: 99-108.

Mulè, G., Susca, A., Stea, G. and Moretti, A. 2004. A species-specific PCR assay based on the calmodulin partial gene for identification of *Fusarium verticillioides*, *F. proliferatum* and *F. subglutinans*. European Journal of Plant Pathology 110: 495-502.

Musanezhad, S. 2002. Determination of vegetative compatibility groups and mating type of rice blast fungus, *Magnaporthe grisea* in Guilan province. Thesis for Master's Degree, Faculty of Agriculture, Tarbiat Modarres University, Tehran, Iran.

Nelson, P.T. and Marasas, W.F.O. 1983. *Fusarium* species: an illustrated manual for identification: The Pennsylvania State University.

Nirenberg, H. 1976. Untersuchungen uber die morphologische und biologische Differenzierung in der *Fusarium*-Sektion Liseola. Biologischen Bundesanstalt für Land- und Forstwirtschaft 169: 1-117.

O'Donnell, K., Kistler, H.C., Cigelnik, E. and Ploetz, R.C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences, 95: 2044-2049.

Padasht Dehkaei, F. 1993. Evaluation of rice foot rot disease (*Gibberella fujikuroi*) in Guilan. Thesis for Master's Degree, College of Agriculture, University of Tehran, Tehran.

Qiu, J.Y., He, D., Lee, Y.W., Fang, J.F., Xu, J. and Shi, J. 2020. *Fusarium fujikuroi* species complex associated with rice, maize, and soybean from Jiangsu province, China: phylogenetic, pathogenic, and toxigenic analysis. Plant Disease 104: 2193-2201. DOI: org/10.1094/PDIS-09-19-1909-RE.

Sharifnabi, B. 2016. Diseases of crop plants in Iran. Isfahan University Industry, Isfaham, Itan.

Snyder, WC. and Hansen, H. 1945. The species concept in *Fusarium* with reference to discolor and other sections. American Journal of Botany 32: 657-666.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S. and Fisher, M.C. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal genetics and biology 31: 21-32.

Wulff, E.G., Sørensen, J.L., Lübeck, M., Nielsen, K.F., Thrane, U. and Torp, J. 2010. *Fusarium* spp. associated with rice bakanae: ecology, genetic diversity, pathogenicity and toxigenicity. Environmental Microbiology 12: 649-657.

Zhong, S. and Steffenson, B.J. 2001. Virulence and molecular diversity in *Cochliobolus sativus*. Phytopathology, 91: 469-476.

گونه‌های *Fusarium* همراه ریشه، طوقه، ساقه و غلاف برگ برنج در ایران

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چکیده: برنج مهم‌ترین غذا برای بخش قابل توجهی از جمعیت جهان به ویژه در آسیا است. آلودگی به گونه‌های *Fusarium* یکی از مشکلات عمده‌ای است که گیاه برنج در سطح جهانی با آن مواجه است. برای شناسایی ریخت‌شناختی و تبارزایی گونه‌های فوزاریوم همراه برنج، نمونه‌برداری گسترده‌ای از ریشه، طوقه، غلاف برگ و ساقه گیاه برنج در مناطق مختلف کشت برنج در استان‌های فارس، گلستان، گیلان، اصفهان، خوزستان و مازندران انجام شد. آغازگرهای مخصوص گونه برای شناسایی سه گونه نزدیک به هم شامل *F. fujikuroi*، *F. proliferatum* و *F. verticillioides* مورد استفاده قرار گرفت که تنها بر اساس معیارهای ریخت‌شناسی قابل تمایز نیستند. با ترکیب ویژگی‌های ریخت‌شناختی و داده‌های توالی نوکلئوتیدی نواحی ژنی *TEF1-α* و بتاتوبولین، نه گونه شامل *F. fujikuroi*، *F. verticillioides*، *F. proliferatum*، *F. globosum*، *F. andiyazi*، *F. anthophilum*، *F. incarnatum*، *F. culmorum* و *F. oxysporum* در بین ۲۴۲ جدایه شناسایی شدند. در این مطالعه *F. andiyazi* اولین بار روی برنج از ایران گزارش می‌شود. علاوه بر عوامل شناخته شده پوسیدگی طوقه برنج، آزمایش بیماریزایی نشان داد که دو گونه *F. culmorum* و *F. anthophilum* نیز می‌توانند از عوامل این بیماری روی برنج در نظر گرفته شوند.

کلمات کلیدی: پوسیدگی طوقه برنج، غیربیماری‌زا، بیمارگر، تبارشناسی، *Oryza sativa*.

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