Molecular identification of *Fusarium oxysprum* species complex isolated from cotton in Iran

***Khosrow Chehri[1] and Mozhgan Fatahi Dehpahni[2]**

[1]Department of Biology, Faculty of Sciences, Razi University, Kermanshah, Iran
[2]Department of Anatomy School of Medicine, Tehran University of Medical Sciences, Iran
**Corresponding author's email: khchehri@gmail.com*

Abstract

Molecular analysis and experimental inoculations showed *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) and *F. redolens* as the causal agents of wilting of cotton in Iran. Twenty-seven *Fusarium* isolates were obtained from necrotic and discolored root and crown tissues of cotton in Iran. Morphological features and molecular analysis with species-specific primers showed that 20 isolates belonged to FOV race 3 and *F. redolens*. Phylogenetical analysis based on translation elongation factor-1α (tef1) sequences dataset clearly classified 7 Iranian FOV genotypes in race 4, 7 or 8 group. In addition, molecular study and pathogenicity tests showed that *F. redolens*, like FOV race 3 genotype, also caused discolorations on the root and crown of cotton.
Keywords: Cotton, Fusarium species complex, Iran, Wilt.

Introduction

Cotton with the scientific name of *Gossypium* is an annual and heat-loving plant. Due to the high economic importance of cotton, this plant is known as white gold and is cultivated in more than 70 countries. Therefore, identifying the pathogens of this plant can be of great help in improving the quantity and quality of this precious product (Sanei *et al*., 2013). *Verticillium* spp. and *Fusarium* spp. are usual pathogens of cotton worldwide (Skovgaard *et al*., 2001; Sanei *et al.*, 2013; Dongzhen *et al*., 2020). Fusarium wilt has been recorded as parasitizing many economically important agricultural crops including cotton (Dongzhen *et al.*, 2020). Fusarium wilt has been reported on different crops such as watermelons, tomato, peas, crucifers, bananas, and cotton in many international production regions and causes annual damages worth billions of dollars (Dita *et al.*, 2018). There are eight races of *F. oxysporum* f. sp. *vasinfectum* throughout the world (Diaz *et al.*, 2021). Based on pathogenicity assays races 1 and 2 were first reported in the USA and Tanzania, race 3 in Egypt and China, race 4 in India, race 5 in Sudan, race 6 in Paraguay and Brazil, and races 7 and 8 in China (Armstrong and Armstrong, 1978; Chen *et al.*, 1985).

Vascular wilt is a limiting factor in cotton cultivation in many cotton growing regions (Zhang *et al*., 2016). *F. oxysporum* Schlect. f. sp. *vasinfectum* was first described as the causal agent of cotton wilting (Chen *et al.*, 1985). The disease agent caused the growth of infected cotton plants to decrease. Fusarium disease symptoms are similar to root knot nematode disease (Garber and Paxman, 1963). Skovgaard *et al*. (2001) demonstrated the existence of eight races of FOV on the basis of phylogenetical analysis and pathogenicity tests. Previous studies showed that this disease was reported from Egypt and China in Asia (Fahmy, 1927; Chen *et al.*, 1985). However, no effort has been made to identify *F. oxysporum* species complex (FOSC) isolated from cotton in Iran. Therefore, the objective of our research was to perform molecular identification of *Fusarium* spp. isolated from cotton wilt and assess the genetic differentiation of FOV isolates in Iran.

Materials and Methods

Cotton with the scientific name of *Gossypium* is an annual and heat-loving plant. Due to the high economic importance of cotton, this plant is known as white gold and is cultivated in more than 70 countries. Therefore, identifying the pathogens of this plant can be of great help in improving the quantity and quality of this precious product (Sanei *et al*., 2013). *Verticillium* spp. and *Fusarium* spp. are usual pathogens of cotton worldwide (Skovgaard *et al*., 2001; Sanei *et al.*, 2013; Dongzhen *et al*., 2020). Fusarium wilt has been recorded as parasitizing many economically important agricultural crops including cotton (Dongzhen *et al.*, 2020). Fusarium wilt has been reported on different crops such as watermelons, tomato, peas, crucifers, bananas, and cotton in many international production regions and causes annual damages worth billions of dollars (Dita *et al.*, 2018). There are eight races of *F. oxysporum* f. sp. *vasinfectum* throughout the world (Diaz *et al.*, 2021). Based on pathogenicity assays races 1 and 2 were first reported in the USA and Tanzania, race 3 in Egypt and China, race 4 in India, race 5 in Sudan, race 6 in Paraguay and Brazil, and races 7 and 8 in China (Armstrong and Armstrong, 1978; Chen *et al.*, 1985).

Vascular wilt is a limiting factor in cotton cultivation in many cotton growing regions (Zhang *et al*., 2016). *F. oxysporum* Schlect. f. sp.

vasinfectum was first described as the causal agent of cotton wilting (Chen *et al.*, 1985). The disease agent caused the growth of infected cotton plants to decrease. Fusarium disease symptoms are similar to root knot nematode disease (Garber and Paxman, 1963). Skovgaard *et al*. (2001) demonstrated the existence of eight races of FOV on the basis of phylogenetical analysis and pathogenicity tests. Previous studies showed that this disease was reported from Egypt and China in Asia (Fahmy, 1927; Chen *et al.*, 1985). However, no effort has been made to identify *F. oxysporum* species complex (FOSC) isolated from cotton in Iran. Therefore, the objectives of our research were to perform molecular identification of *Fusarium* spp. isolated from wilted cotton plants and to assess the genetic differentiation of FOV isolates in Iran.

Results and Discussion

Sixty-seven isolates belong to *Verticillium* spp. (40 isolates) and *Fusarium* spp. (27 isolates) were obtained by direct culturing of infected cotton roots and stems. Morphological features showed that a large number of fungal isolates belong to FOSC (21 isolates) and *F. redolens* (6 isolates) (Leslie and Summerell, 2008). In this research, *Verticillium* spp., *F. redolens* and *F. oxysporum* f. sp. *vasinfectum* were frequently isolated from cotton-growing areas of Iran (Table 1). In previous studies, Verticillium wilt was reported as the major disease affecting cotton in Iran and our results in this research are consistent with the results obtained by previous researchers (Hamdollah-Zadeh, 1993; Sanei *et al*., 2013).

Isolates of *F. redolens* and FOV race 3 were distinguished molecularly using Redolens-F/Redolens-R and FOV-BT-AS-R3/BT5 primers, respectively. Use of molecular techniques for identification of fungal pathogen is considered more reliable strategy than identification on mere morphological characteristics (Khan *et al.*, 2021; Khan and Javaid, 2023). The specific primers of Redolens-F/Redolens-R, produced fragments of 386 bp in all *F. redolens* isolates (Fig. 1). Also, the specific primers of FOV-BT-AS-R3/BT5 produced fragments of 321 bp only in 14 FOSC isolates (Fig. 2). No successful amplification was observed among 7 isolates included in Table 1. Also, partial fragment of tef1 gene (650 bp), from 7 isolates that were not identified based on specific primers, were amplified and identified as FOV race 4. GenBank numbers of the selected strains are presented in Table 1. There are many reports of highly pathogenic strains of FOV isolated from cotton in previous studies worldwide (Fernandez *et al.*, 1994; Skovgaard *et al.*, 2001), However, so far, no research has been done to identify FOSC members related to cotton wilt in Iran, and the results obtained in this research were consistent with the results obtained by previous studies. FOV and *F. redolens* are reported for the first time as the causal agent of crown and root rot of cotton from different regions of Iran.

All the FOSC and *F. redolens* isolates were evaluated for their pathogenicity on the healthy cotton plants in the green house. Five weeks after inoculation, all the cotton plants were either healthy or wilted. The results of the pathogenicity test demonstrated that all the 14 isolates belonged to FOV race 3 and 6 isolates and *F. redolens* were considered as a virulent group (WI = 100) and 7 isolates that based on tef1 sequence analysis were classified in FOV races 4 group, showed no external symptoms and were considered as non-virulent group. Control plants showed no external symptoms.

F. redolens isolates cause a wide range of different diseases such as root and stem wilting in different plants. Our study confirmed again the presence of *F. redolens* as a dangerous pathogen in Iran (Chehri, 2015; Abi Saad *et al*., 2022). In this study, genetic diversity within members of FOSC isolated from cotton collected from the north and west of Iran was observed. Pathogenicity assay showed all FOV race 3 genotypes and *F. redolens* isolates were pathogenic in nature. While 7 FOV isolates that based on tef1 sequence analysis belonged to FOV race 4 were not pathogens. Also, classical fungal taxonomy is based on morphological features that were not useful for differentiation within FOSC isolates. Therefore, results of this study confirmed using pathogenicity test, specific-PCR, and phylogenetic (Leslie and Summerell, 2008).

Conclusion

Molecular identification using the specific primers of FOV-BT-AS-R3/BT5 and sequence analysis based on tef1 gene showed two clearly separated groups within members of Iranian FOV genotypes. In this study, no successful specific amplification was observed among 7 FOV strains, which based on tef1 sequence analysis are placed in separate groups namely race 4. Pathogenicity assay showed that all FOV race 3 genotypes and *F. redolens* isolates were pathogenic towards cotton.

Acknowledgements

Authors appreciate Razi University of Kermanshah for providing the necessary facilities to conduct this study.

Contribution of authors

KC and MFD designed and carried out this experiment, and also wrote the manuscript.

Conflict of interest

Authors declare that there is no conflict of interest.

Table 1: Morphological characteristics and GenBank accession numbers of selected strains of FOSC isolated from cotton in Iran.

Culture no.	Species identified	Shape of microconidia	Shape of basal cell and apical cell	Types of conidigenious cells	Length x width of macroconidia (µm)[a]		[b]*tef1*
					3- and 4-septate	5-septate	
FredNorth11	*F. redolens*	oval, elongated oval and often pointed on one end	Foot shaped and hooked	monophialidic	45.5 ± 2.5 × 4.5 ± 0.5	49.5 ± 2.5 × 5.9 ± 0.2	-
FredNorth13	*F. redolens*	oval, elongated oval and often pointed on one end	Foot shaped and hooked	monophialidic	46.0 ± 1.5 × 5.0 ± 0.5	51.5 ± 2.5 × 5.9 ± 0.2	-
FredNorth14	*F. redolens*	oval, elongated oval and often pointed on one end	Foot shaped and hooked	monophialidic	43.5 ± 1.5 × 4.5 ± 0.5	48.5 ± 2.5 × 5.9 ± 0.2	-
FovNorth310	*F. oxysporum* f. sp. *vasinfectum* (FOV) race 3	oval, elliptical	Foot shaped to pointed and curved	monophialidic	40.5 ± 1.5 × 4.8 ± 0.2	42.5 ± 2.5 × 5.6 ± 0.2	-
FovNorth352	FOV race 3	oval, elliptical	Foot shaped to pointed and curved	monophialidic	39.5 ± 1.5 × 5.0 ± 0.2	41.5 ± 2.5 × 5.5 ± 0.2	-
FovWest413	FOV race 3	oval, elliptical	Foot shaped to pointed and curved	monophialidic	38.5 ± 1.5 × 4.5 ± 0.2	40.5 ± 2.5 × 5.4 ± 0.2	-
FovWest222	FOV race 4, 7, 8	oval, elliptical	Foot shaped, hooked and curved	monophialidic	43.5 ± 1.5 × 4.2 ± 0.2	45.5 ± 2.5 × 5.2 ± 0.2	KX451126
FovWest220	FOV race 4, 7, 8	oval, elliptical	Foot shaped, hooked and curved	monophialidic	44.5 ± 1.5 × 4.3 ± 0.2	45.5 ± 2.5 × 5.3 ± 0.2	KX451128
FovNorth247	FOV race 4, 7, 8	oval, elliptical	Foot shaped, hooked and curved	monophialidic	44.5 ± 1.5 × 4.3 ± 0.2	45.5 ± 2.5 × 5.3 ± 0.2	KX451129
FovWest165	FOV race 4, 7, 8	oval, elliptical	Foot shaped, hooked and curved	monophialidic	44.5 ± 1.5 × 4.3 ± 0.2	45.5 ± 2.5 × 5.3 ± 0.2	KX451124
FovNorth181	FOV race 4, 7, 8	oval, elliptical	Foot shaped, hooked and curved	monophialidic	44.5 ± 1.5 × 4.3 ± 0.2	45.5 ± 2.5 × 5.3 ± 0.2	KX451125
FovNorth246	FOV race 4, 7, 8	oval, elliptical	Foot shaped, hooked and curved	monophialidic	44.5 ± 1.5 × 4.3 ± 0.2	45.5 ± 2.5 × 5.3 ± 0.2	KX451127
FovNorth155	FOV race 4, 7, 8	oval, elliptical	Foot shaped, hooked and curved	monophialidic	45.5 ± 1.5 × 4.4 ± 0.2	46.5 ± 2.5 × 5.4 ± 0.2	KX451123

[a]Mean values of 30 random conidia ± standard deviation; [b]GenBank numbers for *tef1* gene sequences

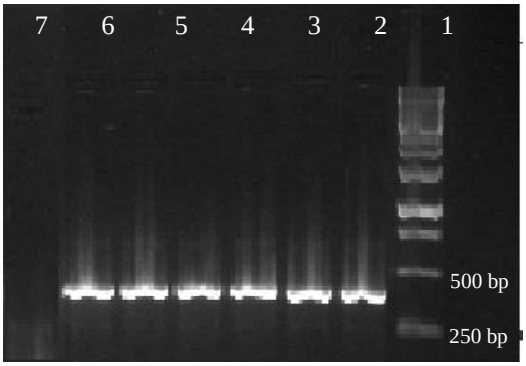

Fig. 1: PCR products obtained with specific primer pairs Redolens-F/Redolens-R (band, 386 bp) from 6 isolates of *Fusarium redolens* in this research. Lane M: GeneRuler 1 kb DNA Ladder. (**1** = FredNorth11, **2** = FredWest12, **3** = FredNorth13, **4** = FredNorth14, **5** = FredWest15, **6** = FredNorth16, **7** = *F. oxysporum* (negative control).

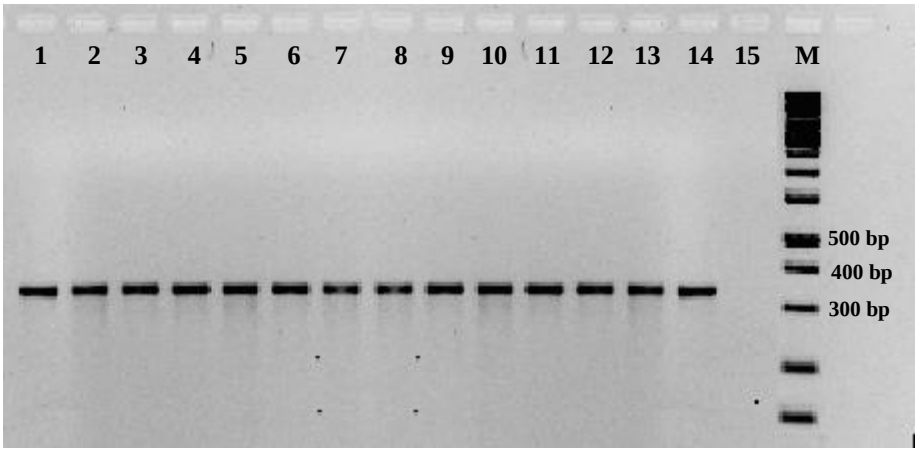

Fig. 2: PCR products obtained with specific primer pairs FOV-BT-AS-R3/BT5 (band, 321 bp) from 14 isolates of *F. oxysporum* f. sp. *vasinfectum* in this research. Lane M: GeneRuler 100 bp DNA Ladder. **1** = FovNorth310, **2** = FovWest132, **3** = FovWest313, **4** = FovNorth342, **5** = FovNorth352, **6** = FovNorth136, **7** = FovWest317, **8** = FovNorth328, **9** = FovNorth139, **10** = FovWest, **11** = FovNorth411, **12** = FovNorth412, **13** = FovWest413, **14** = FOSCfov444, **15** = *F. redolens* (negative control).

References

Abi Saad C, Masiello M, Habib W, Gerges E, Sanzani SM, Logrieco AF, Moretti A, Somma S, 2022. Diversity of *Fusarium* species isolated from symptomatic plants belonging to a wide range of agri-food and ornamental crops in Lebanon. *J. Fungi*, **8**: 897.

Armstrong G, Armstrong JK, 1978. A new race (race 6) of the cotton-wilt Fusarium from Brazil. *Plant Dis. Rep*., **62**: 421-423.

Bogale M, Wingfield BD, Wingfield MJ, Steenkamp ET, 2007. Species-specific primers for *Fusarium redolens* and a PCR-RFLP technique to distinguish among three clades of *Fusarium oxysporum*. *FEMS Microbiol. Lett.*, **271**: 27-32.

Bugbee W, Sappenfield WP, 1968. Varietal reaction of cotton after stem or root inoculation with *Fusarium oxysporum* f .sp .*vasinfectum*. *Phytopathology*, **58**: 212-214.

Chehri K, 2015. First report of postharvest fruit rots of tomato caused by *Fusarium oxysporum* in Iran. *Archiv. Phytopathol. Plant Prot.*, **48**: 537-544.

Chehri K, Ghasempour HR, Karimi N, 2014. Molecular phylogenetic and pathogenetic characterization of *Fusarium solani* species complex (FSSC), the cause of dry rot on potato in Iran. *Microbial Pathogen*., **67**: 14-19.

Chen Q, Ji X, Sun W, 1985. Identification of races of cotton wilt *Fusarium* in China. *Agric. Sci. China*, **6**: 1-6.

Diaz J, Garcia J, Lara C, Hutmacher RB, Ulloa M, Nichols RL, Ellis ML, 2021. Characterization of current *Fusarium oxysporum* f. sp. *vasinfectum* isolates from cotton in the San Joaquin Valley of California and Lower Valley El Paso, Texas. *Plant Dis.*, **105**: 1898-1911.

Dita M, Barquero M, Heck D, Mizubuti ESG, Staver CP, 2018. *Fusarium* Wilt of Banana: current knowledge on epidemiology and research needs toward sustainable disease management. *Front. Plant Sci.*, **9**: 1468.

Dongzhen F, Xilin L, Xiaorong C, Wenwu Y, Yunlu H, Yi C, Jia C, Zhimin L, Litao G, Tuhong W, 2020. *Fusarium* species and *Fusarium oxysporum* species complex genotypes associated with yam wilt in South-Central China. *Front. Microbiol.*, **11**: 1964.

Egamberdiev S, Salahutdinov I, Abdullaev A, Ulloa M, Saha S, Radjapov F, Mullaohunov B, Mansurov D, Jenkins J, Abdurakhmonov I, 2014. Detection of *Fusarium oxysporum* f. sp. v asinfectum race 3 by single-base extension method and allele-specific polymerase chain reaction. *Can. J. Plant Pathol.*, **36**: 216-223.

Fahmy T, 1927. The Fusarium wilt disease of cotton and its control. *Phytopathology*, **17**: 749-767.

Fernandez D, Assigbese K, Dubois MP, Geiger JP, 1994. Molecular characterization of races and vegetative compatibility groups in *Fusarium oxysporum* f. sp. *vasinfectum. Appl. Environ Microbiol*., **60**: 4039-4046.

Follin JC, 1986. La sélection du cotonnier (*Gossypium hirsutum* L.) pour la résistance aux maladies présentes en Afrique au sud du Sahara. Coton et Fibres Tropicales. Série Documents, Etudes et Synthèses **7**: 30.

Garber RH, Paxman GA, 1963. Fusarium wilt of cotton in California. *Plant Dis. Rep*., **47**: 398-400

Geiser DM, del Mar Jiménez-Gasco M, Kang S, Makalowska I, Veeraraghavan N, Ward TJ,

Zhang N, Kuldau GA, O'donnell K, 2004. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying Fusarium. *Eur. J. Plant Pathol.*, **110**: 473-479.

Hamdollah-Zadeh A, 1993. Properties of defoliant and non-defoliant strains of *Verticillium dahliae*, the causal agent of cotton wilt in northern Iran. *Iran. J. Plant Pathol*., **29**: 3-4.

Khan IH, Javaid A, Naqvi SF, 2021. Molecular characterization of *Penicillium expansum* isolated from grapes and its management by leaf extract of *Chenopodium murale. Int. J. Phytopathol.*, **10**: 29-35.

Khan IH, Javaid A, 2023. *Penicillium citrinum* causing postharvest decay on stored garlic cloves in Pakistan. *J. Plant Pathol.*, **105**: 337.

Leslie JF, Summerell BA, 2008. The Fusarium laboratory manual, John Wiley & Sons.

Sanei S., Razavi S., Lotfalinezhad E, 2013. Epidemiology of cotton *Verticillium* wilt in Golestan province, the North of Iran. *Annu. Res. Rev. Biol.*, 564-573.

Skovgaard K, Nirenberg HI, O'Donnell K, Rosendahl S, 2001. Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. *Phytopathology*, **91**: 1231-1237.

Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, **24**: 1596-1599.

Zhang Z, Zhao J, Ding L, Zou L, Li Y, Chen G, Zhang T, 2016. Constitutive expression of a novel antimicrobial protein, Hcm1, confers resistance to both Verticillium and Fusarium wilts in cotton. *Sci. Rep.*, **6**: 20773.