J. Crop Prot. 2018, 7 (2): 161-170

Research Article

Identification of *Fusarium solani* f. sp.*cucurbitae* races using morphological and molecular approaches

Sadra Rezaee[1], Shahrokh Gharanjik[1*] and Shideh Mojerlou[2]

1. Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran.
2. Department of Horticulture and Plant Protection, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran.

Abstract: *Fusarium solani* f. sp.*cucurbitae* races cause crown, fruit and root rots of cucurbits and economic problem for farmers. The aim of this study is identification of *Fusarium solani* f. sp. *cucurbitae* races which collected in Shahrood, Jiroft and Varamin, provinces of Iran using morphological characteristics and fungal-specific primers. Symptomatic cucurbit plants were collected from different fields in three provinces. After isolation and purification of the causal agents, pathogenicity test and DNA extraction were performed. Specific primers for TEF-1α gene were used to distinguish among different species of *Fusarium* and *Fusarium solani*. The isolates that showed a specific 658 bp amplicon on agarose gel were selected for subsequent PCR assay in order to detection of *F. solani* f. sp. *cucurbitae* race 1 and 2. PCR assays were performed with taxon-specific primers race 1 (505bp PCR product) and race 2 (425bp PCR product). Some samples amplified a 505bp amplicon which is specific for race 1 and was in accordance with morphological characteristics. In these isolates, 5 septate macroconidia were dominant that is specific to *Fusarium solani* f. sp.*cucurbitae* race 1 macroconidia. None of the isolates were amplified by race 2 taxon-specific primers. Our study showed that, race 1 of *F.solani* is the dominant race of the pathogen in Shahrood, Jiroft and Varamin provinces which can help to implement effective disease management strategies.

Keywords: *Fusarium solani* f.sp. *cucurbitae*, macroconidia, taxon-specific primers

Introduction

Plant disease is a threat to food production in the world, so that approximately 15% of the food destroyed by plant diseases. Phytopathogenic fungi cause more than 70% of yield loss, a threat that continues to increase (Fisher *et al.*, 2012).

Cucurbitaceae family includes vegetable crops, among them the pumpkins and squash (*Cucurbita* spp.), watermelons (*Citrullus lanatus*), melons (*Cucumis melo*) and cucumbers (*Cucumis sativus*) are most important crops. *Fusarium* is one of the important phytopathogenic genera of microfungi causing serious losses on cucurbit plants in Iran. *Fusarium solani*, *F. oxysporum* f.sp. *melonis*, *F. oxysporum* f.sp. *niveum*, *F. equiseti* were identified as causal agents of infected cucurbits in Shahrood, Iran (Iragi *et al*., 2014). *Fusarium solani* f. sp. *cucurbitae* race 1 (Fsc1) and race 2 (Fsc2) cause fusarium fruit rot (FFR). This pathogen is a hemibiotrophic pathogen that behaves as both biotrophs and necrotrophs and

Handling Editor: Naser Safaie

* **Corresponding author**, e-mail: gharanjik@shahroodut.ac.ir
Received: 5 March 2018, Accepted: 30 April 2018
Published online: 12 May 2018

can shift together in different stages of infection progress depending on conditions. Biotrophic pathogens obtain their nutrient from live host cells (Glazebrook, 2005) while necrotrophic fungal pathogens kill plant tissues and then feed (Williamson *et al*., 2007). FFR can develop as a cortical rot of the crown and roots of the plant. Sporodochia and macroconidia are formed in infected plants, then infestation of the surrounding soil will occur (Samac and Leong, 1989). Conidia of Fsc1 usually form chlamydospores, which can survive for several years in soil (Champaco *et al*., 1993). Fruit cortex that is in contact with wet soil is an appropriate place for germination of chlamydospores and conidia. Fruit infection characterized by dry rot and sporodochia formation. Fsc1 penetrates into fruit cortex and then the flesh, it progress towards the seed cavity and penetrates the seed. Generally *F. solani* f. sp. *cucurbitae* does not damage the cotyledons because it remains between the seed coat and the cotyledons therefore does not reduce seed viability (Tousson, 1961). Wilting of the leaves is the first symptoms of infected plants and followed by plant death within several days.

Fsc2 race was identified by Toussoun and Snyder, as causal agent of *Fusarium* crown and root rot (Tousson *et al.*, 1961). They classified Fsc2 as a different race because it only causes a fruit rot, whereas Fsc1 causes fruit, crown and root rot (Hawthorne *et al.*, 1994; Hawthorne *et al.*, 1992).

Both races directly penetrate into cucurbit fruit, while formation of an appressorium has not been observed. When the cuticle has been removed, isolates of both races cause disease on all plant parts (Hawthorne *et al.*, 1994). The plant cuticle is the first barrier against phytopathogenic fungi. Fungi such as *F. solani* must pass through this barrier before invade and colonize the hosts. The plant cuticle composed majority of cutin. Cutin can be hydrolyzed by the serine esterase, cutinase genes. Genes encoding cutinase previously have been cloned from several fungi including *Fusarium solani* f. sp. *pisi* (Soliday *et al.*, 1989).

Detection and identification of microbial strains in a region is necessary for disease management to achieve higher yield under specific ecological and environmental conditions. The morphological characters such as morphology of the macroconidia, the elongate or short monophialides, color of colonies on PDA and presence or absence of chlamydospores which also help distinguish between different forma specialis (f. sp.) of fungi.

Easy and rapid molecular techniques can be employed in microbial characterization. Some of sequences such as 28S rDNA, internal transcribed spacer (ITS) rDNA and transcription elongation factor (TEF)-1α have proved that the *F. solani* species complex can be classified into 50 sub-specific lineages (O'Donnell, 2000). The TEF 1-α gene has been used for phylogenetic analysis in several studies (Sampietro *et al.*, 2010; Takashima *et al.*, 2015; Wu *et al.*, 2014). This gene encodes a part of the protein translation machinery. The successful PCR assays using DNA extracted from field cucurbit tissues would drastically reduce the time and expertise required to identify and quantify causal agents of diseased plants. The aim of this study is isolation and identification of *F. solani* f. sp. *cucurbitae* races using morphological characteristics and molecular assay in some parts of Iran including Shahrood region.

Materials and Methods

Sample collection

Infected cucurbit plant and fruit samples were collected during the whole autumn 2016 from field and greenhouse of three major cucurbit production areas of Iran, including Jiroft, Varamin and Shahrood. Advanced disease symptoms show destroyed plant tissue leaving only fibrous vascular strands. Observation of pink to white fungal mycelium is common on the soil surface beside the lesion. Also, darkened soil around the crown can be evident. The stem just above the crown, root and fruit of diseased samples were washed in running tap

water and cut into small segments (1.5cm) for pathogen isolation.

Isolation and identification

The small segments of plant were surface-sterilized with 1% hypochlorite sodium for 3 min, washed twice in sterile distillate water, placed in petri plates containing potato dextrose agar (PDA) medium and kept at 25 °C. Then the isolates were purified using single spore and hyphal tip methods and cultured on PDA medium. Isolates initially were studied by morphological and microscopic approaches, including color of colonies on PDA and undersurface pigments and macroconidial characteristics. A piece of purified *Fusarium* colonies placed on potato dextrose broth (PDB) medium. Mycelium was grown in 100 mL of PDB on a rotary shaker at 180 rpm for 3 days at 25 °C and placed under the light for a week so that fungus was ready for DNA extraction.

Molecular identification of *Fusarium* species

Fungal biomass of each isolate in PDB medium was used for DNA extraction after vacuum filtration. Extraction of DNA performed as described by Safaie *et al* (2005) method. In order to distinguish between *F. solani* and *F. oxysporum* species two pair primers were employed in polymerase chain reaction (PCR) assay. First pair primers Fc-1 (5'CATACC ACTTGTTGCCTC 3') and Fc-2 (5'ATTAA CGCGAGTCCCACC3') previously designed according to *F. oxysporum* internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) (Zhang *et al.*, 2012). Second pair primers TEF-Fs4f (5′-ATCGGCCACGTCGACTCT-3′) and TEF-Fs4r (5′-GGCGTCTGTTGATTGTTAGC-3′), were designed based on *Fusarium solani* transcription elongation factor (TEF-1α) gene by Arif *et al* (2012). PCR conditions for both were: initial denaturation at 94 °C for 5 min followed by 40 cycles of 94 °C for 1min, 60 °C for 1 min, and 72 °C for 2 min. The reaction was ended with a 5 min extension at 72 °C. PCR product was electrophoresed in a 1.2% agarose gel with 0.1% ethidium bromide in 1X TBE buffer, separated for 30 min at 100V, and visualized using UV gel documentation system. PCR reaction repeated 3 times for each experiment.

Molecular identification of *F. solani* races

Two races of *F. solani* have been reported previously. These races of the *F.solani* can be identified by taxon-specific primers. Two pair primers were used in PCR assay. The first primers Fsc1-EF1 (5' GCTAACAATCATCT ACAGAC-3') /Fsc1-EF-2 (5'-GACGGA TGA GAGAGCAAC-3') are specific to race 1 and second pair primers Fsc2-EF1 (5′-GTTGGTGA CATATCTCCC-3′)/Fsc2-EF3 (5′-GAGTGAG AGACATGACGG-3′) are specific to race2 (Mehl and Epstein, 2007). Reaction conditions were 94 °C for 10 min followed by 35 cycles of 94 °C for 1 min, 56 °C for l min, and 72 °C for 1 min. The reaction was ended with a 5min extension at 72 °C.

Morphololological identification of *F. solani* races

Microscopic characteristics were employed to monitor the macroconidia of the isolates. Distinguish between races was undertaken based on Matuo and Snyder (1973) method. They sorted nine forma specialis and two races of *F. solani* into four groups (A, B, C, D) by the morphological characters of macroconidia.

Pathogenicity tests of the isolates

Pathogenicity test of the isolates was performed by three protocols on two cultivars of *Cucumis sativus* -khasib and Negin- which are selected cultivars for field cultivation in Jiroft and Varamin regions, respectively. The First method of fungi inoculation was spray inoculum suspension over sprouted seeds (Navi and Yang, 2005). Second method was a cut in the stem or root created by sterilized scalpel. 2 ml spore suspension of fungi prepared from 7-8 days old culture on PDA medium was injected in the cut and the inoculated portion was wrapped with parafilm (Rajput *et al.*, 2008). In the third method; cucumber seedlings were grown in autoclaved soil and after two weeks inoculation

was done with spore suspensions (1.8×10^6 spores/ml) of isolate and examined for disease symptoms 4 weeks later (Bourbos *et al.*, 1997). The experiments were done in a growth chamber under the following conditions: 25/20 °C, 60/80% relative humidity and 16/8 h day/night cycle (300 µE m^{-2} s^{-1}) for four weeks. Each experiment was conducted in three replicates for inoculated and non-inoculated plants.

Results

Isolation and identification of causal agents

50 samples of infected cucurbit plants were collected from Jiroft, Shahrood and Varamin and based on their morphological characteristics, these isolates were identified as *Rhizoctonia* sp. (Fig. 1a), *Alternaria* sp. (Fig. 1b), *Fusarium* spp. (Fig. 1c, d) genus. Isolates were obtained from root, stem, leaf and fruit of symptomatic cucurbit.

Results showed that *Fusarium* species were the most abundant genus among the isolates, so that approximately 46 percent of total (23 isolates) was belonged to this genus. Two species, *F. solani* (14 isolates) and *F. oxysporum* (9 isolates) were identified among the *Fusarium* spp. isolates based on morphological characters such as type of colony, macroconidia, color of colonies on PDA and undersurface pigments. All isolates were included in the morphological studies and then molecular study was used for identification isolates of *F. solani*.

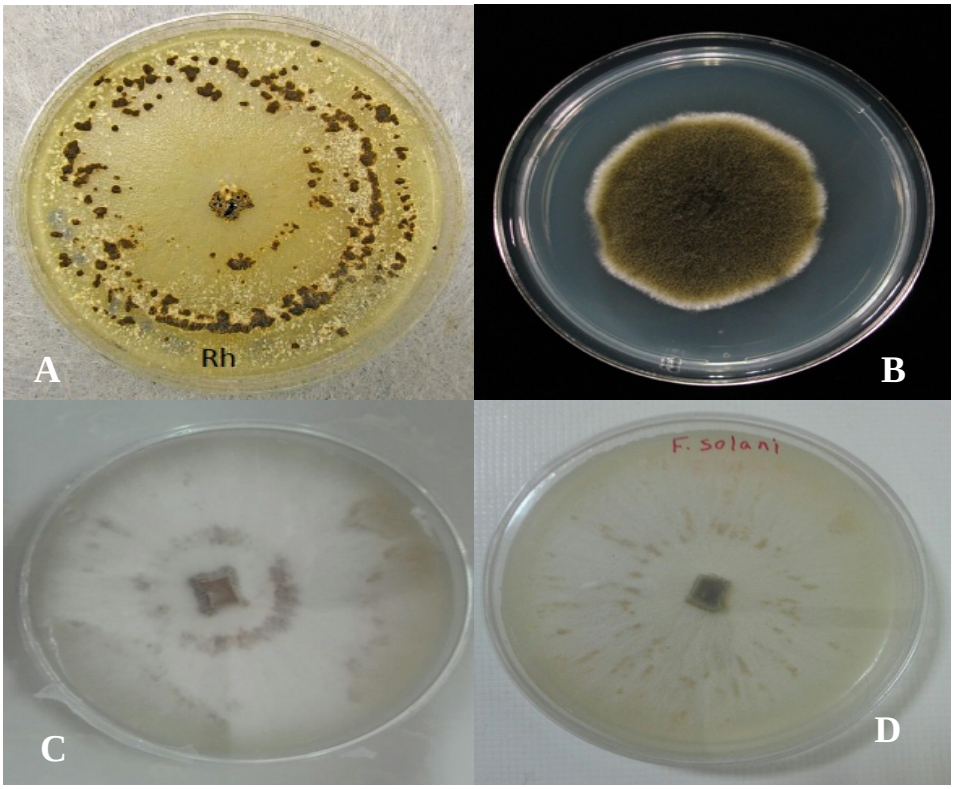

Figure 1 Colonies of *Rhizoctonia* sp. (A), *Alternatia* sp. (B) and *Fusarium* spp. (C, D) isolated from infected tissues on PDA medium after 6 days.

Molecular identification of *Fusarium* species

In order to confirm the morphological results, PCR assay was employed for *F.solani* isolates (14 isolates). PCR was done by TEF-Fs4f and TEF-Fs4r primers which are specific for *F. solani* TEF gene. Nine isolates out of 14 isolates showed a specific 658bp amplicon on agarose gel (Fig. 2). These isolates tested by Fc-1 and Fc-2 primers

which are specific primers for *F. oxysporum*. No amplification was observed in these isolates.

Molecular identification of *F. solani* races

Two races of *F. solani* were differentiated using taxon-specific primers in PCR assay. Fsc1-EF1 and Fsc1-EF2 had an amplicon size of 505 bp for some isolates which is specific for race 1 (Fig. 3). No amplification products were observed using race 2 specific primers. PCR repeated 3 times for each experiment.

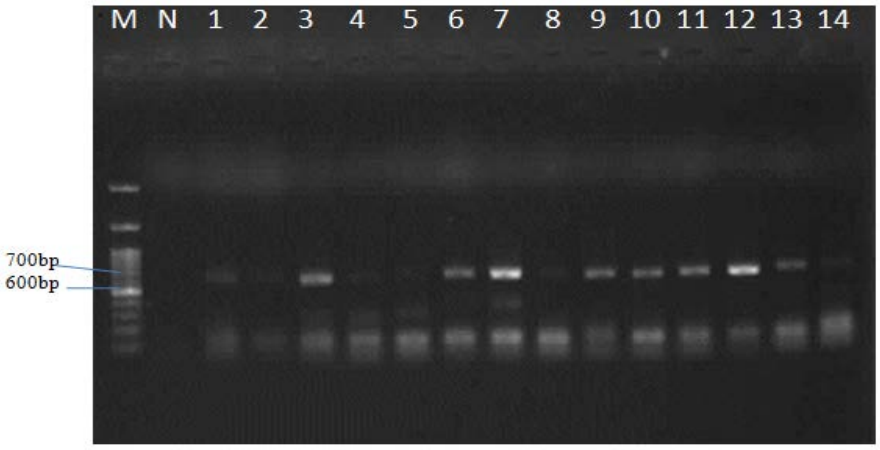

Figure 2 Agarose gel electrophoresis of PCR products amplified with primers TEF-Fs4f and TEF-Fs4r. M, 1kb ladder, N, negative control, lines1-14, studied isolates.

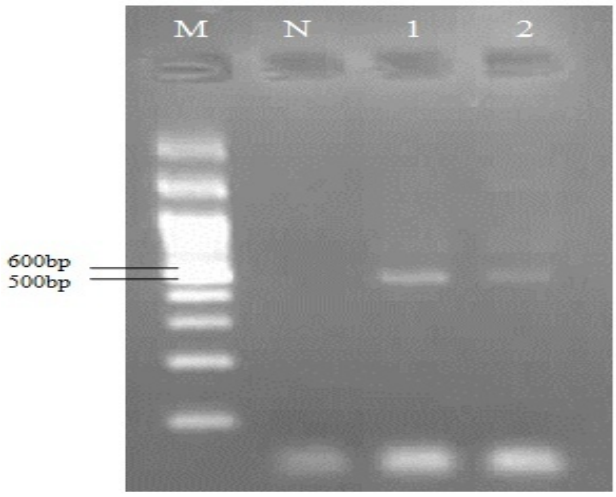

Figure 3 Agarose gel electrophoresis of PCR products amplified with Fsc1 EF1/Fsc1 EF2 primers (race 1 specific primers). M, 1kb ladder, N, negative control, line 1 and 2 studied isolates.

Morphological identification of *F. solani* races

F. solani races are sorted into two groups by the morphologic characters of macroconidia. We investigated these morphologic characters in order to distinguish between *F. solani* races. Isolates with positive PCR results using race 1 specific primers (Fsc1-EF1/Fsc1-EF2) were selected to investigate the macroconidia characteristics. Based on microscopic evidence these isolates showed 5-septate macroconidia which were predominant. This type of macroconidia is the proprietary characteristic of the *Fusarium solani* f. sp. *cucurbitae* race 1 (Fig. 4).

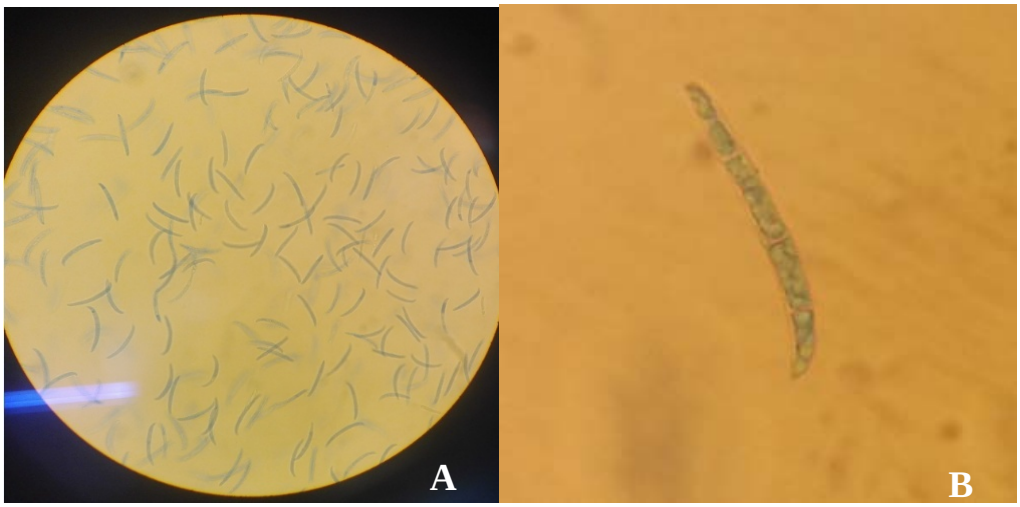

Figure 4 5-septate macroconidia of *F. solani* f.sp. *cucurbitae* race 1 under optical microscope, 40X magnification (A) and 100X magnification (B).

Pathogenicity test of the *Fusarium solani* isolates

The results of the pathogenicity test revealed that among three methods of inoculation, adding spore suspension to soil of two weeks old plants was more suitable than other methods. Khasib and Negin cultivars are the most cultivated cucumber in Jiroft and Varamin, respectively. Khasib showes high level of resistance to downy mildew (*Pseudo peronosporacubensis*) in cucumber cultivars (Marx *et al.*, 2010). Khasib cultivare is resistant to *Fusarium oxysporum* f. sp. *radicis-cucumerinum* whereas Negin cultivare is susceptible to this pathogen (Molavi *et al.*, 2009).

Plants roots detached and surface sterilized 4 weeks after inoculation and then placed on PDA medium. The progress of the fungal colonies size around the roots was observed daily. Resistance to pathogen was determined based on comparison of the colonies size on PDA around the roots (Nagao *et al*., 1994). We observed that growth rate of the fungal colonies size around the Negin cultivar's roots were significantly faster than Khasib cultivar, which means Khasib cultivar is more resistant to this pathogen.

Discussion

Fusarium species survive in the roots and stems of most plants, including cucurbits. They may exist on plant tissues as saprophytes. Many species of *Fusarium* recognized as opportunistic or weak pathogens, therefore they attack only plants that were weakened previously by some other environmental stress such as drought, wind and insects. These stresses affect the amount and disease severity (Palmer and Kommedahl, 1960).

The present study considered *Fusarium solani* pathogen associated with cucurbit diseases in Iran, including morphology, pathogenicity and molecular data. *Fusarium* species have been reported from several parts of the world and they are known to be pathogenic to many plants, especially to cucurbits. So far 2 races of *F. solani* have been associated with cucurbit crops worldwide. This includes *Fusarium solani* f. sp. *cucurbitae* race 1 and race 2 which race 1 causes crown and foot rot of cucurbit. Primary symptoms at the crown include light colored, water soaked areas that can become darker.

All isolates were included in the morphological studies. Among them *Fusarium* spp. colonies were selected for molecular study. *F. solani* colonies growing rapidly on PDA medium and they often have aerial mycelium. Hereafter the surface covered with confluent sporodochia that give the appearance cream, blue-green, or blue surface but never orange. Some colonies may show a dark purple color on

the upper surface. The undersurface is generally colorless, but some colonies produce a dark violet pigment. The morphology of the macroconidia helps to distinguish it from *F. oxysporum*. The elongate monophialides bearing microconidia and cream, blue-green or blue color of colonies on PDA are characteristic of *F. solani* (Nelson *et al.*, 1983). The morphology and macroconidia characteristics of the current study isolates were in accordance with those described previously (Leslie and Summerell, 2006).

Identification of *F. solani* species based on morphological characteristics requires considerable expertise in *Fusarium* taxonomy. Therefore, development of a method as a tool to be rapid, trustworthy and specific diagnosis of *Fusarium* species is needed. Techniques based on nucleic acid including PCR are most important tools for molecular detection of plant pathogenic fungi (Vincelli and Tisserat, 2008). Morphological evidences were confirmed by PCR assay.

DNA sequence data were effectively used for identification of *Fusarium* species in this study. The TEF gene has high utility for identification of *Fusarium* species because it is highly informative at the species level in Fusarium, doesn't have any orthologous copies of the gene in the genus and universal primers have been designed. These primers can distinguish among species of the genus *Fusarium* (Cho *et al.*, 1995). Therefore the TEF gene was used to distinguish between *F. solani* and *F. oxysporum* and also among *F.solani* races isolates. ITS region didn't select for this purpose because there is insufficient variation in ITS region (Hawthorne *et al.*, 1994).

Using morphological characteristics to identify forma specialis of *F. solani* developed by Matuo *et al*. (1973). After molecular identification of Fsc1, we studied morphological characters of Fsc1 isolates. According to Matuo *et al*., (1973) classification, Fsc1 is belong to A group which 5-septate macroconidia is predominant in this group, while Fsc2 is characterized by predominant 3-septate macroconidia, less than 5µ in width (Matuo and Snyder, 1973). In our study the isolates with positive PCR results to Fsc1 specific primers, had 5-septate macroconidia (Fig. 4) which is in accordance with Fsc 1 characteristics (Matuo and Snyder, 1973).

After identification of *F. solani* species and its races using molecular and morphological approaches, pathogenicity test was done and confirmed pathogenicity of the isolates. Fsc1and Fsc2 were reported in Tunisia (Boughalleb *et al*., 2005), while Fsc1 isolated from diseased cucurbits in Spain (García-Jiménez *et al*., 1997) and three provinces (Khorasan Razavi, Northern Khorasan and Fars) of Iran (Falahati Rastegar *et al*., 2009). We provided the first report of the Fsc1 in Shahrood, Varamin and Jiroft provinces.

Fusarium solani f. sp. *cucurbitae* has caused serious crop loss of cucurbit in the greenhouse and field in Iran. Race 1 is the dominant race of the pathogen in Shahrood, Varamin and Jiroft region, which can help to implement effective disease management strategies. The results showed that the specific primers are rapid tools to distinguish species and races of fungi and TEF gene is a good region in order to identification of *F. solani* species and races.

Author's contribution

This project is carried out as part of master thesis in Biotechnology at Shahrood University of Technology, the first author is master student and the second and third authors are thesis supervisors.

Acknowledgements

We are so grateful to the Faculty of Agriculture at Shahrood University of Technology for procuring laboratory facilities and equipment.

References

Arif, M., Chawla, S., Zaidi, M., Rayar, J., Variar, M. and Singh, U. 2012. Development of specific primers for genus *Fusarium* and *F. solani* using rDNA sub-unit and transcription elongation factor (tef-

1α) gene. African Journal of Biotechnology, 11: 444-447.

Boughalleb, N., Armengol, J. and El Mahjoub, M. 2005. Detection of races 1 and 2 of *Fusarium solani* f. sp. *cucurbitae* and their distribution in watermelon fields in Tunisia. Journal of Phytopathology, 153: 162-168.

Bourbos, V., Skoudridakis, M., Darakis, G. and Koulizakis, M. 1997. Calcium cyanamide and soil solarization for the control of *Fusarium solani* f. sp. *cucurbitae* in greenhouse cucumber. Crop Protection, 16: 383-386.

Champaco, E. R.; Martyn, R. D.; Miller, M. E. 1993. Comparison of Fusarium solani and F. oxysporum as causal agents of fruit rot and root rot of muskmelon.HortScience, 28: 1174-1177.

Chehri, K., Salleh, B., Yli-Mattila, T., Reddy, K. and Abbasi, S. 2011. Molecular characterization of pathogenic *Fusarium* species in cucurbit plants from Kermanshah province, iran. Saudi Journal of Biological Sciences, 18: 341-351.

Cho, S., Mitchell, A., Regier, J. C., Mitter, C., Poole, R. W., Friedlander, T. P. and Zhao, S. 1995. A highly conserved nuclear gene for low-level phylogenetics: Elongation factor-1 alpha recovers morphology-based tree for heliothine moths. Molecular Biology and Evolution, 12: 650-656.

Falahati Rastegar, M., Jafarpour, B. and Mahdikhani Moghadam, E. 2009. Genetic diversity in the fungus *Fusarium solani* f. sp. *cucurbitae* race 1, the causal agent of root and crown rot of cucurbits in iran, using molecular markers. Pakistan Journal of Biological Sciences, 12: 836-845.

Fisher, M. C., Henk, D. A., Briggs, C. J., Brownstein, J. S., Madoff, L. C., McCraw, S. L. and Gurr, S. J. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature, 484: 186.

García-Jiménez, J., Armengol, J., Moya, M. and Sales Jr, R. 1997. First report of *Fusarium solani* f. Sp. *cucurbitae* race 1 in spain. Plant Disease, 81: 1216-1216.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review of Phytopathology., 43: 205-227.

Hawthorne, B., Ball, R. and Rees-George, J. 1994. Genetic analysis of variation of pathogenicity in *Nectria haematococca* (*Fusarium solani*) on *Cucurbita* sp. Mycological Research, 98: 1183-1191.

Hawthorne, B., Rees-George, J. and Broadhurst, P. 1992. Mating behaviour and pathogenicity of New Zealand isolates of *Nectria haematococca* (*Fusarium solani*). New Zealand Journal of Crop and Horticultural Science, 20: 51-57.

Iraqi, Z., Rahnama, K., Mamarabadi, M. and Ommati, F. 2014. Identification and pathogenicity of fungal agents of root and crown rot of some cucurbit crops in shahrood region. Quarterly Journal of Research in Plant Pathology 2: 1-14.

Leslie, J. F. and Summerell, B. A. 2006. The Fusarium Laboratory Manual. John Wiley & Sons, 388 pp.

Marx, P., Garber, U. and Schmitt, A. 2010. Downy mildew in organically grown cucumber-selection of cultivars. Julius-Kuhn-Archiv: 451-452.

Matuo, T. and Snyder, W. C. 1973. Use of morphology and mating populations in the identification of formae speciales in *Fusarium solani*. Phytopathology, 63: 562-565.

Mehl, H. and Epstein, L. 2007. Identification of *Fusarium solani* f. Sp. *Cucurbitae* race 1 and race 2 with PCR and production of disease-free pumpkin seeds. Plant Disease, 91: 1288-1292.

Molavi, E. Aminian, H. Etebarian, H. and Shahriari, D. 2009. Investigation the resistance of greenhouse-cucumber cultivars to *Fusarium* stem and root rot disease (*Fusarium oxysporum* f. sp. *radicis-cucumerinum*).Journal of Agriculture, 17: 177-189.

Nagao, H., Sato, K. and Ogiwara, S. 1994. Susceptibility of *Cucurbita*spp. to the cucurbit root-rot fungus, *Fusarium solani* f sp*cucurbitae race* 1.Agronomie,14: 95-102

Navi, S. S. and Yang, X. B. 2005. Seedling inoculation screening technique to identify

resistance to *Fusarium solani* f. sp. *glycines* a causal agent of soybean sudden death syndrome. Plant Pathology abd Microbiology, 11: 25-29.

Nelson, P. E., Toussoun, T. A. and Marasas, W. 1983. *Fusarium* Species: An illustrated manual for identification.Pennsylvania State University. 206 pp.

O'Donnell, K. 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. Mycologia, 92: 919-938.

Palmer, L. T. and Kommedahl, T. 1960. Root-infecting *Fusarium* species in relation to rootworm infestations in corn. Phytopathology, 59: 1613-1617.

Rajput, N., Pathan, M., Jiskani, M., Rajput, A. and Arain, R. 2008. Pathogenicity and host range of *Fusarium solani* (mart.) sacc., causing dieback of shisham (*Dalbergia sissoo* roxb.).Pakistan Journal of Botany, 40: 2631-2639.

Safaei, N., Alizadeh, A., Saeidi, A., Adam, G. and Rahimian, H. 2005. Molecular characterization and genetic diversity among iranian populations of *Fusarium graminearum*, the causal agent of wheat headblight.Iranian Journal of Plant Pathology, 41: 171-189.

Samac, D. A. and Leong, S. A. 1989. Disease development in *Cucurbita maxima* (squash) infected with *Fusarium solani* f. sp. *cucurbitae*. Canadian Journal of Botany, 67: 3486-3489.

Sampietro, D. A., Marín, P., Iglesias, J., Presello, D. A., Vattuone, M. A., Catalán, C. A. N. and Jaen, M. G. 2010. A molecular based strategy for rapid diagnosis of toxigenic *Fusarium* species associated to cereal grains from argentina. Fungal Biology, 114: 74-81.

Soliday, C., Dickman, M. and Kolattukudy, P. 1989. Structure of the cutinase gene and detection of promoter activity in the 5'-flanking region by fungal transformation. Journal of Bacteriology, 171: 1942-1951.

Takashima, M., Manabe, R.-i., Iwasaki, W., Ohyama, A., Ohkuma, M. and Sugita, T. 2015. Selection of orthologous genes for construction of a highly resolved phylogenetic tree and clarification of the phylogeny of *Trichosporonales* species. PloSone, 10: e0131217.

Tousson, T. 1961. The pathogenicity, distribution, and control of two races of *fusarium* (hypomyces) *solani*f.sp cucurbitae. Phytopathology, 51: 17-22.

Vincelli, P. and Tisserat, N. 2008. Nucleic acid–based pathogen detection in applied plant pathology. Plant Disease, 92: 660-669.

Williamson, B., Tudzynski, B., Tudzynski, P. and van Kan, J. A. 2007. *Botrytis cinerea*: The cause of grey mould disease. Molecular Plant Pathology, 8: 561-580.

Wu, G., Feng, B., Xu, J., Zhu, X.-T., Li, Y.-C., Zeng, N. K., Hosen, M. I. and Yang, Z. L. 2014. Molecular phylogenetic analyses redefine seven major clades and reveal 22 new generic clades in the fungal family boletaceae. Fungal Diversity, 69: 93-115.

Zhang, S., Zhao, X., Wang, Y., Li, J., Chen, X., Wang, A. and Li, J. 2012. Molecular detection of *Fusarium oxysporum* in the infected cucumber plants and soil. Pakistan Journal of Botany, 44: 1445-1451.

شناسایی نژادهای *Fusarium solani* f. sp. *cucurbitae* با استفاده از روش‌های ریخت‌شناسی و مولکولی

صدرا رضائی[1]، شاهرخ قرنجیک[1*] و شیده موجرلو[2]

۱- گروه زراعت و اصلاح نباتات، دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران.
۲- گروه باغبانی و گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران.
* پست الکترونیکی نویسنده مسئول مکاتبه: gharanjik@shahroodut.ac.ir
دریافت: ۱۴ اسفند ۱۳۹۶؛ پذیرش: ۱۰ اردیبهشت ۱۳۹۷

چکیده: نژادهای *Fusarium solani* f. sp.*cucurbitae* عامل پوسیدگی طوقه، ریشه و میوه‌ی کدوئیان می‌باشد که یک مشکل اقتصادی برای پرورش‌دهندگان این گیاهان است. هدف این تحقیق شناسایی نژادهای *F. solani* f. sp.*cucurbitae* با استفاده از آغازگرهای اختصاصی و بررسی‌های مورفولوژیکی قارچ در نواحی شاهرود، جیرفت و ورامین ایران است. گیاهان آلوده از مناطق مختلف این نواحی جمع‌آوری شدند. پس از جداسازی و خالص‌سازی عامل بیماری، آزمون بیماری‌زایی و استخراج DNA از آن‌ها انجام شد. آغازگر اختصاصی ژن TEF-1α برای تمایز بین گونه‌های مختلف فوزاریوم و نژادهای *F. solani* استفاده شد. جدایه‌هایی که یک قطعه تکثیرشده ۶۵۸ جفت بازی را بر روی ژل آگارز نشان دادند، برای آزمون PCR بعدی به‌منظور شناسایی نژاد ۱ و ۲ *F. solani* f. sp.*cucurbitae* انتخاب شدند. آزمون PCR با آغازگرهای ویژه‌ی نژاد ۱ (با محصول ۵۰۵ جفت بازی) و نژاد ۲ (با محصول ۴۲۵ جفت بازی) انجام شد. تعدادی از جدایه‌ها یک قطعه‌ی ۵۰۵ جفت بازی را تکثیر کردند که این قطعه اختصاصی برای نژاد ۱ است و این یافته با ویژگی‌های ریخت‌شناسی مطابقت داشت. ماکروکنیدی‌های ۵ قسمتی در این جدایه‌ها به‌صورت غالب بود که نتایج قبلی را تأیید می‌کند. هیچ‌کدام از جدایه‌ها توسط آغازگر نژاد ۲ تکثیر نشدند. بررسی حاضر نشان می‌دهد که نژاد ۱ به‌عنوان نژاد غالب در منطقه‌ی شاهرود، ورامین و جیرفت است که این یافته می‌تواند به اجرای استراتژی‌های مؤثر برای مدیریت بیماری کمک کند.

واژگان کلیدی: *Fusarium solani* f. sp.*cucurbitae*، ماکروکنیدی، آغازگر اختصاصی تاکسون