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ORIGINAL ARTICLE

Molecular characterization of pathogenic *Fusarium* species in cucurbit plants from Kermanshah province, Iran

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Abstract *Fusarium* is one of the important phytopathogenic genera of microfungi causing serious losses on cucurbit plants in Kermanshah province, the largest area of cucurbits plantation in Iran. Therefore, the objectives in this study were to isolate and identify disease-causing *Fusarium* spp. from infected cucurbit plants, to ascertain their pathogenicity, and to determine their phylogenetic relationships. A total of 100 *Fusarium* isolates were obtained from diseased cucurbit plants collected from fields in different geographic regions in Kermanshah province, Iran. According to morphological characters, all isolates were identified as *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium equiseti*, *Fusarium semitectum* and *Fusarium solani*. All isolates of the five *Fusarium* spp. were evaluated for their pathogenicity on healthy cucumber (*Cucumis sativus*) and honeydew melon (*Cucumis melo*) seedlings in the glasshouse. *F. oxysporum* caused damping-off in 20–35 days on both cucurbit seedlings tested. Typical stem rot symptoms were observed within 15 days after inoculation with *F. solani* on both seedlings. Based on the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) restriction fragment length polymorphism (RFLP) analysis, the five *Fusarium* species were divided into two major groups. In particular, isolates belonging to the *F. solani* species complex (FSSC) were separated into two RFLP types. Grouping among *Fusarium* strains derived from restriction analysis was in agreement with criteria used in morphological classification. Therefore, the PCR-ITS-RFLP method provides a simple and rapid procedure for the differentiation of

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Fusarium strains at species level. This is the first report on identification and pathogenicity of major plant pathogenic *Fusarium* spp. causing root and stem rot on cucurbits in Iran.

1. Introduction

Cucurbit plants (*Cucurbitaceae*) are the main agricultural crops, particularly in the Kermanshah province in Iran. Annually it is estimated that over 3000 ha of agricultural land in the province are under cucurbits. Root and stem rots of cucurbits have significantly increased in incidence and severity in the past 20 years and they are a yield-limiting factor in many intensive cucurbit production, especially in cucumber (*Cucumis sativus*), watermelon (*Citrullus lunatus*) and honeydew melon (*Cucumis melo*), resulting sudden death and complete destruction of these economic plants (Alymanesh et al., 2009). Diseased plants were characterized by yellowing of the leaves, stem necrotic lesions, phloem discolorations, and collapse. There are many pathogens capable of producing these vine decline symptoms in cucurbits (Boughalleb et al., 2005).

The most important pathogens that cause root and stem rots in cucurbit plants are *Fusarium* spp., which are responsible for vascular wilts, such as those on melons (cantaloupe and muskmelon) caused by *Fusarium oxysporum* f. sp. *melonis*. *Fusarium proliferatum* and *Fusarium solani* f. sp. *cucurbitae* cause crown and foot rots of summer squash, melon, pumpkin, and a fruit rot of pumpkin (Pivonia et al., 1997; Namiki et al., 1994). There are two ''races'' of *F. solani* f. sp. *cucurbitae* causing fruit, crown and foot rots in cucurbit plants. *F. solani* f. sp. *cucurbitae* race 1 (Fsc1) causes crown, fruit, and root rots of cucurbits whereas *F. solani* f. sp. *cucurbitae* race 2 (Fsc2) causes only a fruit rot (Hawthorne et al., 1992). Fsc1 and Fsc2 are not easily distinguished morphologically, but identification requires mating tests, pathogenicity tests and molecular assays (Mehl and Epstein, 2007; Hawthorne et al., 1994; Boughalleb et al., 2005). *F. solani* f. sp. *cucurbitae* race 1 has been reported as the causal agent of melon root and foot rot from Khorasan province in the eastern part of Iran (Alymanesh et al., 2009).

Characterization of the population structure of fungal pathogens is important for understanding the biology of the organism and for development of disease-control strategies (Malvick and Percich, 1998), and for molecular studies among individuals, which is one of the components of population structure (Leung et al., 1993). Universally, *F. solani* species complex (FSSC) has an extensive host range and very high levels of diversity in pathogenicity and morphology (Brasileiro et al., 2004). However, the classification system based only on morphology has not provided an accurate tool for the identification of FSSC, neither has morphological classification system resolved the relationship of isolates within FSCC. So, a molecular approach is promising in establishing the objective (O'Donnell and Gray, 1995; Zhang et al., 2006; O'Donnell et al., 2008). Among the methods which researchers have used to analyze the phylogenetics of *F. solani* species are rDNA-IGS, rDNA-ITS regions, large submit RNA gene and translation elongation factor-alpha (tef) (Zhang et al., 2006). Internal transcribed spacer (ITS) region is probably the most widely sequenced region of DNA in fungi. rDNA-ITS and rDNA-IGS (intergenic spacer) regions show a higher degree of diversity than other ribosomal regions such as small subunits (SSU) and large subunits (LSU) (O'Donnell and Gray, 1995; Depriest and Been, 1992; O'Donnell, 2000; Brasileiro et al., 2004). Therefore, the objectives of this study were: (i) to isolate and identify disease causing *Fusarium* spp. from infected cucurbit plants in Kermanshah province; (ii) to determine their pathogenicity; and (iii) to determine phylogenetic relationships and usefulness of the PCR-ITS-RFLP as a genetic marker within the *Fusarium* spp.

2. Materials and methods

2.1. Sample collection

Infected cucurbit plants were collected from different regions of Kermanshah province, Iran (Table 1). Each sample were stored in a paper envelope and kept in a cool box with dry ice. In the laboratory, roots and stems of diseased samples were washed in running tap water and cut into small blocks (1.5 cm) for isolation.

2.2. Isolation and identification

For isolation of *Fusarium* spp., the blocks were surface-sterilized with 1% sodium hypochlorite for 3 min and rinsed with several changes of sterile water. The sterilized samples were placed onto a general medium (water agar) (Burgess et al., 1994) and a semi-selective medium for *Fusarium*, i.e., peptone-pentachloronitrobenzene agar (PPA) plates (Nash and Snyder, 1962), and incubated under a standard growth condition (Salleh and Sulaiman, 1984). The resulting *Fusarium* colonies were single-spored and transferred onto potato dextrose agar (PDA), carnation leaf agar (CLA) (Fisher et al., 1982), spezieller nahrstoffarmer agar (SNA) (Nirenberg, 1976), and potassium chloride agar (KClA) plates (Fisher et al., 1983) for morphological identification (Leslie and Summerell, 2006).

2.3. Pathogenicity test

All isolates of the *Fusarium* species were tested for their pathogenicity on apparently healthy and uniform 20 days-old seedlings of cucumber (*C. sativus*) and honeydew melon (*C. melo*) in the glasshouse. Roots and stems of the cucumber and honeydew melon seedlings were washed in running tap water before inoculation. Conidial suspension of each individual isolate was prepared by pouring sterile distilled water and gently scraping the conidia of 7 days-old cultures on PDA plates grown under the standard growth condition (Salleh and Sulaiman, 1984). The concentration of the pooled suspension was adjusted to 2×10^6 conidia/ml by using a haemocytometer. The roots of the seedlings were soaked in 20 ml conidial suspension for 20 min for root inoculation technique. For stem inoculation technique, 20 ml of the conidial suspension of each *Fusarium* species was sprayed on the stems. The control plants were inoculated by booth techniques with 20 ml of sterile distilled water. Three replicates were performed for each isolate and the experiment was repeated twice. All the