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Morphological, cultural and pathogenic variability among the different isolates of *Fusarium oxysporum* f. sp. *radicis cucumerinum* causing root and stem rot of cucumber: A review

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Abstract

Fusarium root and stem rot is one of the most damaging diseases of greenhouse cucumber. The causative organism, *Fusarium oxysporum* f. sp. *radicis cucumerinum* is widespread in cucumber growing areas resulting in considerable economic losses. The importance and variability of different isolates is hereunder reviewed briefly.

Keywords: *Fusarium oxysporum* f.sp. *radicis cucumerinum* (FORC), morphological, variability

Introduction

Cucumber (*Cucumis sativus* L.) belongs to family cucurbitaceae and most important vegetable, which is a major source of human edible products and useful fibers. Cucumber probably originated in the foothills of the Himalayas and have been cultivated for at least 3,000 years (Kroon *et al.*, 1979) [18]. *Fusarium* root and stem rot of cucumbers caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum* (T. D. Vatchev, 2015, Vakalounakis, 1996) [45, 44]. FORC is a relatively new disease first reported in Greece by Vakalounakis (1996) [44] who described it in detail. It has been reported that various *F. oxysporum* pathotypes can survive successfully either in the soil or above ground by means of thick-walled chlamydospores that are either free or embedded in infected plant debris (Shlevin *et al.*, 2003; Suarez-Estrella *et al.*, 2004) [39, 38]. The disease has also been reported from Canada, China, France, Israel, The Netherlands, Spain and United States (Punja and Parker, 2000; Cercauskas *et al.*, 2001; Moreno *et al.*, 2001; Rose and Punja, 2004; Pavlou and Vakalounakis, 2005) [34, 4, 29, 37, 35] in China in 1999, and in Spain in 2000, causing significant losses in the yield (Punja & Parker, 2000) [34]. Symptoms of *Fusarium* root and stem rot include large basal stem lesions on which abundant sporulation is often observed.

Cultural Characters

Mycelial colour varied from white to dull white with slightly yellowish to pinkish tinge in among 20 isolates of *F. oxysporum* f.sp. *pisi* (Gupta *et al.* 2011) [13]. Singh *et al.* (2011) [40] observed that out of 12 isolates of *F. oxysporum* f.sp. *ciceris*, 7 isolates expressed appressed type of growth pattern on PDA. Mycelial colour of isolates exhibited wide range colour of variation from creamy white to dark purple. Patel *et al.* (2011) [33] observed that the dry mycelial weight of different isolates of *F. oxysporum* f.sp. *lini* ranged from 221.00 to 494.00 mg. Wagh *et al.* (2010) [43] observed that isolate SGFOL-5 was recorded as fast growing (82.00 mm) while remaining isolates showed moderate mycelial growth ranging from 71.60 mm to 78.10 mm. Honnareddy and Dubey (2007) [14] observed sporulation count among 21 isolates of *F. oxysporum* f.sp. *ciceris* ranging from 0.4×10^6 to 2.3×10^6 conidia/ml. Based on this, the isolates were grouped into abundant, moderate and low sporulating. Rupe, 1989 gave the method of isolation, according to him, the roots were washed in running tap water and divided into lateral roots, taproot epidermal tissue, taproot cortical tissue, and tissues from the 5 cm. of stem with vascular discoloration. The epidermis of lower stem were also removed, plant parts were cut into 1cm. segments and surface- disinfested by dipping in 95% ethanol, soaking in 0.5% sodium hypochloride for 5 min, and rinsing in sterile water. The segments were placed on 2% water agar amended with 50mg/l of streptomycin sulfate. Segments were placed in Petri dishes (five segments per dish) and incubated at room temperature. Vakalounakis *et al.*, (1996) [44] identified *F. oxysporum* f.sp. *cucumerinum* based on the disease symptoms on cucumber plants and differing pathogenicity on different plant spp. of the cucurbitaceae. This disease

caused by the fungus *Fusarium oxysporum* f.sp. *radicis-cucumerinum*, was first observed in Greece and the Netherlands. It was reported in British Columbia (10% losses), Canada (25% losses) in 1994 and later in Ontario (35% losses) in 2000. Mishra *et al.*, (2009) [28] collected diseased sample of tomato from different geographical regions of India and isolated ten different isolates of *F. oxysporum* f. sp. *lycopersici*. Karaca *et al.* (2009) [17] tested for Pathogenicity host range by inoculations on *Cucumis sativus*, *Luffa acutangula*, *L. aegyptiaca*, *Cucurbita maxima*, *C. moschata*, *Cucurbita ficifolia*, *Cucumis melo* and *Citrullus lanatus* plants in Turkey. Three weeks after inoculation, cucumber plants presented severe root and stem rot symptoms and died. *Luffa aegyptiaca*, *Cucumis melo* and *Citrullus lanatus* plants showed the same symptoms, whereas *Luffa acutangula* and *Cucurbita* species were healthy. Two formae speciales of *F. oxysporum* infect cucumber; f. sp. *cucumerinum* causes vascular wilting, whereas f. sp. *radicis-cucumerinum* causes wilting accompanied by root and stem rot. In addition to cucumber, the latter fungus can also infect melon (*Cucumis melo*), watermelon (*Citrullus lanatus*) and sponge gourd (*Luffa aegyptiaca*). Amini and Sidovich, (2010) [1] isolated *F. oxysporum* f. sp. *lycopersici* from tomato plants showing wilting symptoms. Dubey *et al.* (2010) [7] reported *F. oxysporum* f. sp. *ciceri* isolates were highly variable in their colony growth pattern, size of colony and pigmentations. One hundred and twelve isolates were grouped into 12 categories on the basis of their radial growth, size of macro conidia and growth pattern. Majority of the isolates were highly pathogenic causing more than 50 per cent wilt in chickpea cultivar JG 6. Nirmaladevi and Srinivas (2012) [31] observed significant variations among the 114 isolates of *F. oxysporum* f.sp. *lycopersici* in tomato with respect to rate and type of growth, colony colour, mycelial growth pattern, sporulation, septation of the conidia, number and pattern of chlamydospore formation. Kumar and Upadhyay (2014) [16] observed cultural, morphological and pathogenic variability in fifteen isolates of *F. oxysporum* f.sp. *udum* of pigeonpea collected from four major crop growing states. All the isolates showed great variations. Gupta *et al.* (2014) [12] in Himachal Pradesh isolated 19 isolates of *Fusarium oxysporum* f.sp. *zingiberi* caused *Fusarium* yellows in ginger and obtained wide variations in respect of morphological, cultural and pathological among the all isolates. Chopada *et al.* (2015) [5] studied cultural and morphological variability among 10 isolates of *Fusarium oxysporum* f.sp. *lycopersici* in tomato from Gujarat. They reported that all the isolates showed wide variations in respect of mycelial colour, mycelial growth, dry mycelial weight, sporulation, conidial size and formation of chlamydospores. The isolates produced moderate, profuse fluffy, thin flat to slight fluffy and submerged growth with white, yellow, light pink, dark pink, orange and purple orange pigmentation. Liaquat *et al.* (2016) [21] isolated different isolates from grape fruit in Pakistan and studied morphological characters in respect to colony colour *i.e.*, creamy white and pink or purple tinge, and their margins slightly lobed or smooth. Micro conidia were single, oval to reniform and monophialides type Moon crest shape macro conidia were produced in sporodochium with multiseptum. Short aerial conidiophores were unbranched, producing one-cell conidia in false head (Hussain *et al.*, 2012). The microscopic structure was similar to that of *Fusarium oxysporum*. Nath *et al.* (2017) [30] observed cultural, morphological, physiological and pathogenic variations among the nine isolates of *F.*

oxysporum f.sp. *ciceri* in the chick pea from Bangladesh and found that maximum mycelial growth and sporulation of the pathogen was at 25 °C temperature after 7 days of inoculation in all the isolates which are reduced drastically below 15 °C temperature and above 35 °C temperature. Among these 9 isolates only one (FOC 1) isolate found to be highly virulent on the variety of BARI Chola-1 of Chick pea.

Morphological Characters

Mohammed *et al.* (2016) [26] performed morphological identification of different strains of *F. oxysporum* based on the characteristics of micro conidia, macro conidia, phialides and chlamydospores formation. Mahdikhani (2016) [22] studied morphological, pathological and molecular variability among the 120 isolates *F. oxysporum*, *Fusarium acuminatum*, *Fusarium graminearum*, *Fusarium proliferatum* collected during 2013 to 2015 in Iran. Kumar and Upadhyay (2014) [16] observed cultural, morphological and pathogenic variability in fifteen isolates of *F. oxysporum* f.sp. *udum* of pigeonpea collected from four major crop growing states. All the isolates showed great variations. Gupta *et al.* (2014) [12] in Himachal Pradesh isolated 19 isolates of *Fusarium oxysporum* f.sp. *zingiberi* caused *Fusarium* yellows in ginger and obtained wide variations in respect of morphological, cultural and pathological among the all isolates. Patel *et al.* (2011) [33] showed that 21 isolates of *F. udum* varied in sporulation, size of macro conidia and micro conidia while studying for morphological characteristics. The micro conidia were round to oval in shape and had 0 to 1 septa. The size of micro conidia ranged from 2.9-7.7 X 1.4-2.8 µm in FU-14 and 2.9-8.8 X 1.8-2.9 µm in FU-21 to 8.2-9.4 X 1.2-2.5 µm in FU-7. Macro conidia were spindle as well as sickle shaped with 2 to 6 septa. The size of macro conidia ranged from 10.2-17.6 X 2.8-3.4 µm in FU-19 to 32.5-62.5 X 3.9-5.2 µm in FU-15. (Wagh DR, Verma KP, Dantre RK, Baghel A, Chaliganjewar SD, 2010) [43]. Prasad *et al.* (2008) [36] observed that proportion of macroconidia and microconidia varied in different isolates of *F. oxysporum* f.sp. *ricini*. Macroconidia were of 2–7 septate, straight to curve, sickle shaped or linear to broad. The average size of macroconidia ranged from 23.2 × 4.1 in For 22 to 64.5 × 5.4 µm in For 29. Microconidia were hyaline, round to oval in shape ranged from 9.5 × 3.2 in For 22 to 23.4 × 6.8 µm in For 29. Gupta *et al.* (2011) [13] found that 20 different isolates of *F. oxysporum* f. sp. *pisi* collected from different parts of Himachal Pradesh showed variation in morphological characteristics, micro conidia varied from 3.16 x 3.16 to 9.13 x 5.44 µm and macro conidia varied from 11.77 x 3.16 to 24.60 x 5.91 µm in size they have also reported that all the isolates formed chlamydospores on PDA medium. Honnareddy and Dubey, (2007) [14] noticed morphological variability in all 25 isolates of *F. oxysporum* f. sp. *ciceri* causing chickpea wilt, where minimum size of micro conidia ranged from 5.50 to 13.50 X 2.50 to 3.50 µm and that of macro conidia 15.00 to 37.50 X 3.50 to 4.50 µm as well as isolate from 1 to 14 produced 1-3 septate micro conidia where as other produce 1-2 septate macro conidia. Sinha *et al.* (2007) reported that 69 isolates of *F. udum* collected from major states of India, showed variation in conidial length and septation however micro conidia Length and septa did not vary among isolates. Susan Groenewald, (2005) [42] divided the different *F. oxysporum* f. sp. *cubense* isolates, in accordance to their morphology, grouped them into three morphological types, namely sporodochial, cottony, and slimy pinnotal, out of these sporodochial types was the most dominant morphological type. Lugo and Sanabria, (2001)

found that all the 16 isolates of *F. oxysporum* f.sp. *lycopersici* showed variation in conidia and chlamydospore size and morphology. Snyder and Hansen, 1940, found that *Fusarium oxysporum* is comprised of fungal strains which are morphologically and physiologically similar. Gupta *et al.* 1986^[11], studied on six isolates of *F. oxysporum* f. sp. *ciceris* that revealed variation in size of macro conidia and micro conidia. The micro conidia ranged from 3.88-8.75 X 2.33-3.33 µm to 6.66-9.99 X 1.66-4.99 µm with no septation and size of macro conidia varied from 16.55-33.30 X 3.33-6.66 µm to 33.30-66.60 X 3.30-4.99 µm with 1-6 septation. Mc Millan (1986)^[23] reported variations among *Fusarium* isolates from cucumber.

Pathogenic Variability

Owen (1955)^[32] found through cross inoculation studies with *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *niveum* isolated from cucumber and watermelon in Florida, that *Fusarium* isolates were specifically pathogenic. A change from one forma specialis (*F. oxysporum* f. sp. *niveum*) to another forma specialis (*F. oxysporum* f. sp. *melonis*) has been reported (Bouhot, 1981) and one isolate of *F. oxysporum* f. sp. *cucumerinum* from the Netherlands is pathogenic to cucumber, muskmelon and watermelon (Geriagh and Blok, 1988)^[10]. Kawai *et al.*, 1958^[20] and Matuo & Yamamoto, 1957 reported that six formae speciales, *F. oxysporum* f. sp. *niveum*, *F. oxysporum* f. sp. *melonis*, *F. oxysporum* f. sp. *cucumerinum* and *F. oxysporum* f. sp. *radicis-cucumerinum* are globally distributed and more important pathogens from an economic point. The other two formae speciales, *F. oxysporum* f. sp. *lagenaria* and *F. oxysporum* f. sp. *luffae* have only been recognized in a restricted geographic area of Japan. Komada and Ezuka, (1974) also reported *Fusarium* wilts and foot rot of cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* has been reported from many parts of the world. El-sayed *et al.*, (2008)^[8] isolated 23 isolates of *Fusarium oxysporum*, eight isolates of *Fusarium solani*, two isolates of *Verticillium dahliae* and four isolates of *Rhizoctonia solani* from tomato plant showing wilting and root rot symptoms at different localities in Egypt. Mishra *et al.*, (2009)^[28] collected diseased sample of tomato from different geographical regions of India and isolated ten different isolates of *F. oxysporum* f. sp. *lycopersici*. Sharma *et al.* (2011)^[24] isolated 24 isolates of *F. oxysporum* f. sp. *lycopersici* from tomato plants showing typical wilt symptoms from fourteen different regions including different agroclimatic condition in India. Garibaldi *et al.* (2016)^[9] observed for the first time in Verona, (Italy) in commercial farm where 5 month old cucumber plant in plastic house were affected with chlorosis, yellowing, and wilting of stem. On the basis of affected stems, a whitish-light orange mycelium appeared while vessels were discolored, finally affected tissues collapsed and plants died. The forma sp. *radicis cucumerinum* was confirmed by Amplification with the specific primer FORCF1 and FORCR2 designed by Lievens *et al.* (2007)^[3]. Kiprof *et al.* (2002)^[15] revealed that all 79 isolates of *F. udum* isolated from wilted chickpea plants showed high significant variation in virulence. Desai *et al.* (2003)^[6] reported that six out of 15 isolates of *F. oxysporum* f. sp. *ricini* isolated from castor wilt, collected from different areas of Gujarat proved highly virulent against VP-1 and VP-9. Majdah *et al.* (2015)^[25] tested eight isolates of *Fusarium oxysporum* f.sp. *cucumerinum* for virulence against the susceptible cucumber cultivar Beit alpha from different localities in Egypt. Isolate No. 3 was the most virulent isolate,

it recorded percent disease index (PDI) (77.33%), followed by isolate No. 4 (74.66%). Highest Resistant isolate no 7 with 95.99% PDI.

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