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VARIABILITY IN INDIAN ISOLATES OF *FUSARIUM OXYSPORUM* F. SP. *CUCUMERINUM* CAUSING CUCUMBER WILT

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The variability in cultural characteristics and the virulence among ten isolates of *Fusarium oxysporum* f. sp. *cucumerinum* causing vascular wilt in cucumber was studied in the glasshouse and under laboratory conditions. There were strong differences in cultural and morphological characters *viz.* colour of mycelium, microconidia, macroconidia and chlamydospore. Pathogenic variation among the *Fusarium oxysporum* f. sp. *cucumerinum* was analyzed by pathogenicity tests with cucumber cultivars. Based on pathogenicity to cucumber cvs. (Long Green, Poinsett, W.B.C-37 and DM-Dr-2) isolates were divided into four groups. Among tested cultivars Long Green was found susceptible to almost all the isolates while DM-Dr-2 was resistant to II, III and IV pathogenic groups.

Keywords: Cucumber; *Fusarium;* India; Pathogenic variability.

Introduction

Cucumber is a crop of high economic importance in many countries[1]. Wilt of cucumber caused by *Fusarium oxysporum* (Schlechtend: Fr) f sp. *cucumerinum* (Owen) Snyder & Hansen is an important vascular disease worldwide[2-5]. The cucumber wilt disease is one of the major limiting agents for production of cucumber and from another side the highly resistant varieties have not been developed and effective control measures are not available. Wide cultural, morphological and pathogenic variations have been observed in *Fusarium oxysporum*. This paper reports cultural, morphological and pathogenic variations in *Fusarium oxysporum*. f. sp. *cucumerinum*.

Material and Methods

Fungal isolates-Wilt infected cucumber plants were collected from different states of India (Delhi, Haryana, Punjab and Rajasthan). All strains were obtained from single spore and maintained on potato dextrose agar (PDA). Identification was performed on the basis of mycological characters described by Nelson *et al.*[6]. The isolates from different geographical regions were coded according to their source and area of collection (Table 1).

Cultural and morphological variability-Cultural and morphological variability was studied from 7 day old mycelium cultured on potato dextrose agar medium (PDA) at 25°C under dark. The isolates from different geographical regions were characterized on the basis of macro-and microscopic characteristics like colour of mycelium, shape of macroconida, growth rate etc[6].

On the basis of growth rate on PDA medium at 25±2°C, the radial growth of each isolate was measured in millimeter (mm) at an interval of 24 hours for a period of four days. Average cumulative growth rate per day was calculated by using the following formula:

Growth rate= Final growth - inoculating block/ time.

Pathogenic variability-Each isolate was grown in potato dextrose broth at 28°C for 4-5 days on an orbital shaker (120 rpm). The mycelial mats were removed by passing through 4 layers of cheesecloth, and the conidial concentration was adjusted to 5×105 conidia per ml. Virulence test of all isolates was performed on the susceptible cucumber cultivar 'Long Green' using the root dip method described by Jacobson and Gordon[7]. For further classification of pathogenic strains, cucumber cultivars (Long Green, Poinsett, W.B.C.-37 and DM-DR-2) were used as differential cultivars. (All trials carried out in a glasshouse at temperature 25-30°C and relative humidity 50-90%). The inocula was applied to the plant roots by dipping root cuttings for 30 minutes in the conidial suspensions, after which seedlings were transplanted on sterilized organic substrate in plastic pots (8.5-10 cm in diameter). Seedlings treated similarly and dipped in tap water serve as a control. Plants were watered daily without fertilizers. Seedlings were rated every 4-5 days for 30 days.

After four weeks of transplanting, each plant was uprooted and cut lengthwise to evaluate symptoms of Fusarium wilt. Disease rating was done by using 0-4 disease rating scale (0=Healthy plant, 1=Initial symptoms

Table 1. Isolates of *Fusarium oxysporum f. sp. cucumerinum* collected from different places of India.

Isolate No.	Isolate Code*	Geographical origin
1	CRK	Kotputli (Rajasthan)
2	CRT-1	Tonk I (Rajasthan)
3	CRD	Delhi
4	CRG	Gurgaon (Haryana)
5	CRB-1	Bagpat (Uttar Pradesh)
6	CRB-2	Banas (Rajasthan)
7	CRA	Alipur (Uttar Pradesh)
8	CRU	Ujha (Uttar Pradesh)
9	CRT-2	Tonk II (Rajasthan)
10	CRS	Sikar (Rajasthan)

C= cucumber, R= root 3rd alphabetic= Geographic origin

of leaf chlorosis and internal browning of the lower vessels, 2=Severe symptoms of wilting and initial symptoms of leaf necrosis and 4= Plant totally wilted and leaves completely necrotic) developed by Jacobson and Gordon[7].All isolates were considered pathogenic if the mean disease rating was ≥ 1 after 4 weeks. The experiment was conducted as a randomized complete block design with three replicates and five plants per replicate.

Results and Discussion

Cultural and morphological variability- Morphological variability in *Fusarium oxysporum* f. sp. *cucumerinum* was high in the isolates collected from different geographical regions. For the macroscopic characteristics, the color of the isolates went from whitish (isolate CRG), brownish-white (isolate CRK),pinkish(isolate CRS) pinkish-white (isolate CRD), purplish pink (isolate CRU and CRT-2) purplish-brownish-white (isolate CRB-1 and CRT1) to creamish pink(CRB-2 and CRA).

Macroconidia varied from straight, slightly curved, sickle and boat shaped. Numbers of septa were 1-3 in most of the isolates but different *viz.* 1-5 in CRB1, 3-6 in CRA and 2-5 in CRU isolates. Microconidia were abundant, unicellular, oval and but absent in CRB-1 and CRU isolates. Chlamydospores had a thick wall, were spherical and had an intercalary or terminal location. Their texture varied from smooth to rough walled in different isolates (Table 2).

Isolates were grouped as slow (growth < 7 mm day^{-1}), moderate (growth 7-11 mm day^{-1}) and fast (growth > 11 mm day^{-1}). Growth rate varied from 4.75 mm in isolate 9 from Tonk (Rajasthan) to 14.25 mm in isolate 7 from Alipur (Uttar Pradesh). Moderate growing isolates comprised 50 % of population followed by slow growing 30 % and Fast growing rate 20 % (Table 3).

According to morphological variability of FOC, our results coincide with those obtained by Trujillo *et al*[8], who also detected a high degree of phenotypic and genomic variability in the strains of *Fusarium* spp. responsible for wilting in carnations. *Fusarium oxysporum* has been reported to vary in color on the PDA growth medium[9]. The aerial mycelium is white and can change to a variety of colors -from violet to dark purple depending on the strain of *F. oxysporum*. The macro- and microscopic characteristics of the FOC strains, the phenotypic variation, coincides with that reported in other studies which identify *F. oxysporum* as one of the most variable species in its genus[10-11].

Pathogenic variability-The 10 pathogenic isolates collected from various locations in India showed variable but high disease index in range 2.7-4.Maximum disease index of 4.0 was present in isolate no. 7 (CRA) (Table 4). These were classified into four groups on the basis of differences in pathogenicity to cucumber cvs. DMDR-2, W.B.C-37, Long Green and Poinsett). These results indicate that at least four pathogenic groups occur in the Indian population of *F. o. f. sp. cucumerinum* (Table 5).

Isolates of group I (code no. 6,7) were pathogenic to all four cultivars. Isolates of group II (code no. 2,8,9) were pathogenic to cvs. Long Green and Poinsett but not on DMDR-2 and W.B.C-37. Isolates in group III (code no. 3,10) were pathogenic only to Long Green but not on other three cultivars and isolates in group IV (code no. 1,4,5) were pathogenic to cvs. Long Green and W.B.C-37 but not on DMDR-2 and Poinsett.

Table 2. Cultural and morphological characteristics of *Fusarium oxysporum f. sp. cucumerinum* isolates.

Isolate No	Name of the isolate	Colour of mycelium	Macroconidia Shape	Microconidia No. of Septa	Chlamydospore Shape	Chlamydospore Texture
1	CRK	Cream to buff	Boat shaped, occasionally curved at one end,pointed ends	3	Oval	Smooth walled
2	CRT-1	White to cream	Straight to slightly curved, rounded ends	1 to 3	Oval	Smooth to rough walled
3	CRD	White to pink	Straight to slightly curved, rounded ends	1 to 3	Oval	Smooth walled
4	CRG	White	Straight to slightly curved, rounded ends	1 to 3	Oval	Smooth walled
5	CRB-1	White to cream	Slightly curved to sickle shaped, rounded to pointed ends	1 to 3	Absent	Absent
6	CRB-2	Cream to pink	Straight to slightly curved, rounded ends	1 to 5	Oval-round	Smooth to rough walled
7	CRA	Cream to pink	Sickle to boat shaped, pointed ends	3 to 6	Oval	Smooth walled
8	CRU	Pink to dull cream	Straight to slightly curved, rounded ends	2 to 5	Absent	Absent
9	CRT-2	Pink to dull cream	Straight to slightly curved, rounded to pointed ends	1 to 3	Oval	Smooth to rough walled
10	CRS	Pink	Sickle to boat shaped, pointed ends	1 to 3	Absent	Absent

Table 3. Growth rate (mm/day) of *F. oxysporum f. sp. cucumerinum.*

Isolate No.	Isolate Code*	Growth	Growth Rate (mm)
1	CRK	M	10.25
2	CRT-1	S	5.75
3	CRD	S	5.75
4	CRG	M	7.25
5	CRB-1	M	8.75
6	CRB-2	M	8.25
7	CRA	F	14.25
8	CRU	M	7.25
9	CRT-2	S	4.75
10	CRS	F	12.75

S-Slow, M-Moderate, F-Fast

Table 4. Disease index of Isolates of *F. oxysporum f. sp. cucumerinum* on cucumber cv Long green.

Isolate No.	Isolate Code*	Disease Index**
1.	CRK	3
2.	CRT-1	3.3
3.	CRD	2.1
4.	CRG	1.8
5.	CRB-1	2.7
6.	CRB-2	3.6
7.	CRA	4.0
8.	CRU	3.6
9.	CRT-2	3.3
10	CRS	3.4

*Disease rating scale 0-4 (0=Healthy plant,1=Initial symptoms of leaf chlorosis and internal browning of the lower vessels, 2=Severe symptoms of wilting and initial symptoms of leaf necrosis and 4= Plant totally wilted and leaves completely necrotic)

Table 5. Classification of Indian isolates of *F. oxysporum f. sp. cucumerinum* according to reaction of different cultivars of cucumber.

Pathogenic groups	(Isolate Code No.)	Cucumber cultivars Long green	Poinsett	W.B.C-37	DM-Dr-2
I	(6,7)	S	S	S	S
II	(2,8,9)	S	S	R	R
III	(3,10)	S	R	R	R
IV	(1,4,5)	S	R	S	R

S= Susceptible R= Resistant

The classification system of races within *Fusarium oxysporum* f. sp. *cucumerinum* proposed by Armstrong *et al.*[12] generally has been accepted. However it is difficult to use this system for Indian strains, because seeds of the race differential cultivars are not commercially available in India. Similar problem was reported by Namike *et al*[13] and Wright *et al*[14] in case of F.o f. sp. *meloni* and dianthii in Spain and Australia from melon and carnation, respectively. In this study we found Indian cultivars (Long Green, Poinsett, WBC-37 and DMDR-2) could be substituted for the group differential cultivars used by Armstrong *et al.*[12]. Among tested cultivars Long Green was found susceptible to almost all the isolates while DM-Dr-2 was resistant to II, III and IV pathogenic groups. From this study it can be concluded that there appears to be correlation between the vegetative growth of pathogen isolates with its virulence as highly virulent isolates showed lesser growth rates.

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