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Studies of pathogenic variability, Mycelial Compatibility and Incompatibility Groups of *Fusarium Oxysporum* F. Sp. *Ciceri* Isolates

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Abstract
Studies of Pathogenic Variability, Mycelial Compatibility and Incompatibility Groups of *Fusarium Oxysporum* F. Sp. *Ciceri* Isolates Laboratory studies of fifteen isolates of *Fusarium oxysporum* f. sp. *ciceri*, obtained from different locations were categorized into various groups based on cultural characters and pathogenic variability. Results of this study shows that the isolates varied in colony colour (white, dirty white and milky white), type of growth (fluffy and compact), growth rate (fast and slow), Pathogenecity (weakly pathogenic, moderately pathogenic, highly pathogenic). Based on cultural characters and relative Pathogenecity of 15 isolates of Foc studied were finally characterized into 5 groups. Five isolates viz., 16, 60, 80, 105 and 88 were found highly pathogenic with medium growth i.e., 70-79 mm, while 3 isolates exhibited fast growth and highly pathogenic too. Seven isolates belonging to moderately pathogenic group which are either medium or slow growing (<70mm). Mycelial compatibility among the isolates was also observed and out of 105 combinations only 72 combinations (68.5%) showed compatible reaction and 22 (25.9%) whereas vegetative incompatibility groups were identified. Almost 31.42 per cent combinations i.e. 33 showed antagonistic reaction. Microscopic observations on mycelial interaction revealed protoplast lysis, vacuole formation, hyphal thickness and hyphal lysis..

Keywords: Pathogenic Variability, Mycelial, *Fusarium Oxysporum* and Isolation

Introduction
Chickpea (*Cicer arietinum* L.) is an important pulse crop, ranking third after dry beans. India is the largest producer of chickpea contributing around 70 per cent (6.2 m. tones) of the world's total production. Chickpea is affected by several seed, soil and air borne diseases which is responsible for lowering its yield. Soil borne pathogens like *Sclerotium rolfsii* (Collar rot), *Fusarium oxysporium* f. sp. *ciceri* (Vascular wilt) and *Rhizoctonia bataticola* (Dry root rot) are responsible for causing diseases from seedling to flowering and pod formation stage. (Haware 1990; Nene *at el*. 1987). Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is the most important disease of chickpea.
The disease can occur at all the stage of plant growth right from seedling to maturity and causes annual yield losses of 10-90 per cent annually (Jalali and Chand, 1992, Jimmez *et al*. 1989).Gupta *et al*. (1986) reported the difference in growth pattern, pigmentation, sporulation and virulence of six isolates of *Fusarium oxysporum* f. sp. *ciceri*. This study was carried out to know the pathogenic variability, mycelial compatibility and incompatibility groups of *Fusarium oxysporum* f. sp. *ciceri* isolates.

Material and Methods
The present investigations have been undertaken to study the pathogenic variability, mycelial compatibility and incompatibility groups of *Fusarium oxysporum* f. sp. *ciceri* isolates and identity the wilt resistant sources. Chickpea entries/varieties were evaluated for wilt resistance in the multiple diseases sick field under AICRP on Chickpea located at seed Breeding farm J.N.K.V.V. Jabalpur. Infected plants showing vascular wilt symptoms were collected during the month of October to December 2012 from different chickpea fields in the vicinity of Jabalpur. Samples were brought into the chickpea pathology laboratory under AICRP on chickpea in Department of Plant Breeding and Genetics for isolation and further studies.Variability in isolates the cultural characters of different chickpea isolates of *Fusarium oxysporum* were studied on PDA medium. These isolates were categorized into various groups according to type of growth (fluffy and compact), colour variation of colony (white, milky white, dirty white), growth rate (slow, medium and fast) at an interval of 24 hrs.

Result and Discussion

Mycelial compatibility For the study of mycelial compatibility between the 15 isolates, viz, I-60 (Porbonder, GJ), I-50 (Seoni, MP), I-58 (Bilaspur, CG), I-15 (Jabalpur, MP) I-80 (Kawardha, CG), I-54 (Raisen, MP), I-3 (Narsinghour, MP), I-57 (Raisen, MP), I-16 (Jabalpur, MP), I-105 (Surat, GJ), I-87 (Amravati, MH), I-100 (Sagar, MP), I-10 (Narsinghpur, MP), I-12 (Jabalpur, MP), I-88 (Amravati, MH), mycelial disc of 5 mm diameter were cut from the edge of an actively growing colony (3 to 4 days old) of each isolate, which placed approximately 25 to 35 mm apart on opposite sides of 90 x 15 mm petridishes and incubated at 25± 2^0C. Three isolates were usually paired in one petridish. Observation on antagonistic reaction i.e. formation of interaction zone/barrase formation was observed in all the pairing up to 7 days of incubation. The pairing was examined microscopically after 5 days of incubation for interaction in the region of mycelial contact. OpTo know the variability in the cultural characters namely; colony colour, type of growth, and growth rate within the 15 Foc isolates have been studied on PDA and presented in Tables– 1, 2,

Table 1: Colony characters of various isolates of *Fusarium oxysporum* f. sp. *ciceri* on Potato dextrose agar medium

Isolates	Colony colour	Type of growth	Location	State
60	Milky white	Fluffy	Porbonder	GJ
50	White	Fluffy	Seoni	MP
58	Dirty White	Compact	Bilaspur	CG
15	White	Fluffy	Jabalpur	MP
80	White	Compact	Kawardha	CG
54	Dirty White	Compact	Raisen	MP
3	White	Fluffy	Narsinghpur	MP
57	Dirty White	Compact	Raisen	MP
16	White	Compact	Jabalpur	MP
105	Dirty White	Compact	Surat	GJ
87	White	Compact	Amravati	MH
100	Dirty White	Fluffy	Sagar	MP
10	Milky White	Fluffy	Narsinghpur	MP
12	White	Fluffy	Jabalpur	MP
88	Dirty White	Compact	Amravati	MH

On the basis of colony colour the isolates were categorized as milky white, dirty white and white whereas, type of growth was categorized as fluffy and compact type. It is evident from the data presented in Table – 2 that out of the 15 isolates, 7 (46.6%) isolates were found to be white, 2 (13.33%) isolates were dirty white and 6 (40%) isolates were milky white. Similarly the type of growth pattern also varied within the isolates, 7 (46.6%) showed fluffy growth pattern and 8 (53.33%) were found to be of compact type growth pattern.(Table 1 & 2, i, ii, iii) on the basis of colony colour the isolates are grouped in three group, while 2 groups falls under either fluffy or compact type growth.

Table 2: Grouping of isolates of *Fusarium oxysporum* f. sp. *ciceri* on the basis of colony characters on PDA

Colony colour

Colony colour	Isolates	Total
White	3,12,15,16,50,80,87	7
Dirty white	10,60	2
Milky white	54,57,58,88,100,105	6
	Total	15

Type of growth

Colony pattern	Isolates	Total
Fluffy	3,10,12,15,50,60,100	7
Compact	16,54,57,58,80,87,88,105	8
	Total	15

Growth rate

Category	Isolates	Total
Slow growing (<70.0mm)	3,15,50,60,87	5
Medium growing (70.0-79.0mm)	10,80,88,100,105	5
Fast growing (>79.0mm)	12,16,54,57,58	5
	Total	15

Fifteen isolates of *Fusarium oxysporum* were grouped into 3 main groups on the basis of growth rate, on potato dextrose agar medium at 25±1°C (Table 3). Five isolates were categorized as slow growing having <70 mm, five isolates an medium growing (70-79 mm) and five isolates were fast growing (>79 mm) within seven days. Further, the isolates may be grouped with three groups as they fall in slow, medium and fast growing.

Pathogenic variability

Pathogenecity tests for 15 isolates were conducted under pot condition using sick soil method and susceptible variety. Observation on plant mortality due to wilt were recorded weekly and days taken for maximum disease development is presented in Table-3.

Table 3: Number of days taken for maximum disease development caused by *Fusarium oxysporum* f. sp. *ciceri* isolates on chickpea variety JG 62

Isolates No.	Percent wilt	Days for maximum disease development
60	100	19
50	100	17
58	100	19
15	100	15
80	90	23
54	100	19
03	100	14
57	100	19
16	100	10
105	71.8	17
87	75.7	24
100	94	22
10	100	14
12	100	10
88	79.4	24

In order to see the pathogenic variability with regard to number of days taken for disease initiation and causing maximum mortality after inoculation by different isolates of *F. oxysporum*, a highly susceptible chickpea variety JG 62 was used. The data exhibited in table No. 3 revealed that maximum seedling mortality (percent) occurred due to different isolates after disease initiation ranged from 10-24 days. Isolates 12 and 16 resulted 100 percent wilting within 10 days. Whereas isolates 87 and 88 registered for maximum mortality in 24 days.

Table 4: Grouping of *Fusarium oxysporum* f. sp.*ciceri* isolates based on the Pathogenecity

Category	Isolates	Total
Weakly pathogenic (<30% wilt)		-
Moderately pathogenic (31-50% wilt)	3,10,15	3
Highly pathogenic (>50% wilt)	12,16,50,54,57,58,60, 80,87,88,100,105	12
	Total	15

Based on the Pathogenecity wilt incidence Foc isolates were grouped 3 categories. 3 isolates were moderately pathogenic and 12 isolates were highly pathogenic presented in Table 4

Table 5: Grouping of *Fusarium oxysporum* f. sp. *ciceri* isolates based on the culture and Pathogenecity

Category	Isolates	Total
Highly pathogenic, fast growing	54,57,58	3
highly pathogenic, medium growing	16,60,80,88,105	5
Highly pathogenic, slow growing	12,100	2
Moderately pathogenic, medium growing	10,50,87	3
Moderately pathogenic, slow growing	3,15	2
	Total	15

Based on cultural characters, and relative Pathogenecity of 15 isolates of Foc studied on the basis of were finally characterized in 5 groups (Table 5) Among 15 isolates 5 isolates viz., 16, 60, 80, 105 and 88 were found highly pathogenic with medium growth i.e., 70-79 mm, while 3 isolates exhibited fast growth and highly pathogenic too. Seven isolates belonging to moderately pathogenic group with either medium or slow Growing (<70mm).Variability within population in a geographical region are important because these document changes occurring in the population. Sporadic work has been carried out on geographical variability of *Fusarium oxysporum* f. sp. *ciceri* isolated from various hosts. There are few reports which indicate the variability in the population of *Fusrium oxysporum* of different hosts and same hosts (Mishra *et al.* 1977; Salem *et al.*, 1991; Gupta *et al.* 2002; Chaudhary *et al.* 2007). Hence, the present investigations were undertaken with a view to know the pathogenic variability, mycelial compatibility and incompatibility groups of *Fusarium oxysporum* f. sp. *ciceri*.In the present investigation, growth pattern among the isolates varied from fluffy (46.6%) to compact (53.3%). Similar findings were also made by Sharma *et al.* (2002) who reported 18 isolates of fluffy type and 8 of compact type out of 26 isolates of *F. oxysporum* from different hosts. Patil *et al.* (2005) recorded white mycelium with different growth pattern in aquatic isolates of *F. oxysporum.*After critically going through the data on growth rate recorded within seven days, it denotes that the rate of growth in isolates, also varies five isolates were categorized as slow growing (<70mm), five isolates were medium growing (70-79 mm) and five isolates were fast growing (>79 mm). Isolates 57 and 58 gave significantly higher growth than other isolates within seven days of incubation and slow growth has been observed in isolates 60 and 87, which indicate differential growth rate in isolates. These results are in coincidence with Brake *et al.* (1990) and Primo *et al.* (2001) who reported differences in mycelial growth rate of *F. oxysporum* f. sp. *ciceri* in chickpea. Mycelial compatibility among the isolates of *Fusarium oxysporum* led to the conclusion that the isolates possess three combinations i.e. compatible reaction, antagonistic reaction and the vegetable incompatibility groups. The highest percentage of compatible reactions (68.5%) further showed the extent of diversity among these isolates thus suggested the presence of genetically distinct mycelia. Formation of vacuole, hyphal thinning followed by protoplast and mycelia lysis at the interaction zone is attributed to the heterokaryotic condition of the nuclei but involvement of toxins cannot be ruled out (Primo *et al.* 2001; Khan *et al.* 2002; Moncada *et al.* 2009).

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