

Studies on variability in the growth of twenty isolates of *Fusarium oxysporum* F. Sp. ciceri causing vascular wilt of chick pea on different solid media

ASHISH SHRIVASTAVA AND VIJAY AGRAWAL

International Journal of Plant Protection (April, 2010), Vol. 3 No. 1 : 120-123

See end of the article for authors' affiliations

Correspondence to :
VIJAY AGRAWAL,
Precision Farming
Development Centre,
Central Institute of
Agricultural
Engineering, BHOPAL
(M.P.) INDIA

SUMMARY

Field survey was undertaken and 71 samples of chickpea wilted plants, were collected from 23 locations in different districts namely Bhopal, Raisen Rajgarh, Sagar, Sehore and Vidisha of Vaindhyan Plateau Zone of Madhya Preadesh. Out of 71 isolates, only 20 were found pathogenic to chickpea. These isolates were categorized into six different groups on the basis of growth pattern, colony diameter and number of micro and macro conidia. The physiological studies of the representative isolates of these 6 groups were made on seven solid media. All the media differed significantly from each other. The minimum mean growth (6.08cm) was recorded in group 5 of isolates Ri4, Ri5 and V2 and maximum (7.86cm) in group I consisting of B2, B3, Se6 and Se8. The maximum (6.28cm) on yeast extract agar. These isolates exhibited three types of growth pattern namely fluffy, partially submerged and submerged. The maximum number of micro conidia were produced on PDA (7.61million/ml) and minimum (2.60million/ml) on nutrient agar. Similarly, the maximum number of macro conidia were produced on PDA (2.50million/ml) and on nutrient agar.

Key words :

Management, Pea
mosaic virus,
Medicinal plants

Chickpea (*Ciceri aretinum* L.) is an important pulse crop of India and suffers with various diseases caused by fungi, bacteria and virus of which vascular wilt caused by *Fusarium oxysporum* f. Sp ciceri is much dangerous than other diseases (Singh and Dahiya, 1973). The incidence of the disease varies from 10-100 per cent depending on the locality. In Madhya Pradesh, its incidence has been reported from 0-60 per cent (Gupta *et al.*, 1983). In order to find suitable lines resistant to this disease, it is necessary to study the variability of the pathogen. The isolates of the chickpea wilt pathogen obtained from various locations of Madhya Pradesh, were grouped into six groups on the basis of morphological, cultural characters (Gupta *et al.*, 1986) and physiological basis (Kushwaha *et al.*, 1974). The variability in the growth of twenty isolates on seven solid media is reported in this paper.

MATERIALS AND METHODS

All the pathogenic isolates were grown on seven solid media namely Potato dextrose agar, Rice extract agar, Richards agar, Maize extract agar, Czapecks agar, Nutrient agar and Yeast extract agar. All the media were adjusted at pH 6.5 and auto claved at 15 lbs psi for 20 minutes. A quantity of 20 ml of the medium

was poured in each Petriplate and allowed to solidify. Five mm disc was cut through sterilized cork borer from the margin of seven days old culture of *F. oxysporum* f.sp. ciceri. One disc was placed in the centre of each plate and incubated at $28 \pm 1^\circ\text{C}$ for seven days. Three replication were maintained for each medium for each isolate on tenth day of inoculation, five mm disc of the fungus was cur randomly from each petriplate, suspended in 2ml of sterilized water and shaken well. This was examined under low power of microscope on haemocytometer and the number of micro and macro conidia per ml of suspension were calculated using following formula:

$$\text{Conidia/ml} = X \times 25000$$

X = Number of conidia/square of haemocytometer of 1/25 mm²

RESULTS AND DISCUSSION

Observations were recorded on the diameter of the colony. Growth pattern of each colony were recorded as fluffy, partially submerged and the numbers of micro and macro conidia/ml was recorded. It is evident from the data given in Table 1, that all the groups of isolates of *F. oxysporum* f. sp. ciceri differed significantly from each other in their growth

Accepted :
March, 2010

Table 1 : Colony diameter of 20 isolates of *Fusarium oxysporum* f. sp. ciceri on different solid media

Group no.	Medium							Mean (cm)	Isolates included
	Potato dextrose agar	Richard's agar	Rice extract agar	Maize extract agar	Czapeck's agar	Nutrient agar	Yeast extract		
1.	9.00	8.46	7.46	7.93	7.26	8.13	6.80	7.86	B2,B3, Se5 and Se8
2.	8.86	8.16	7.30	7.67	7.67	7.36	7.27	7.62	R1 and Sa2
3.	8.38	7.66	7.56	7.80	7.66	8.03	6.52	7.66	B5, Ri8, Sa4 and Se3
4.	7.83	6.96	6.50	6.96	5.46	6.66	6.18	6.65	B8, Rj4, V4 and V5
5.	8.16	6.40	4.93	7.16	4.50	5.80	5.66	6.08	Ri4, Ri5 and V2
6.	8.42	7.36	7.56	6.66	6.40	5.66	6.28	6.90	Se2, Se5 and V6
Mean (cm)	8.44	7.50	6.87	7.36	6.44	7.00	6.28	-	-

C.D. (P=0.05) for media 0.16 for strain 0.14

B – Bhopal, Ri = Raisen, Sa = Sagar, Se = Sehore and V = Vidisha

on seven solid media. The minimum mean growth (6.08 cm) was obtained in case of group 5 consisting of Ri4, Ri5, and V2, while maximum (7.86 cm) in group 1 of B2, B3, Se6 and Se8. Growth of the pathogen was significantly different on most of the media but yeast extract agar and Czapeck's agar were not significant of each other. In the same way maize extract agar and Richard's agar also did not exhibit any significant difference. The maximum mean growth (8.44cm) was observed on PDA and on yeast extract agar (6.28cm). The interaction effect between isolates and media was also significant statistically. As regards the growth pattern only group 1 of B2, B3, Se6 and Se8 isolates was found to produce the same growth pattern (fluffy). Other groups varied in this respect. On the basis of growth pattern, isolates could be placed into six groups as shown in the Table 2.

The number of micro conidia of *F. oxysporum* f. sp. ciceri on seven solid media were recorded in Table 3. The maximum sporulation (7.61 million/ml) was recorded significantly in all the other media. It was minimum on

nutrient agar (2.60 million/ml) followed by Yeast agar, Rice extract agar, Maize extract agar, Richard agar and Czapeck's agar media.

The maximum micro conidia was produced by group 5 of (4.44 million/ml) Ri4, Ri5 and V2 while group 6 of Se2, Se3 and V6 had minimum micro conidia (3.86 million/ml). The interaction effect between isolates and media with regard to micro conidia production was significant statistically indicating the difference among 6 different groups.

The number of macro conidia produced by isolates of *F. oxysporum* f. sp. ciceri on seven solid media is recorded in Table 4. It is evident from data that maximum macro conidia was produced on PDA (2.50 million/ml),

While on other media, the macro conidia production was recorded significantly. The minimum macro-conidia were observed on nutrient agar (0.67 million/ml) followed by Yeast extract agar, Czapeck agar, Rice extract agar, Richard agar and Maize extract agar.

As regard the groups, maximum macro conidia were

Table 2 : Growth pattern of 20 isolates of *Fusarium oxysporum* f. sp. ciceri on solid media

Group No.	Medium							Isolates included
	Potato dextrose agar	Richard's agar	Rice extract agar	Maize extract agar	Czapeck's agar	Nutrient agar	Yeast extract	
1.	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy	Fluffy	B2, B3, Se6 and Se8
2.	Fluffy	Fluffy	Fluffy	Fluffy	Partially Submerged	Partially Submerged	Fluffy	Ri1 and Sa2
3.	Fluffy	Fluffy	Fluffy	Submerged	Partially Submerged	Submerged	Partially Submerged	B5, Ri8 Sa4 and Se3
4.	Partially Submerged	Partially Submerged	Partially Submerged	Submerged	Submerged	Partially Submerged	Partially Submerged	B8, Rj4, V4 and V5
5.	Fluffy	Fluffy	Submerged	Fluffy	Fluffy	Submerged	Fluffy	Ri4, Ri5 and V2
6.	Submerged	Submerged	Partially Submerged	Partially Submerged	Submerged	Submerged	Submerged	Se2, Se5 and V6

B = Bhopal, Ri – Raisen, Sa = Sagar, Se = Sehore and V = Vidisha

Table 3 : Micro conidia million/ml of 20 isolates of fusarium oxysporum f. sp. ciceri on different solid media

Group No.	Medium							Mean (million/ml)	Isolates included
	Potato dextrose agar	Richard's agar	Rice extract agar	Maize extract agar	Czapeck's agar	Nutrient agar	Yeast extract		
1.	7.83	4.08	2.93	3.66	5.33	2.44	2.95	4.17	B2, B3, Se6 and Se8
2.	7.88	4.16	3.26	3.70	5.56	2.82	2.66	4.29	Ri1 and Sa2
3.	7.26	3.91	2.77	3.52	5.22	2.44	2.58	3.96	B5, Ri8, Sa4 and Se3
4.	7.56	4.08	3.10	3.72	5.24	2.72	2.91	4.19	B8, Rj4, V4 and V5
5.	7.91	4.30	3.16	4.05	5.77	2.91	2.99	4.44	Ri4, Ri5 and V2`
6.	7.26	3.72	2.81	3.14	5.32	2.28	2.50	3.86	Se2, Se5 and V6
Mean	7.61	4.04	3.00	3.63	5.40	2.60	2.76	-	-

C.D. (P=0.05) for media 0.08 for strain 0.05

B = Bhopal, Ri = Raisen, Sa = Sagar, Se = Sehore and V = Vidisha

Table 4 : Micro conidia million/ml of 20 isolates of fusarium oxysporum f. sp. ciceri on different solid media

Group No.	Medium							Mean (million/ml)	Isolates included
	Potato dextrose agar	Richard's agar	Rice extract agar	Maize extract agar	Czapeck's agar	Nutrient agar	Yeast extract		
1.	2.38	1.21	1.11	1.38	1.06	0.73	0.85	1.24	B2, B3, Se6 and Se8
2.	2.44	1.37	1.37	1.38	1.08	0.70	0.78	1.30	Ri1 and Sa2
3.	2.32	1.18	1.15	1.27	1.18	0.62	0.72	1.20	B5, Ri8, Sa4 and Se3
4.	2.61	1.53	1.33	1.72	1.22	0.72	0.83	1.42	B8, Rj4, V4 and V5
5.	2.75	1.35	1.16	1.63	1.24	0.65	0.88	1.38	Ri4, Ri5 and V2`
6.	2.49	1.28	1.19	1.44	1.16	0.61	0.72	1.27	Se2, Se5 and V6
Mean	2.50	1.32	1.22	1.47	1.15	0.67	0.80	-	-

C.D. (P=0.05) for media 0.06 for strain 0.03

B = Bhopal, Ri = Raisen, Sa = Sagar, Se = Sehore and V = Vidisha

formed by group No. 4 (1.42 million/ml) of B2, Rj4, V4 and V5 isolates. The minimum macro conidia were produced by group No. 3 (1.20 million/ml) of B5, Ri8, Sa4 and Se3 isolates. The statistically significant effect of interaction between media and isolates with regard to formation of macro-conidia indicated that the isolates differed from each other. In general, it is clear that much less macro conidia were produced as compared to micro conidia irrespective of isolates of the fungus and different media.

The colony diameter of isolates in general was reduced on all 6 media to a variable extent when compared with PDA. On the other hand, the mean radial growth did not differ much from each other. Although, the data was statistically significant interaction with regard to isolates, the significant interaction effect however, showed the variability amongst the isolates. Probably the qualitative characters can only be the best criterion for such type of grouping of isolates.

The 20 isolates exhibited three types of growth

pattern that is fluffy, partially submerged and submerged. As regards the growth pattern only group 1 of B2, B3, Se6 and Se8 isolates had the same growth pattern (fluffy) on all the media. Group 2 of Ri and Sa2 isolates had fluffy mycelium on PDA, Richards agar, Rice extract agar, Maize extract agar and Yeast extract agar, but it changed to partially submerged on Czapecks and nutrient agar. Similarly, group 5 of Ri4, Si5 and V2 isolates were fluffy on PDA and other media except on rice extract agar and nutrient agar on which their growth was submerged.

As observed, the sporulation was also influenced by different solid media. The higher number of micro conidia as well as macro conidia were recorded on PDA. While the numbers were reduced drastically on other media. The number of macro conidia in all the cases were much less as compared to micro conidia. The data on sporulation were statistically significant so as the isolates on the basis of these character (Khare *et al.*, 1975). The results of present investigation agree with it.

Acknowledgement:

Author is grateful to the authorities of College of Agriculture, Sehore (M.P.) for providing necessary facilities during the course of investigation.

Authors' affiliations:

ASHISH SHRIVASTAVA, Department of Plant Pathology, College of Agriculture, VIDISHA (M.P.) INDIA

REFERENCES

- Gupta, Om, Kotasthane, S.R. and Khare, M.N.** (1983). *Int. Chickpea Newst.*, **17** : 21-22.
- Gupta, Om, Khare, M.N. and Kotasthane, S.R.** (1986). Variability among six isolates of *Fusarium oxysporum* f. sp. *ciceri* causing vascular wilt of chickpea. *Indian Phytopath.*, **32** (2) : 279-281.
- Khare, M.N., Agarwal, S.C., Dhingra, O.D. and Kushwaha, L.S.** (1975). Variability in the growth of eight strains of *Fusarium oxysporum* f. sp. *lentis* on different solid media. *Indian Phytopathology*, **5** : 126-128.
- Kushwaha, L.S., Agarwal, S.C., Khare, M.N. and Dhingra, O.D.** (1974). Vsri ability in *Fusarium oxysporum* f. sp. *lentis* for nutritional requirements. *J.N.K.V.V. Res. J.*, **8**.
- Singh, K.B. and Dahiya, B.S.** (1973). Breeding for wilt resistance in chickpea. Symposium on wilt problem and breeding for wilt resistance in Bengal gram. Sept. 1973 at Indian Agriculture Research Institute, New Delhi, India, pp. 13-14 (Abstr.).
