

Effect of culture media on growth and pigmentation of *Fusarium oxysporum* f. sp. *lentis* isolates

R.G. CHAUDHARY and R.K. SINGH

Division of Crop Protection, Indian Institute of Pulses Research, Kanpur, U.P., India 208 024; E.mail: rgc_1234@yahoo.com

ABSTRACT

The present study was undertaken to find out the suitability of culture media for the study of cultural variability among 40 *Fusarium oxysporum* f.sp. *lentis* isolates using Potato dextrose agar (PDA) and Czapek's Dox agar (CDA) medium. Of them 35 isolates showed growth of <7 mm/day (slow growth rate), while 5 isolates showed >7-10 mm/day (medium growth rate) on CDA. On PDA, all three types of growth rates were observed. On CDA medium, appressed and fluffy growth pattern was observed in 30 and 10 isolates, respectively while on PDA, 6 isolates were appressed and 34 fluffy type. Similarly, all isolates expressed white to dull white mycelia colour on CDA but on PDA, 20 isolates showed white to dull white, 13 isolates showed light orange to dark orange and 7 isolates as light purple to dark purple colour mycelia. Similar variations were also observed with respect to substrate colour produced by the isolates. Light buff to buff colour substrate was produced by 32 isolates, light brown to dark brown by 5 isolates and light purple to dark purple by 2 isolates on CDA. However, on PDA medium, light buff to buff colour substrate was produced by 13 isolates, light brown to dark brown by 18 isolates and light purple to dark purple colour substrate by 9 isolates. Thus, potato dextrose agar medium showed higher differential cultural behaviour of *F. oxysporum* f. sp. *lentis* isolates than Czapek's Dox agar medium, hence, the former was better suited for studying the cultural variability in *F. oxysporum* f. sp. *lentis*.

Key words: Cultural behaviour, Czapek's Dox agar, *Fusarium oxysporum* f. sp. *lentis*, Potato dextrose agar

Wilt (*Fusarium oxysporum* f. sp. *lentis* (*Fol*) (Vasudeva and Srinivasan) is a serious soil and seed borne disease causing economic losses to lentil especially in South America, the Mediterranean basin and South Asia (Khare 1980). Great variability exists in lentil wilt pathogen. Khare *et al.* (1975) reported eight strains of *Fol*. Isolates which differed significantly from each other in respect of growth rate and growth pattern on different media. Similar trends of results were reported by Dhingra *et al.* (1974). In the present study, differential behaviour of 40 *Fol* isolates in terms of cultural variability on potato dextrose agar and Czapek's Dox Agar media was studied with an aim to find out suitable medium for the expression of higher cultural variability among the isolates of *F. oxysporum* f. sp. *lentis*.

MATERIALS AND METHODS

Forty isolates of *Fusarium oxysporum* f. sp. *lentis* (*Fol*) were isolated from lentil wilt samples, collected from different

agro climatic zones of Uttar Pradesh and Bihar, during 2004-05 and 2005-06 (Table 1). Isolations were made from ten infected roots and single spore pure culture was used in the study. The isolates apparently similar to *Fusarium oxysporum* f. sp. *lentis* (*Fol*) were retained for further characterization on potato dextrose agar (PDA) and Czapek's Dox agar (CDA) media using factorial completely randomized design with four replications. Pre-sterilized medium was poured separately in sterilized 90 mm Petri dishes @ 25 ml/plate. After the solidification of medium, 7 mm fungal disk cut from the 7 day old pure culture of isolates were inoculated at the centre of the Petri dish. Inoculated Petri dishes were sealed with parafilm to avoid contamination. All the inoculated Petri plates were incubated in BOD at 25±1°C for 7 days. Following cultural characters were recorded to quantify the variability among the isolates:

1. Colony growth rate at 25°C - slow (<7 mm/day), medium (>7 mm to 10 mm/day), fast (>10 mm/day)
2. Colony growth pattern- fluffy, appressed
3. Substrate colour- light buff to buff, light brown to dark brown, light purple to dark purple or crimson
4. Mycelia colour – white to creamy white or dull white, light orange to dark orange, light purple to dark purple

All cultural characters recorded in the study were qualitative except the growth rate which was sub grouped into slow, medium and fast types.

RESULTS AND DISCUSSION

Colony growth rate: Growth rate of these isolates on CDA medium revealed that thirty five isolates were slow growing and five isolates (35, 55, 67, 70 and 75) had medium growth rate. On PDA medium, 15 isolates were slow growing, 21 isolates had medium growth. On contrary to CDA where none was fast growing, 4 isolates (L-34, 45, 55-2, 66) showed fast growth on PDA medium (Table 1). Thus, potato dextrose agar medium favoured better growth showing 4 isolates as fast, 21 as medium and 15 as slow growing as against 0, 5 and 35 on Czapek's Dox agar medium, respectively.

Colony growth pattern: Out of 40 isolates, 30 expressed appressed type growth pattern on CDA medium while 10 isolates produced fluffy growth pattern. On PDA medium, only 6 isolates expressed appressed, while 34 isolates showed fluffy growth (Table 1). This indicated that growth pattern of

Table 1. Growth rate, growth pattern, mycelium and substrate colour of *Fusarium oxysporum* f. sp. *lentis* isolates on PDA and CDA

Isolate	Place of collection	Growth rate		Growth pattern		Mycelium colour		Substrate colour	
		CDA	PDA	CDA	PDA	CDA	PDA	CDA	PDA
L -2A	Chillaghat, Banda	4.8	6.3	Appressed	Fluffy	White	Creamy white	Buff	Purple
L -2B	Chillaghat, Banda	5.8	8.9	Appressed	Fluffy	White	White	Dull white	Buff
L -3	Piprenda, Banda	6.1	8.9	Fluffy	Fluffy	Dull white	White	Light orange	Buff
L -24B	Sumerpur, Hamirpur	5.4	7.6	Appressed	Fluffy	Dull white	Dark orange	Buff	Dark brown
L -28	Pilakhi, Muraul, Muzaffarpur, Bihar	5.8	10.0	Fluffy	Fluffy	White	Creamy white	Buff	Buff
L -31	TCA, Dholi, Bihar	4.4	3.8	Appressed	Appressed	White	White	Buff	Buff
L -32	Sudehi, Gosaiganj, Lucknow	5.7	6.2	Appressed	Fluffy	White	White	Light brown	Light brown
L -33	Gosaiganj, Lucknow	5.5	9.7	Fluffy	Fluffy	White	Orange	Buff	Brown
L -34	Rahmatnagar, Gosaiganj, Lucknow	6.5	11.7	Appressed	Fluffy	White	Light orange	White	Crimson
L -35	Gotauna, Hydergarh, Barabanki	7.2	9.5	Fluffy	Fluffy	White	Light brown	Dull white	Light orange
L -36	Mahua bojhi, Jagdishpur, Sultanpur	5.7	8.5	Appressed	Fluffy	White	Light orange	Light brown	Light brown
L -37	Amhat, Sultanpur	6.4	6.8	Appressed	Appressed	White	White	White	Buff
L -39	Narharpur, sulatanpur	4.9	6.7	Appressed	Fluffy	Dull white	White	Light brown	Buff
L -40	Poora, aashpur, Derara, Pratapgarh	5.7	8.6	Appressed	Fluffy	White	White	Dull white	Purple
L -41	Shadi- Shadi, Ghajipur	4.8	7.8	Appressed	Fluffy	Creamy white	Dull white	Dull white	Buff
L -44	Newada, Bansaon, Gorakhpur	5.3	8.6	Appressed	Fluffy	White	Dark purple	Dull white	Dark brown
L -45	Bheeti Raut, Sahjanwa, Gorakhpur	6.1	10.3	Appressed	Appressed	White	Dark orange	Creamy white	Dark brown
L -47	Nariyan, Khalilabad, Santkabir Nagar	6.9	8.3	Appressed	Fluffy	White	Light orange	Creamy white	Light purple
L -48	Pachmohani, Saltaua, Basti	6.6	6.6	Appressed	Fluffy	White	Light purple	Dull white	Light purple
L -50	Dhankharpur, Dumriyaganj, Sidharthnagar	4.3	10.0	Appressed	Fluffy	White	White	Creamy white	Dark brown
L -55	Helapur, Hamirpur	7.2	6.9	Appressed	Appressed	White	White	Buff	Light orange
L -55-2	Helapur, Hamirpur	4.2	11.1	Appressed	Fluffy	White	Light purple	Creamy white	Dark brown
L -55-3	Helapur, Hamirpur	5.6	7.2	Fluffy	Fluffy	Dull white	Dark orange	Dull white	Light brown
L -55-4	Helapur, Hamirpur	3.3	3.9	Fluffy	Appressed	White	Purple	Buff	Brown
L -55-5	Helapur, Hamirpur	6.2	4.0	Appressed	Fluffy	Dull white	Dull white	Brown	Light yellow
L -58	Gulalpur, Barabanki	5.4	7.7	Appressed	Fluffy	White	White	Dull white	Light yellow
L -59	Bamour, Jhansi	5.0	8.2	Appressed	Appressed	White	White	Brown	Buff
L -62	Chilla, Birgha, Lalitpur	4.8	6.7	Appressed	Fluffy	Creamy white	Pinkish white	Creamy white	Yellow brown
L -63	Mahrauni, Lalitpur	6.9	8.4	Fluffy	Fluffy	Creamy white	Light orange	Buff	Crimson
L -64	Bamour, Jhansi	5.2	6.7	Appressed	Fluffy	Dull white	Dark orange	Dull white	Dark brown
L -66	Mahrauni, Lalitpur	6.2	12.4	Fluffy	Fluffy	White	White	White	Buff
L -67	Bamour, Jhansi	8.1	6.8	Appressed	Fluffy	Dull white	Dark purple	Light orange	Dark brown
L -68	Madwara, Lalitpur	4.9	7.2	Appressed	Fluffy	Dull white	White	Dull white	Buff
L -71	Mau, Jhansi	5.5	9.0	Appressed	Fluffy	White	White	Creamy white	Brown
L -72	Bamour, Jhansi	5.8	6.3	Appressed	Fluffy	Dull white	Dark orange	Dull white	Light brown
L -73	Mau, Jhansi	5.4	7.6	Appressed	Fluffy	White	Dark orange	Dull white	Light brown
L -76	Chilla, Birgha, Lalitpur	5.8	8.4	Fluffy	Fluffy	White	Dark orange	Buff	Light brown
L -77	Mau, Jhansi	5.9	6.8	Appressed	Fluffy	White	White	White	Buff
L -70	Mau, Jhansi	7.7	7.4	Fluffy	Fluffy	White	Light purple	White	Light purple
L -74	Bamour, Jhansi	7.6	4.5	Appressed	Fluffy	Creamy white	Dark orange	Buff	Dark brown

isolates varied with the changing nutrient supply through the two media.

Mycelia colour : It was interesting to record that all 40 *Fol* isolates on CDA medium produced only white to dull white mycelia while on PDA medium, 20 isolates produced white, creamy white or dull white mycelia while 13 isolates produced light orange to dark orange colour mycelium and rest 7 isolates had light purple to dark purple mycelia colour (Table 1). The differential response of isolates over the two media indicated that mycelium colour expression was wider on potato dextrose agar medium while Czapek's Dox agar did not show any mycelium colour variation. Thus, PDA proved better for the differentiation of mycelium colour than CDA.

Substrate colour: Substrate colour was observed as most unstable character and showed great variation on the

two media from light buff-buff substrate and light brown to dark brown substrate colour on CDA isolates. The substrate colour of *Fol* isolates showed more variation on PDA medium (Table 1). On the basis of differential response of isolates on two media, it was concluded that substrate colour was again not a stable character and varied according to nutrition of the two media.

Thus, the cultural variation of 40 isolates of *Fol* on PDA and CDA medium expressed great variation in respect of different cultural characters. Vasudeva and Srinivasan (1952) also advocated *Fol* to grow on PDA. Khare (1980) differentiated 8 isolates of lentil wilt pathogen on the basis of colour of mycelium. *Fol* isolates showed variable preference for different nutrients especially carbon, nitrogen, sulphur and phosphorus (Kushwaha *et al.* 1974) and therefore the

Table 2. Distribution of *Fusarium oxysporum* f. sp. *lentis* isolates in respect of cultural characters on PDA and CDA

Parameter	No. of isolates	
	PDA	CDA
Growth rate		
Slow	15	35
Medium	21	5
Fast	4	0
Growth pattern		
Appressed	6	30
Fluffy	34	10
Mycelium colour		
White, creamy white or dull white	20	40
Light orange to dark orange	13	0
Light purple to dark purple	7	0
Substrate colour		
Light buff to buff	13	33
Light brown to dark brown	8	5
Light purple to dark purple	9	2

two media tested in this study (one semi synthetic and one synthetic) showed variation in the cultural characters of these

isolates. On the basis of above results it can be inferred that the potato dextrose agar medium is better for studying the cultural variability of *Fusarium oxysporum* f.sp. *lentis* isolates than Czapek's Dox agar medium.

REFERENCES

- Dhingra OD, Agrawal SC, Khare MN and Kushwaha LS. 1974. Temperature requirements of eight strains of *Fusarium oxysporum* f. sp. *lentis* causing wilt of lentil. Indian Phytopathology 27: 408-410.
- Erskine W and Saxena MC. 1991. Breeding lentil at ICARDA for southern latitudes. Paper presented in ICAR-ICARDA Seminar 'Lentil in South Asia', New Delhi, 11-15 March 1991, pp. 1-19.
- Khare MN. 1980. 'Wilt of lentil' JNKVV, Jabalpur, pp. 155.
- Khare MN, Agrawal SC and Joshi LK. 1975. Studies on diseases of lentil. Proc. XIV Annual Workshop on Rabi Pulses, APAU, Hyderabad, 29 Sept to 1st Oct., 1975, pp. 1-6.
- Kushwaha LS, Agrawal SC, Khare MN and Dhingra OD. 1974. Variability in *Fusarium oxysporum* f. sp. *lentis* for nutritional requirements. JNKVV Research Journal 8: 80-85.
- Vasudeva RS and Srinivasan KV. 1952. Studies on the wilt disease of lentil (*Lens esculenta* Moench). Indian Phytopathology 5: 25-32.