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STUDIES ON WILT DISEASES OF PULSES
I: VARIATION AND TAXONOMY OF
FUSARIUM SPECIES ASSOCIATED WITH
WILT DISEASE OF PULSES.

By

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A number of isolates of *Fusarium* inciting wilting in gram (*Cicer arietinum* L.), lentil (*Lens esculenta* Moench.) and pigeon pea (*Cajanus cajan* (L.) Millsp.) were studied along with their variants produced on monoconidial cultures on three different media, namely potato dextrose agar, oat meal agar and steamed rice. Variations in cultural characters, e.g. amount of aerial mycelium, texture, strength production of sporodochia and sclerotia were noted among the different isolates and their variants. Some of the monoconidial cultures from gram and lentil isolates produced sporodochia, although these structures were lacking in the original isolates. In view of the non persistence of the characters which are utilized in the classification of *Fusaria* according to Wollenweber and Reinking's (1935) system, new names for gram and lentil *Fusaria* e.g. *Fusarium oxysporum* f. *ciceri* and *Fusarium oxysporum* f. *lentis* respectively have been proposed in place of *F. orthoceras* var. *ciceri* and *F. orthoceras* var. *lentis*. Naming of *Fusarium udum* as *F. oxysporum* f. *udum* proposed by Snyder and Hansen (1940) has been supported.

INTRODUCTION

Wilt diseases of pigeon pea (*Cajanus cajan* (L.) Millsp.), gram (*Cicer arietinum* L.) and lentil (*Lens esculenta* Moench.) caused by *Fusarium* spp. are quite common in West Bengal (India). Wilt of pigeon pea was first reported by Bulter (1910) who named the causal fungus as *Fusarium udum*. Padwick (1940) after a critical study of the *Fusarium* isolated from wilted gram plants considered the incitant to be a new variety of *F. orthoceras* and named it *F. orthoceras* var. *ciceri*. Vasudeva and Srinivasan (1952) reported a *Fusarium* from wilted lentil plants and named it as *F. orthoceras* var. *lentis*. In view of the cultural variations in

Fusarium spp. and the resultant complications in the problem of their taxonomy Snyder and Hansen (1940) advocated a new species concept modifying Wollenweber and Reinking's (1935) system in which they combined all the species in the section *Elegans* into one species, namely *Fusarium oxysporum* and proposed the creation of forma specialis under this species based on pathogenicity.

The investigation reported here were undertaken to find the range of variability in the *Fusaria* inciting wilt diseases of gram, lentil and pigeon pea and their implications on the taxonomic position of the *Fusaria* isolated from these crops.

MATERIALS AND METHODS

Diseased plant materials of gram, lentil and pigeon pea were collected from different parts of West Bengal. From the roots and stems of these materials *Fusarium* spp. were isolated on *potato dextrose agar* slants. The hyphae coming out of the infected tissues were transferred on Petridishes containing *potato dextrose agar*, *oat meal agar* and *steamed rice* respectively and incubated at 25°C for 20 days after which cultural characteristics of the different isolates were studied.

From each of the original isolate obtained on *potato dextrose agar* slants from infected tissues, 20 monoconidial cultures were made following the method described by Hansen and Smith (1932). The monoconidial cultures which showed variations from their parental types were also grown on Petriplates as before along with their corresponding parental types. For pathogenecity test of *Fusarium*, isolates were grown on *sand maize* medium (crushed maize 25.0 gm., fine sand 75.0 gm, distilled water 100 ml.) in 250 ml. Erlenmeyer flasks. After 15 days growth the inoculum from the flask was mixed thoroughly with the soil (sterilized) in pots. After 7 days of inoculation of soil, seeds of respective host plants were sown. Percentage of wilting after six weeks sowing was noted.

Of the different isolates studied, variants were produced in monoconidial cultures from isolates FG8(FG8A), FG12(FG12A, Fg12B), Fg13(FG13A) from gram, FL1(FLIA), FL3 (FL3A) from lentil and FA2(FA2A) and FA3(FA3A, FA3B) from pigeon pea.

RESULTS

The macroscopic growth characters as well as the conidial measurements of the first cultures together with the variants produced and of the cultures of *Fusarium orthoceras* var. *ciceri*, *Fusarium orthoceras* var. *lentis* and *Fusarium udum*, obtained from Indian Agricultural Research Institute, New Delhi are presented in Table 1.

Table 1. *Cultural and Conidial characters of different isolates of Fusarium from Gram, Lentil and Pigeon Pea on three different media after 20 days growth at 25°C.*

Host	Isolate	Media	Cultural characters					Conidial characters			
			Aerial Mycelium	Colour of substratum	Texture	Zonation	Sporodochia	Sclerotia	Length (in μ)	Breadth (in μ)	Septation
Gram	FG ₂	P.D.A. O.M.A. S.R.	Scanty Scanty ++	Champgne Cream Red oxide		+			9.8-29.7(14.0) 9.9-23.1(14.1) 6.6-13.2(9.8)	2.7-3.8(3.0) 3.0-3.8(3.4) 2.3-3.3(2.5)	0-3 0-3 0-2
	FG ₄	P.D.A. O.M.A. S.R.	++ ++ ++	Light biscuit Pale cream Post office red	Cottony Cottony Cottony	+	+		6.6-23.1(13.6) 6.6-23.1(13.6) 6.6-19.8(10.4)	2.8-3.3(2.5) 2.5-3.3(2.9) 2.8-3.3(3.0)	0-2 0-2 0-2
	FG ₈	P.D.A. O.M.A. S.R.	++ ++ ++	Chocolate Pale cream Maroon .	Wooly Wooly Cottony	+	++		6.6-39.6(18.0) 6.6-33.3(17.0) 6.6-26.4(9.6)	2.4-3.7(3.5) 3.0-3.7(3.2) 2.6-3.3(2.8)	0-4 0-4 0-3
	FG _{8A}	P.D.A. O.M.A. S.R.	Scanty Scanty ++	Beige Champgne Imperial brown	Cottony		++ ++ ++		9.9-39.6(21.8) 9.9-39.6(20.8) 9.9-26.1(14.3)	2.4-3.7(3.2) 3.0-3.7(3.2) 2.6-3.3(2.8)	0-4 0-4 0-3
	PG ₁₂	P.D.A. O.M.A. S.R.	++ ++ ++	Chocolate Pale cream Crimson	Cottony Cottony Cottony	+	++ ++ ++	+	9.9-26.4(13.1) 9.9-26.4(14.2) 6.6-19.8(10.9)	3.3-4.2(3.6) 3.3-3.9(3.7) 2.3-3.3(2.9)	0-3 0-3 0-3
	PG _{12A}	P.D.A. O.M.A. S.R.	++ ++ Scanty	Darm brown Light purple brown	Cottony		++ ++ ++		9.9-29.7(15.9) 9.9-29.7(17.1) 6.6-23.1(12.5)	2.4-4.4(3.4) 2.3-4.2(3.3) 2.3-3.8(2.8)	0-4 0-4 0-3
	FG _{12B}	P.D.A. O.M.A. S.R.	Scanty Scanty ++	Beige Pale cream Light brown	Wooly Wooly Wooly		++ ++ ++		9.9-26.4(15.0) 9.9-26.4(16.3) 6.6-23.1(14.5)	2.8-4.1(3.2) 2.7-4.9(3.5) 2.8-4.1(3.0)	0-3 0-3 0-3
	FG ₁₃	P.D.A. O.M.A. S.R.	Scanty Scanty ++	Terracota Deep Indian red Signal red	Cottony			+	6.6-16.5(9.4) 6.6-19.8(10.1) 6.6-13.2(8.1)	3.0-3.3(3.0) 3.3-3.4(3.3) 3.3-3.4(3.3)	0-1 0-1 0-1

Table 1. (Contd.) : Cultural and Conidial characters of different Isolates of *Fusarium* from Gram, Lentil and Pigeon Pea on three different media after 20 days growth at 25°C.

Host	Isolate	Media	Cultural characters					Conidial characters			
			Aerial Mycelium	Colour of substratum	Texture	Zonation	Sporodochia	Sclerotia	Length (in μ)	Breadth (in μ)	Septation
Lentil	FG ₁₃ A	P.D.A.	Scanty	Skashine					6.6-23.1(12.7)	2.7-4.1(3.0)	0-2
		O.M.A.	Scanty	Light straw					6.6-26.4(13.5)	2.6-4.4(3.1)	0-3
		S.R.	++	Maroon	Wooly		+		6.6-19.8(11.3)	2.4-3.9(3.0)	0-3
	<i>F. orthoceras</i> var. <i>Ciceri</i> I.C.A.R.	P.D.A.	Scanty	Pale cream	Cottony				6.6-29.7(12.5)	3.3-4.2(4.0)	0-2
		O.M.A.	Scanty	Pale cream	Cottony				6.6-29.7(13.5)	3.3-3.8(3.7)	0-2
		S.R.	+++	Deep cream	Cottony				6.6-23.1(9.3)	2.9-3.2(3.0)	0-2
	FL ₁	P.D.A.	+++	Deep cream	Cottony				9.9-39.6(17.1)	2.9-3.7(3.2)	0-4
		O.M.A.	+++	Deep cream	Cottony	+	+		9.9-39.6(15.2)	3.3-3.7(3.4)	0-4
		S.R.	+++	Crimson	Cottony				6.6-26.4(11.7)	3.2-3.4(3.3)	0-3
	FL ₄ A	P.D.A.	++	Light stone	Cottony		++		9.9-39.6(19.3)	2.8-4.4(3.7)	0-4
		O.M.A.	Scanty	Light stone	Cottony		++		9.9-39.6(17.1)	2.7-4.0(3.0)	0-4
		S.R.	+++	Chocolate	Cottony				6.6-36.3(11.7)	2.6-3.8(3.0)	0-3
FL ₂	P.D.A.	++	Deep Indian red	Cottony				6.6-29.7(10.8)	3.2-4.0(3.4)	0-3	
	O.M.A.	+++	Beige	Cottony				6.6-26.4(11.5)	2.8-3.8(3.1)	0-3	
	S.R.	+++	Maroon	Cottony		+		6.6-16.5(8.3)	2.7-3.3(2.9)	0-3	
FL ₃	P.D.A.	++	Nut brown	Cottony				9.9-26.4(12.5)	3.0-3.3(3.2)	0-3	
	O.M.A.	+++	Maroon	Cottony				9.9-33.0(13.4)	3.0-3.3(3.1)	0-3	
	S.R.	+++	Maroon	Cottony				6.6-29.7(10.4)	3.1-3.3(3.2)	0-3	
FL ₃ A	P.D.A.	Scanty	Champagne	Cottony		+		9.9-33.0(16.11)	2.6-4.0(3.1)	0-3	
	O.M.A.	Scanty	Light biscuit	Cottony				9.9-33.0(18.8)	2.8-4.1(3.0)	0-3	
	S.R.	+	Deep cream	Cottony				9.9-26.4(13.2)	2.4-3.9(2.8)	0-3	
<i>Fusarium orthoceras</i> Var. <i>lentis</i> (I.A.R.I.)		P.D.A.	Scanty	Pale cream	Cottony			6.6-39.6(12.4)	3.0-3.3(3.2)	0-3	
		O.M.A.	Scanty	Pale cream	Cottony				6.6-36.3(14.2)	3.2-3.3(3.2)	0-3
		S.R.	+	Deep cream	Cottony				6.6-33.3(10.6)	3.0-3.3(3.0)	0-3

Pigeon pea FA ₁	P.D.A. O.M.A. S.R.	++ ++ ++	Deep cream Jasmine yellow Signal red	Cottony Wooly Wooly	6.6-36.3(23.0) 6.6-36.3(25.6) 6.6-29.7(17.6)	3.0-4.4(4.1) 2.7-4.3(4.0) 2.2-3.8(3.4)	0-3 0-3 0-3
FA ₂	P.D.A. O.M.A. S.R.	Scanty Scanty +	Pale cream Jasmine yellow Maroon	++ ++ ++	9.9-46.2(31.1) 9.9-42.9(33.9) 9.9-42.9(23.5)	2.9-4.6(4.1) 2.7-4.3(3.9) 2.3-3.8(2.9)	0-4 0-4 0-4
FA _{3A}	P.D.A. O.M.A. S.R.	+ ++ ++	Service brown Yellow Deep orange	Wooly Wooly Cottony	6.6-33.0(17.1) 6.6-36.3(19.4) 6.6-29.7(17.6)	3.0-4.3(3.9) 3.2-4.1(4.0) 2.8-4.2(3.6)	0-4 0-4 0-4
FA ₃	P.D.A. O.M.A. S.R.	Scanty Scanty +	Pale cream with navy blue sectors Champagne Terracota	Wooly Wooly Wooly	6.6-39.6(26.2) 6.6-39.6(24.7) 6.6-36.3(19.5)	2.8-4.4(4.0) 3.2-4.5(4.2) 2.9-4.4(4.1)	0-4 0-4 0-4
FA _{3A}	P.D.A. O.M.A. S.R.	Scanty Scanty +	Cream Golden yellow	Wooly Wooly Wooly	6.6-39.6(24.3) 6.6-39.6(24.4) 6.6-36.6(19.1)	2.6-4.1(3.9) 2.8-4.6(3.7) 2.4-4.2(3.6)	0-4 0-4 0-4
FA ₃	P.D.A. O.M.A. S.R.	Scanty Scanty ++	Navy blue Navy blue Olive drab	Wooly Wooly Wooly	6.6-39.6(25.2) 6.6-36.3(24.8) 6.6-36.3(20.7)	2.8-4.0(3.9) 2.4-4.4(3.7) 2.5-4.4(3.6)	0-4 0-4 0-4
Pigeon pea FA ₄	P.D.A. O.M.A. S.R.	Scanty Scanty +	Light straw Pale Cream Crimson	++ ++ ++	6.6-36.3(22.6) 6.6-36.3(22.3) 6.6-33.0(16.0)	2.5-4.2(3.8) 2.8-4.0(3.7) 2.4-3.9(3.5)	0-3 0-3 0-3
<i>Fusarium</i> <i>udum</i> (I.A.R.I.)	P.D.A. O.M.A. S.R.	Scanty Scanty ++	Deep cream Pale cream Deep cream	++ ++ ++	6.6-39.6(29.4) 6.6-39.6(27.9) 6.6-33.0(29.9)	2.4-4.0(3.9) 2.4-4.4(4.1) 2.2-3.9(3.3)	0-3 0-3 0-3

P.D.A. = potato-dextrose agar ; O.M.A. = oat meal agar ; S.R. = steamed rice ; + = moderate, present ; ++ = abundant, marked.

Colour of the isolates were compared with the help of "Colours for ready mixed paints" (Published by Indian Standards Institute).

From Table 1 it may be noted that variations exist among the isolates of pulse *Fusaria* regarding the amount of aerial mycelium, texture, colour of substratum, zonation, production of sporodochia and sclerotia. The variant isolates differed considerably from their parental types. The general findings are as follows :—

Cultural characters

(i) Aerial mycelium in most of the isolates from gram and pigeon pea on potato dextrose and oat meal agar are scanty while those from lentil show abundant mycelial growth. Mycelial growth on steamed rice is more abundant.

(ii) Colour of the substratum shows considerable variation in different isolates. It is, however, more pronounced on steamed rice medium.

(iii) Zonation is noted in a few isolates. It is more abundant in oat meal agar.

(iv) None of the original isolates from gram and lentil produce sporodochia, but their variants, FG₈A, FG₁₂A and FG₁₃A (among isolates from gram) and FL₁A (among isolates from lentil) produce these. All the isolates from pigeon pea, except FA₁, however produce sporodochia. *Steamed rice* medium was not found to favour production of sporodochia.

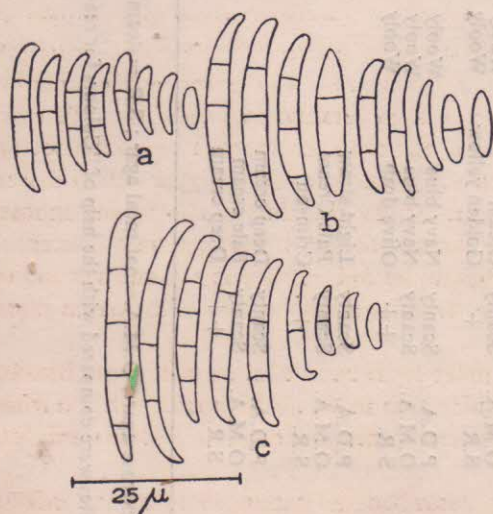
Conidial characters (Text-Fig. 1)

Size :—In their average length and breadth the conidia produced by isolates from gram, lentil and pigeon pea do not show much variation among their

own isolates on *potato dextrose* agar and *oat meal* agar. Some variation is, however, noted on the range of length. The conidia produced on *steamed rice* medium are smaller in size.

The conidia of isolates from pigeon pea are larger in length as compared to those of isolates from gram and lentil which do not show much difference.

The average length of the conidia of the variants of gram and lentil isolates are larger than the parental ones. In case of pigeon pea isolates, however, the variants do not show much difference in conidial size in comparison to their parental types.



TEXT-FIG. 1. Conidia of *F. oxysporum* f. *ciceri* (a), *F. oxysporum* f. *lentis* (b), and *F. oxysporum* f. *udum* (c).

Shape :—The conidia of the isolates from gram and lentil are ovoid to spindle shaped, slightly curved or almost cylindrical at the middle, somewhat, curved at one end into an epical cell with narrowly and smoothly rounded tip,

typically thin walled. In pigeon pea isolates the conidia are kidney shaped to falcate with curvature, long and narrow, typically thin walled, markedly curved at one end to a somewhat hooked apical cell.

Septation :—The range of septation in gram, lentil and pigeon pea isolates varies from 0-4, the mode of septation in case of gram and lentil isolates is 1, while in case of pigeon pea isolates it is 3.

Cross inoculation studies

Cross inoculation tests were undertaken by inoculating *Fusarium* isolates from gram, lentil and pigeon pea on a number of pulse crops, namely gram, lentil, pigeon pea, mung (*Phaseolus aureus* Ham.) urid (*Lythyrus sativus* L.), Pea (*Pisum sativum* L.) and cowpea (*Vigna sinensis* Endl.).

The isolates of *Fusarium* studied are specific on their respective host plants, i.e. they can not infect any other host plant except their own.

DISCUSSION

Fusarium orthoceras var. *ciceri* Padwick, was described by Padwick (1940) as the incitant of wilt of gram (*Cicer arietinum* L.) in the year in which Snyder and Hansen (1940) also propounded the new species concept in the genus *Fusarium*. Snyder and Hansen (1940), according to the new principles in the concept of speciation in the genus *Fusarium*, revised the section *Elegans* and merged all the species belonging to its three sub-sections, namely *Orthocera*, *Constrictum* and *Oxysporum*, into a single species *F. oxysporum* and proposed the creation of biologic forms within this species based on relative pathogenicity. Padwick (1940a) in a later communication stated that even the conception of the section *Elegans*, *Lateritium* and *Liseola* of Wollenweber and Reinking's (1935) system has so altered that they are no longer entirely distinct, but he (1941) was opposed to the mass revision of the genus and did not accept wholly the new concept of Snyder and Hansen (1940). Consequently he did not rename gram wilt organism, but retained the previous name *F. orthoceras* var. *ciceri*. This name is still in use in India.

The views of Padwick (1940a, 1941) in respect of gram wilt organism was upheld by later workers in case of lentil (*Lens esculenta* Moench.) wilt *Fusarium*, when Vasudeva and Srinivasan (1952) named the organism as *F. orthoceras* var. *lentis* Vasudeva and Srinivasan.

Fusarium udum Butler was first described by Butler (1910) as the incitant of wilt of pigeon pea (*Cajanus cajan* (L.) Millsp.). He in a later communication (1926) considered the fungus to be closely akin to *F. vasinfectum* and even *F. oxysporum*. He, however, preferred to retain the name *F. udum*. Wollenweber and Reinking also retained the name *F. udum* under the section *Elgans* in their "Die Fusarien." Snyder and Hansen (1940), however, treated the fungus as *F. oxysporum f. udum* according to their new species concept.

The section *Elegans*, as pronounced by Wollenweber (1917) contains three subsections, namely *Orthocera*, *Constrictum* and *Oxysporum*, based upon the presence or absence of sporodochia and width of macroconidia. The subsection *Orthocera* is distinguished from the others in the absence of sporodochia and the width of macro conidia measuring $3.2-4\mu$. The subsection *Oxysporum* has sporodochia produced and greater width of macroconidia ($3.7-5\mu$). The subsection *Constrictum* is characterized by presence of sporodochia and smaller width of macroconidia.

Isolates of *Fusarium* causing gram and lentil wilt, in the present study, shows cultural variants which produce sporodochia, though the parental type is non-sporodochial. Breadth of conidia in majority of cases are between $2.8-4\mu$, but in a number of cases the average breadth is $3.5-4\mu$. So on the basis of the presence or absence of sporodochia and differences in width of conidia the gram and lentil *Fusaria* cannot be maintained in a separate subsection *Orthocera* as described by Wollenweber (1917). There is hardly any difference among the isolates from gram and lentil in their morphology, though they are selected in their pathogenicity. Breadth of conidia of the isolates from pigeon pea also fall within the same range as those of gram and lentil *Fusaria*.

From the data presented on the study of the different aspects of a large number of monoconidial isolates of *Fusarium* from gram, lentil and pigeon pea and from the preceding discussions it is proposed that *Fusarium orthoceras* var. *ciceri* Padwick should be renamed as *F. oxysporum* f. *ciceri*. Padwick emend Synder and Hansen. Similarly *Fusarium orthoceras* var. *lentis* Vasudeva and Srinivasan should be *F. oxysporum* f. *lentis* Vasudeva and Srinivasan emend Snyder and Hansen.

Padwick's (1940a) contention of *F. udum* to be a distinct species is due to its formation of sporodochia, conidia tending to become hooked at the apices and bright orange or yellow colours produced on steamed rice medium. Substratum colour of the isolates from pigeon pea in the present study varied from terracota to deep cream to narrow orange or red on steamed rice. Similar range of colouration has also been showed on the same substratum by the isolates from gram and lentil. Sporodochial types in the variants of the isolates from gram and lentil has already been indicated earlier. Regarding the predominance of hooked conidia in *F. udum*, i.e. the tendency of the conidia to be curved at the ends, it is very strong in *F. udum* no doubt, but the presence of such conidia has also been noted in some isolates from gram and lentil. In a broader concept Synder and Hansen's (1940) treatment of *Fusarium udum* Bult. as *F.oxysporum* f. *udum*, therefore, appears to be thoroughly justified as evidenced in discussion above.

REFERENCES

- Butler, E. J. (1910). The wilt disease of pigeon pea and the parasitism of *Neocosmospora vasin-gecta*. Mem. Dept. Agric. India, Bot. Ser., 2, 1-64.

- (1926). The wilt disease of cotton and sesamInum n idia. *Agric. Jour. India*, **21**, 268-273.
- Hansen, H. N. and R. E. Smith (1932). The mechanism of variation in imperfect fungi : *Botrytis cinerea*. *Phytopathology*, **22**, 953-964.
- Padwick, G. W. (1940). The genus *Fusarium* III. A critical study of the fungus causing wilt of gram (*Cicer arietinum*) and its related species in the subsection Orthocera, with special reference to the variability of key characters. *Indian Jour. Agric. Sci.*, **10**, 241-284.
- (1940a). The genus *Fusarium* IV. *Fusarium udum* Bult., *Fusarium vasinfectum* Atk., and *Fusarium lateritium* Nees. Var. *uncinatum* Wr. *Indian Jour. Agric. Sci.*, **10**, 863-878.
- (1941). The genus *Fusarium* VI. A recent attempt at mass renision. *Indian Jour. Agric. Sci.*, **11**, 663-674.
- Snyder, W. C. and H. N. Hansen. (1940). The species concept in *Fusarium*. *American Jour. Botany*, **27**, 64-67.
- Vasudeva, R. S. and K. V. Srinivasan (1952). Studies on wilt disease of lentil (*Lens esculenta* Moench.) *Indian Phytopathology*, **5**, 23-32.
- Wollenweber, H. W. (1917). *Fusaria* autographic delineata. *Ann. Micol.* **15**, 1-56.
- Wollenweber, H. N. and O. A. Reinking (1935). *Die Fusarien*, Paul Parey Berlin.

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