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Research Article

VARIABILITY AMONG ISOLATES OF *Fusarium oxysporum* f. Sp. *UDUM* CAUSING PIGEONPEA WILT

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Abstract- Cultural and morphological variability were determined among the eight isolates of *Fusarium oxysporum* f.sp. *udum*. The colony diameter ranged from 80.00 to 90 mm after nine days of incubation at 28 ± 1ºC. The size of macroconidia ranged from 16.5 to 20.6 µm (18.47 µm) × 2.1 to 4.1 µm (2.98 µm) in RFU to 24.0 to 37.0 µm (30.24 µm) × 2.6 to 4.0 µm (3.52 µm) in BeFU. The size of microconidia varied from 4.9 to 11.8 µm (8.10 µm) × 2.2 to 4.0 µm (2.98 µm) in BeFU to 8.3 to 17.4 µm (12.94 µm) × 2.5 to 5.8 µm (4.0 µm) in RFU. The macro conidia were 1 to 4 septate, fusaroid to sickle shaped. The shape of chlamydospores varied from round in KoFU, RFU, RjFU and BeFU isolates to oval in isolate RgFU and long oval in KFU, MFU and DFU. The size of round chlamydospores ranged from 3.8 to 4.7 µm in RFU to 6.4 to 8.7 µm in diameter in RjFU and long oval chlamydospores were 11 to 13 × 3 to 4 µm in MFU to 19 to 21 × 4 to 5 µm (Length × Breadth) in DFU.

Keywords Chlamydospores, Colony, *Fusarium*, Macroconidia

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Introduction

Pigeonpea (*Cajanus cajan* L. Millsp.) is one of the most widely-grown and eaten drought tolerant grain legume in semi-arid tropical and sub-tropical countries of the world [7,12]. It is the fifth prominent pulse crop in the world and second most important pulse crop after chickpea in India [13]. Wilt caused by *Fusarium oxysporum* f. sp. *udum* is the one of the most important soil borne disease of pigeonpea and was first described in 1906 from Bihar state in India [1,2]. The disease is capable of causing 100 per cent yield loss, when it occurs at pre-pod stage. The loss may 67 per cent at pod maturity stage and at pre-harvest stage it can be 29.50 per cent in a susceptible variety [6,11,12]. The pathogen is both seed and soil borne in nature hence management of the disease is very difficult [4,10]. Several strategies have been employed for the management of this disease, among them host plant resistance is the major one. Due to breakdown in the face of high pathogenic variability in the pathogen population, the usefulness of many resistant cultivars is restricted to only a few years [1]. This has led to the attention of possible existence of the races. Keeping this background the present study was designed to know the variability existing in the growth and morphology of the eight isolates collected from different pigeonpea growing regions of Chhattisgarh.

Materials and Methods

Fungal isolates

Disease samples, naturally infected pigeonpea plants showing typical vascular wilt symptoms just after initiation of the disease at different development stages were collected from eight major pigeonpea growing districts of Chhattisgarh [Table-1]. Collected samples were brought to laboratory and the pathogen was successfully isolated. Pure culture of the isolates of *Fusarium udum* was obtained by implying single spore isolation method [3]. All of the isolates were maintained on PDA medium at 28±1°C for further studies.

Pathogenicity test

Pathogenecity of the isolated organism, *F. oxysporum* f. sp. *udum* was proved in glass house condition by implying Koch's postulate. The isolated fungus was multiplied in 100 ml Potato Dextrose broth medium in 250 ml conical flask. A five mm disk of seven days old fungal culture was placed in each flask containing 100 ml broth and incubated at 25 ± 2°C for seven days. After incubation the entire fungal mat bearing conidia from each flask was macerated in mortar-pestle and macerated fungal growth was suspended in 100 ml distilled water and spores concentration was maintained at 2 × 10^6 conidia per ml. Test seedlings of 8-10 days old, grown in pots containing sterilized soil: sand in the ratio of 7:3 were removed very carefully and dipped in the spore suspension for at least 5 minutes [5]. The seedlings were then transplanted back in their respective pots. Seedlings dipped in sterilized water for five minutes and transplanted back in same pots served as control. Three replications were maintained for culture and control. Observations of wilt were recorded after appearance of symptoms.

Cultural and morphological variability studies

Potato Dextrose Agar medium was used as basic medium for cultural and morphological variability studies among different isolates of test fungus. Twenty ml of PDA media was poured aseptically into petriplates of 90 mm diameter. Before pouring it was supplemented with streptomycin sulphate in lukewarm medium in order to check the bacterial contamination. Five mm disc from an actively growing zone of eight days old culture was placed upside down at the centre of the solidified medium and were incubated at 28 ± 1ºC in BOD incubator for maximum 22 days. Total three culture plates of each isolate were maintained and observations for the cultural characteristics and pigmentation were recorded after third, fifth, seventh and nineth days of inoculation.

Size, shape, septation and colour of macro conidia, micro conidia and chlamydospore were recorded for morphological studies. A clear slide was prepared from 12 days old culture, stained with cotton blue and observed under the calibrated compound microscope. The length and breadth of twenty macro

conidia and micro conidia for each of three replications were measured. The size and shape of chlamydospores were recorded by using 22 days old culture.

Results and Discussion

Cultural and morphological characters studied on Potato Dextrose Agar at room temperature showed variation among eight isolates of *F. oxysporum udum.* Variation observed among the isolates with respect to colony diameter, colony characters, pigmentation, conidial and chlamydospore characters. Colony diameter of pathogen was recorded at four different sets of incubation period *viz.* 3 DAI, 5 DAI, 7 DAI and 9 DAI. It was revealed from the experimental data presented in [Table-1], [Fig-1(a)] that colony diameter increased with increasing the days of incubation. Maximum colony diameter (90 mm) was observed in case of KFU, RjFU, KoFU and BeFU isolates followed by isolates DFU (87 mm), RgFU (85 mm) and MFU (83 mm) while least colony diameter (80 mm) was of RFU after 9 days of incubation.

Table-1 *Cultural characters of different isolates of Fusarium oxysporum udum*

Isolate designation	Isolate collection place (Village / place)	Colony diameter (mm) at different days of incubation				Colony characters	Pigmentation
		3	5	7	9		
RFU	Raipur IGKV Research farm	22	39	60	80	White suppressed growth with smooth margin	Light brown with centre pink
RgFU	Raigarh (Karpipali)	24	42	73	85	White to light yellow, partially appressed with concentric rings	Dark yellow
KFU	Kawardha (Kapa)	30	53	80	90	White dense cottony fluffy with serrated margin	Light yellow
RjFU	Rajnandgaon (Mohara)	32	54	83	90	White sparse with smooth margin	Pinkish brown
KoFU	Korea (Navagaon)	30	53	84	90	White dense partially appressed with serrated margin	Light yellow to brown
BeFU	Bemetara (Chherkapur)	39	57	86	90	White fluffy with serrated margin	Yellow
MFU	Mungeli (Dabo)	26	43	63	83	Whitish to light yellow fluffy with serrated margin	Brown with centre black to dark brown
DFU	Durg (Nandvai)	27	46	74	87	Whitish to light yellow fluffy with serrated margin	Dark pink
Mean		28.75	48.38	75.38	86.88		
SEm (±)		1.19	1.44	1.34	0.41		
C.D. (5%)		3.60*	4.36*	4.05*	1.23*		

* Significant at 5% level of significance

Isolate RFU produced white suppressed mycelial growth, smooth margin with light brown (centre pink) pigmentation, while isolate RgFU produced white to light yellow mycelia, partially appressed with concentric rings and dark yellow pigmentation. Isolate KFU produced white dense cottony fluffy mycelia with serrated margin and light yellow pigmentation while isolate RjFU produced white sparse mycelia with smooth margin and pinkish brown pigmentation. Isolate KoFU produced white dense partially appressed mycelia with serrated margin and light yellow to brown pigmentation. Isolate BeFU produced white fluffy mycelia with serrated margin and yellow pigmentation while isolate MFU produced whitish to light yellow fluffy mycelia with serrated margin and brown with centre black to dark brown pigmentation and isolate DFU produced whitish to light yellow fluffy mycelia with serrated margin and dark pink pigmentation [Table-1], [Fig-1(b)]. Existence of such variability among the isolates is well documented by many workers. Nene *et al.* [11] have classified *F. oxysporum* f. sp. *udum* in to 12 distinct groups based on cultural characters. Reddy and Choudhary [15] grouped six isolates of *F. oxysporum* f. sp. *udum* into three distinct groups based on colony characters. Kiprop *et al*. [7] characterized the causal agent of wilt disease of pigeonpea, from Kenya, India and Malawi according to their cultural characteristics, pathogenicity and vegetative compatibility group (VCG). Mahesh *et al*. [10] studied the existence of variation among forty one *Fusarium oxysporum* f. sp. *udum* isolates collected from different parts of India and with respect to mycelial colour, pigmentation and colony characters. Pande *et al*. [12] reported that *F. oxysporum* f. sp. *udum* isolates have great variation in mycelial color, substrate color, mycelial growth and sporulation. Some isolates of *F. oxysporum* f. sp. *udum* also show great variation in conidial length, conidial septation and growth rate.

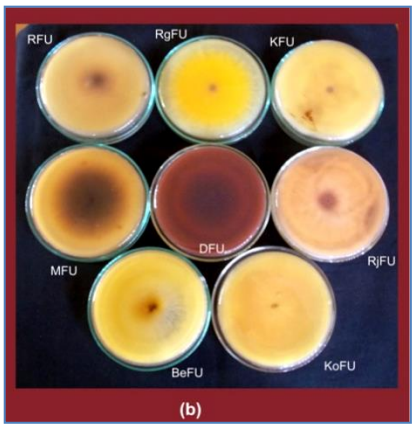

Fig-1 *Fusarium oxysporum* f. sp. *udum* (a): Cultural characteristics (b): Colony pigmentation

Morphological studies also revealed the variation of micro and macro conidia among eight isolates of *F. oxysporum* f. sp. *udum*. Microconidia of isolate RFU was long and curved with narrow rounded ends, while it was long elliptical, slightly curved with rounded ends in RgFU and elliptical with slight narrow in KFU isolate. Isolate RjFU and MFU were having oval with rounded ends microconidia, isolate KoFU microconidia were long oval with rounded ends. Microconidia of BeFU isolate was oval curved with slight narrow ends, while it was elliptical curved with slight narrow ends in isolate DFU. The micro conidia were 0-1 septate, hyaline, round to oval in shape. The size of micro conidia varied from 4.9-11.8 µm (8.10 µm) × 2.2-4.0 µm (2.98 µm) in BeFU to 8.3-17.4 µm (12.94 µm) × 2.5-5.8 µm (4.0 µm) in RFU. The macro conidia were 1 to 4 septate, fusaroid to sickle shaped. The size of macroconidia ranged from 16.5-20.6 µm (18.47 µm) × 2.1-4.1 µm (2.98 µm) in RFU to 24.0-37.0 µm (30.24 µm) × 2.6-4.0 µm (3.52 µm) in BeFU [Table-2, [Fig-2 (a) and (b)]. Macroconidia of isolate RFU, RgFU and RjFU was long sickle shaped, while it was long, slightly curved with rounded ends in KFU isolate. Isolate KoFU was having fusaroid macroconidia with pointed ends, while it was long fusaroid with pointed ends in BeFU and DFU. Isolate MFU was having long fusaroid conidia. The shape and size of chlamydospores were also varied among all the isolates of *F. oxysporum* f. sp. *udum*. The chlamydospores were thick walled, single or chains and terminal or intercalary. The shape varied from round

in KoFU, RFU, RjFU and BeFU isolates to oval in isolate RgFU and long oval in KFU, MFU and DFU. The size of round chlamydospores ranged from 3.8-4.7 µm in RFU to 6.4-8.7 µm in diameter in RjFU and long oval chlamydospores were 11-13 × 3-4 µm in MFU to 19-21 × 4-5 µm (Length × Breadth) in DFU [Table-2], [Fig-2 (a) and (b)]. The present investigation was coincided with the findings of Kumar and Upadhyay [8]. They obsreved considerable variations in cultural and morphological characters fifteen isolates of *Fusarium oxysporum* f. sp. *udum*. The colony diameter ranged from 29.6 to 57.3 mm after eight days of incubation at 27±2 ºC. Six isolates produced white mycelia colour, serrated margin with fluffy growth with light and dark yellow pigmentation while four isolates produced white mycelia colour, serrated margin with partially appressed growth with dark yellow to brown pigmentation in substrate. Remaining five isolates produced white mycelia color, serrated margin with appressed growth with light yellow to brown pigmentation. The size of macro conidia and micro conidia ranged from 15.4-45.0 × 2.1-6.2 µm and 2.5-17.5 × 2.1-6.2 µm, respectively. Madhukeshwara and Seshadri [9] studied on existence of variability, with respect to morphological, cultural, nutritional, physiological, spore germination, isozyme and pathogenicity characters revealed considerable variation among six isolates. The size of macro and microconidia varied from 18-21 × 4-5 µm to 23-26 × 4-5 µm respectively. Chlamydospores measured from 10-17 µm in diameter and varied pigmentation was noticed from white to dusky red. Rangaswamy *et al*. [14] studied on morphological and cultural characters of twenty six isolates of *Fusarium oxysporum* f. sp. *udum.* The size of micro- and macro-conidia varied from 7.27 × 2.88 µm to 13.25 × 2.68 µm and 23.37 × 3.17 µm to 40.05 × 4.71 µm respectively. Macroconidia of all the isolates showed 2 to 6 septations. Diameter of chlamydospores measured between 9.47 µm to 15.57µm. Colony characters ranged from sparse to dense mycelial growth. The colony diameter of isolates considerably differed. The isolates were with either yellow, pale yellow, reddish yellow, brownish yellow, pink or light pink pigmentation. Shinde *et al*. [16] studied on cultural and morphological variation among twenty isolates of *Fusarium oxysporum* f. sp. *udum.* They observed wide variability among the isolates with respect to mycelial growth, spore size, mycelial width, and colony pigmentation. The average size of macro and micro conidia was varied from 23.80-37.26 × 3.10-5.52 µm and 6.10-9.67 × 2.65-4.35 µm with absence of macro-conidia in two isolates. Anjaneyareddy and Saifulla [1] recorded that among morphological studies, the size of macroconidia varied from 13.03 × 3.6 µm (Bangalore isolate) to 20.6 × 2.1 µm (ICRISAT isolate), while the size of microconidia varied between 5.26 × 1.78 µm (ICRISAT isolate) to 9.09 × 1.94 µm (Gulbarga isolate).

Table-2 *Morphological characters (Size, shape, septation and colour of the macro and micro conidia) of different isolates of Fusarium oxysporum f.sp. udum*

Isolates	Size (µm) Length × Breadth				Shape		Septation		Colour	Chlamydospore	
	Macro		Micro		Macro	Micro	Macro	Micro		Shape	Size (µm)
	L	B	L	B							
RFU	16.5-20.6 (18.47)	2.1-4.1 (2.98)	8.3-17.4 (12.94)	2.5-5.8 (4.0)	Long, sickle shaped	Long, oval, curved with narrow round ends	2-3	0-1	Hyaline	Round	3.8-4.7
RgFU	15.8-24.1 (20.22)	2.2-3.7 (3.05)	4.7-12.3 (8.72)	2.5-4.1 (3.2)	Long, sickle shaped	Long, elliptical, slightly curved with rounded ends	3-4	0-1	Hyaline	Oval	5.1-6.7
KFU	19.5-29.0 (24.30)	2.4-4.1 (3.18)	6.1-13.2 (9.68)	2.5-4.7 (3.58)	Long, slightly curved with rounded ends	Elliptical with slight narrow ends	2	1	Hyaline	Long oval	18-21×5-6
RjFU	17.4-25.0 (21.23)	2.4-4.0 (3.12)	5.0-15.1 (10.07)	2.2-5.1 (3.62)	Long, sickle shaped	Oval with round ends	3	0-1	Hyaline	Round	6.4-8.7
KoFU	19.1-22.3 (20.86)	2.5-5.1 (3.64)	6.7-16.9 (11.68)	2.5-6.0 (4.18)	Fusaroid with pointed ends	Long, oval with rounded ends	1	1	Hyaline	Round	4.5-5.9
BeFU	24.0-37.0 (30.24)	2.6-4.0 (3.52)	4.9-11.8 (8.10)	2.2-4.0 (2.98)	Long, fusaroid with pointed ends	Oval, curved with slight narrow ends	2	1	Hyaline	Round	4.8-6.3
MFU	21.0-32.0 (25.98)	2.6-4.0 (3.28)	7.5-17.4 (12.25)	2.5-5.0 (3.74)	Long, fusaroid	Oval with round ends	3	0-1	Hyaline	Long oval	11-13×3-4
DFU	19.0-26.5 (22.43)	2.4-4.9 (3.22)	6.9-16.9 (11.88)	2.4-5.6 (3.98)	Long, fusaroid with pointed nds	Elliptical, curved with slight narrow ends	2	1	Hyaline	Long oval	19-21×4-5

* Values within the parentheses are average of twenty conidia

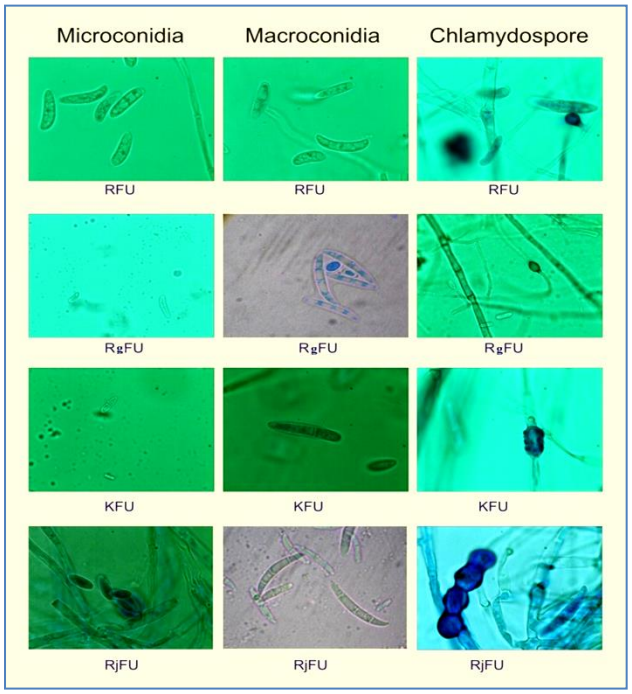

Fig-2(a) Microphotograph of micoconidia, macroconidia and chlamydospore of four isolates of *Fusarium oxysporum* f. sp. *udum*

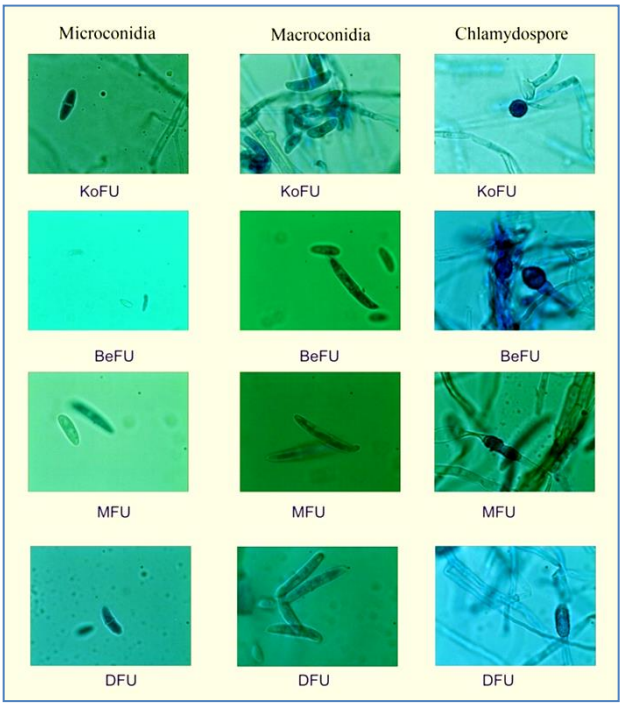

Fig-2(b) Microphotograph with micoconidia, macroconidia and chlamydospore of four isolates of *Fusarium oxysporum* f. sp. *Udum*

Conclusion

All the isolates of *Fusarium oxysporum* f.sp. *udum* exhibited considerable variations in cultural and morphological characters. The colony diameter ranged from 80.00 to 90 mm after nine days of incubation at 28 ± 1ºC. Isolate RFU produced white suppressed mycelial growth, smooth margin with light brown (centre pink) pigmentation, while isolate RgFU produced white to light yellow mycelia, partially appressed with concentric rings and dark yellow pigmentation. Isolate KFU produced white dense cottony fluffy mycelia with serrated margin and light yellow pigmentation while isolate RjFU produced white sparse mycelia with smooth margin and pinkish brown pigmentation. Isolate KoFU produced white dense partially appressed mycelia with serrated margin and light yellow to brown pigmentation. Isolate BeFU produced white fluffy mycelia with serrated margin and yellow pigmentation while isolate MFU produced whitish to light yellow fluffy mycelia with serrated margin and brown with centre black to dark brown pigmentation and isolate DFU produced whitish to light yellow fluffy mycelia with serrated margin and dark pink pigmentation. The macro conidia of three isolates of *Fusarium oxysporum* f.sp. *udum* were long, sickle shaped, two isolates produced long, fusaroid with pointed ends, while remaining three isolates produced long, slightly curved with rounded ends, fusaroid with pointed ends and long, fusaroid macro conidia respectively. The size of macroconidia ranged from 16.5-20.6 µm (18.47 µm) × 2.1-4.1 µm (2.98 µm) in RFU to 24.0-37.0 µm (30.24 µm) × 2.6-4.0 µm (3.52 µm) in BeFU. The size of micro conidia varied from 4.9-11.8 µm (8.10 µm) × 2.2-4.0 µm (2.98 µm) in BeFU to 8.3-17.4 µm (12.94 µm) ×2.5-5.8 µm (4.0 µm) in RFU. The macro conidia were 1 to 4 septate, fusaroid to sickle shaped. The shape of chlamydospore varied from round in KoFU, RFU, RjFU and BeFU isolates to oval in isolate RgFU and long oval in KFU, MFU and DFU. The size of round chlamydospores ranged from 3.8-4.7 µm in RFU to 6.4-8.7 µm in diameter in RjFU and long oval chlamydospores were 11-13×3-4 µm in MFU to 19-21×4-5 µm (Length × Breadth) in DFU.

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Author Contributions

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Abbreviations

BOD: Biological Oxygen Demand, DAI: Days After Inoculation, °C: Degree Celsius, PDA: Potato Dextrose Agar, µm: Micrometer, VCG: Vegetative Compatibility Group

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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