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Pathogenic variability of *Fusarium udum* causing pigeonpea wilt

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Abstract

Pathogenic variability among the twenty one isolates of *Fusarium udum* of pigeonpea collected from different locations of India was studied in respect of pathogenicity. Screening of *F. udum* isolates by pot Culture method on susceptible variety ICP-2376 observed high degree of variation for wilt incidence (40–95%) and average expression of wilting was observed from 37 to 45 DAS. In moderately resistance variety PKV Tara showed variation for wilt incidence (0–65%) and average expression of wilting was observed from 44 to 58 DAS. In resistance variety Asha found variation for wilt incidence (0-55%) and average expression of wilting was observed from 55 to 65 DAS. And Screening of *F. udum* isolates by water culture method on susceptible variety ICP-2376 found high degree of variation for wilt incidence (33.33–100%) and average expression of wilting was observed from 5 to 10 DAI. In moderately resistance variety PKV Tara showed variation for wilt incidence (0–100%) and Average expression of wilting was observed from 0 to 15 DAI. In resistance variety Asha found variation for wilt incidence (0-50%) and average expression of wilting was observed from 0 to 22 DAI.

Keywords: *Fusarium udum*, incidence, Pigeonpea, variability

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp) is an important pulse crop cultivated in the tropics and subtropics. The crop represents about 5% of world's legume production with more than 70% being produced in India (Damaris 2007) [3]. Pigeonpea yield is significantly reduced by vascular wilt disease caused by *Fusarium udum*, with estimated yield losses of US\$36 million in India and \$5 million in eastern Africa (Kannaiyan *et al.* 1984) [9]. Identification of several stable resistance sources and their subsequent utilisation in breeding programme have resulted in development and release of wilt resistant varieties for different regions of India. However, some of these genotypes have exhibited differential reactions possibly due to pathogenic variability in wilt fungus (Reddy *et al.* 1996). Limited studies on variability in *F. udum* have indicated that the fungus exhibits physiologic specialisation (Baldev and Amin 1974; Shit and Sen Gupta 1978; Reddy and Chaudhary 1985; Sahoo 1987; Rajendra and Patil 1992; Reddy and Raju 1993) [1, 18, 15, 17, 13, 14]. Understanding the physiological races and genetic diversity of the pathogen is essential to guide the development of appropriate strategies for breeding that will enhance durability of *Fusarium* wilt resistance.

The discovery of the perfect state, *Gibberella indica* (Rai and Upadhyay, 1982) [21] of the pathogen has provided the substantial knowledge about the pathogen and has opened a new avenue for research on the disease and its causal organism. Hence, it would be necessary to ascertain the pathogenic variability amongst the pathogen. In the present investigation, studies were carried out to see the variability among the different isolates of *Fusarium udum*.

Material Methods

Present studies on pathogenic variability (on pigeonpea varieties, ICP-2376, PKV Tara and Asha) work were conducted in the Department of Plant Pathology Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Pot culture (soil inoculation) Method

The pathogenic variability of *Fusarium udum* isolates was tested by using soil inoculation method suggested by Nene *et al.* (1981) [9]. The inoculum of *F. udum* was prepared in sorghum grain media (3 part sorghum grain + 1 part sand + distilled water to moisten the media). The media was prepared by mixing sorghum grains with clean sand in plastic tub followed by

moistening with distilled water. About 200 g mixture was transferred in 500 ml Erlenmayer flask, plugged using non-absorbent cotton and sterilized at 1.04 kg/cm² for 30 minutes. The flasks containing the sterilized media were inoculated with mycelial disc of *F. udum* (5 mm diameter) and incubated at 27 ± 2 °C for 15 days. The inoculum was used for soil inoculation at 25 g kg⁻¹ soil in pot experiments. 10 seeds/per pot of wilt susceptible ICP-2376, moderately resistance PKV Tara, and resistance Asha were sown in each pot and with 3 replications. In case of control, pigeonpea seeds were sown in pots containing uninoculated soil. Plants were observed periodically upto 65 days after sowing (DAS) for days of initiation of wilt symptoms and per cent disease incidence.

Water culture inoculation method

The pathogenic variability of selected isolates was tested by water culture technique on wilt susceptible ICP-2376, moderately resistance PKV Tara, and resistance Asha as per procedure suggested by Nene and Haware (1980)^[8]. The pure cultures of respective *Fusarium udum* isolates were multiplied on 100 ml PDB in 250 ml flasks on a rotary shaker for seven days at 27 ± 2 °C. The PDB grown culture was diluted with sterilized distilled water to get final concentration of 1.5 x 10⁵ conidia per ml. The inoculum load was counted under compound microscope by using Haemocytometer. 10 ml of conidial suspension of each *F. udum* isolate was transferred to separate tube. Eight days old seedlings grown on sterilized sands in plastic tray were carefully uprooted and roots were washed under running water to remove excess of sands adhering to root system. The root tips around 0.5 cm long were cut off to facilitate the entry of the pathogen into the roots. Individual seedlings were transferred to each tube. The inoculated seedling in test tube was kept under greenhouse condition. In case of control seedlings were grown in distilled water without inoculation of pathogen for comparison. Sterilized distilled water was filled into tubes every 48 hour to make up the loss of water. Observation regarding days of initiation of wilting of seedlings and wilt incidence was recorded at 24 hours interval upto 22 days after transplanting.

Disease assessment and data analysis

After every second day, inoculated seedlings were observed for initiation of disease symptoms and per cent wilt incidence. Wilt incidence were calculated by using formula,

$$\text{Percent disease incidence} = \frac{\text{Number of plant showing wilt symptoms}}{\text{Total number of plant examined}} \times 100$$

The isolates of *Fusarium udum* were tentatively divided into five groups on the basis of virulence as follows.

Category

Per cent wilt

- i) Non-pathogenic isolates (NPI) 0%
- ii) Weakly pathogenic isolates (WPI) 1-20%
- iii) Moderately pathogenic isolates (MPI) 21-50%
- iv) Strong pathogenic isolates (SPI) 51-70%
- v) Highly pathogenic isolates (HPI) >70%

Results and Discussion

Pathogenicity and pathogenic variability

An experiment was set up to study the pathogenic variability existing in different isolates of *Fusarium udum*, collected from different pigeonpea growing regions of India. Twenty one isolates of *F. udum* were screened with highly wilt susceptible variety ICP-2376, moderately resistant variety

PKV- Tara and resistant variety Asha by pot culture (soil inoculation) as well as water culture (spore suspension) method.

Pot culture (Soil inoculation) method

The data on disease incidence recorded in wilt susceptible variety ICP-2376 in response to twenty one respective *F. udum* isolates are presented in Table 1. Among twenty one isolates, all were found pathogenic in nature. Wide range of variation in wilt incidence was recorded. The data revealed that variation in wilt incidence in test isolates were 40 to 95 per cent. Isolate Fu-7 proved to be most virulent, produced mean disease incidence of 95 per cent. Isolates Fu-4 and Fu-11 were caused average wilt incidence 90 per cent followed by Fu-9, and Fu-13 (85.00%). Out of twenty one *F. udum* isolates twelve isolates viz., Fu-1, Fu-2, Fu-4, Fu-5, Fu-7, Fu-9, Fu-10, Fu-11, Fu-13, Fu-14, Fu-15 and Fu-18, were observed highly pathogenic, produced more than 70 per cent wilt incidence while five isolates of *F. udum* viz., Fu-3, Fu-6, Fu-12, Fu-16 and Fu-21 were found strongly pathogenic showed 51 to 70 per cent wilt. Whereas, four viz., Fu-8, Fu-17, Fu-19 and Fu-20 were found moderately pathogenic with average 21-50 per cent wilt while. Wilt symptom was not observed in control plants and plants remain healthy. Expression of wilting in individual isolates varied from each other. Average expression of wilting was observed from 37 to 45 days after sowing. There was no relation between virulence and expression of symptoms. Most of the virulent isolates express the symptoms upto 40-42 days after sowing.

Table 1: Pathogenic variability among *Fusarium udum* isolates by pot culture method on susceptible variety ICP-2376

Sr. No.	Isolates Code	Wilted plants	Wilt Incidence (%)	Days for initiation of Wilting (DAS)	Wilt reaction
1	Fu-1	15	75.00	43	HPI
2	Fu-2	16	80.00	40	HPI
3	Fu-3	12	60.00	43	SPI
4	Fu-4	18	90.00	40	HPI
5	Fu-5	15	75.00	40	HPI
6	Fu-6	12	60.00	43	SPI
7	Fu-7	19	95.00	41	HPI
8	Fu-8	10	50.00	43	MPI
9	Fu-9	17	85.00	44	HPI
10	Fu-10	15	75.00	37	HPI
11	Fu-11	18	90.00	45	HPI
12	Fu-12	13	65.00	40	SPI
13	Fu-13	17	85.00	43	HPI
14	Fu-14	16	80.00	45	HPI
15	Fu-15	16	80.00	45	HPI
16	Fu-16	13	65.00	42	HPI
17	Fu-17	10	50.00	42	MPI
18	Fu-18	15	75.00	40	HPI
19	Fu-19	08	40.00	45	MPI
20	Fu-20	10	50.00	44	MPI
21	Fu-21	13	65.00	45	SPI

The disease incidence was recorded in moderately resistant variety to wilt PKV-Tara in response to twenty one respective *F. udum* isolates and the data was presented in table 2. Among twenty one isolates, 20 showed pathogenic in nature and one isolate found non-pathogenic in nature. Wide range of variation in wilt incidence was recorded. The data revealed that variation in wilt incidence in test isolates ranges between 0 to 65 per cent.

Table 2: Pathogenic variability among *Fusarium udum* isolates by pot culture method on moderately resistant variety PKV-Tara

Sr. No.	Isolates Code	Wilted plants	Wilt Incidence (%)	Days for initiation of Wilting (DAS)	Wilt reaction
1	Fu-1	13	65.00	50	SPI
2	Fu-2	02	10.00	48	WPI
3	Fu-3	04	20.00	51	WPI
4	Fu-4	04	20.00	47	WPI
5	Fu-5	08	40.00	47	MPI
6	Fu-6	05	25.00	50	MPI
7	Fu-7	05	25.00	46	MPI
8	Fu-8	04	20.00	46	WPI
9	Fu-9	04	20.00	52	WPI
10	Fu-10	12	60.00	47	SPI
11	Fu-11	01	05.00	55	WPI
12	Fu-12	02	10.00	58	WPI
13	Fu-13	06	30.00	46	MPI
14	Fu-14	04	20.00	45	WPI
15	Fu-15	00	00.00	00	NPI
16	Fu-16	02	10.00	50	WPI
17	Fu-17	03	15.00	44	WPI
18	Fu-18	04	20.00	45	WPI
19	Fu-19	05	25.00	50	MPI
20	Fu-20	06	30.00	51	MPI
21	Fu-21	05	25.00	49	MPI

Isolate Fu-1 proved to be most virulent, produced mean disease incidence of 65 per cent. Isolates Fu-10 were caused average wilt incidence 60 per cent followed by Fu-5 (40.00%). Out of twenty one *F. udum* isolates one Fu-15 was found non-pathogenic. Eleven isolates of *F. udum* viz., Fu-2, Fu-3, Fu-4, Fu-8, Fu-9, Fu-11, Fu-12, Fu-14, Fu-16, Fu-17 and Fu-18 were observed weakly pathogenic (WPI) with average 1-20 per cent wilt incidence, while seven isolates of *F. udum* viz., Fu-5, Fu-6, Fu-7, Fu-13, Fu-19, Fu-20 and Fu-21 were found moderately pathogenic showed 21 to 50 per cent wilt incidence. Two isolates viz., Fu-1, and Fu-10 were observed strongly pathogenic with average 51-70 per cent wilt incidence (Table 2) and highly pathogenic isolates was not found. No wilt symptoms was observed in control plants.

Expression of wilting in individual isolates was varied among all. Average expression of wilting was observed from 44 to 58 days after sowing. There was no relation between virulence and expression of diseased symptoms. Most of the virulent isolates express the symptoms upto 46-50 days after sowing. Among twenty one isolates fifteen showed pathogenic in nature and six isolates found non-pathogenic in nature. Wide range of variation in wilt incidence was recorded. The data presented that variation in wilt incidence in test isolates ranges between 0 to 55 per cent.

Table 3: Pathogenic variability among *Fusarium udum* isolates by pot culture method on resistant variety Asha

Sr. No.	Isolates Code	Wilted plants	Wilt Incidence (%)	Days for initiation of Wilting (DAS)	Wilt reaction
1	Fu-1	08	40.00	60	MPI
2	Fu-2	02	10.00	60	WPI
3	Fu-3	04	20.00	62	WPI
4	Fu-4	04	20.00	57	WPI
5	Fu-5	00	00.00	--	NPI
6	Fu-6	03	15.00	62	WPI
7	Fu-7	02	10.00	60	WPI
8	Fu-8	01	05.00	65	WPI
9	Fu-9	02	10.00	62	WPI
10	Fu-10	11	55.00	60	SPI
11	Fu-11	02	10.00	57	WPI
12	Fu-12	02	10.00	58	WPI
13	Fu-13	00	00.00	--	NPI
14	Fu-14	00	00.00	--	NPI
15	Fu-15	01	05.00	65	WPI
16	Fu-16	01	05.00	62	WPI
17	Fu-17	00	00.00	--	NPI
18	Fu-18	03	15.00	58	WPI
19	Fu-19	04	20.00	55	WPI
20	Fu-20	00	00.00	--	NPI
21	Fu-21	00	00.00	--	NPI

Isolate Fu-10 proved to be most virulent, produced mean disease incidence of 55 per cent. Isolates Fu-1 were caused average wilt incidence 40 per cent followed by Fu-3, Fu-4 and Fu-19 (20.00%). Out of twenty one *F. udum* isolates six Fu-5, Fu-13, Fu-14, Fu-17, Fu-20 and Fu-21 were found non-pathogenic. Thirteen isolates of *F. udum* viz., Fu-2, Fu-3, Fu-4, Fu-6, Fu-7, Fu-8, Fu-9, Fu-11, Fu-12, Fu-15, Fu-16, Fu-18 and Fu-19 were observed weakly pathogenic isolates with average 1-20 per cent wilt, while one isolates of *F. udum* viz., Fu-1 was found moderately pathogenic showed 21 to 50 per cent wilt. One isolates viz., Fu-10 was observed strongly pathogenic with average 51-70 per cent wilt incidence (Table 3) and highly pathogenic isolates was not found. No wilt symptoms were observed in control plants. Expression of wilting in individual isolates was varied among all. Average

expression of wilting was observed from 55 to 65 days after sowing. There was no relation between virulence and expression of symptoms. Most of the virulent isolates express the symptoms upto 57-60 days after sowing.

Similar result was found Parmar and Kathiria (2015) [12] screened out eight pigeonpea genotypes were subjected for pot culture screening using artificial inoculation. The wilt incidence was ranged between 0-90% in the wilt sick pot screening technique. The results were indicate in screening for wilt disease and the genotype ICP 8863 had shown resistance against *F. udum* in the screening techniques. Sushreeta Naik *et al.*, (2017) [20] soil inoculation disease incidence were recorded up to 30 DAS. Maximum 100% PDI was acquired by FU-6 followed by 83.33% by FU-7 at 135 DAS. Minimum PDI of 25% was recorded by FU-10 at 135

DAS. Similar studied and reported by many workers in past Dhar *et al.*, (2012)^[5] Panwar *et al.*, (2017)^[11], Birajdar *et al.*, (2018)^[2], Singh *et al.*, (2010)^[19] and Nikam *et al.*, (2011)^[10].

Water culture (Spore suspension) method

During the present investigations pathogenicity of all twenty one isolates of *Fusarium udum* were tested by water culture method which wilt susceptible variety (ICP-2376), moderately resistant variety (PKV- Tara) and resistant variety (Asha) were used. The seedling maintained in sterilized water served as control.

Testing pathogenicity wilt on susceptible variety ICP-2376, the result given in Table 4 revealed that wilt observed in water culture experiment showed variation from 33.33 to 100 per cent. Maximum wilt was observed (100%) in isolates Fu-9 and Fu-10. The minimum wilt incidence (33.33%) was observed in Fu-3, Fu-11 and Fu-18 (Fig. 8). Out of twenty one, three isolates *viz.*, Fu-3, Fu-11 and Fu-18 were found moderately pathogenic with average 21-50 per cent wilt incidence, three isolates *viz.*, Fu-5, Fu-7 and Fu-8 strongly pathogenic and remain fifteen *viz.*, Fu-1, Fu-2, Fu-4, Fu-6, Fu-9, Fu-10, Fu-12, Fu-13, Fu-14, Fu-15, Fu-16, Fu-17, Fu-19, Fu-20 and Fu-21 were found highly pathogenic.

Table 4: Pathogenic variability among *Fusarium udum* isolates by water culture method on susceptible variety ICP-2376

Sr. No.	Isolates Code	Wilt Incidence (%)	Days for initiation of Wilting (DAI)	Wilt reaction
1	Fu-1	83.33	09	HPI
2	Fu-2	83.33	10	HPI
3	Fu-3	33.33	10	MPI
4	Fu-4	83.33	07	HPI
5	Fu-5	66.66	10	SPI
6	Fu-6	83.33	07	HPI
7	Fu-7	66.66	11	SPI
8	Fu-8	66.66	10	SPI
9	Fu-9	100.0	05	HPI
10	Fu-10	100.0	05	HPI
11	Fu-11	33.33	08	MPI
12	Fu-12	83.33	05	HPI
13	Fu-13	83.33	05	HPI
14	Fu-14	83.33	05	HPI
15	Fu-15	83.33	05	HPI
16	Fu-16	83.33	05	HPI
17	Fu-17	83.33	05	HPI
18	Fu-18	33.33	07	MPI
19	Fu-19	83.33	07	HPI
20	Fu-20	83.33	07	HPI
21	Fu-21	83.33	07	HPI

Testing pathogenicity on wilt moderately resistant variety PKV-Tara, the result given in table 5 revealed that, wilt observed in water culture experiment found variation range between 0 to 100 per cent wilt incidence. Maximum wilt was observed (100%) in isolates Fu-8. The minimum wilt incidence (16.66%) was observed in Fu-17, Fu-18 and Fu-19 (Fig. 10). Out of twenty one, five isolates *viz.*, Fu-2, Fu-3, Fu-9, Fu-11 and Fu-16 were recorded non-pathogenic. Three isolates *viz.*, Fu-17, Fu-18 and Fu-19 were weakly pathogenic with average 1-20 per cent wilt incidence, seven isolates *viz.*, Fu-6, Fu-7, Fu-13, Fu-14, Fu-15, Fu-20 and Fu-21 were found moderately pathogenic with average 21-50 per cent wilt incidence, four isolates *viz.*, Fu-1, Fu-4, Fu-5 and Fu-12 were strongly pathogenic and two isolates *viz.*, Fu-8 and Fu-10 were found highly pathogenic.

Table 5: Pathogenic variability among *Fusarium udum* isolates by water culture method on moderately resistant variety PKV-Tara

Sr. No.	Isolates Code	Wilt Incidence (%)	Days for initiation of Wilting (DAI)	Wilt reaction
1	Fu-1	66.66	08	SPI
2	Fu-2	00.00	00	NPI
3	Fu-3	00.00	00	NPI
4	Fu-4	66.66	07	SPI
5	Fu-5	66.66	07	SPI
6	Fu-6	50.00	07	MPI
7	Fu-7	50.00	08	MPI
8	Fu-8	100.0	09	HPI
9	Fu-9	00.00	00	NPI
10	Fu-10	83.33	10	HPI
11	Fu-11	00.00	00	NPI
12	Fu-12	66.66	07	SPI
13	Fu-13	33.33	07	MPI
14	Fu-14	33.33	08	MPI
15	Fu-15	50.00	07	MPI
16	Fu-16	00.00	00	NPI
17	Fu-17	16.66	10	WPI
18	Fu-18	16.66	12	WPI
19	Fu-19	16.66	15	WPI
20	Fu-20	50.00	08	MPI
21	Fu-21	50.00	07	MPI

Testing pathogenicity on wilt resistant variety Asha, the results depicted in table 6 revealed that, wilt observed in water culture experiment showed variation range between 0 to 50 per cent wilt incidence. Maximum wilt was observed (50%) in isolates Fu-5, Fu-9, Fu-14, Fu-15 and Fu-17. The minimum wilt incidence (16.66%) was observed in Fu-18, Fu-19 and Fu-20 (Fig 12). Out of twenty one, eight isolates *viz.*, Fu-1, Fu-2, Fu-3, Fu-4 Fu-6, Fu-10, Fu-11 and Fu-16 were recorded non-pathogenic. Three isolates *viz.*, Fu-18, Fu-19 and Fu-20 were weakly pathogenic with average 1-20 per cent wilt incidence and remaining ten isolates *viz.*, Fu-5, Fu-7, Fu-8, Fu-9, Fu-12, Fu-13, Fu-14, Fu-15, Fu-17 and Fu-21 were found moderately pathogenic with average 21-50 per cent wilt incidence.

Table 6: Pathogenic variability among *Fusarium udum* isolates by water culture method on resistant variety Asha

Sr. No.	Isolates Code	Wilt Incidence (%)	Days for initiation of Wilting (DAI)	Wilt reaction
1	Fu-1	00.00	00	NPI
2	Fu-2	00.00	00	NPI
3	Fu-3	00.00	00	NPI
4	Fu-4	00.00	00	NPI
5	Fu-5	50.00	08	MPI
6	Fu-6	00.00	00	NPI
7	Fu-7	33.33	08	MPI
8	Fu-8	33.33	10	MPI
9	Fu-9	50.00	15	MPI
10	Fu-10	00.00	00	NPI
11	Fu-11	00.00	00	NPI
12	Fu-12	33.33	20	MPI
13	Fu-13	33.33	08	MPI
14	Fu-14	50.00	09	MPI
15	Fu-15	50.00	09	MPI
16	Fu-16	00.00	00	NPI
17	Fu-17	50.00	09	MPI
18	Fu-18	16.66	22	WPI
19	Fu-19	16.66	20	WPI
20	Fu-20	16.66	09	WPI
21	Fu-21	33.33	08	MPI

Similar results were observed by Madhukeshwara and Seshadri (2001) ^[7] in virulence behavior of six isolates of *Fusarium udum* by test tube and modified root injury method on a set of host differential of pigeonpea. Kiprof *et al.*, (2002) ^[6] studied that 79 isolates of *F. udum* were pathogenic to the wilt-susceptible pigeonpea variety KAT 60/8, although they showed high significant variation in virulence. *Fusarium* wilt symptoms on pigeonpea plants were observed from the eighth day of inoculation and the fungus re-isolated from the infected plants on the 14th day. The wilt incidence on infected plants ranged from 35% to 100%. The isolates were grouped into two categories; those causing up to 60% wilt were moderately virulent and those causing more than 60% wilt were highly virulent.

The results in the present studies also supported the finding of pathogenic variability and virulence of *F. udum*. Reported by many workers in past Roy (2002) ^[16] proved the variability among four isolates *F. udum* collected from Vidarbha region and tested by spore suspension technique under high inoculum density (10^7 spore/ml). He recorded the differentials on the basis of pathogenic ability of different isolates and categorized as highly and moderately pathogenic. Dhage *et al.*, (2014) ^[4] studied that using susceptible variety TAT-10 was found variation in the wilting percentage from 33.3% to 100% at concentration of 1.5×10^5 spore/ml in the isolates collected from different Vidarbha region. Parmar and Kathiria (2015) ^[12] screened out of eight pigeonpea genotypes *viz*: ICP 8863, ICPL 84060, BSMR 853, AGT 2, GT 101, T 15-15, AVPP 1 and LRG 41 by water culture screening using artificial inoculation. The wilt incidence was ranged between 0-100% in the water culture screening technique.

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