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CULTURAL, MORPHOLOGICAL AND PATHOGENIC VARIABILITY IN *PHYTOPHTHORA DRECHSLERI* F.SP. *CAJANI* ISOLATES CAUSING STEM BLIGHT OF PIGEON PEA (*CAJANUS CAJAN* L.)

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ABSTRACT

Phytophthora blight of pigeon pea is caused by *Phytophthora drechsleri* f.sp. *cajani*. It is a potentially important disease of pigeon pea in India after fusarium wilt and sterility mosaic disease. The present study was carried out at Department of Plant Pathology, ICAR-Indian Institute of Pulses Research, Kanpur. 30 isolates of *Phytophthora drechsleri* f.sp. *cajani* were collected from different parts of central and eastern Uttar Pradesh. Cultural, morphological and pathogenic variability was determined of 30 isolates and pathogenic variability determined follow inoculation on two susceptible varieties, UPAS120 and ICP7119 at the seedling stage. On the basis of cultural variability, colour and texture of colony, 30 isolates were categorized into 5 groups *i.e.*, cottony white fluffy colony (18 isolates), cottony white mat type colony (3 isolates), creamy white fluffy colony (2 isolates), creamy white mat type colony (6 isolates) and light pink fluffy colony (1 isolates). Based on the morphology of sporangia were categorized into 3 types *i.e.*, papillate (14 isolates), semi-papillate (11 isolates) and non-papillate (5 isolates). On the basis of pathogenicity experiments carried out of 30 isolates, 7 isolates (*Pdc12*, *Pdc14*, *Pdc16*, *Pdc18*, *Pdc21* and *Pdc22*) showed 100% mortality on pigeon pea cultivars. The maximum size of sporangia was recorded in isolate *Pdc17* (320.92 µm^2).

Key words : *Cajanus cajan*, Pathogenicity, *P. drechsleri* f.sp. *cajani*, Sporangia, Variability.

Introduction

Pigeon pea [*Cajanus cajan* (L.) Millsp.], an often cross-pollinated, diploid perennial grain legume, is the fourth most important food legume in the world after dry bean (*Phaseolus vulgaris* L.), field pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.) (Pande *et al*., 2011). In India, it is the second most Important food legume crop after chickpea. It is grown under a wide range of cropping systems in the Deccan Plateau in India (Reddy *et al*., 1998). It is rotated with cereal crops increase the yield of cereals by enhancing soil nitrogen and helps in breaking the disease cycle of important cereal pathogens (Jadesha *et al*., 2019). Pigeon pea is susceptible to many diseases and insect pests but only a few of them are economically important (Nene *et al*., 1996; Vishwa Dhar *et al*., 2004). Phytophthora stem blight caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* is a potentially important disease of pigeon pea in India after fusarium wilt and sterility mosaic disease (Kanaiyan *et al*., 1984). The first suspected occurrence of Phytophthora blight on pigeon pea in India was reported in 1966 by Williams *et al.* (1968). He first isolated a PB-causing pathogen from wilted pigeon pea plants with stem canker symptoms at New Delhi, India. Since its appearance, the disease had spread to most pigeon pea growing areas in Asia (Pal *et al*., 1970; Williams *et al.* (1975), Africa, America (Kannaiyan *et al*., 1984), Australia (Weaning and Birch, 1988), Dominican Republic, Kenya, Panama and Puerto Rico (Nene *et al*., 1996). Amin *et al.* (10) described it is a new species *Phytophthora cajani*. Singh and Dube (2005) has been reported cultural and morphological variability in *Phytophthora* spp. and proved the pathogenicity of the isolates was tested on pigeon pea cv. pusa-33 at 35 days

old plants. The present study was carried out the determine the cultural, morphological and pathogenic variability of *PDC* isolates; this may and future PSB breeding programme and variation in the *Phytophthora drechsleri* f.sp. *cajani* isolates collected from different parts of central and eastern U.P.

Materials and Methods

The experiment was conducted at the Department of Plant Protection, ICAR-Indian Institute of Pulses Research, Kanpur, U.P.

Collection of disease sample, purification and isolation of the fungus

The samples of pigeon pea plants depicting typical symptoms of Phytophthora blight were collected in the paper bags from different parts of central and eastern U.P. The diseased sample were brought to the laboratory and isolated on potato dextrose agar medium. *P. drechsleri* f sp. *cajani* isolates collected from various parts of country was reported by Singh *et al.* (12).

Table 1 : Isolates collected from Different parts of Central and Eastern U.P.

Isolates No.	District	Place of collection	Pigeon pea cultivar
Pdc1	Kanpur	ICAR-IIPR, Main Research Farm	Bahar
Pdc2		ICAR-IIPR, Main Research Farm	Pusa-33
Pdc3		ICAR-IIPR, Main Research Farm	UPAS-120
Pdc4		ICAR-IIPR, Main Research Farm	Bahar
Pdc5		ICAR-IIPR, Main Research Farm	ICP7119
Pdc6		ICAR-IIPR, Main Research Farm	ICP7119
Pdc7		ICAR-IIPR, Main Research Farm	UPAS-120
Pdc8		ICAR-IIPR, Main Research Farm	Bahar
Pdc9	Kanpur	ICAR-IIPR, New Research Campus (NRC)	Bahar
Pdc10		ICAR-IIPR, New Research Campus (NRC)	UPAS120
Pdc11		ICAR-IIPR, New Research Campus (NRC)	UPAS120
Pdc12		ICAR-IIPR, New Research Campus (NRC)	PUSA-33
Pdc13		ICAR-IIPR, New Research Campus (NRC)	ICP7119
Pdc14	Hamirpur	Kurara	Bahar
Pdc15		Sumerpur	LOCAL
Pdc16		Muskura	UPAS-120
Pdc17		Mandhaha	Bahar
Pdc18		Jhalokhar	LOCAL
Pdc19	Mirzapur	Adalpura	ICP7119
Pdc20		Adalpura	UPAS-120
Pdc21		Adalpura	MAL-13
Pdc22		Adalpura	Bahar
Pdc23	Varanasi	BHU Reasearch Farm	UPAS120
Pdc24		BHU Reasearch Farm	ICP7119
Pdc25		BHU Reasearch Farm	MAL-13
Pdc26	Varanasi	Suswahi	Bahar
Pdc27		Suswahi	ICP7119
Pdc28	Varanasi	Ramnagar	BDN-2
Pdc29		Ramnagar	MAL-13
Pdc30		Ramnagar	UPAS120

Isolation of fungus was done according to tissue segment method (Rangaswami, 1958). The disease sample washed thoroughly with distilled water, dried and cut with sharp sterilized blade into small bits (5 mm), keeping half healthy and half diseased portion intact. These pieces were surface sterilized with 1 per cent sodium hypochlorite (NaOCl) for 60 seconds. These surface sterilized leaf and stem bits were then inoculated on the solidified PDA poured petri plates and inoculated plates were incubated in BOD incubator at 26 ± 2^{0}C temperature for 2 to 3 days. The isolates were purified by single hyphal tip method (Tutte, 1969).

Cultural and morphological variability

30 isolates of *Phytophthora drechsleri* f.sp. *cajani* for the study cultural and morphological characteristics on potato dextrose agar media. Seven days old culture of isolates were used to study of cultural and morphological characteristics *viz*. growth, colour, diameter of colony and sporulation on PDA media. PDA Petri dishes were inoculated with uniform inoculation bits at the centre and incubated at 28 ± 2°C for seven days. Colony diameter and type of colony growth were recorded after 7 days of inoculation. Based on colony colour and growth isolates are categorized in to different groups *i.e.*, cottony white, creamy white, light pink and fast growing, moderate growing, slow growing. These isolates are also categorized in to fluffy and mat type colony. Morphological and cultural characteristics the isolates were categorized into 6 groups (Singh *et al*., 2008). Morphological characters, Sporangia were observed in all the 30 isolates and based on the morphology of sporangia were categorized into three types *i.e.* papillate, semi- papillate and non- papillate and observed sporangia size (length, width), hyphal swellings.

Pathogenic variability

The pathogenicity of the isolated fungus was tested following Koch's postulates in a pot experiment on pigeon pea variety UPAS 120 and ICP 7119 under greenhouse conditions. The surface sterilized healthy seeds were sown 10 seeds/pot in pots filled with sterilized soil. The pathogenicity test was proved by soil drenching method. The pathogen was mass multiplied on potato dextrose broth (100 ml) in flasks and incubated at 25^{0}C for 15 days. This fungus inoculum (mycelial mat + broth) was macerated in a blender for 1-2 min. Diluted this suspension with tap water to get a final volume of 200 ml. Then 35 days of sowing the plants were inoculated by pouring 100 ml inoculum around the base of the seedlings in a pot (Chand *et al*., 2015). The plant mortality percent data was recorded after 5th days of inoculation. Mortality percent calculate the following formula.

$$Mortality\ \% = \frac{Total\ no.of\ Diseased\ Plants}{Total\ no.\ of\ plants} \times 100$$

Results and Discussion

Cultural and morphological characterization

All 30 isolates of *Phytophthora drechsleri* f.sp. *cajani* on the basis of mycelial growth categorized in to three groups; 3 isolates were fast growing (81.0 – 90.0 mm), 21 isolates were moderate growing (50.0 – 80.0 mm) and 6 isolates were categorized in to slow growing (below 50.0 mm), similar results also reported by Singh *et al*. (12), radial growth of *Phytophthora drechsleri* f.sp. *cajani* on PDA after 96 h of inoculation at 28^{0}C as fast growing (85.0 - 90.0 mm), moderate growing (50.0 - 84.9 mm) and slow growing (<50.0 mm). Singh and Dube (2005) characterized the *Phytophthora drechsleri* f.sp. *cajani* isolates from the north-western plain of India into two groups based on the mycelial growth. Based on morphology, radial growth, colony colour and mycelial characters, 39 isolates of *Phytophthora drechsleri* f.sp. *cajani* from different location of U.P., were characterized into three groups: fast growing, moderate growing and slow growing.

The differences in radial growth of isolates grown on same medium and incubated at same temperature (28 ±2^{0}C), after 7 days observed maximum radial growth was measured in isolates *Pdc26 (*90.0 mm) followed by *Pdc1* (86.0 mm) and minimum radial growth of isolates *Pdc10* (40.0 mm). Based on texture of colony and colour of colony of different isolates of *Phytophthora drechsleri f.sp. cajani* were categorized in to 5 groups (Table, 2 and Fig. 1) *i.e.* 18 isolates were exhibited cottony white with fluffy colony (*Pdc1, Pdc3, Pdc4, Pdc5, Pdc6, Pdc8, Pdc14, Pdc17, Pdc18, Pdc20, Pdc22, Pdc21, Pdc24, Pdc25, Pdc26, Pdc27, Pdc28,* and *Pdc30*) while 3 isolates (*Pdc7, Pdc29* and *Pdc16*)

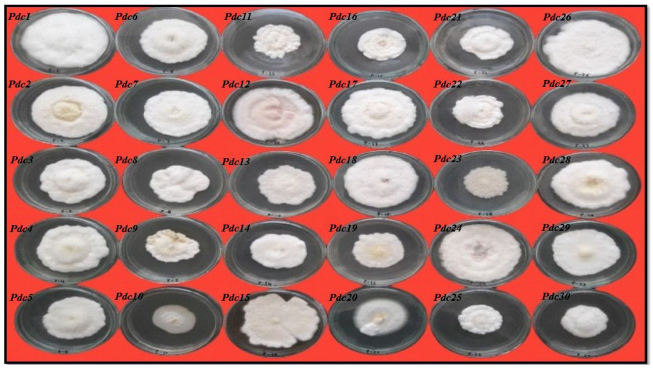

Fig. 1 : Cultural variability among the *Phytophthora drechsleri* f.sp. *cajani* isolates.

Table 2 : Cultural and morphological variability of *phytophthora drechsleri* f.sp. *cajani* isolates.

S. no.	Name of isolates	*Radial growth of the colony (mm) after 7 days	Colour of the colony	Texture of colony	Nature of sporulation	Type of sporangia	Shape of sporangia	Hyphal swellings	Abundance of mycelium
1	*Pdc1*	86.01	Cottony white	Fluffy	Fast	Papillate	Ovoid	Present	Sparse
2	*Pdc2*	72.00	Creamy white	Fluffy	Moderate	Papillate	Ovoid	Absent	Sparse
3	*Pdc3*	70.10	Cottony white	Fluffy	Moderate	Papillate	Ovoid	Present	Profuse
4	*Pdc4*	72.00	Cottony white	Fluffy	Moderate	Papillate	Globose	Absent	Sparse
5	*Pdc5*	70.22	Cottony white	Fluffy	Moderate	Semi- papillate	Globose	Absent	Sparse
6	*Pdc6*	74.00	Cottony white	Fluffy	Moderate	Papillate	Globose	Present	Sparse
7	*Pdc7*	70.00	Cottony white	Mat type	Moderate	Semi- papillate	Ovoid	Absent	Profuse
8	*Pdc8*	44.13	Cottony white	Fluffy	Moderate	Semi- papillate	Ovoid	Absent	Sparse
9	*Pdc9*	50.00	Creamy white	Fluffy	Slow	Semi- papillate	Ovoid	Absent	Profuse
10	*Pdc10*	40.08	Creamy white	Mat type	Slow	Semi- papillate	Ovoid	Present	Sparse
11	*Pdc11*	44.15	Creamy white	Mat type	Slow	Semi- papillate	Ovoid	Present	Sparse
12	*Pdc12*	74.00	Light pink	Fluffy	Moderate	Semi- papillate	Ovoid	Absent	Sparse
13	*Pdc13*	60.16	Creamy white	Mat type	Moderate	Papillate	Globose	Absent	Profuse
14	*Pdc14*	56.00	Cottony white	Fluffy	Moderate	Semi- papillate	Ovoid	Present	Profuse
15	*Pdc15*	74.02	Creamy white	Mat type	Moderate	Semi-papillate	Ovoid	Absent	Sparse
16	*Pdc16*	48.00	Cottony white	Mat type	Slow	Papillate	Ovoid	Absent	Profuse
17	*Pdc17*	80.22	Cottony white	Fluffy	Moderate	Papillate	Ovoid	Absent	Sparse
18	*Pdc18*	80.00	CottonyWhite	Fluffy	Moderate	Semi- papillate	Ovoid	Present	Sparse
19	*Pdc19*	50.00	Creamy white	Mat type	Slow	Non- papillate	Ovoid	Absent	Profuse
20	*Pdc20*	60.24	Cottony white	Fluffy	Moderate	Non- papillate	Ovoid	Absent	Sparse
21	*Pdc21*	56.13	Cottony white	Fluffy	Moderate	Papillate	Globose	Absent	Sparse
22	*Pdc22*	54.00	Cottony white	Fluffy	Moderate	Non- papillate	Ovoid	Absent	Sparse
23	*Pdc23*	46.14	Creamy white	Mat type	Slow	Non- papillate	Ovoid	Absent	Profuse
24	*Pdc24*	84.00	Cottony white	Fluffy	Fast	Non- papillate	Ovoid	Absent	Profuse
25	*Pdc25*	42.09	Cottony white	Fluffy	Slow	Papillate	Ovoid	Present	Sparse
26	*Pdc26*	90.00	Cottony white	Fluffy	Fast	Papillate	Ovoid	Absent	Sparse
27	*Pdc27*	70.00	Cottony white	Fluffy	Moderate	Papillate	Ovoid	Absent	Sparse
28	*Pdc28*	76.05	Cottony white	Fluffy	Moderate	Papillate	Globose	Absent	Sparse
29	*Pdc29*	70.00	Cottony white	Mat type	Moderate	Semi- papillate	Globose	Present	Sparse
30	*Pdc30*	50.00	Cottony white	Fluffy	Slow	Papillate	Ovoid	Absent	Profuse

*****Average of three replications.

Table 3 : Sporangia size (length and width) of different isolates of *P. drechsleri* f.sp. *cajani.*

Name of isolates	Sporangia		
	Length (µm)	Width (µm)	Size (µm^2)
Pdc1	18.8	11.4	207.48
Pdc2	20.1	12.4	249.24
Pdc3	19.8	10.8	213.84
Pdc4	19.6	11.3	221.48
Pdc5	19.1	12.3	234.93
Pdc6	22.1	13.6	300.56
Pdc7	21.3	14.1	300.33
Pdc8	19.4	11.4	221.16
Pdc9	18.9	12.1	228.69
Pdc10	20.2	11.8	238.36
Pdc11	22.4	13.2	295.68
Pdc12	20.3	13.8	280.14
Pdc13	19.8	12.6	249.48
Pdc14	20.7	12.9	267.03
Pdc15	21.8	13.1	285.58
Pdc16	19.9	12.2	242.78
Pdc17	22.6	14.2	320.92
Pdc18	21.4	14.4	308.16
Pdc19	20.3	13.7	278.11
Pdc20	19.7	12.8	252.16
Pdc21	19.4	11.7	226.98
Pdc22	20.5	11.9	243.95
Pdc23	20.8	12.2	253.76
Pdc24	19.8	11.6	229.68
Pdc25	21.2	13.5	286.20
Pdc26	20.6	12.4	255.44
Pdc27	21.9	11.8	258.42
Pdc28	22.4	13.3	297.92
Pdc29	20.7	12.3	254.61
Pdc30	19.6	11.9	233.24

exhibited white mat type colony and 2 isolates showed creamy white fluffy colony (*Pdc2* and *Pdc9*), Other 6 isolates exhibited creamy white mat type colony (*Pdc10, Pdc11, Pdc13, Pdc15, Pdc19, Pdc23*) and 1 isolate showed light pink fluffy colony (*Pdc12*), similar results also found by Singh *et al.* (12), he collected thirty-nine isolates of *Phytophthora drechsleri f.sp. cajani* from different locations of Uttar Pradesh and categorized on the basis of cultural and morphological variability. The isolates were categorized into six groups i.e., fast growing creamy white (10 isolates), fast growing cottony white (6 isolates), moderate growing creamy white (12 isolates), moderate growing cottony white (6 isolates), slow growing creamy white (2 isolates) and slow growing cottony white (3 isolates).

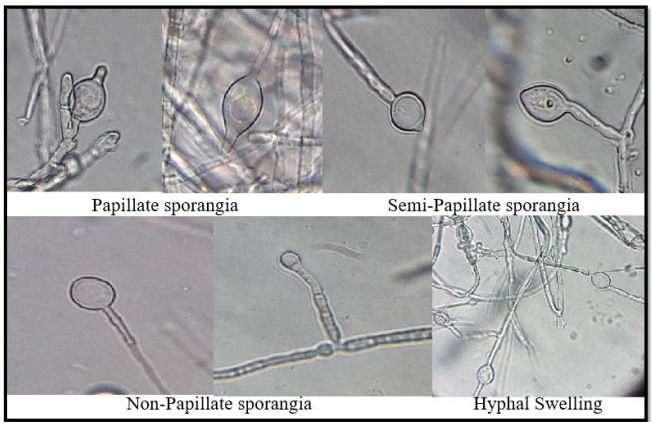

Fig. 2 : Morphological variability among the *Pdc* isolates.

Fig. 3 : Pathogenic variability of *Phytophthora drechsleri* f.sp. *cajani* isolates on pigeon pea cultivars, UPAS120 and ICP7119.

Sporangia were observed in all the 30 isolates and based on the morphology, Sporangia were categorized in to three types *i.e.*, papillate, semi- papillate and non-papillate (Table, 02 and Fig. 02). Papillate sporangia were observed in 14 isolates, these isolates were *Pdc1, Pdc2, Pdc3, Pdc4, Pdc6, Pdc13, Pdc16, Pdc17, Pdc21, Pdc25, Pdc26, Pdc27, Pdc28 and Pdc30*. Semi-papillate sporangia found in eleven isolates were *Pdc5, Pdc7, Pdc8, Pdc9, Pdc10, Pdc11, Pdc12, Pdc14, Pdc15, Pdc18* and *Pdc29*. In five isolates sporangia were found non papillate *Pdc19, Pdc20, Pdc22, Pdc23* and *Pdc24*. Chlamydospores were not found any isolates. Nine isolates were produced hyphal swellings (*Pdc1, Pdc3, Pdc6, Pdc10, Pdc11, Pdc14, Pdc18, Pdc25* and *Pdc29*). Similar results also found by Pande (1) observed that, growth of *P. drechsleri f.sp. cajani* on selective media shows terminal and outer calary hyphal swelling. Agrawal *et al.* (16) observed non-papillate sporangia during present investigation were also reported in *P. drechsleri f. sp. cajani* isolates.

Size of sporangia and variation in length, width was studied on potato dextrose agar for all isolates of *P. drechsleri f. sp. cajani.* In all 30 isolates, maximum length of sporangia was recorded in *Pdc17* (22.6 µm) and

Table 4 : Pathogenicity test of *Phytophthora drechsleri* f.sp. *cajani isolates* on pigeon pea seedlings.

S. no.	Name of the Isolates	Disease Mortality of Pathogenon two cultivar		Nature of Pathogenicity
		UPAS120	ICP7119	
1	*Pdc1*	44.44	42.12	Pathogenic
2	*Pdc2*	85.71	70.50	Highly pathogenic
3	*Pdc3*	24.50	22.33	Less pathogenic
4	*Pdc4*	87.50	70.21	Highly pathogenic
5	*Pdc5*	66.37	64.28	Pathogenic
6	*Pdc6*	80.00	85.71	Highly pathogenic
7	*Pdc7*	11.11	14.22	Less pathogenic
8	*Pdc8*	37.50	40.00	Pathogenic
9	*Pdc9*	55.26	44.44	Pathogenic
10	*Pdc10*	50.00	55.60	Highly pathogenic
11	*Pdc11*	42.00	37.50	Pathogenic
12	*Pdc12*	100	86.22	Highly pathogenic
13	*Pdc13*	44.44	46.52	Pathogenic
14	*Pdc14*	80.56	100	Highly pathogenic
15	*Pdc15*	48.72	42.85	Pathogenic
16	*Pdc16*	100	82.85	Highly pathogenic
17	*Pdc17*	45.00	40.00	Pathogenic
18	*Pdc18*	100	83.00	Highly pathogenic
19	*Pdc19*	35.28	33.33	Pathogenic
20	*Pdc20*	80.00	72.00	Highly pathogenic
21	*Pdc21*	100	80.22	Highly pathogenic
22	*Pdc22*	90.00	100	Highly pathogenic
23	*Pdc23*	46.33	42.85	Pathogenic
24	*Pdc24*	100	81.33	Highly pathogenic
25	*Pdc25*	71.42	68.22	Pathogenic
26	*Pdc26*	20.00	22.60	Less pathogenic
27	*Pdc27*	78.85	80.00	Highly pathogenic
28	*Pdc28*	0.00	0.00	Non-pathogenic
29	*Pdc29*	50.00	48.28	Pathogenic
30	*Pdc30*	54.54	55.33	Highly pathogenic
31	Control	0.00	0.00	

minimum length observed in *Pdc1* (18.2 µm), maximum width of sporangia was 14.2 µm in *Pdc17* and minimum width were observed 11.2 µm in *Pdc24*, maximum size of sporangia was observed in isolate *Pdc17* in 320.92 μm^2, while minimum size of sporangia was recorded in *Pdc1* in 207.48μm^2. The sporangia size, length and width are presented in Table 3.

Pathogenic variability

The pathogenicity of the 30 isolates were tested on pigeon pea cv. UPAS120 and ICP7119 at 35 days old plants in pot. On the basis of pathogenicity experiment all the 30 isolates of *Phytophthora* were categorized into 0-4 rating scale (Singh *et al*., 2016).

Category	Mortality %
Non- pathogenic	0
Less pathogenic	1-25
Pathogenic	26-50
Highly pathogenic	>50

Out of 30 *Pdc* isolates, 7 isolates (*Pdc12, Pdc14, Pdc16, Pdc18, Pdc21, Pdc22*) showed 100% mortality of pigeon pea plants. In all 30 isolates, 14 isolates (*Pdc2, Pdc4, Pdc6, Pdc10, Pdc12, Pdc14, Pdc16, Pdc18, Pdc20, Pdc21, Pdc22, Pdc24, Pdc27* and *Pdc30*) showed >50% mortality of pigeon pea plants, so they were placed in highly pathogenic category, 12 isolates (*Pdc1, Pdc5, Pdc8, Pdc9, Pdc11, Pdc13, Pdc15, Pdc17, Pdc19, Pdc23, Pdc25* and *Pdc29*) had the mortality range between 26-50% so they were placed in pathogenic category. 3 isolates (*Pdc3, Pdc7* and *Pdc26*) showed 1-25% of pigeon pea plant mortality so they are placed under less pathogenic category. *Pdc*28 did not show any mortality and it was found to be non-pathogenic. The results are depicted in Table 4 and Fig. 3. Similar results also found by Singh *et al*. (17). The isolate PDC013-1 and PDC014-3 showed highest 47.0% plant mortality on ICP 7119 genotype. Among the isolates PDC014-3 killed 28.6% plants after 4th day of inoculation.

Conclusion

All 30 isolates exhibited great variability when cultured on PDA medium with colony colour varying from cottony white and creamy white. The texture of colonies was fluffy and mat type. On the basis of cultural variability, colour and texture of colony, 30 isolates were categorized into 5 groups *i.e.*, cottony white fluffy colony (18 isolates), cottony white mat type colony (3 isolates), creamy white fluffy colony (2 isolates), creamy white mat type colony (6 isolates) and light pink fluffy colony (1 isolates). Based on the morphology of sporangia were categorized into 3 types *i.e.*, papillate (14 isolates), semi-papillate (11 isolates) and non- papillate (5 isolates). The isolates of *Phytophthora drechsleri* f.sp. *cajani*, grown on PDA medium, developed sporangia lengths ranging between 18.80 - 22.60 µm and breadth between 10.80 – 14.40 µm. The sporangia size ranged between 207.48 - 320.92 μm^2. The pathogenicity test of *Phytophthora drechsleri* f.sp. *cajani* isolates was determined on susceptible pigeon pea cultivar UPAS120 and ICP7119. Among 30 isolates, 14 isolates showed >50% mortality of pigeon pea plants, so they were placed in highly pathogenic category, 12 isolates exhibited the mortality range between 26-50%

so they were placed in pathogenic category, 3 isolates (*Pdc3, Pdc7* and *Pdc26*) showed 1-25% of pigeon pea plant mortality so they are placed under less pathogenic category. 1 isolate did not show any mortality. It was found to be non-pathogenic.

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Competing interests

Authors have declared that no competing interests exist.

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