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Characterization of *Phytophthora* Isolates from the Pigeonpea Field of Bundelkhand Region of Uttar Pradesh, India

MONIKA MISHRA, RANJANA BHATI* AND RAJ KUMAR MISHRA[1]

Department of Microbiology, Bundelkhand University, Jhansi-284 128 (U. P.), India
**(e-mail: ranjana.iitkgp@gmail.com; Mobile: 9453039734)*

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ABSTRACT

Phytophthora stem blight (PSB), caused by *Phytophthora drechsleri* f. sp. *cajani*, is re-emerging as a serious soil-borne disease of pigeonpea (*Cajanus cajan*) in India. In the present investigation, 25 isolates of *P. drechsleri* were obtained from diseased pigeonpea plants and characterized for their cultural, morphological, physiological and pathogenic features. Considerable variability was observed in colony appearance, pigmentation and radial growth, leading to classification of isolates into fast, moderately fast, moderate, slow and very slow-growing groups. Sporangial morphology also differed markedly, with papillate, semi-papillate, non-papillate and chain-like forms documented. Pathogenicity tests under greenhouse conditions revealed significant variation in virulence: isolates P_{20} and P_{21} induced complete plant mortality, while others caused moderate to mild symptoms. Physiological studies indicated that optimal mycelial growth occurred at 26-30°C and pH 6.5-7.0, whereas growth was restricted under extreme acidic or alkaline conditions. Overall, the study demonstrated substantial variability among *P. drechsleri* isolates in Bundelkhand and provided a basis for resistance breeding and the development of effective management strategies against PSB in pigeonpea.

Key words: Pigeonpea, Phytophthora stem blight, variability, pathogenicity

INTRODUCTION

Pigeonpea [*Cajanus cajan* (L.) Millsp] is the most important grain legume crop in the world. Globally pigeonpea is grown in 6.23 million hectares with a production of 4.67 million tonnes (FAO, 2019). India is the largest producer of pigeonpea in the world sharing approximately 70% of the production and covering 74% of the cultivated area. Pulses make up 32% of total agricultural produce and cover about 33.6% of region's gross crop area (Sah *et al.,* 2024). Bundelkhand region accounts for over 50% of Uttar Pradesh's pulse cultivation area, its yield is less than the state average. Pigeonpea is the most important pulse crop in the Bundelkhand region followed by urad, lentil, pea and mungbean (Kumar *et al.,* 2022). The main constraint in boosting the yield of pigeonpea crop is its narrow genetic base for yield and its attributing characters coupled with susceptibility to large number of plant pathogens including fungi, bacteria, viruses, mycoplasma like organisms and nematodes. Global trade increases the risk of invasive *Phytophthora* spp., making rapid and accurate identification vital for disease management (Barwell *et al.*, 2021).

Phytophthora stem blight (PSB) ranks among the most significant soil-borne diseases affecting pigeonpea, following wilt and sterility mosaic disease. Under favouurable biotic conditions, PSB can cause yield losses up to 100% in susceptible cultivars, typically due to continuous rainfall, high humidity and waterlogging, fostering oospore growth in the soil. Rain droplets near the stem facilitate stem infection. The disease is most severe in the early stages of crop growth, especially with intermittent rains from June to September. The substantial crop yield losses from this disease underscore the pressing need for additional research to develop effective disease management methods. In the recent past, an alarming resurgence of PSB in pigeonpea was observed irrespective of cropping system, soil types and cultivars especially, when excessive rains fall within a short span of time and hot and humid weather persists during the crop season. However, the basic and fundamental need for devising disease management practices is to have a thorough understanding

[1]ICAR-Indian Institute of Pulses Research, Kanpur-208 024 (U. P.), India.

of pathogen biology, including its characteristics and precise identification of the pathogen up to the species level. Various studies based on laboratory tests have pointed out the effect of culture medium and temperature on variability in Phytophthora growth and formation of its reproductive structures. Detailed information on mycelial growth, characteristics of oospores and sporangia, differences in size and shape of reproductive structures can contribute to identification and specification of biology of *Phytophthora* sp. in specific conditions. It is noteworthy that, till date, no detailed investigation has been carried out on the variability and characterization of *P. drechsleri* f. sp. *cajani* causing Phytophthora stem blight disease in pigeonpea from the Bundelkhand region. Reports on other *P. drechsleri* fungus causing PSB disease are very scarce and only a few researchers have worked on this disease or aspect. Therefore, this investigation was carried out to define the morphology, cultural characteristics and pathogenic behaviour of *P. drechsleri*, in addition to examining the impact of temperature and pH variations on its mycelia development.

MATERIALS AND METHODS

Sampling from affected plants was conducted from July to September at various locations within pigeonpea growing areas of the Bundelkhand region (Table 1), where the incidence of PSB disease was high. Aseptically, 50% plants exhibiting typical symptoms of Phytophthora stem blight were collected and placed in sterilized poly bags. The infection was recorded on different parts of the stem using the disease rating scale following standard protocol.

The pathogen was isolated following the tissue segment method (Sharma and Ghosh, 2016). First, the plants were thoroughly washed with distilled water, dried and the stem was cut into 2-4 cm segments with sharp sterilized blade, keeping half healthy and half diseased portion intact. These stem segments were then surface-sterilized by immersion in 1% sodium hypochlorite (NaOCl) for 60 sec. and subsequently rinsed three times with sterile distilled water. The sterilized stem bits were blot-dried and plated onto Petri dishes containing V8 juice agar medium (Himedia, Mumbai, India), amended with a PARP antibiotic solution in 1 l of medium. The antibiotic solution consisted of: 400 ìl of pimaricin, 250 mg of ampicillin, 1000 ìl of rifampicin and 5 ìl of pentachloronitrobenzene (PCNB). The plates were then incubated under a 12-h light / dark photoperiod at 28±2°C for five days. After incubation, 25 putative *Phytophthora* colonies were selected and subcultured onto PDA (Potato Dextrose Agar) slants. Colony identification was confirmed based on their cultural and morphological characteristics.

The mycelial structure and growth of individual *Phytophthora* isolates were examined. Seven days old culture of isolates was used to study of cultural and morphological characteristics i. e. growth, colour, diameter of colony and sporulation. Mycelial discs, 5 mm in diameter, were taken from 7-day-old cultures of the 25 isolates and transferred to the center of fresh Petri plates containing V8 medium using a sterilized cork borer. The Petri plates were incubated at 28±2°C. The growth area of each isolate was measured based on the radius of the colony at 24 h intervals until the Petri plates were fully covered by mycelial growth. The fungal morphology was observed using an Olympus CX41 phase contrast microscope.

The pathogenicity of 25 isolates of *Phytophthora drechsleri* f. sp. *cajani* was evaluated in a greenhouse using a soil-drench inoculation

Table 1. Sample collected from different parts of Bundelkhand Region, U. P.

District	Place of collectionn	Pigeonpea cultivar	Geographical area
Hamirpur	Farmer field	UPAS-120	31.68° N, 76.52° E
Chitrakoot	-do-	Local Arhar	25.17° N, 80.86° E
Banda	-do-	Bahar	25.47° N, 80.33° E
Jalaun	-do-	IPA-203	26.14° N, 79.32° E
Orai	-do-	IPA-203	25.98° N, 79.44° E
Lalitpur	-do-	IPA-15-06	24.69° N, 78.41° E
Mahoba	-do-	IPA-206	25.29° N, 79.88° E
Jhansi	-do-	IPA-15-2	25.44° N, 78.56° E

method, following the Koch's postulates. The surface-sterilized of healthy seed was sown (10 seeds/pot) in pots filled with sterilized soil. The pathogen was mass multiplied on potato dextrose broth (100 ml) in flasks and incubated at 28^0C±2°C for 15 days. The resulting fungus inoculum (mycelial mat + broth) was macerated in a blender for 1-2 min and diluted with tap water to a final volume of 200 ml. Thirty-five days after sowing, the plants were inoculated by pouring 100 ml of inoculum around the base of the seedlings in each pot. The experiment was repeated three times. To confirm pathogenicity, the pathogen was re-isolated from symptomatic plants on potato dextrose agar (PDA), identified based on morphological traits, and re-inoculated to reproduce typical disease symptoms.

To determine the impact of temperature on the mycelial growth of *Phytophthora* isolates, mycelial discs of 5 mm diameter were taken from the actively growing edges of five-day-old cultures. Each disc was centrally placed on a potato dextrose agar (PDA) plate. The inoculated plates were incubated at a range of temperatures from 20 to 30°C at 2°C intervals in BOD incubators. After 96 h of incubation, the colony diameter was measured along two perpendicular axes, and the average radial growth was calculated after subtracting the size of the original inoculum. To determine the effect of different pH levels, pH of PDA medium was adjusted from 5.0 to 8.5 at an interval of 0.5 using 1N HCl or 1N NaOH before sterilization. The plates were incubated at 28/ ±/ 2°C in a BOD incubator, and each treatment was replicated three times. Among the isolates, P_7 was chosen for detailed temperature and pH studies based on its consistent radial growth and served as the representative strain for physiological evaluation.

RESULTS AND DISCUSSION

Blight symptoms first appear as water soaked lesions on leaves which become necrotic within a week. Symptoms appear on stem as dark brown to black lesions distinctly different from healthy green portions on main stem, branches and petiole. The lesions on stems and branches increase rapidly and extend up to 20 cm, girdle, and cracks and dry out the stem.

A total of 25 isolates of *P. drechsleri* f. sp. *cajani* were studied for their cultural and morphological characteristics. Based on their radial growth on PDA medium after seven days of incubation (Table 2), the isolates were grouped into five distinct categories: fast growing (4 isolates), moderately fast growing (5 isolates), moderate growing (5 isolates), slow growing (7 isolates) and very slow growing (4 isolates). Among these, isolate P_7 exhibited the highest radial growth (90.5 mm), followed by P_{25} (89.5 mm) and P_1 (87.0 mm). The minimum radial growth was recorded in isolate P_{10} (60.0 mm), which was classified as very slow-growing. Colony colour and texture varied substantially across isolates. Creamy white colonies were predominant, though some isolates displayed cottony white or whitish pink colouration (Table 2). Textural differences were also evident, with isolates producing either mat-type colonies or fluffy colonies.

Sporangial morphology was one of the most variable traits. Four distinct types of sporangia were observed: papillate, semi-papillate, non-papillate and chain-like sporangia (Fig. 1). Papillate sporangia were detected in seven isolates, while 11 isolates produced semi-papillate sporangia. Four isolates showed non-papillate sporangia, whereas chain-like sporangia were observed in three isolates. The shape of sporangia was predominantly ovoid or globose across isolates. Hyphal swellings, an important taxonomic characteristic, were present in several isolates, whereas other isolates lacked this feature (Table 2). Overall, the variability in radial growth, colony morphology and sporangial structure clearly indicated a high degree of morphological and cultural diversity among the *P. drechsleri* f. sp. *cajani* isolates. These findings align with earlier reports of researchers that show significant variability among isolates of *P. drechsleri* f. sp. *cajani* across different geographical locations, where differences in colony morphology, growth rate and sporulation patterns among isolates were observed (Singh *et al.*, 2017; Sumi *et al.,* 2024). Similarly, Kaur *et al*. (2019) documented substantial variability in cultural and pathogenic characteristics of *P. drechsleri* isolates obtained from multiple regions, suggesting that local environmental factors and host genotype interactions may contribute to this observed heterogeneity. Such variability may be attributed to

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

Isolate No.	Radial growth of the colony (mm) after seven days	Colony colour	Texture of colony	Nature of sporulation	Sporangia		Hyphal swellings
					Type	Shape	
P_1	87.0±4.31^{g}	Creamy white	Mat type	Fast growing	Papillate	Ovoid	Present
P_2	77.0±3.56^{d}	Creamy white	Fluffy	Moderate growing	Non-papillate	Globose	Present
P_3	86.5±4.23^{fg}	Cottony white	Fluffy	Moderately fast growing	Chain like sporangial	Ovoid	Present
P_4	71.0±3.12^{c}	Cottony white	Mat type	Slow growing	Papillate	Ovoid	Absent
P_5	66.0±2.80^{b}	Creamy white	Fluffy	Very slow growing	Semi- papillate	Globose	Absent
P_6	79.5±3.80^{d}	Cottony white	Fluffy	Moderate growing	Papillate	Ovoid	Present
P_7	90.5±4.89^{h}	Cottony white	Mat type	Moderate	Chain like sporangial	Ovoid	Present
P_8	74.0±3.67^{cd}	Cottony white	Fluffy	Slow growing	Semi- papillate	Ovoid	Present
P_9	84.5±3.99^{f}	Creamy white	Fluffy	Moderately fast growing	Semi- papillate	Globose	Absent
P_{10}	60.0±2.90^{a}	Creamy white	Mat type	Very slow growing	Papillate	Globose	Absent
P_{11}	69.0±3.21^{bc}	Creamy white	Mat type	Slow growing	Semi- papillate	Ovoid	Absent
P_{12}	77.5±3.61^{d}	Cottonywhite	Fluffy	Moderate growing	Semi- papillate	Globose	present
P_{13}	86.0±4.20^{f}	Creamy white	Mat type	Moderately fast growing	Non-papillate	Ovoid	Present
P_{14}	73.0±3.50^{c}	Cottony white	Fluffy	Slow growing	Semi- papillate	Ovoid	absent
P_{15}	81.0±4.12^{e}	Creamy white	Mat type	Moderate growing	Semi-papillate	Globose	Present
P_{16}	65.0±3.11^{b}	Cottony white	Fluffy	Very slow growing	Chain like sporangial	Ovoid	Present
P_{17}	82.0±4.09^{e}	Creamy white	Fluffy	Moderately fast growing	Non-papillate	Ovoid	Present
P_{18}	68.5±3.20^{bc}	Cottonywhite	Fluffy	Slow growing	Semi- papillate	Ovoid	Absent
P_{19}	75.5±3.51^{cd}	Creamy	Fluffy	Slow growing	Papillate	Globose	Absent
P_{20}	70.5±3.09^{c}	Creamy white	Fluffy	Slow growing	Semi-papillate	Ovoid	Absent
P_{21}	86.6±4.23^{fg}	Whitish pink	Fluffy	Fast growing	Papillate	Globose	Present
P_{22}	85.0±4.11^{f}	Creamy white	Mat type	Moderately fast growing	Semi-papillate	Ovoid	Absent
P_{23}	78.5±3.67^{d}	Cottony white	Fluffy	Fast growing	Non-papillate	Ovoid	Present
P_{24}	67.0±3.21^{b}	Creamy	Mat type	Very slow growing	Semi-Papillate	Globose	Present
P_{25}	89.5±4.43^{h}	Creamy white	Mat type	Fast growing	Papillate	Globose	Absent

Superscripts by different letters are significantly ($P < 0.05$) different from each other.

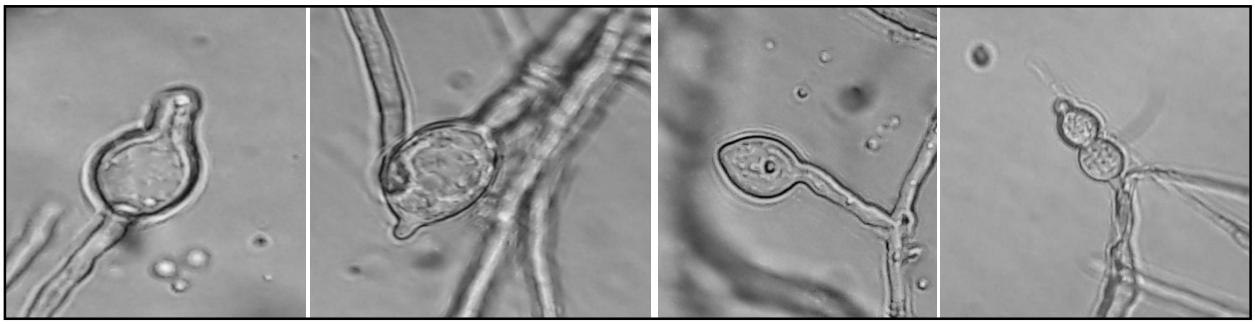

Fig. 1. Morphological variation for sporangial shape and behaviour in *Phytophthora drechsleri* (A) Papillate sporangia, characterized by a pronounced apical thickening (papilla), typical of indirect zoospore discharge; (B) Non-papillate sporangia, exhibiting a less prominent apical papilla; (C) Semi-papillate sporangia and (D) Chain like sporangial behaviour.

differential host-pathogen interactions, ecological conditions and local adaptation strategies of the pathogen.

The pathogenicity test of the 25 isolates carried on pigeonpea variety UPAS-120 showed distinct differences in pathogenic potential. According to the pathogenicity scale, the isolates were grouped into five categories: (1) Non-pathogenic (0-1%), (2) less pathogenic (2-25%), (3) moderately pathogenic (26-50%), (4) pathogenic (51-80%) and (5) highly pathogenic (>80%). This categorization revealed considerable variability in virulence, ranging from avirulent to highly aggressive isolates. Three isolates were found to be non-pathogenic, while two isolates showed low

Table 3. Pathogenicity of *Phytophthora* isolates in pigeonpea variety UPAS-120

Isolate No.	Disease incidence (%)	Level of pathogenicity	Scale (1-5)
P_1	40.00	Moderate pathogenic	3
P_2	78.22	Pathogenic	4
P_3	58.00	Pathogenic	4
P_4	47.00	Moderate pathogenic	4
P_5	0.00	Non-pathogenic	1
P_6	75.39	Pathogenic	4
P_7	33.98	Moderate pathogenic	3
P_8	80.30	Pathogenic (highly)	5
P_9	75.26	Pathogenic	4
P_{10}	60.00	Pathogenic	4
P_{11}	46.00	Moderate pathogenic	3
P_{12}	52.00	Pathogenic	4
P_{13}	23.44	Less pathogenic	2
P_{14}	50.56	Pathogenic	4
P_{15}	20.72	Less pathogenic	2
P_{16}	52.00	Pathogenic	4
P_{17}	40.00	Moderate pathogenic	3
P_{18}	52.00	Pathogenic	4
P_{19}	36.28	Moderate pathogenic	3
P_{20}	100.00	Pathogenic (highly)	5
P_{21}	98.50	Pathogenic (highly)	5
P_{22}	76.71	Pathogenic	4
P_{23}	0.00	Non-pathogenic	1
P_{24}	75.81	Pathogenic	4
P_{25}	0.00	Non-pathogenic	1

virulence and were classified as less pathogenic. Six isolates exhibited moderate pathogenicity. The largest group consisted of 11 isolates, which induced 52-78% disease incidence and were placed in the pathogenic category. Notably, three isolates i.e. P_8 (80.3%), P_{20} (100%) and P_{21} (98.5%) were found to be highly pathogenic (Table 3). To establish the pathogenic role of P. drechsleri Koch's postulates were fulfilled: the pathogen was re-isolated from artificially inoculated plants, and typical stem blight symptoms were reproduced upon reinoculation. These observations confirmed the pathogenic nature of the isolates and corroborate earlier findings reported by Sharma and Ghosh (2018).

Growth of the pathogen varied significantly with temperature. Maximum mycelial growth was observed at 28°C followed by 30°C (Table 4). Moderate mycelial growth was observed at 24°C and 26°C, fungal development was noticeably slower at 20°C and 22°C. These results suggest that *P. drechsleri* prefers moderately warm temperatures for active colonization. The findings are consistent with earlier reports indicating that many *Phytophthora* species exhibit optimal growth

Table 4. Effects of temperature on mycelial growth of *Phytophthora drechsleri*

Temperature (°C)	Mycelial growth (mm)
20	19.33±0.81[a]
22	22.33±1.22[a]
24	74.33±2.92[b]
26	79.67±2.36[b]
28	89.33±3.23[c]
30	86.30±3.10[c]

Superscripts by different letters are significantly ($P < 0.05$) different from each other.

between 24°C and 28°C, depending on the species and host-pathogen interaction. The optimum temperature for the growth of the *Phythophthora* isolates was found to be between 26°C and 30°C, favouring the development of PSB (Singh *et al.,* 2017). Temperature, pH and humidity are also known to affect the survival of fungal pathogens in soil and identified as important factors in disease occurrence (Singh *et al.,* 2017). Temperature affects the growth rate of mycelium and the extent of sporangial formation in other *Phytophthora* species and thereby influences, at least to some extent, the rate of disease development (Scagel *et al.,* 2023). Similar temperature dependent growth patterns have also been reported for other *Phytophthora* species, including *P. capsici* and *P. infestans*, which tend to show reduced development at low temperatures and thermal inhibition at higher extremes (McLay *et al.,* 2025).

The pathogen showed significant variation in growth across the different pH levels. The results revealed that the maximum mycelial growth (89.40 mm) was observed at pH 7.0 after seven days of inoculation. However, further increases in pH (up to 8.5) resulted in decreasing growth and sporulation (Table 5). From this, it can be concluded that fungus prefers a slightly acidic to neutral environment for rapid sporulation and growth. Soil pH plays a significant role in the development of *Phytophthora* root rot in avocado and acidic soil conditions intensified disease severity, particularly when accompanied by high soil moisture levels. This suggests that pH, along with other environmental factors, can substantially influence the growth, sporulation and pathogenic activity infectivity of *Phytophthora* spp. The survival and pathogenicity of *Phytophthora* zoospores are highly influenced by the pH of their

Table 5. Effect of pH on the mycelial growth of *Phytophthora drechsleri*

pH	Mycelial growth (mm)
5.0	48.67±2.31[a]
5.5	57.90±2.82[b]
6.0	78.81±3.26[e]
6.5	89.33±4.23[g]
7.0	89.40±4.28[g]
7.5	80.56±3.87[f]
8.0	65.53±2.81[d]
8.5	58.63±2.54[c]

Superscripts by different letters are significantly ($P < 0.05$) different from each other.

environment. The findings revealed that zoospore viability was greatest under near-neutral conditions (pH 6.0-7.0), whereas both acidic (pH <5) and alkaline (pH >8) conditions significantly reduced survival. This emphasizes the importance of pH as a key environmental factor regulating the survival and disease potential of *Phytophthora* species.

CONCLUSION

The study demonstrated significant morphological, cultural and pathogenic diversity among *P. drechsleri* f. sp. *cajani* isolates from Bundelkhand region, Uttar Pradesh. Twenty-five isolates of *P. drechsleri* varied in radial growth, colony traits and sporangial morphology. The study delineated the effect of temperature and pH strongly influencing growth and sporulation. Pathogenicity tests confirmed all isolates caused typical stem blight symptoms, though virulence levels differed. These findings provide valuable insights for breeding resistant pigeonpea cultivars and their utilization in enhancing disease resistance through breeding initiatives.

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