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Population Structure of *Phomopsis vexans* and its Virulence Status Associated with Brinjal Fruit Rot Disease in Kashmir

Ali Anwar, Mudasir Bhat, M.N. Mughal, Efath Shahnaz* and G.M. Jeelani
Division of Plant Pathology, Faculty of Horticulture,
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir,
Shalimar, Srinagar-190025, (J&K), India.

(Corresponding author: Efath Shahnaz)*
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ABSTRACT: *Phomopsis* fruit-rot of brinjal is one of the most important diseases that deteriorate quality and market value of fruits. A number of studies have been conducted regarding the aetiology, epidemiology and management of the fungus but studies regarding variability are few and far between. The present study was an effort to bridge this important gap in the pathogenic nature of the causal organism, *Phomopsis vexans.* The pathogenicity of the isolated fungus was established by proving Koch's postulates. The inoculated seedlings produced symptoms of the disease after 7 days of inoculation. The culture of the pathogen produced characteristic disease symptoms after 10 days of inoculation on injured fruits and after 18 days of inoculation on uninjured fruits. Isolates of *P. vexans* were collected from different locations of three districts viz. Budgam, Ganderbal and Srinagar. Significant variation was revealed in pathogen population on their cultural, morphological and pathogenic behaviour. Culture variations ranged from fluffy to embedded with regular to irregular margins. Most of the isolates showed white colour, however, colour of the remaining isolates varied from pinkish to blackish brown or grey with regular to irregular margins and varied in their growth rates. PV_{23} and PV_{28} with mean radial growth of 84.10 mm were found fastest while as least growth rate of 29.15 mm was recorded in isolate PV_{26}. All the isolates of *P. vexans* were also varied with respect to their spore germinability. Maximum germination of 68.27 per cent was recorded in isolate PV_{30}, whereas, minimum (11.15%) was observed in isolate PV_{19}.

Keywords: Brinjal, fruit-rot, *Phomopsis vexans*, pathogenicity, variation, isolates.

INTRODUCTION

Brinjal (*Solanum melongena* L.), also known as eggplant or aubergine belongs to nightshade family Solanaceae and order Solanales. It is a budget friendly vegetable crop and an important constituent of diet, particularly in the developing countries like India and China (Doganlar *et al.*, 2002). It is believed to have originated from India (Ahmad, 1987; Bothara, 2003), where its wild relatives can still be found. It is an important crop of tropics, sub-tropics and warm temperate regions (Sihachakr *et al.*, 1994; Rai, 1995) and is grown commercially in India, China, Japan, Philippines, Southern Europe and South America. Asia has largest brinjal producing area of 90 per cent with 87 per cent of the world production coming from this area (Choudhary and Gaur, 2009). In India, it is grown all-round the year in different parts except in the of Kashmir Valley of Union Territory of Jammu and Kashmir. Here it is grown only in Kharif season under warm temperate conditions when its climatic requirements are met. Transplantation of the crop is done in May and the crop is ready for harvest from July to October.

An important bottleneck in realising the full genetic potential of the crop in Kashmir is the development of various fungal, bacterial, viral and nematode diseases which find a favourable environment during the active crop season. However, fungal diseases caused by various species of *Alternaria, Ascochyta, Cercospora, Colletotrichum, Fusarium, Phomopsis* and *Phytophthora* are economically important and cause more havoc than non-fungal diseases (Divinagracia, 1972; Ali *et al.*, 2017). Phomopsis blight and fruit rot caused by *Phomopsis vexans* (anamorphic stage of *Diaporthe vexans*) is considered to be the most destructive and one of the major constraints of successful brinjal cultivation, causing severe losses in different parts of the world (Ashrafuzzaman, 2006; Islam *et al.*, 2010; Jayaramaiah *et al.*, 2013; Bhat *et al.*,2019). Primary infection is caused by the fungus surviving in crop debris, seeds and soil, whereas, the secondary infection is caused by α-conidia, released from pycnidial fruiting body and dispersed by splashing rain, insects and contaminated equipment (Howard and David, 2007). Initially small, circular spots appear on the leaves which later turn grey to brown with a lighter colored centre. Later whole leaves turn yellow and

eventually die. Lesions on petiole and stem can cause blighting of the affected portions. On fruits also, pale to light brown sunken spots are clearly visible. Later these spots enlarge showing conspicuous concentric rings and eventually the fruits are rotten and mummified (Kumar *et al.*, 1986).

Variability is an important characteristic of pathogens which imparts immense benefits to the pathogenic population. Variability has been reported in *Alternaria porri* (Shahnaz *et al.*, 2013); *Phomopsis vexans* (Jamir *et al.*, 2018); *Sclerotinia sclerotiorum* (Chaudhary *et al*., 2020); *Sacrocladium oryzae* (Kumar and Rai, 2021) and *Fusarium udum* (Bindhu *et al.*, 2021), among others. The present study was an effort to study variability among the isolates of *Phomopsis vexans* obtained from different locations of the Union Territory of Jammu & Kashmir.

MATERIALS AND METHODS

Pathogenicity test. Pathogenicity test was performed on seeds, one month old seedlings and on detached fruits of most susceptible brinjal variety 'Pusa Purple Long' using the method adopted by Sanders and Koreston (2004).

Variability among the isolates. Three districts from the Union Territory of Jammu & Kashmir were selected for the collection of thirty isolates of *P. vexans.* From each of these districts two locations were selected *viz.* Palpora and Tailbal from Srinagar, Chadoora and Narkora from Budgam and Lar and Zazuna from Ganderbal. These isolates of *P. vexans* obtained from different sites of the said locations were named as PV_1 to PV_{30} and were used for further cultural, morphological and physiological studies.

Pathogenic variability. There were five isolates of the pathogen (PV_1, PV_{11}, PV_{16}, PV_{17} and PV_{21}) evaluated against 10 germplasm lines of brinjal (Pusa purple cluster, Kashi Tauro, Pusa kranti, Local Long, Pusa Purple Long, KS-224, Swarn mani, SK-BL-01, SK-BL-08 and Pb. Sadabahar) and the reaction of different brinjal lines against different isolates was recorded after inoculation of these isolates on the selected brinjal lines.

Germinability of conidia. Alpha (α) and beta (β) conidial germination of all the 30 isolates of *P. vexans* was studied to observe whether the isolates varied in their spore germinability by using the 30-days old culture grown on PDA. The pycnidia were taken in sterilized distilled water and crushed with the help of sterilized needle, the spore suspension so obtained was filtered through muslin cloth. Subsequently, the concentration of conidia was adjusted to 50-60 conidia per microscopic field with the help of haemocytometer. 10 µl of conidial suspension of each isolate was placed on cavity glass slides. The slides were then placed in petriplates having moist blotting sheets to maintain humidity and incubated at 25±1°C for 24 hours. The slides of each isolate were examined microscopically after 24 hours of incubation. Total three replications were taken for each isolate. Before observations, the slides were stained using cotton blue lactophenol. Per cent germination was observed after 24 hours and was calculated using formula given by Vincent (1947):

Per cent spore germination =

$$\frac{\text{No. of spores germinated}}{\text{Total no. of spores observed}} \times 100$$

RESULTS

Isolation and purification. Diseased samples of brinjal consisting of seed, leaf, stem and fruit were used for the isolation of the pathogen. The pathogen then was purified by single spore technique and transferred to fresh slants containing potato dextrose agar medium.

Pathogenicity test. Pathogenicity of *Phomopsis vexans* was determined on seedlings and injured/un-injured detached fruits of highly susceptible Pusa Purple Long (PPL) cultivar of brinjal. Observations regarding the pathogenicity of the test pathogen revealed that the initiation of typical symptoms of the disease were recorded after 7 days of inoculation on seedlings and 10 days after inoculation on pin pricked fruits (Plate 1). However, in case of uninjured fruits, the disease symptoms developed 18 days after inoculation. Re-isolation from infected seedlings and fruits produced the typical culture of the pathogen, thus confirming Koch's postulates.

Control Inoculated Control Inoculated

Plate 1: Pathogenicity.

Pathogenicity test on seeds was conducted on apparently healthy seeds of variety 'Pusa Purple Long' by rolling them on actively growing culture of *P. vexans*. During this test it was found that seeds inoculated with *P. vexans* showed germination failure and produced poor and weak seedlings which resulted in damping off symptoms on tender stems as compared to normal seedlings produced by healthy seeds (Table 1), thereby confirming its pathogenic nature to cause the disease. Hence it has been proved that the pathogen is able to reduce the germination of seeds.

Table 1: Effect of *Phomopsis vexans* on germination and vigour of seedlings.

Treatments	Normal seedlings	Infected seedlings	Ungerminated seeds
Healthy seeds (Control)	46	-	4
Contaminated seeds (*P. vexans*)	14	15	21
Result based on 50 seeds in each case			

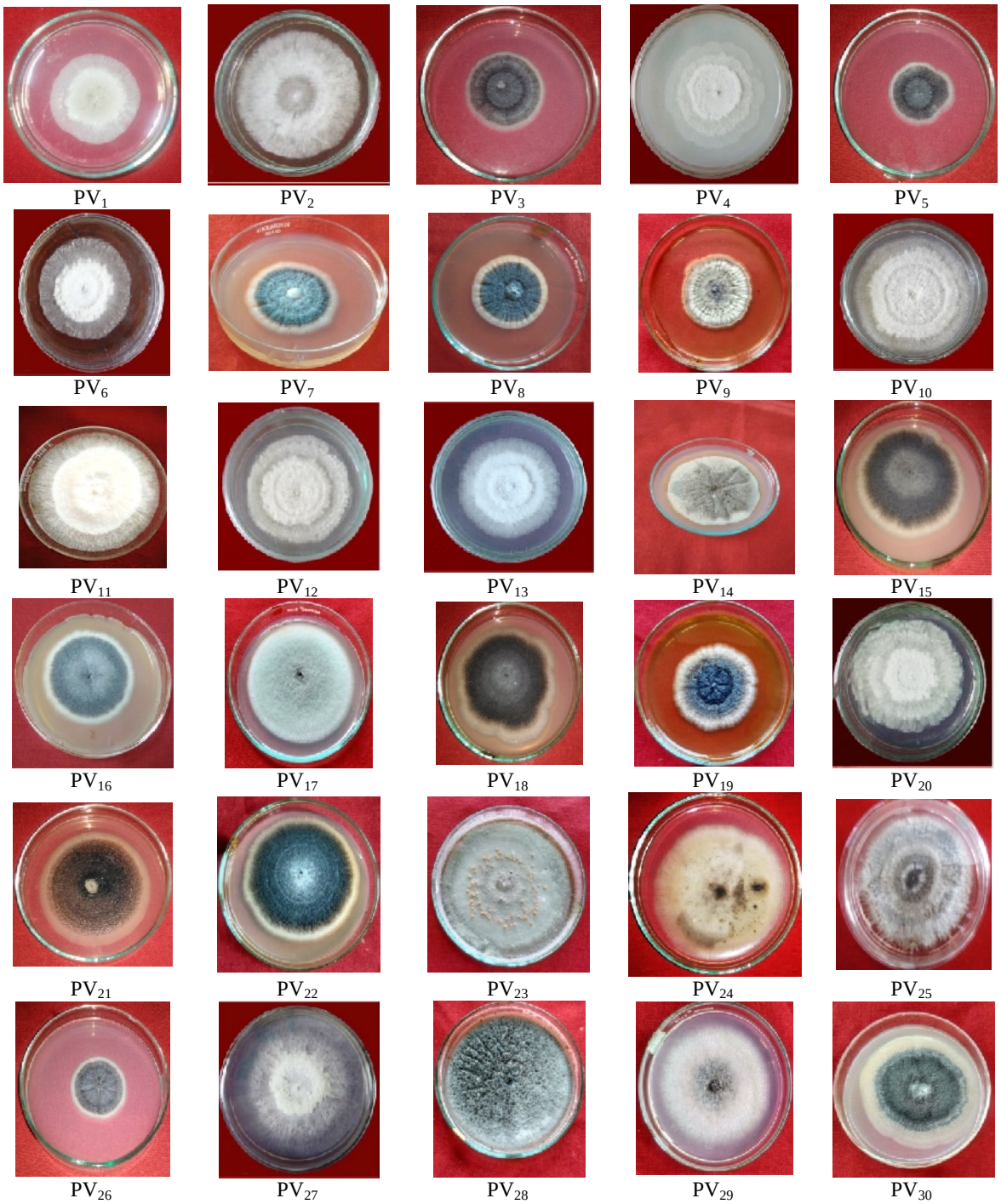

Plate 2: Cultural variability in isolates of *Phomopsis vexans.*

Variability among the isolates of *Phomopsis vexans.* The different cultures of *P. vexans* differed significantly in their cultural characteristics and there was a distinct variation in shape and color of colony, type, radial growth and mycelial weight. The colony colour varied from snowy white to creamy/milky white among most of the isolates viz. PV_1, PV_2, PV_4, PV_6, PV_9, PV_{10}, PV_{11}, PV_{12}, PV_{13}, PV_{20}, PV_{24} and PV_{27}, while black to brown in PV_3, PV_5, PV_7, PV_8, PV_{15} and PV_{18}, whereas, PV_{14}, PV_{16}, PV_{17}, PV_{23}, PV_{26} and PV_{28} were greyish in colour. Some of the isolates namely PV_{21} and PV_{29} were pink in colour. Most of the isolates had fluffy mycelial growth with regular to irregular margins. While fluffy growth was observed in PV_1, PV_2, PV_6, PV_7, PV_8, PV_{11}, PV_{12}, PV_{13}, PV_{19}, PV_{20}, PV_{23}, PV_{24}, PV_{25}, PV_{27}, PV_{28} and PV_{29}, remaining isolates namely PV_3, PV_4, PV_5, PV_9, PV_{10}, PV_{14}, PV_{15}, PV_{16}, PV_{17}, PV_{18}, PV_{21}, PV_{22}, PV_{26} and PV_{30} showed

embedded mycelial growth. The development of zonation also varied considerably among the isolates with some isolates viz. PV_4, PV_5, PV_6, PV_8, PV_{10}, PV_{12}, PV_{13} and PV_{24} showing clear and distinct zonation. In rest of the isolates namely PV_1, PV_2, PV_3, PV_7, PV_9, PV_{11}, PV_{12}, PV_{14}, PV_{15}, PV_{16}, PV_{17}, PV_{18}, PV_{19}, PV_{20}, PV_{21}, PV_{22}, PV_{23}, PV_{25}, PV_{26}, PV_{27}, PV_{28}, PV_{29} and PV_{30} zonation did not develop or if developed it was not clear (Plate 2). After 12 gays of growth, there was a significant variation in the radial growth (mm) of different isolates. The fastest mean radial growth of 84.10 mm was recorded in PV_{23} and PV_{28} followed by PV_{10}, PV_2 and PV_{25} showing radial growth of 83.36, 83.18, 83.02 mm, respectively. The lowest mycelial growth of 29.15 mm was recorded in PV_{26}. With respect to mycelial dry weight, maximum dry weight of 408.90 mg was found in PV_{28} followed by PV_{10} (406.32 mg) and minimum dry weight of 107.20 mg was produced by isolate PV_{26}.

Density and germinability of conidia. Minimum spore count of α-conidia i.e. 1.47×10^4 per ml was recorded in isolate PV_{20} while as maximum spore count of 37.25 $\times 10^4$ per ml was observed in isolate PV_{16}. In rest of the isolates sporulation density ranged between 1.47-37.25 (10^4) per ml (Table 2). All the isolates of *P. vexans* were examined for their spore germinability to observe the variation. Maximum germination of 68.27 per cent was recorded in isolate PV_{30} however minimum (11.15%) was observed in isolate PV_{19}. The results further revealed that in remaining isolates, spores germinated between 11.15-68.27 per cent. The β-conidia of all the isolates did not germinate irrespective of the incubation periods.

Table 2:Variability in conidia of different isolates with respect to spore density and germination.

Isolate	Concentration* of α-conidia ($\times 10^4$)/ ml	Germination* (%)	Concentration of β-conidia ($\times 10^4$)/ ml	Germination (%)
PV 1	21.13	62.05	0.00	0.00
PV 2	19.37	47.15	0.00	0.00
PV 3	10.45	39.28	0.00	0.00
PV 4	21.33	46.12	0.00	0.00
PV 5	8.71	49.20	0.00	0.00
PV 6	30.42	57.43	0.00	0.00
PV 7	26.59	41.35	0.00	0.00
PV 8	18.55	49.66	0.00	0.00
PV 9	28.09	16.40	0.00	0.00
PV 10	19.46	45.53	0.00	0.00
PV 11	26.11	60.10	0.00	0.00
PV 12	23.15	50.14	0.00	0.00
PV 13	21.75	54.04	0.00	0.00
PV 14	31.10	48.66	0.00	0.00
PV 15	18.39	32.57	0.00	0.00
PV 16	37.25	61.38	0.00	0.00
PV 17	36.17	66.31	0.00	0.00
PV 18	33.16	57.33	0.00	0.00
PV 19	4.28	11.15	0.00	0.00
PV 20	1.47	53.22	0.00	0.00
PV 21	20.36	60.45	0.00	0.00
PV 22	12.34	56.13	0.00	0.00
PV 23	14.55	41.66	0.00	0.00
PV 24	26.18	45.00	0.00	0.00
PV 25	27.15	51.74	0.00	0.00
PV 26	32.71	55.61	0.00	0.00
PV 27	22.62	59.02	0.00	0.00
PV 28	29.03	24.53	0.00	0.00
PV 29	6.58	30.29	0.00	0.00
PV 30	4.35	68.27	0.00	0.00

*Mean of three replicates

DISCUSSION

The perfect stage of the pathogen was not observed during the course of present investigations. Similar results have been reported by other workers (Islam *et al.*, 2010; Ganaie, 2014). The pathogenicity of *P. vexans* was successfully established on seeds, young seedlings and detached fruits of brinjal by different methods. Mir, (1992) proved pathogenicity of *P. vexans* by inoculation, with conidial suspension of the pathogen, of healthy seeds and detached injured brinjal fruits. Similar technique on fruits was also adopted by Thind and Jhooty (1990); Ganaie (2014) to prove the pathogenicity of *P. vexans*. Ahmad (1987) observed that fruits inoculated with *P. vexans* by pin prick method developed symptoms like rot lesions after 15 days of incubation at 25°C in BOD incubator. Singh and Chand, (1986) while investigating the pathogenicity of *P. vexans* revealed that un-injured mature fruits did not produce any symptoms but the fruits given half centimeter injury produced clear symptoms. Similarly, Lal *et al.* (1981) have also

confirmed and reported that the plant injury means of various biotic (Verma and Anwar, 1997-98) and abiotic injury is necessary for disease development and ripe fruits are more susceptible than green ones. These observations are in agreement with the present findings. Results of the pathogenicity on seedlings were in support of Bhardwaj and Vineeta, (2013) who observed that symptoms on brinjal seedlings appeared after 7-9 days after inoculation of *P. vexans*. Besides colony character and its growth characteristics formed major inputs to confirm identity of the *P. vexans*. Our results found during the present study are also in agreement with the above findings**.**

The present study on spore count and germinability of α-conidia of different isolates was made by recording concentration of spores per ml of the spore suspension and the germinability after 24 hours of incubation. All the isolates distinguished significantly with respect to spore count as very little spore count was recorded in isolate PV_{20} while as maximum spore count was found in isolate PV_{16}. Similarly, significant variation was found with respect to germinability of α-conidia in different isolates of *P. vexans*. Maximum germination of 68.27 per cent was recorded in isolate PV_{30} while as minimum germination of 11.15 per cent was found in isolate PV_{19}. The results attained are in conformity to those of Islam (2006); Akhtar (2006); Akhtar and Chaube, (2006).

A successful disease management programme depends on a number of factors, foremost among which is an understanding of pathogen population structure and mechanism by which variation arises within populations. These variations in populations of pathogens can be discerned on the basis of different morphological, cultural and pathogenic characteristics. The present work was a step towards ascertaining the prevalence of variation amongst the isolates of *P. vexans* obtained from different locations of the valley. Variation among different locations in furnishing the isolates might be due to variation in environmental conditions like temperature, relative humidity, rainfall etc. and also due to different disease status at different locations. High variability at some locations corresponded to high disease incidence and intensity in those locations. This can be attributed to the use of contaminated seeds, susceptible varieties, microclimate (modified by variation in plant spacing) besides faulty cultural practices and neglected spray programme. Variability in *P. vexans* might also be due to genetic factors like mutation, recombination, parasexuality etc.

In the present study isolates of *P. vexans* varied in their cultural characteristics viz. colony type, colony colour and germinability and density of spores. Colonies were fluffy and embedded with colour ranging from white, pinkish to blackish brown and greyish. The extent and magnitude of differences, invariably and significantly do establish the existence of variability in natural population of *P. vexans.* Several workers have also reported cultural variability among the isolates of *P. vexans* (Akhtar and Chaube, 2006; Islam *et al.*, 2010; Rohini *et al.*, 2016; Ahmad *et al*., 2017; Thesiya *et al.*, 2020). Heng *et al.*, (2021) analysed the genome sequence of *Phomopsis vexans*: a fungal pathogen causing Phomopsis blight of eggplant and found that the complete genome was about 59.78 Mb with 51.24% G+C content and 4.93 Mb contig. N50. Rajput *et al.*, (2021) created three groups A, B and C through neighbour joining cluster analysis based on the morpho-cultural characteristics of twelve *Phomopsis* isolates collected from various oilseed crops. Thus, it is clear that variability exists in different isolates of *Phomopsis vexans* isolates of Jammu & Kashmir. However, further studies are needed to ascertain the genetic diversity of this species under the agro-climatic conditions of Kashmir.

Conflict of Interest. None.

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