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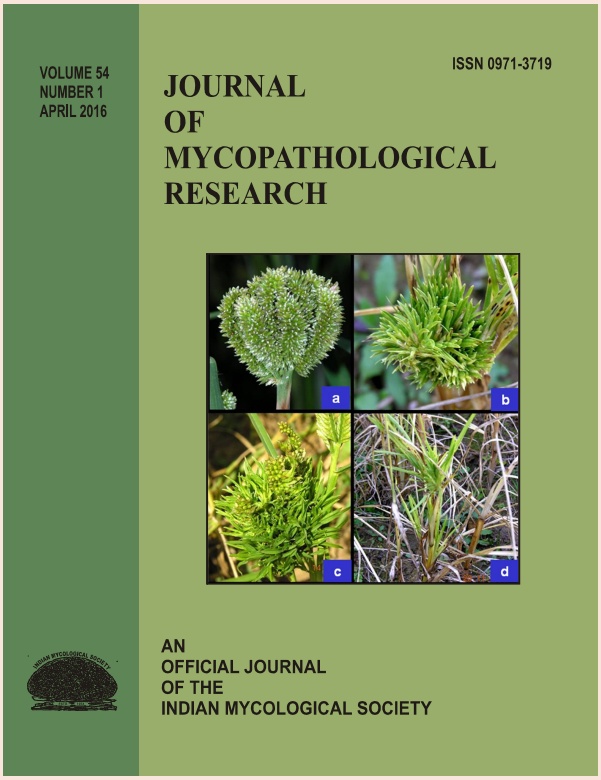

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Variation in spore morphology and relative virulence among isolates of *Phytopthora parasitica* causing Foot rot and Leaf rot of betelvine (*Piper betle* L.)

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Five isolates of *Phytophthora* spp. (P1, P6, P7, P8 and P9) of betelvine were studied from infected leaves and stems of betelvine. The cultural study among the isolates had dull white to white colour of mycelium with compact growth. In morphometric study, P9 had highest length, breadth and P1 had shortest length, breadth of sporangia and they were pear, lemon or spherical in shape. The relative dimension of chlamydospore revealed that P8 and P1 isolates were the largest and smallest respectively. Attachment was terminal and /or intercalary. Pathogenicity test was also carried out and it was observed that P8 was highly virulent and P1 was less virulent.

Key words: Spore morphology, relative virulence, foot rot and leaf rot, *Phytophthora parasitica,* Betelvine

INTRODUCTION

The betelvine favours tropical fresh conditions which provide cool shaded, considerable humidity and adequate supply of moisture in soil, diffused light. Ironically, these conditions are also ideal for the growth of pathogenic fungi and bacteria which cause the several destructive diseases. A number of diseases have been reported from India. The major ones among these are Foot rot and Leaf rot caused by *Phytophthora parasitica* Butler causing losses from 30%-100% in case of foot rot and 20%-40% in case of leaf rot leading to almost total crop failure (Dasgupta *et al*, 2000).The extent of losses varies may be due to the presence of different isolates with same species in different plant barejas of West Bengal. The present investigation has been carried out to study variation in spore morphology and relative virulence among isolates of *Phytophthora parasitica* causing Foot rot and Leaf rot of betelvine

MATERIALS AND METHODS

Isolation of Pathogen from Infected Stem/Leaf

The samples of Foot rot and Leaf rot caused by *Phytophthora parasitica* were collected from different barejas of Nadia and 24-pargana (N) where incidence of foot rot and leaf rot were high. Infected leaf samples with actively progressing lesions were selected and washed thoroughly in tap water. The leaf samples were then cut into small pieces having some infected and some healthy portion, surface sterilized with 0.1% $HgCl_2$ solution for one minute and then washed thoroughly for three to four times in sterile distilled water under aseptic condition. The leaf samples were then placed aseptically in sterile petri plates containing V8 juice agar medium and incubated at 28±°C. After 72 hrs. the growing fungus was examined under microscope. Small discs of initial mycelial growth of pathogen next transferred to OMA (Oat Meal Agar me-

dium) and incubated for 4-5 days at 28±°C. The culture was also maintained in OMA slants at 5°C and sub culturing was done at 15 days intervals. One set of isolates was preserved in liquid paraffin at 5^0C.

Isolate characteristics of the pathogen

All the isolates of *Phytophthora parasitica* were grown on OMA for 7 to 21 days. Semi-permanent mounts were prepared from 7 to 21 days old cultures. Length, breadth, length: breadth andshape of the sporangia and chlamydospores and their type of formation were observed under microscope. They were photographed where ever possible.

Cultural characteristics of pathogen

The Petri plates containing OMA was subsequently inoculated at the centre with the mycelial discs taken from 5 days old culture and incubated at 25° ± 1°C in a BOD incubator starting from the first day of incubation. At 24 hrs. of interval, data were recorded on colony diameter till a full plate growth was obtained. Any variations in culture morphologies and growth characters were found, also recorded for all isolates.

Morphometric study of the isolates

The morphometric measurement i.e. length, breadth, length: breadth and shape of the sporangia and chlamydospores of the all the isolates were done. The measurements were taken by using standard technique i.e. by the use of ocular and stage micrometer.

Virulence of the pathogen

The virulence test of the isolates was conducted on leaf of Ghanagette variety by using different isolates of *Phytophthora parasitica* on excised leaves by inoculating the leaves with their petiole kept in a flask containing water. The test was conducted for all the isolates with 4 replications each and 5 leaves per replication. Suitable control was maintained by spraying water.

A spore (sporangia) suspension ($3.5x10^5$ sporangia/ml) was prepared from 8 days old culture grown on OMA media was sprayed on leaves, with an all glass atomizer and the whole set up was placed in the humid chamber. The disease was allowed to develop and the lesions appeared after 2-3 days of inoculation were observed and the virulence rating was done as Percent Disease Index i.e. the number of leaves infected was counted after 7 days of spraying. The intensity was rated on an empirical scale where 0 = no infection; 1 = 1 – 25 % area affected; 2 = 26 – 50 % area affected; 3 = 51 – 75 % area affected and 4 = more than 75 % area affected. Re-isolation was made from the artificially produced lesions. Percent Disease Index was calculated with the formula:

Per cent Disease Index = Sum of all numerical ratings x 100 / Total number of leaves x maximum score

The virulence test of the isolates causing foot rot was conducted in the same variety by placing 3 months old 5 betelvine healthy stem pieces side by side maintaining humid condition inside Petriplates. A sporangial suspension ($5x10^5$ sporangia/ml) was prepared and sprayed using an all glass atomizer on to the stem pieces and kept at room temperature for 3 – 4 days. The stem pieces were injured lightly by rubbing with carborurdum power. Suitable control was maintained by spraying water. Irregular, dark brown to black, water soaked lesions developed on the stem pieces. In stem rot the severity of disease was rated after 10 days. Re-isolation was made from the artificially produced lesions. Percent Disease incidence (PDI) was calculated as mentioned earlier.

RESULTS AND DISCUSSION

Five isolates of *Phytophthora parasitica* were isolated from infected betelvine stems and leaves. The isolates were collected from different parts of Nadia and North 24 Parganas districts of West Bengal (Table 1).

Cultural characteristics

Characteristics of 5 isolates were studied. It showed considerable variation in characteristics among the different isolates. The general morphological features of different isolates grown on OMA medium are presented in Table 2.

The characters were similar to those reported by Tiwari, 1973; Maiti and Sen, 1977; Mohanty, 2003.

Morphometric characteristic

Table 1 : Sources of isolates of pathogen

Isolates	Place of collection	Source	Variety
P_1	Kalyani (BCKV, Gene Bank, Barja-1, Nadia)	Leaf	Gol Bhabna
P_6	Simurali (AizalMondal, Nadia)	Leaf	Simurali Deshi
P_7	Simurali (SaidulMondal, Nadia)	Stem	Simurali Bhavna
P_8	Simurali (SelimMondal, Nadia)	Stem	Ghanagette
P_9	Mandauri Bareja, Mohanpur, Kanchrapara, North 24 Pargana	Leaf	Simurali Deshi

Table 2 : Cultural characteristics of pathogen

Isolates	Colony characters (grown on OMA)
P_1	Colony white in colour, compact growth, very fast mycelial growth.
P_6	Colony dull white in colour, moderately compact growth, slow mycelial growth.
P_7	Colony white in colour, no aerial mycelium, compact mycelial arrangement.
P_8	Colony white in colour, fast growth rate, regular margin, aerial mycelium absent.
P_9	Colony dirty white in colour, moderately compact growth of mycelium, fast rate of mycelial growth.

Table 3 : Relative size of sporangia of different isolates of *Phytophthora parasitica*

Isolates	Sporangial Measurement (mm)		Length : breadth	Shape
	Length	Breadth		
P_1	17.00 – 22.10 (19.32)*	14.20 – 15.50 (15.20)	1.27 : 1	Pear
P_6	27.10 – 29.00 (28.12)	16.50 – 18.60 (17.42)	1.61 : 1	Pear
P_7	18.70 – 23.20 (21.00)	15.10 – 15.90 (15.40)	1.36 : 1	Lemon
P_8	31.20 – 37.00 (34.36)	21.20 – 24.20 (21.66)	1.58 : 1	Lemon
P_9	35.30 – 41.60 (37.84)	23.30 – 25.80 (24.48)	1.54 : 1	Spherical

Morphometric characteristics of the test pathogens were recorded and are presented herewith.

Sporangial characteristics of different isolates

All the isolates were grown on OMA and semi-permanent mount was prepared from 7 days old cultures and observed under microscope. The sporangial measurements of different isolates did not show any significantly observable difference. The length, breadth, ratio of length and breadth and shape of sporangia of different isolates are presented in Table 3.

The results (Table 3) revealed that the average length of sporangia was highest in isolates P9- (37.84 mm) and shortest in P1 (19.32 mm). The other isolates were in between them in respect to length of sporangia. The average breadth of sporangia was maximum in isolate P9 (24.48 mm) and shortest in P1 (25.20 mm). The rests of the isolates were in between them. Highest length breadth ratio of sporangia was recorded in P6 (1.61:1) and lowest length breadth ratio was recorded in P1 (1.27: 1). The rest of the isolates with respect to L: B ratios were intermediary.

From the microscopic observation it was also found that sporangia were pear shaped in P1, P6, lemon shaped in P-7 and P8 and spherical in P9 with small apical papilla.

Relative dimensions of chlamydospore of different isolates of *Phytophthora parasitica*.

All the isolates were grown on OMA (Oat Meal Agar) and the size of chlamydospore was measured after 21 days under light microscope. The measurements of chlamydospores of different isolates showed observable differences. The range, average diameter and attachment of the chlamydospore of different isolates are presented below:

From the microscopic observations, it was found that all the chlamydospore were of thick walled double layered, spherical to rounded shape . From the table (Table 4) it was revealed that the largest chlamydospore were found in P8 (30.6m) and smallest was P7 (23.8m). The remaining isolates

were intermediate into the dimension of chlamydospore.

Attachment of the chlamydospore was intercalary or / and terminal type. Terminal chlamydospores was found in P1, P8, P9 and intercalary chlamydospores were noticed in P6 and P7 (Table 4).

Virulence test of isolates of P. parasitica towards betelvine stem

Virulence of different isolates of *Phytophthora parasitica* was tested on healthy, betelvine stems following the method described earlier and the result (Table 5) showed a marked variation in the virulence of the different isolates towards the stems of the host (betelvine). The result showed that the isolate P6 was highly virulent causing 90% damage, it did not differ significantly with P1 and P8. The least virulent was P9 causing 10% damage which was statistically at par with P7. In general the isolates of *Phytophthora parasitica*can be arranged in the following order of their virulence towards stem: P6, P1, P8 P7, P9.

Table 4 : Dimensional characteristics of chlamydospore of *Phytophthora prasitica*

Isolates	Chlamydospores		
	Diameter range (μ)	Average diameter (μ)	Attachment
P_1	(23.0 – 29.3)*	26.6	Terminal
P_6	(23.4 – 30.2)	26.85	Intercalary
P_7	(21.2 – 25.5)	23.8	Intercalary
P_8	(27.5 – 33.5)	30.6	Terminal
P_9	(25.0 – 30.5)	28.0	Terminal

Table 5: Relative virulence of isolates of *Phytophthora parasitica* towards betelvine stems and leaves

Isolates	PDI on stem*	PDI on leaf*
P_1	51.87 (46.07)	42.75 (40.79)
P_6	60.47 (51.14)	78.52 (62.88)
P_7	37.85 (37.62)	67.10 (55.15)
P_8	45.55 (42.43)	80.17 (63.86)
P_9	29.55 (32.88)	49.60 (44.76)
SEm (±)	2.99	3.82
CD at 5%	9.18	11.73
CD at 1%	12.87	16.45
CV %	10.08	10.11

* Present disease index (PDI) are mean of 3 replications, figure of parentheses are angular transformed values.

Virulence test of isolates of P. parasitica towards betelvine leaves

Different isolates of *Phytophthora parasitica* was tested on healthy betelvine leaves for virulence following the method described earlier and the result (Table 5) showed that a marked variation in the virulence of the different isolates towards the leaves of the same host as indicated by the percent disease incidence (PDI). Isolate P8 showed highest level of virulence and it was statistically at par with P6 and P7. The least virulent isolate was P1 which did not differ significantly with P9-.In general, the isolates tested can be arranged in the following order of virulence:P8, P6, P7, P9, P1.
Finally, the isolates of *Phytophthora parasitica* can be categorized in the following way based on virulence towards leaves and stem:

P6 = Highly virulent towards stem.
P1 = Highly virulent towards stem and weekly virulent to leaf.
P8 = Highly virulent towards leaf and stem.
P7 = Moderately virulent towards leaf and weekly virulent to stem.
P9 = Very weakly virulent towards leaf and stem.

Variable percentage of disease incidence (PDI) recorded from pathogenecity test in isolates of *Phytophthora parasitica* might be due to uniqueness of the isolates collected from various sources. Besides, virulence towards leaf and stem of betelvine was interchangeable and would be a function of minor shifts in the biotic-abiotic micro-ecological conditions.

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