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ISOLATION AND IDENTIFICATION OF *PHYTOPHTHORA CAPSICI* AND ITS MATING TYPE DETERMINATION

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Abstract: An investigation was conducted at the laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh during the period from January to April 2006 to isolate Phytophthora capsici from the field soils affected with collar and root rot of chilli. Soil and plant samples were collected from affected chilli fields in horticulture centre, BAU, Brahmaputra river side and villages near BAU campus where 42.86% tissue and 14.26% soil samples from horticulture centre, BAU; 50% tissue and 25% soil samples from Brahmaputra river sides; and 16.66% samples in both cases (tissue and soil) from villages near BAU campus were involved. All the sides showed positive result for P. capsici. The isolated P. capsici was found to be heterothallic.
Key words: *Phytophthora*, Collar rot, Root rot, Isolation, Identification, Mating type.

Introduction

Collar and root rot of pepper (*Capsicum annum*) caused by *Phytophthora capsici*, is one of the most economically destructive soil-borne diseases of pepper throughout the world. *P. capsici* was first described by Leonian on pepper in New Mexico in 1992 (Leonian, 1992). In 1931, Tucker (1931) classified it as a species of the genus *Phytophthora* and considered *P. capsici* as a host-specific fungus pathogenic to pepper. The most common symptoms associated with the disease are wilting followed by root and collar rot with dark brown stem lesion extending upward from the soil line (Black *et al*., 1991). The advancing lesion eventually girdles the main stem and kills the whole plant (Bowers and Mitchell, 1991). The disease often called chilli wilt, often differs from vascular wilts caused by *Verticillium dahliae* and *Fusarium oxysporum*. Large plants wilt and die, leaving brown stalks and leaves. If the fungus enters the roots, the plants cannot obtain enough water, may suddenly wilt, and eventually die. When the disease is severe, the fungus may attack main stems and branches, causing brown or black spots that kill that portion of the plant. Survey conducted by Kim *et al.* (1990) in Suwon, South Korea, indicated that plants receiving numerous injuries due to cultivation developed the highest incidence of *P. capsici* blight. Phytophthora root rot of New Mexican-type peppers has been observed to increase immediately after cultivation. The disease caused by the soil borne fungus is widespread in furrow-irrigated fields. This disease generally occurs under excessively wet conditions, usually in heavy soil or low spots in a field. The disease may occur in patches in a field, or follow rows, and is spread in presence of water. Infected plants exhibit severe wilting, die and turn straw-colored. Plants may defoliate. Roots become dead and the root barks sluff off easily. *P. capsici* commonly occurs in temperate, tropical and sub-tropical environments. Although root and collar rot like symptom on chilli plant are common in Bangladesh, but unfortunately no detailed study was conducted on collar and root rot associated with *P. capsici.* Therefore, this study was initiated to isolate and identify *Phytophthora capsici* and its mating type.

Materials and Methods

Diseased samples (both tissue and soil) were collected from horticultural centre of Bangladesh Agricultural University (BAU) campus, Brahmaputra riverside and around the villages near BAU. Isolations were made by two methods- Tissue planting method and Baiting method.

Isolation by tissue planting method: The collected tissue samples were first washed in tap water to make them free from soil and sand. Then small pieces of infected stem, leaf and root were cut out from the juncture of the healthy and diseased tissues. These pieces were rinsed in sterile water and blotted to dry. Then the tissue segments were placed on commercial agar amended with pimaricin, ampicillin, rifamficin, pentachloronitrobenzene (PCNB) and hymexazol (RPARH) medium. This semi selective culture medium was used for *P. capsici* isolation from infected tissues placed in RPARH media and allowed for incubation in the dark place. Then mycelial tips from the edge of the growing colony of *P. capsici* were transferred on carrot agar medium.

Baiting on fruits: Fifteen grams of each of the collected soil sample was taken in to 30 petri plates for the experiment. About 40 ml sterile distilled water was taken into these petri plates. Ten petri plates for Brinjal fruits, 10 for Tomato and the rest 10 for chilli fruits. The fruits were placed on the petri plates and kept at room temperature for 2 to 3 days and observations were made twice a day for any fungal growth. For confirmatory diagnosis small portion of infected lesions were cut out and placed on sterile water and was observed under microscope. Then the infected fruit surface area was placed in RPARH medium kept in dark place for sporangia production or mycelial growth of *Phytopthora*, if any.

Baiting on leaves: Fifteen grams of infected soil was taken into 30 plastic bottles and soil was covered with pieces of white cloths. Sufficient amount of water was added to the bottles so that the water level was just 1 cm above the cloth. Ten pieces of Chilli, 10 pieces of Brinjal, 10 pieces of Tomato leaves were taken and cut with cork borer. Six pieces of leaves were placed per bottle and kept at room temperature for 24 hours. After 24 hours of incubation the pieces of leaves were taken out from the bottle and placed on tissue paper for blotting. The blotted pieces of leaves were placed on RPARH medium at the rate of 6 disks per plates. The plates were then kept at dark condition for 2 days and observation was made at every 24 hours.

Fresh culture plates of *Phytophthora* were prepared in carrot agar by transferring single hyphal tip from RPARH medium and transfer on to a carrot agar medium.

Identification: The fungus was identified by observing colony characters such as linear growth, colour and sporulation following the available literature.

Temperature test*:* In this study carrot agar media was used. Agar plates were incubated with a 4 mm mycelial disc of test fungus and incubated at different temperature from 10^0 to 35ºC at an interval of 5ºC. Five plates were used for each temperature. After 24 hours, diameter of the colonies were measured.

Sporangia production: For the production of sporangia, 4 mm diameter mycelial block was placed on moistened chamber; sterile distilled water was added into the petri dishes and incubated at 18^0 c for 24 hours. After 24 hours, the morphology (length and breath) were studied using ocular micrometer.

Mating type determination: Isolates of *P. capsici* were grown on 20% clarified V8 juice (CV8) agar and crossed with known tester for A1 (Obtained from Professor Dr. Robart L. Wick, Department of Plant Soil and Insect Sciences, University of Massachusetts, Amherst MA, USA) and A2 (Cap1 to Cap11). A 4 mm diameter plug of non colonized medium was sandwiched between a plug of an A1 tester mycelium and the isolate of unknown compatibility type. After 4 days incubation at 24^0C the middle plug was examined under the microscope for oospore formation.

Data analysis: Data were subjected to statistical analysis and mean of all treatments were compared for difference among them with Duncan's Multiple Range Test (DMRT).

Results and Discussions

Isolation of *P. capsici* was made from Collar and root rot affected chilli plants. *P. capsici* were obtained from 21 of 50 collar and root rot affected samples.

Out of 50 soil and tissue samples collected from horticulture centre (14), Brahmaputra rive side (24) and villages (12) near BAU campus, 15 samples (30.00%) were found positive for *P. capsici* infection (Table 1), whereas 35 (70.00%) were negative for *P. capsici*. Out of 7 tissue samples and 7 soil samples collected from horticulture centre, 3 (42.86%) tissue samples and 1 (14.26%) soil samples of *P. capsici* were isolated. But 4 (57.14%) tissue samples and 6 (85.71%) soil samples showed negative results (Table 2). At Brahmaputra river side out of 12 tissue samples and 12 soil samples 6 (50%) and 3 (25%) samples were positive for *P. capsici* isolation respectively and 6 (50%) and 9 (75%) samples showed negative result respectively (Table 1). Out of 6 tissue samples and 6 soil samples collected from villages near BAU campus, 1 (16.66%) showed positive result for *P. capsici* in both cases (Table 1). The colony characters as observed in this investigation (Plate 3) do agree with those of Babadoost (2002) who described growth patterns of colonies as cottony, pettaloid, rosaceous and satellite.

Optimum temperature for mycelial growth of fungus was found to be 20^0C followed by 25^0C. The mycelial growth was totally absent at a temperature of 15^0C and 30^0C (Table 2). The results are in accordance with those of Tucker (1931), Waterhouse (1974) and Gopalachari (1984).

Sporangia of *P. capsici* were variable in shape and were papillate with long pedicles in the laboratory culture plate (Plate 1 and 2). Different shape of sporangia such as subspherical, ovoid, obovoid, ellipsoid, fusiform and pyriform was observed under microscope. Part of Chilli stem, Tomato fruit, Brinjal, and Chilli fruit cultured on RPAR developed typical cottony type colony of *P. capsici* (Plate 3). Disc of RPAR media with colony *P. capsici* placed on water media produced sporangia after 24 hour inculcation. The sporangia of the 4 isolates of *P. capsici* differed significantly in pedicel length, length and width. Isolate from tomato flesh had the highest pedicel length while brinjal flesh isolate was the widest. The ratio of length to width of the sporangia varied from 1.40:1 to1.82:1 (Table 3).

Out of all isolates 7 isolate of *P. capsici* (Cap 3, Cap 5, Cap 7, Cap 8, Cap 9, Cap 10 and Cap 11) showed A2 type of oospores (A2-isolated *P. capsici*)(Plate 4 a, b) and rest 4 isolates showed no oospores production (Table 4). So, the isolated *P. capsici* was heterothallic and shows A2 type of mating. The pathogen is heterothallic and both A1 and A2 types exist in processing field in Illinois (Babadoost, 2006). These findings were similar to the findings of Ploetz *et. al*. (2002) who also isolated both mating types from the same squash plant in Florida. Measurement of oospores and oogonia were taken from 7 isolated *P. Capsici* (Table 5). The average size of 12 oospores and oogonia of Cap3 was 107.7 and 91.37 µm, respectively. Similarly, average size of 27 Oospores and oogonia of Cap 5 was 123.8 and 100.4 µm, respectively. Twenty one oospores and oogonia from a culture plate of Cap 7 isolate measured 108.20 and 76.23 µm respectively. Cap 8 isolate produced oospore and oogonia with an average size of 125.0 and 106.22 µm, respectively. Likewise Cap 9 produced 24 oospore and oogonia with an average size 89.1and 68.00 µm, respectively. Eighteen oospore and oogonia produced from Cap 10 isolate showed an average size 92.36 and 65.43 µm, respectively. The average size of 16 oospore and oogonia of Cap11 was 142.0 and 122.2 µm, respectively.

In the study, the pathogen isolated from infected pepper plants showing collar and root rot was identified as *P. Capsisi* being the causal organism of the disease. The isolated pathogen was heterothallic and had A2 type of mating.

Table 1. Isolation of *P. capsici* from tissue and soil samples collected from different locations

Areas of sampling	Type of samples	Number of samples	Number of positive samples (%)	Number of negative samples (%)
Horticultural Centre, BAU	Tissue	7	3 (42.86)	4 (57.14)
	Soil	7	1(14.26)	6(85.71)
Brahmaputra River Side	Tissue	12	6 (50.00)	6(50.00)
	Soil	12	3(25.00)	9(75.00)
Villages Near BAU Campus	Tissue	6	1(16.66)	5(83.33)
	Soil	6	1(16.66)	5(83.33)
Total		50	15(30.00)	35(70.00)

Table 2. Effect of temperature on vegetative growth of *P. capsici*

Sl. No.	Temperature ^{0}C	Colony Diameter (mm)
1	10	0
2	15	1
3	20	2
4	25	2
5	30	1
6	35	0

Table 3. Measurement of size of Sporangia

Isolate	Pedicel length	Length	Breadth	Ratio of length and breath
IS1	18.86ab	18.86ab	13.43ab	1.40:1
IS2	32.14a	23.00a	12.64ab	1.82:1
IS3	4.71b	22.14a	14.29a	1.55:1
IS4	17.43ab	16.00b	11.00b	1.45:1

Same letter (s) in a column do not differ significantly at 1% level of probability

Table 4. Result of mating type determination of Isolated *P. capsici*.

Known Isolate of P. capsici	Unknown P. capsici	Reaction	Result
A1	Cap1	-ve	A1
A1	Cap2	-ve	A1
A1	Cap3	+ve	A2
A1	Cap4	-ve	A1
A1	Cap5	+ve	A2
A1	Cap6	-ve	A1
A1	Cap7	+ve	A2
A1	Cap8	+ve	A2
A1	Cap9	+ve	A2
A1	Cap10	+ve	A2
A1	Cap11	+ve	A2

Table 5. Measurement of Oospore and Oogonia

Isolate	Average size of Oospore (µm)	Average size of Oogonia (µm)	Number Oospore/ Plate
Cap3	107.7	91.37	12
Cap5	123.8	100.4	27
Cap7	108.20	76.23	21
Cap8	125.0	106.22	20
Cap9	89.1	68.00	24
Cap10	92.36	65.43	18
Cap11	142.0	122.2	16

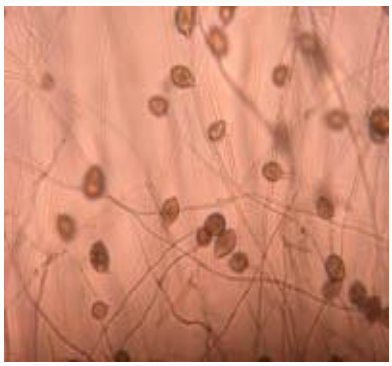

Plate 1. Sporangia of *P. capsici* under microscope (10 X)

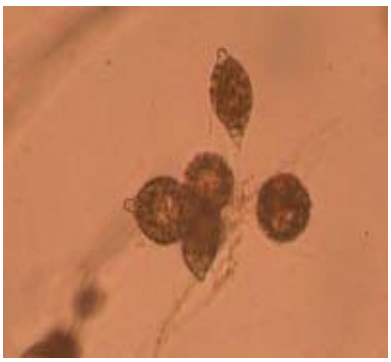

Plate 2. Sporangia of *P. capsici* under microscope (40 X)

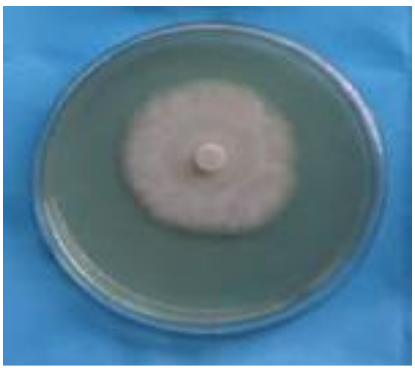

Plate 3. Pure culture of *Phytophthora capsici* in RPAR media

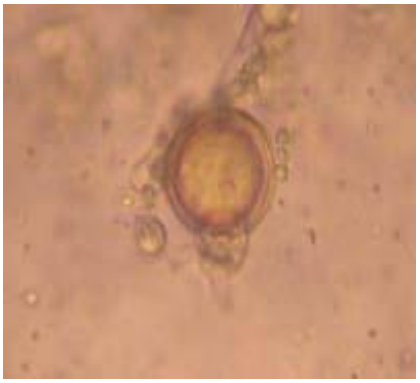

Plate 4a. A1 and A2 type isolate at mating stage		**Plate 4b. Newly formed Oospore of *P. capsici* after mating**

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