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Morphological and pathogenic variability of *Alternaria brassicae* causing Alternaria blight of Rapeseed-Mustard

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The pathogenic, morphological and cultural variability exist among five different isolates, collected from five different places of Uttar Pradesh namely Chaubepur (CHR), Ghatampur (GHA), Billhaur(BIL), Shivali (SHI) and Sarsaul (SAR). The variation in radial growth sporulation and conidial septation revealed that the maximum radial growth (52.5mm) and good sporulation were observed in isolates from Sarsaul and the minimum growth and fair sporulation was observed in isolates of Billhaur Kanpur district showing dark brown colony characteristics. As far as conidial septation is concerned, the horizontal septation varied from 4-13 and vertical from 0 – 6. The septum distance between two septa and length of beak also showed some variation. Pathogenic variability also existed in all the isolates showing 44.4 - 64.8% infection. In regard to disease severity on leave and pods it was found that maximum disease severity on leaves was caused by Ghatampur isolate but on pod it was by Bilhaur isolate.

Key words : *Alternaria brassicae,* Rapeseed-Mustard, morphological, pathogenic variability

INTRODUCTION

Rapeseed-mustard is a crop of tropical as well as temperate climate and require cool and dry weather for its satisfactory growth and development. In India, they are grown during *Rabi* season. The optimum sowing time is in the month of September-November in Uttar Pradesh. Rapeseed-mustard is capable of growing under a wide-range of soil conditions but they thrive best on light loam soils. The total production of rapeseed and mustard in India is about 6.7 m tones from 5.53M ha of land. The crop contributes 28.3% and 19.8% in world acreage and production and the productivity of crop is 6.41q/ha (Anonymous, 2007). However, both production and productivity of the crop in India is very low as compared to other country which is mainly due to biotic factors. Among the biotic factors Alternaria blight caused by *Alternaria brassicae* is one of the most yield limiting factor in India. The pathogen causes 10-70% yield loss in different parts of the country. The pathogenic, morphological and cultural variability existed in nature as reported by several workers. (Singh and Gupta, 1953; Chatterjee and Biswas, 2002; Awashti and Kolte, 1989, Kolte, 1986). The present study has been undertaken to understand the extent of variability of the pathogen concerned.

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MATERIALS AND METHODS

Survey and collection of disease samples

Disease samples were collected from farmers field of five different places of Kanpur district i.e: Chaubepur, Ghatampur, Billhaur, Shivli and Sarsaul during the month of December to March,2009-10. The affected leaves of mustard showing characteristic symptoms of Alternaria blight were collected and brought to the laboratory, Department of Plant Pathology for isolation and purification of pathogen.

Isolation and purification of pathogen

The leaf spot showing the initial and distinct char-

acteristic symptoms of Alternaria blight were selected for isolation of the pathogen. The selected leaves were washed in sterile water, cut into small bits along with some healthy green portion with a sterilized blade. The cut pieces were surface sterilised with in 0.1 per cent of mercuric chloride for 30 second and washed 3-4 times with sterilized water and excess moisture was dried between two folds of sterilized blotting paper under aseptic conditions in a inoculation chamber. The leaf pieces were then transferred with sterilized forceps to Petri dish poured with 2 % PDA medium. The Petridishes were incubated at 25° ± 1°c. As soon as mycelia growth was visible around the pieces, hyphal tip from the advancing mycelium were transferred aseptically in sterilized culture tubes containing 2% PDA medium.

The culture was then purified by single spore isolation technique. The purified cultures were then designated as CHR (Chaubepur), GHA (Ghatampur), BIL (Billhaur), SHI (Shivali) and SAR (Sarsaul).

Cultural and Morphological character

The cultural characters and colour of growth on PDA and Morphological characters like :

Colony character, Mycelium character, Conidiophore character and Conidial character were noted.

Pathogenic variability

The pathogenic variability of isolated pathogen was studied by grow out test. In a glass house of the Department, healthy leaves of variety *varuna* were inoculated with different isolates of pathogen in order to establish the pathogenic nature of the fungus.

Leaves were thoroughly washed by spraying sterilized water with an atomizer, slightly injured with sterilized needle keeping other remains uninjured. The plants were then inoculated with spore suspension of different isolates collected from different places of Uttar Pradesh. The concentration of the spore was maintained at 10^5 conidia/ml.

After inoculation, the plants were kept under moist chamber for 48 under polythene bags. Uninoculated plants were kept as check. Three replications were maintained. The pots were then out from the glasshouse and were watched for the appearance of the disease symptoms.

Measurement of severity of disease

Fifteen leaves were randomly collected from each replicate of treatment during the course of study. These leaves were arranged into six groups from zero to five, on the basis of the percentage leaf affected. The per cent disease index was calculated by the following formula as suggested by Conn *et al.* (1990).

$$PDI = \frac{\text{Sum of all numerical rating x class frequency}}{\text{Total no. of leaves x maximum class rating}} \times 100$$

RESULTS AND DISCUSSION

Cultural and Morphological characters

The results in all the cases showed that the colony in culture (PDA) is ashy grey, fluffy and circular in the beginning and later turned dark brownish due to sporulation, Mycelium is septate, branched and light brown at initial stage and in advanced stage, it becomes darker with slender radiating, branched, filamentous, measuring 3.0 to 5.9 mm wide. Conidiophores are simple, septate (0-8), epigenous with slightly swollen base and rounded apex, erect, geniculate with a prominent scar at each geniculation, 34.5-18.4 mm in length. Conidia are muriform, obclavate, elongate to oval with long beak, olive buff and 0-5 longitudinal septa, measuring 86.4 to 240.5 × 15.5 to 30.0 mm and produced in acropetal succession on conidiophores (Fig 1). These morphological characters closely resemble the description by several workers (Wiltshire, 1947; Singh and Gupta, 1953; Changsri and Weber, 1963; Siddiqui, 1963; Groves and Skolko, 1944 and Prasada *et al.*, 1970)

Data presented in Table-1 revealed that the isolates varied in radial growth sporulation and conidial septations. The Sarsaul isolate showed maximum radial growth (52.5 mm) and good sporulation and Billhaur isolates showed minimum growth and fair sporulation showing dark brown colony. As far as conidial septation is concerned, the horizontal septation varied from 4-13 numbers and vertical from 0 – 6. The septum distance between two septa beak, length and width also showed variation. Chaubepur (CHR) isolate

Table 1 : Morphological and cultural variation on radial growth and conidial characteristics of *Alternaria brassicae*.

Place	Radial growth (mm)	Sporulation	Number of 10 conodia/isolate septation	
			Horizontal	Vertical
Chaubepur	48.5	Fair	8.5(7-11)	1.1(1-4)
Ghatampur	45.6	Fair	7.2(6-10)	1.2(0-3)
Bilhaur	41.3	Fair	6.8(5-9)	1.0(0-4)
Shivli	40.7	Poor	6.5(4-9)	2.1(3-6)
Sarsaul	52.5	Good	8.4(7-13)	2.0(0-4)

Table 2: Pathogenic variability of *Alternaria brassicae* collected from different places of U.P.

Location (in farmers' field at)	Avg.spot size(mm)	Disease incidence (%)	
		on leaves	on pods
Farmer field Chaubepur, Kanpur.	8.4	58.6	30.8
Farmer field Ghatampur, Kanpur.	7.9	64.8	26.4
Farmer field Bilhaur, Kanpur.	6.8	64.5	36.3
Farmer field Shivli, Kanpur.	6.2	53.8	28.4
Farmer field Sarsaul, Kanpur.	10.6	44.4	30.6

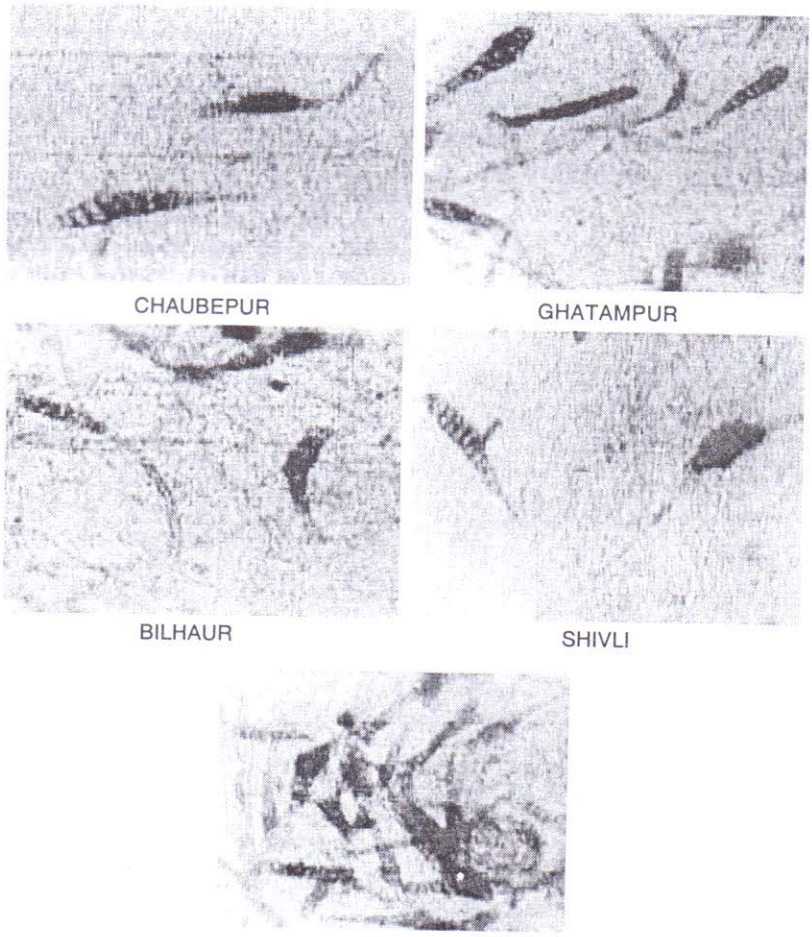

Fig. 1: Conidial characteristics of *Alternaria brassicae*, collected from different places of Uttar Pradesh

showed growth of 48.5 mm radius fair sporulation and number horizontal & vertical septation numbers was 8.5 (7-11) and 1.1(1-4), respectively.

Pathogenic variability

The characteristics of symptoms presented in the Table 2 showed that the size of spot varies from 6.2 to 10.6 mm in diameter. Isolate collected from Sarsaul have produced spot of maximum size (10.6mm.), followed by Chaubepur isolate (8.4mm.). The isolate collected from Chaubepur (CHR) showing average spot size 8.4 mm. The data in the table showed that all the isolates were able to cause 44.4 - 64.8% infection. The per cent disease severity on both leaves and pods was presented in Table 2. It was found that maximum disease severity on leaves was caused by Ghatampur isolate but on pod Bilhaur isolate was severe. There two isolates appered are not virulent. The minimum disease severity on leaves was by Sarsaul isolate and on pod by Ghatampur. Awasthi and Kolte (1989) have also distinguished three isolates of *Alternaria* spp. collected from different places of Uttar Pradesh. Kolte *et al.* (1985, 1986) have reported that Pantnagar isolates have resemblance with Bihar and Kanpur isolates in their morphological characters. Singh *et al.* (2009) also reported cultural and morphological variability of *A. brassicae* in Hisar.

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