

# Cultural and morphological variability in *Alternaria brassicae* isolates of Indian mustard (*Brassica juncea* L. Czern & Coss.)

Dhiraj Singh<sup>1</sup>, Rajender Singh<sup>1</sup>, Harbinder Singh<sup>1</sup>, Ram Chander Yadav<sup>2</sup>, Neelam Yadav<sup>2</sup>, Martin Barbetti<sup>3</sup>, Phil Salisbury<sup>4</sup>, Somveer Nimbal<sup>1</sup>, Chirantan Chattopadhyay<sup>5</sup>, Arvind Kumar<sup>5</sup>

<sup>1</sup>Deptt. of Plant Breeding, C.C.S. Haryana Agricultural University, Hisar 125004, India

<sup>2</sup>Deptt. of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar 125004, India.

<sup>3</sup>School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, W.A. 6009, Australia

<sup>4</sup>School of Agriculture and Food Systems, The University of Melbourne, Victoria 3010 Australia

<sup>5</sup>National Research Centre on Rapeseed-Mustard, Sewar, Bharatpur 321303, India

Email: oilseeds@hau.ernet.in; dhiraj542004@yahoo.com

## Abstract

*Alternaria* blight caused by *Alternaria brassicae* (Berk.) Sacc. is among the serious fungal diseases of Indian mustard (*Brassica juncea* L. Czern & Coss.). None of the cultivated species of oilseeds Brassica are observed free from the incidence of *Alternaria* blight. *Alternaria brassicae* is considered to be most virulent on all brassicaceous plants and cause adverse effect on both quality and quantity of the crop. The present investigation was carried out to know the cultural and morphological variability in *Alternaria brassicae* causing *alternaria* blight of oilseeds Brassica. One hundred and five disease samples were collected from 18 Districts of Haryana (India) at 20 to 25 km intervals. The spot/lesion size on leaves of the collected samples ranged from 3.0 to 11.5 mm. These samples were isolated and purified by single spore technique to study the morphological, cultural and radial growth behavior at 20 and 25°C. The radial growth varied from 34.6-81.1 mm with creamish, light brown to dark brown in colour and compressed to fluffy mycelial growth. The average conidial length ranged from 117.0 to 192.0 µm and breadth from 14.0 to 24.0 µm. The conidial beak length varied from 42.0 to 116.0 µm, number of horizontal/longitudinal septa ranged from 6 to 9 and vertical/transverse septa ranged from 1 to 3 and average distance between two septa have also been determined.

**Key words:** *Alternaria brassicae*, morphological variability, cultural variability, symptoms

## Introduction

*Alternaria* blight caused by *Alternaria brassicae* (Berk) Sacc. is an economically important disease of oilseed brassica in many parts of the world which cause very severe losses both in terms of quantity and quality. In India, the yield losses to the extent of 70 percent have been reported (Chahal:1986, Kolte:1985, Saharan:1991). Out of four species of *Alternaria* known to occur on this crop. *Alternaria brassicae* is more severe one (Verma and Saharan, 1994.). Preliminary reports on variability in *Alternaria* species were made from Holland (Van Schreven, 1953) and UK (Mridha 1983.) There are number of reports on the existence variability in *A brassicae* in India on the basis of morphology, speculation, growth and cultural characteristics and reaction on set of host differentials. However, complete information is not available on Morphological variability of *A brassicae* isolates collected from all over Haryana state. Therefore present investigation was undertaken to find out the cultural and morphological variability in *A brassicae* isolated collected from all over Haryana.

## Material and Methods

*Alternaria* blight infected 105 samples were collected from 18 districts of Haryana at an interval of 20-25 Km. The pathogen *A brassicae* (Berk) Sacc. was isolated from diseased leaves as per method described by Dhingra and Sinclair (1985). The fungal colonies showing characteristics of *A brassicae* was picked up and sub cultured in Petri plates containing PDA supplemented with rose Bengal. These plates were incubated in BOD incubator at 25±1°C for 4 to 5 days. Isolates were then purified by single spore technique (Toussoun and Nelson, 1976). Isolates were maintained on PDA slants in a refrigerator at 5°C for further studies. The spot/lesion size on leaves of each collected sample was measured. Cultural characteristics of each isolates such as radial growth at 20 and 25°C, colony colour characteristics and growth behavior was observed. Morphological characteristics of each isolates such as conidia length, breadth, no. of septations, beak length and average distance between septum were recorded.

## Results

The collected *Alternaria* blight samples have variation in spot/lesion size and symptoms produced. The spot size varies from 3.0 to 11.5 mm in diameter having light brown to dark brown coloration with less to prominent concentric rings. The sample collected from Yamuna Nagar district have minimum spot size (3.0 mm) having light brown spot with less visible rings. The maximum spot size was observed from Sirsa district i.e. 11.5 mm with medium to large dark brown spots with well developed concentric rings.(Table 1) The cultural characteristics such as growth behavior and colony character were studied at

20 and 25°C. The pure culture of isolates collected from Yamuna Nagar have maximum growth i.e. 73.6 and 81.1 mm both at 20°C and 25°C followed by isolates collected from Hisar with creamy to dark brown colony character. Isolates collected from Bhiwani district had minimum growth i.e. 34.6 and 40.5 mm with dark brown colony characteristics both at 20 and 25°C (Table 3). Morphological observations recorded on each isolate revealed that isolates differed in their conidial size. The range of conidial length varies from 81.0 to 293.5 µm. Isolates collected from Jind have minimum where as isolates collected from Rohtak have maximum conidial length. The conidial breadth also ranges from 8.1 to 31.5 µm. The thickest conidium was of Isolate Kurukshetra and thinnest of isolate Sonapat. The horizontal septation varied from 4-12 and vertical from 0 to 5. The septum distance between two septa also showed some variation which ranges from 6.7 to 14.8µm. Some variation also recorded in beak length and width. The average beak length varied from 26.2 to 225.2 µm. The longest beak was of isolate Rohtak and smallest of Ambala. The beak septation no. ranges from 0-9, (Table 2:Fig. 1).

**Table 1. Symptoms and spot size of *Alternaria* samples collected from different Districts of Haryana (India)**

District	Avg. Spot size (mm)	Symptoms
Jind	7.8	Dark brown irregular shape spot with concentric ring
Panipat	5.4	Brown spots with yellowish margin
Sonapat	5.1	Brown spot with less visible concentric rings
Fatehabad	3.8	Dark brown spot with yellowish margin
Kurukshetra	4.8	Irregular, brown spots with less visible concentric rings
Karnal	8.1	Light brown spots with whitish margin, less visible concentric rings
Yamuna nagar	3.0	Light brown spots, less visible rings
Hisar	4.8	Dark brown spot with concentric rings
Bhiwani	6.2	Medium light to dark brown spots with concentric rings
Rohtak	5.3	Small greenish brown spots with irregular shape
Jhajjar	9.4	Light brown spots, concentric rings with papery growth
Rewari	5.5	Small dark brown spots with concentric rings
Kaithal	6.5	Light brown spots with yellowish margin
Mahender Garh	7.0	Dark brown spots with well developed concentric rings
Sirsa	11.5	Medium to large dark brown spots with concentric rings
Faridabad	5.5	Dark brown spots
Gurgaon	8.2	Brown spots with concentric rings
Ambala	5.2	Whitish to brown irregular spots

**Table 2 Morphological variation in conidial size of *Alternaria brassicae* collected from different Districts of Haryana (India)**

District	Conidial length (µm)	Conidial width (µm)	No. of Septation		Beak		Septum distance (µm)
			Horizontal	Vertical	Length (µm)	Septation No.	
Ambala	118.5(94.5-148.5)	13.8(11.5-19.2)	6.2(5-7)	0.7(0-3)	41.5(27.0-54.0)	1.8(0-5)	6.7(5.4-9.4)
Jind	117.1(81.0-163.4)	16.2(12.1-20.2)	5.6(4-8)	0.8(0-3)	41.8(30.4-51.5)	2.2(1-6)	13.5(12.8-16.2)
Fatehabad	151.2(108.0-192.5)	16.2(13.5-18.9)	5.7(5-8)	1.2(1-5)	54.0(27.0-94.5)	2.1(1-5)	8.1(5.4-10.8)
Gurgaon	191.7(135-233.5)	18.3(14.8-18.3)	7.8(5-12)	1.3(0-4)	54.0(40.5-82.3)	2.7(0-4)	9.4(8.1-13.5)
Y. Nagar	120.2(87.5-154.6)	16.2(14.8-21.6)	5.5(4-7)	1.0(0-4)	42.5(27.0-67.5)	2.2(1-4)	14.8(13.5-16.2)
Faridabad	191.6(121.0-293.5)	18.9(14.8-22.95)	8.4(7-12)	1.3(1-3)	60.7(27.0-82.7)	2.1(0-5)	10.8(9.4-13.5)
Panipat	141.7(94.5-192.5)	17.5(13.5-27.0)	6.8(5-9)	1.1(1-4)	52.5(40.5-67.5)	1.6(1-4)	10.8(8.1-12.7)
Karnal	121.5(91.5-148.5)	16.2(13.5-21.6)	5.5(5-8)	0.8(0-3)	67.5(40.5-87.7)	1.2(1-5)	11.7(8.1-13.5)
Kurukshetra	135.0(94.0-168.5)	21.5(16.5-31.5)	6.8(5-9)	1.0(0-4)	62.5(33.5-81.0)	1.4(1-3)	13.5(9.4-16.2)
Sonapat	148.5(108-186.5)	13.5(8.1-20.2)	5.6(5-8)	0.8(0-3)	54.0(40.5-94.5)	1.2(1-4)	8.1(5.4-13.5)
Hisar	117.3(81.8-161.6)	15.3(9.0-27.1)	6.5(5-9)	1.5(0-3)	47.1(26.2-81.8)	2.2(1-3)	9.4(7.5-12.4)
Bhiwani	137.7(89.9-180.8)	16.8(10.1-25.7)	7.2(6-10)	1.2(0-3)	67.1(45.2-116.1)	4.7(2-8)	10.7(9.2-15.5)
Rohtak	192.5(112.0-290.9)	20.5(9.3-27.2)	6.2(5-9)	2.1(0-4)	116.0(34.3-225.2)	5.6(3-9)	6.9(5.3-10.2)
Jhajjar	165.1(87.8-206.0)	18.2(12.5-25.2)	6.5(4-9)	2.1(0-4)	65.1(35.3-116.1)	1.7(0-3)	12.2(8.7-15.8)
Rewari	157.5(90.9-241.4)	14.4(12.8-18.1)	7.3(5-9)	2.3(0-4)	80.7(35.2-136.3)	3.2(2-5)	13.6(11.8-14.9)
Kaithal	132.9(86.8-207.8)	17.5(16.6-20.8)	7.7(6-11)	2.6(0-5)	42.9(27.2-86.9)	1.8(0-3)	9.8(7.3-12.3)
M. Garh	135.5(90.9-180.8)	17.2(9.9-24.7)	7.4(6-11)	1.2(0-3)	65.2(45.2-118.2)	4.7(2-9)	9.9(7.5-13.4)
Sirsa	146.2(89.9-189.7)	18.1(17.3-21.7)	8.5(7-11)	2.0(0-4)	43.7(31.2-90.9)	2.7(1-5)	11.1(9.8-14.5)

## Discussion

Earlier workers also described morphological variation in *A. brassicae* isolates (Awasthi and Kolte, 1989, Saharan and Kadian, 1983). The current results also describe that collected *A. brassicae* isolate have lot of morphological and cultural variation. The conidial length ranges from 81.0 to 293.5 µm and breadth from 8.1 to 31.5 µm. Kumar *et al* (2003) observed average conidial length from 118.62 to 194.52 µm and breadth from 14 to 23µm. They further observed some variation in beak length and number of septation. Mehta *et al* (2003) categorized *A. Brassicae* isolates in four groups i.e. small (<100µm.), Medium (101-150 µm.), long (151-200 µm.) and very long (>200 µm.). These variation in conidial size may be due to

variation in medium concentration and location/site of different field.

## Conclusion

The cultural and morphological difference in *A. brassicae* isolates indicates significant variation in the pathogen, it may be attributed due to environmental variation and racial differences.

**Table 3. Radial Growth, Colour and Growth pattern of different isolates of *A. brassicae* isolates collected from different districts of Haryana (India) on Indian mustard.**

District	Radial Growth		Colour	Growth pattern
	20°C	25°C		
Karnal	41.3	45.5	Dark brown	Compressed
Panipat	44.6	48.3	Brownish	Compressed
Fatehabad	38.3	44.6	Light Brown	Compressed
Bhiwani	34.6	40.5	Dark Brown	Compressed
Mahendergarh	42.7	50.0	Brown	Compressed
Rewari	51.3	60.0	Light Brown	Slightly Compressed
Gurgaon	45.5	56.6	Creamy whitish	Fluffy
Faridabad	55.5	66.7	Rough whitish	Fluffy
Hisar	57.6	68.6	Brown to dark brown	Compressed
Kaithal	51.3	56.6	Light brown to rough whitish	Fluffy
Sirsa	37.3	41.3	Light brown	Fluffy
Jind	40.0	46.6	Light brown	Compressed
Rohtak	46.6	55.5	Whitish	Compressed to Fluffy
Ambala	43.3	48.3	Creamy whitish	Fluffy
Jhajjar	46.6	50.0	Creamy white to light brown	Fluffy
Sonepat	53.3	60.0	Whitish	Fluffy
Kurukshetra	45.5	52.5	Light brown	Compressed
Yamuna Nagar	73.6	81.1	White to creamy chocolate	Compressed to Fluffy



Morphological variability in *Alternaria brassicae* isolates

## References

- Awasthi, R.P. and Kolte, S.J. 1989. Variability in *Alternaria brassicae* affecting rapeseed and mustard. *Indian Phytopath.* 42:275 (Abstr.)
- Chahal, A.S. 1986. Losses and Chemical control of *Alternaria* in rapeseed-mustard in Punjab. *Plant Dis. Res.* 1: 46-50
- Dhingra, O.D. and Sinclair, J.B. 1985. *Basic Plant Pathology Methods*. CRC Press, Inc. Boca Raton, Florida. pp 335.
- Kolte, S.J. 1985. *Diseases of Annual Edible Oilseed Crops. Vol II, Rapeseed-Mustard and Sesame Diseases*, CRC Press, Inc. Florida. 135 pp.
- Kumar. Satish, Sangwan M.S.; Mehta Naresh and Kumar Rakesh 2003. Pathogenic diversity in Isolates of *Alternaria brassicae* infecting rapeseed and mustard. *J. Mycol. Pl. Pathol.*; 33(1):59-64.
- Mehta. Naresh, Sangwan M.S. and Srivastava. M.P. 2003. Morphological and pathological variations in rapeseed and mustard isolates of *Alternaria brassicae*. *Indian Phytopath.* 56(2): 188-190.
- Mridha, M.A.U. 1983. Virulence of different isolates of *Alternaria brassicae* on winter oilseeds rape cultivars. 6<sup>th</sup> Int. Rapeseed Conf.; Paris, France. Pp 1025-1029.
- Saharan, G.S. 1991. Assessment of losses, epidemiology and management of black spot disease of rapeseed-mustard, GRIC 8<sup>th</sup> Proc. Int. Rapeseed Congr.; July 9-11, Saskatoon, Canada, p84 (Abstract) Proc. Vol II: 465-470.
- Saharan, G.S. and A.K. Kadian. 1983. Physiological specialization in *Alternaria brassicae*. *Cruciferae Newsletter* 8:32-33.
- Tousson, T.A. and Nelson, P.E. 1976. *A Pictorial guide to the Identification of Fusarium spp. According to the Taxonomic system of Synder and Hanson*. University Park, Pennsylvania, USA; Pennsylvania State University Press. P 43.
- Van Shreven, D.A. 1953. *Alternaria, Stemphylium and Botrytis* infection of colza (*Brassica napus*). *Tizdschr. Planterzickten* 59: 105-136.
- Verma, P.R. and Saharan, G.S. 1994. *Monograph on Alternaria Diseases of Crucifers*. Research Branch, Agriculture and Agriculture Food Canada, Saskatoon Res. Centre, Canada, pp 162.