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Ashwini Kumar

Department of Plant Pathology,
Jawaharlal Nehru Krishi Viswa
Vidyalaya, Jabalpur, Madhya
Pradesh, India

SN Singh

Department of Plant Pathology,
Jawaharlal Nehru Krishi Viswa
Vidyalaya, Jabalpur, Madhya
Pradesh, India

Morphological variability among isolates of *Alternaria brassicae* (Berk.) Sacc. causing leaf blight of mustard

Ashwini Kumar and SN Singh

Abstract

Indian mustard (*B. juncea*) is a crop of tropical as well as temperate zone, which is grown during rabi season. *Alternaria* blight disease caused by *A. brassicae* (Berk.) Sacc. is one of the most important disease of Indian mustard. Twenty isolates of *A. brassicae* were collected from different divisions of Madhya Pradesh to identify the morphological variation. All the isolates showed high level of variability *in vitro* in respect of conidial length, width, beak length and number of septa. The conidial length and width ranged from 61.6 to 126.3 μm and 9.7 to 16.9 μm respectively and beak length of conidia ranged from 43.0 to 74.3 μm in different isolates of *A. brassicae*. The number of horizontal septa and vertical septa ranged from 4.6 to 7.6 and 0.3 to 2.6 respectively.

Keywords: Morphological variability, isolates, *Alternaria brassicae* (Berk.) Sacc. causing leaf blight

Introduction

Indian mustard (*Brassica juncea* (L.) Czernj. Cosson) is the most important oilseed crop of rapeseed mustard group and is the most dominating rabi oilseed crop of Northern M.P. It comprises more than 80% share in the production of mustard from five districts of Madhya Pradesh including Morena, Bhind, Gwalior, Sheopur and Datia.

In India rapeseed mustard occupy an area of 63.40 lakh ha with a production of 78.20 mt and productivity of 1234.40 kg / ha (Anon, 2014) [1]. Madhya Pradesh covered area 8.00 lakh hectares with 11.40 mt production and 1425 kg / ha productivity. Districts of Gwalior contributes 0.563 lakh hectare of area with 0.612 mt production and 1088 kg/ ha productivity (Anon, 2014) [1].

Yield gap between the potential yield and the yield realized at the farmers field is very wide which is largely because of several diseases, which adversely affects the crop. Among diseases, *Alternaria* blight caused by *Alternaria brassicae* (Berk.) Sacc. and *Alternaria brassicicola* (Schwein) Wiltshire is one of the most common and destructive disease of Indian mustard. However, *Alternaria brassicae* is predominantly present in most of the mustard growing area. It causes up to 47 percent yield loss depending on the intensity and environmental conditions (Chattopadhyay, 2008) [2]. Lack of resistant varieties indicates the presence of several variants in the pathogen. Special attention was focused on morphological variability among twenty isolates of *Alternaria brassicae* collected from different divisions of M.P.

Materials and Methods

Collection, Isolation and Purification of disease sample

During Dec.- Jan., of 2015-17 survey was conducted in mustard growing areas and markets of different divisions of Madhya Pradesh including Gwalior, Morena, Bhind, Datia, Sheopur and Shivpuri etc. and it was observed that there is severe infection of *Alternaria* blight in mustard. Special emphasis was given for occurrence and study of symptoms of the disease during different stages of plant growth at JNKVV, College of Agriculture, Jabalpur. *Alternaria brassicae* culture was isolated from diseased mustard plant showing concentric ring like symptoms on leaves from different areas. Isolates were identified microscopically by their characteristic shape of conidia. Isolates of the pathogen were maintained in culture tubes containing PDA medium and used as stock culture of the target organism throughout the study.

Correspondence

Ashwini Kumar

Department of Plant Pathology,
Jawaharlal Nehru Krishi Viswa
Vidyalaya, Jabalpur, Madhya
Pradesh, India

The leaves of affected mustard plants showing typical symptoms of *Alternaria* blight were cut (sterilized scalpel) and isolation were made for the presence or absence of the causal organism. These selected infected spots were washed 3-4 times in sterilized distilled water followed by surface sterilization by dipping in 4% NaOCl solution for 1 min, followed by washing with sterilized water 3-4 times. Surface sterilized pieces were then aseptically transferred into 9 cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25±2°C for seven days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces were aseptically transferred into another Petri plate containing PDA medium, where it was grown for 15 days at 23±2°C in the BOD incubator. On the basis of their conidiophore and conidial morphology as described by Simmons (2007) [9], the pathogen was identified as *Alternaria brassicae* (Berk.) Sacc. and purified by single spore isolation method. The culture was preserved in the refrigerator (4°C) for further studies. The detailed location and coding of the isolates has been presented in table 1.

Morphological variability

The morphological characters of 20 isolates of *A. brassicae* was studied by growing isolates on PDA medium for 12 days at 25°C. After incubation a loop full of spore were suspended in a drop of distilled sterilized water kept on a clean glass micro slide. The observation on size of conidia (length & width), septation of conidia (horizontal and vertical), were recorded. The measurements were done by Micrometer under high power objective (40X) and 10x eye piece. In total 200 conidia were measured for each isolate. The average was used to calculate the conidial length, width, beak length and number of horizontal and vertical septa.

Table 1: List of *Alternaria brassicae* isolates collected from different locations

S. No.	District	Location	Previous crop	Variety	Isolates code
1.	Gwalior	Gwalior	Bajra	Varuna	I ₁
		Bhitarwar	Jowar	Rohini	I ₂
2.	Morena	Morena	Bajra	Varuna	I ₃
		Abhah	Bajra	NRC-2	I ₄
		Porsa	Jawar	Kranti	I ₅
		Joura	Bajra	Kranti	I ₆
3.	Bhind	Bhind	Jawar	JM -3	I ₇
		Lahar	Urd	JM -3	I ₈
		Gohad	-	JM-3	I ₉
		Atair	sesame	Urvasi	I ₁₀
		Mehgoan	Bajra	Arpan	I ₁₁
4.	Datia	Datia	Sesame	Varuna	I ₁₂
		Seondha	-	Varuna	I ₁₃
		Bhander	Sesame	Varuna	I ₁₄
5.	Sheopur	Sheopur	Urd	NRC-2	I ₁₅
		Karahal	Soybean	JM-3	I ₁₆
		Vijaypur	-	Varuna	I ₁₇
6.	Shivpuri	Pohri	-	Kranti	I ₁₈
		Karera	Urd	Varuna	I ₁₉
7.	Jabalpur	Jabalpur	Soybean	Pusa bold	I ₂₀

Result and Discussion

Morphological variability in respect of conidial length,

conidial width, beak length and number of horizontal and vertical septa (Table 2) showed significant variation. It was observed that out of 20 isolates, the conidial length and width ranged from 61.6 to 126.3 µm and 9.7 to 16.9 µm respectively. The conidial length was maximum in I₁ (126.3 µm) followed by I₆ (121.3 µm) and I₅ (118.3 µm), whereas minimum was recorded in isolate I₁₅ (61.6 µm). There was no significant difference among isolates I₆ (121.3), I₅ (118.3), I₁₁ (116.6), I₂ (115.3), I₁₇ (114.0), I₂₀ (104.3), I₃ (102.6), I₁₆ (98.6), I₄ (96.3), I₉ (95.0), I₇ (94.3), I₁₄ (77.3) and I₈ (77.3). However, isolates I₁ (126.3) was significantly differed from other isolates. The conidial width was maximum in isolate I₁ (16.9 µm) followed by I₆ (15.6 µm) and I₁₇ (15.2 µm). Isolates I₆ (15.6), I₁₇ (15.2), I₁₉ (14.9), I₁₁ (14.2), I₅ (13.6), I₁₃ (13.5), I₂ (13.4), I₈ (12.8), I₁₄ (12.5), I₁₆ (11.8), I₉ (11.8), I₃ (11.6), I₁₂ (10.9), I₂₀ (10.9), I₈ (10.8) and I₁₅ (10.6) were at par to each other. However, isolates I₁ (16.9) was statistically different with all other isolates. Average conidial beak length varied from 43.0 to 74.3 µm. Maximum conidial beak length was observed in isolate I₂₀ (74.3 µm) followed by I₁₁ (71.0 µm) and I₁ (69.0). However, minimum conidial beak length was recorded in isolate I₁₅ (43.0 µm). There was no significant difference among the isolates I₂₀ (74.3), I₁₁ (71.0), I₁ (69.0), I₁₂ (67.0), I₁₃ (65.0), I₄ (61.6), I₉ (61.3), I₃ (61.0), I₁₈ (59.0), I₂ (54.6), I₁₆ (54.3), I₆ (53.0), I₁₀ (52.6), I₅ (52.0), I₁₄ (51.6), I₁₉ (51.6), I₁₀ (51.3), I₇ (47.3), I₈ (46.0), and I₁₅ (43.0).

Average number of horizontal septa varied from 4.6 to 7.6 no. The maximum horizontal septa was noted in isolate I₃ (7.6 no.) followed by I₁₃ (7.3 no.), I₁₈ (7.3 no.) and I₁₅ (7.0 no.). However, minimum horizontal septa was recorded in isolate I₁₄ (4.6 no.). Isolates I₁₅ (7.6), I₁₃ (7.3), I₁₈ (7.3), I₇ (6.6), I₁₉ (6.6), I₁ (6.3), I₅ (6.3), I₁₁ (6.3), I₂₀ (6.3), I₁₆ (6.0), I₆ (6.0), I₄ (5.6), I₉ (5.6), I₁₀ (5.6), I₁₇ (5.6), I₂ (5.3), I₈ (5.3), I₁₂ (5.3) showed no significant differences among each other. Vertical septa ranged from 0.3 to 2.6 no. Maximum vertical septa were recorded in isolate I₁₂ (2.0 no.), However minimum in I₇ (0.3 no.). Isolates I₁₄ (2.6), I₂ (2.0), I₁₂ (2.0), I₁₀ (1.6), I₁₃ (1.6), I₁ (1.3), I₄ (1.3), I₁₅ (1.3), I₁₇ (1.3), I₂₀ (1.3), I₃ (1.0), I₈ (1.0), I₁₆ (1.0), I₁₈ (1.0), I₅ (0.6), I₆ (0.6), I₉ (0.6), I₁₁ (0.6), I₁₉ (0.6), and I₇ (0.3) were not found significantly different on the basis of average number of vertical septa.

Earlier reports regarding characterization of different *A. brassicae* isolates on the basis of morphological characters viz. conidial length, conidial width, number of horizontal and vertical septa, beak length (Mehta *et al.*, 2003; Varma, *et al.*, Kaur *et al.*, 2007; Meena *et al.*, 2012; Selvamani *et al.*, 2013; Pramila, *et al.* 2014; Saha *et al.*, 2015) [4, 10, 7, 6, 8, 5] have reported in the same manner as in the findings of present study. They have reported conidial length from 51.4- 481.2 µm, width from 6.9- 285.9µm, beak length 16.3-266.9 µm, number of horizontal and vertical septa from 3.0-9.0 and 1-2 respectively. The present finding will help in establishing the pathogenic role of *A. brassicae* in mustard and subsequently the development of *Alternaria* blight in major mustard growing areas of Madhya Pradesh.

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Table 2: Morphological variability of twenty isolates of *Alternaria brassicae*

Isolates	Conidial length (µm)	Conidial width (µm)	Conidial beak length (µm)	Horizontal septa (No.)	Vertical septa (No.)
I ₁	126.3	16.9	69.0	6.3	1.3
I ₂	115.3	13.4	54.6	5.3	2.0
I ₃	102.6	11.6	61.0	7.6	1.0
I ₄	96.3	9.7	61.6	5.6	1.3
I ₅	118.3	13.6	52.0	6.3	0.6
I ₆	121.3	15.6	53.0	6.0	0.6
I ₇	94.3	9.8	47.3	6.6	0.3
I ₈	77.3	10.8	46.0	5.3	1.0
I ₉	95.0	11.8	61.3	5.6	0.6
I ₁₀	102.3	12.8	51.3	5.6	1.6
I ₁₁	116.6	14.2	71.0	6.3	0.6
I ₁₂	108.3	10.9	67.0	5.3	2.0
I ₁₃	103.3	13.5	65.0	7.3	1.6
I ₁₄	77.3	12.5	51.6	4.6	2.6
I ₁₅	61.6	10.6	43.0	7.0	1.3
I ₁₆	98.6	11.8	54.3	6.0	1.0
I ₁₇	114.0	15.2	52.6	5.6	1.3
I ₁₈	104.3	9.9	59.0	7.3	1.0
I ₁₉	89.6	14.9	51.6	6.6	0.6
I ₂₀	104.3	10.9	74.3	6.3	1.3
Sem±	1.3	2.4	0.3	0.4	0.4
C.D. at 5%	3.8	7.0	1.0	1.1	1.2

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