

## VARIABILITY AMONG THE ISOLATES OF *ALTERNARIA* SPECIES CAUSING BLIGHT IN RAPE-SEED MUSTARD (*BRASSICA JUNCEA* L.)

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### Abstract

Variation in the mycelial growth was observed within the isolates of *Alternaria brassicae* and *A. brassicicola* on PDA medium. The isolates were also found to differ from each other in respect to cultural and conidial characters. The colonies were cottony or fluffy in appearance with varied colour from olivaceous green, white, off white, light brown to brown. Colony margins were smooth, rough or wavy and zonation was either present or absent. In *Alternaria brassicae* isolates, conidial length varied from 92.5 to 116.2  $\mu\text{m}$ , conidial width varied from 11.8 to 17.2  $\mu\text{m}$ , horizontal septa varied from 3.4 to 5.3, vertical septa varied from 0.3 to 1.4  $\mu\text{m}$  and beak length varied from 68.4 to 88.4  $\mu\text{m}$ . However, in *A. brassicicola* isolates, conidial length varied from 46.8 to 52.8  $\mu\text{m}$ , conidial width varied from 12.8 to 14.6  $\mu\text{m}$ , horizontal septa varied from 3.2 to 3.7 and vertical septa varied from 0.2 to 0.8  $\mu\text{m}$ . Beak was absent in the isolates of *A. brassicicola*. In the isolates of *A. brassicae* and *A. brassicicola*, maximum per cent disease intensity and spot size on all the genotypes was observed in Ab<sub>11</sub> and Abl<sub>2</sub>, respectively.

### Introduction

Rapeseed-mustard (*Brassica juncea* L.) is an economically prodigious and useful oilseed crop of the world. Rapeseed-mustard is exposed to white rust, powdery mildew, downy mildew and Alternaria blight. Among these, Alternaria blight caused by *Alternaria brassicae* (Berk) Sacc. and *A. brassicicola* (Schw.) has been reported from all the continents of the world causing severe economic yield loss (Meena *et al.* 2010).

Variability studies are important to document the changes occurring in the population and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes. Many reports on the existence of variability among different *Alternaria* species have been reported by earlier workers (Verma *et al.* 2006, Kaur *et al.* 2007, Kumar *et al.* 2008, Goyal *et al.* 2011, Sharma *et al.* 2013, Singh *et al.* 2015). The present study was conducted to find out the variability in *Alternaria brassicae* and *A. brassicicola*.

### Materials and Methods

The pathogens were isolated and purified by single spore isolation technique described by Singh *et al.* (2014). Petriplates containing PDA medium were inoculated with a 5 mm diameter plug. Colony characters recorded on 12<sup>th</sup> day after inoculation while conidial variability of the Alternaria isolates was observed by the method described by Meena *et al.* (2005).

A conidial suspension of each isolates was prepared by scrapping mycelia and spores from the plates of actively growing fungal cultures into sterilized water and filtered the suspension through four layers of cheese cloth. The filtered spore suspension was centrifuged at 2000 x g for five min. Spore count was adjusted by using haemocytometer and number of spores per ml was calculated by the formula of Tuite (1969).

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The pathogenic variability was studied on the three genotypes of *B. juncea* viz. Varuna, RSPR-03 and Kranti. Each genotype was sown in sterilized soil in pots under control conditions. Spore suspension of  $2 \times 10^3$  spores/ml of each isolate was prepared according to Giri *et al.* (2013). Such spore suspension of *A. brassicae* i.e. Ab<sub>1</sub>, Ab<sub>2</sub>, Ab<sub>4</sub>, Ab<sub>7</sub>, Ab<sub>10</sub>, Ab<sub>11</sub> and *A. brassicicola* i.e. Abl<sub>2</sub>, Abl<sub>4</sub> and Abl<sub>5</sub> was sprayed on 3<sup>rd</sup>/4<sup>th</sup> true leaves of 30 days old plant of *B. juncea* by drop plus agarose artificial inoculation method (Giri *et al.* 2013). Each inoculated site of the leaf was covered with agarose. The agarose drops appeared as clear dome on the leaf surface. The agarose solidified and fixed the inoculums on the target site within few minutes. The leaves were kept in sealed Petriplates and placed in growth chamber at 25°C and 70% relative humidity. The disease intensity and average spot size (mm) were recorded after five days of inoculation.

### Results and Discussion

The radial growth ranged from 64 to 89 mm after 12 days of inoculation. Maximum radial growth (89 mm) was observed in Ab<sub>11</sub> followed by Ab<sub>10</sub> (87 mm). However, minimum radial growth (64 mm) was recorded in Ab<sub>2</sub>. The maximum mean radial growth was recorded in Ab<sub>11</sub> (67.66 mm) followed by Ab<sub>10</sub> (64.83 mm) and Ab<sub>3</sub> (59.50) (Table 1). Maximum radial growth was recorded from the isolate Abl<sub>2</sub> (89 mm) followed by Abl<sub>7</sub> and Abl<sub>3</sub> (80 mm) after 12 days of inoculation. The maximum mean radial growth (67 mm) was recorded in the isolate Abl<sub>2</sub> followed by Abl<sub>7</sub> (58.71 mm). Considering mean radial growth, twelve isolates of *A. brassicae* were finally categorized into three groups i.e. fast growing (Ab<sub>3</sub>, Ab<sub>6</sub>, Ab<sub>10</sub>, Ab<sub>11</sub>), moderate growing (Ab<sub>1</sub>, Ab<sub>7</sub>, Ab<sub>8</sub>, Ab<sub>9</sub>, Ab<sub>12</sub>) and slow growing (Ab<sub>2</sub>, Ab<sub>4</sub> and Ab<sub>5</sub>). Isolates of *A. brassicicola* were categorized on the basis of radial growth into three groups i.e. fast growing (Abl<sub>2</sub>, Abl<sub>3</sub>, Abl<sub>7</sub>), moderate growing (Abl<sub>1</sub>, Abl<sub>4</sub>, Abl<sub>9</sub>) and slow growing (Abl<sub>5</sub>, Abl<sub>6</sub>, Abl<sub>8</sub>).

After grouping, six isolates of *A. brassicae* i.e. Ab<sub>1</sub>, Ab<sub>2</sub>, Ab<sub>4</sub>, Ab<sub>7</sub>, Ab<sub>10</sub>, Ab<sub>11</sub> and three isolates of *A. brassicicola* i.e. Abl<sub>2</sub>, Abl<sub>4</sub>, Abl<sub>5</sub> were taken for further studies.

The colony colour varied from white, off-white, olivaceous green, light brown to brown. Most of the colonies were brown, light brown or olivaceous green in colour except Ab<sub>10</sub>, and Abl<sub>2</sub> where colony colour was off white and white, respectively. Cottony growth with rough margins were observed in the isolates Ab<sub>1</sub>, Ab<sub>4</sub>, Ab<sub>7</sub>, Ab<sub>10</sub>, and Abl<sub>4</sub> whereas, fluffy growth with smooth margins were in Ab<sub>11</sub>, Abl<sub>2</sub> and Abl<sub>5</sub>. Fluffy growth with wavy margin was observed in Ab<sub>2</sub>. As far as colony shape and zonation was concerned, isolates Ab<sub>1</sub>, Ab<sub>4</sub>, Ab<sub>7</sub>, Ab<sub>11</sub> and Abl<sub>4</sub> had circular shape with no zonation whereas in Ab<sub>2</sub>, Ab<sub>10</sub>, Abl<sub>2</sub> and Abl<sub>5</sub> colony shape was circular with zonation (Table 2). These results are in agreement with earlier workers (Meena *et al.* 2005, Patni *et al.* 2005, Kaur *et al.* 2007, Singh *et al.* 2007, Sharma *et al.* 2013).

The data presented in Table 3 showed that the isolates of Alternaria blight pathogens exhibited morphological variability in respect of conidial length, breadth, number of horizontal septa, vertical septa and length of the beak. The average conidial length of six isolates of *A. brassicae* ranged from 92.5 to 116.2 µm. Out of the six isolates, Ab<sub>11</sub>, Ab<sub>2</sub> and Ab<sub>10</sub> showed maximum average conidial length viz. 116.2, 114.2 and 112.4µm, respectively. It was followed by Ab<sub>4</sub> and Ab<sub>7</sub> having average conidial length viz. 106.2 and 98.5 µm, respectively. The minimum conidial length (92.5 µm) was recorded in the isolate Ab<sub>1</sub>. On the other hand, isolates of *A. brassicicola* Abl<sub>4</sub>, Abl<sub>2</sub> and Abl<sub>5</sub> had average conidial length viz. 52.8, 47.6 and 46.8 µm, respectively. Average conidial breadth of *A. brassicae* isolates ranged from 11.8 to 17.2 µm. Maximum average conidial breadth was observed in Ab<sub>2</sub> (17.2 µm) and Ab<sub>11</sub> (16.8 µm) and minimum in Ab<sub>7</sub> (11.8 µm). In case of *A. brassicicola*, the maximum (14.6 µm) conidial breadth was observed in Abl<sub>5</sub> and minimum (12.8 µm) in Abl<sub>4</sub>.



Table 2. Cultural and conidial variation among different isolates of *Alternaria* spp. on PDA medium.

Isolate No.	Color	Colony cultural characteristics				Conidial characteristics								
		Appearance	Margin	Zonation	Conidial length (µm)	Conidial breadth (µm)		No. of Horizontal septa		No. of Vertical septa		Beak length in µm		
					Average	Range	Average	Range	Average	Range	Average	Range	Average	Range
Ab <sub>1</sub>	Light brown	Cottony	Rough	Absent	92.5	84 - 104	14.2	12 - 16	3.4	3 - 5	0.3	0 - 1	68.4	52 - 72
Ab <sub>2</sub>	Olivaceous green	Fluffy	Wavy	Present	114.2	92 - 120	17.2	16 - 20	4.8	4 - 6	1.2	0 - 2	82.2	76 - 88
Ab <sub>4</sub>	Brown	Cottony	Rough	Absent	106.2	92 - 112	15.2	12 - 20	3.9	3 - 5	0.6	0 - 1	81.6	72 - 88
Ab <sub>7</sub>	Light brown	Cottony	Rough	Absent	98.5	84 - 108	11.8	10 - 14	3.6	3 - 5	0.3	0 - 1	72.8	64 - 84
Ab <sub>10</sub>	Off white	Cottony	Rough	Present	112.4	92 - 124	13.4	12 - 16	4.2	4 - 6	0.8	0 - 1	88.4	76 - 92
Ab <sub>11</sub>	Olivaceous green	Fluffy	Smooth	Absent	116.2	96 - 128	16.8	12 - 20	5.3	4 - 6	1.4	0 - 2	85.6	76 - 96
Mean	-	-	-	-	106.67	84 - 128	14.77	10 - 20	4.2	3 - 6	0.77	0 - 2	79.83	52 - 96
Abl <sub>2</sub>	White	Fluffy	Smooth	Present	47.6	44 - 48	14.4	12 - 16	3.5	3 - 5	0.6	0 - 2	Absent	Absent
Abl <sub>4</sub>	Brown	Cottony	Rough	Absent	52.8	48 - 56	12.8	10 - 16	3.7	3 - 5	0.2	0 - 1	Absent	Absent
Abl <sub>5</sub>	Olivaceous green	Fluffy	Smooth	Present	46.8	44 - 48	14.6	12 - 16	3.2	2 - 4	0.8	0 - 2	Absent	Absent
Mean	-	-	-	-	49.07	44 - 56	13.93	10 - 16	3.47	2 - 5	0.53	0 - 2	Absent	Absent

\* Average of 30 conidia in each isolates.

The number of horizontal septa of *A. brassicae* isolates was found to range from 3.4 to 5.3. The maximum number of horizontal septa was recorded in Ab<sub>11</sub> (5.3), which was followed by Ab<sub>2</sub> (4.8) and Ab<sub>10</sub> (4.2). However, minimum number of horizontal septa was observed in Ab<sub>1</sub> (3.4). In the isolates of *A. brassicicola*. The maximum number of horizontal septa (3.7) was recorded in Abl<sub>4</sub> and minimum (3.2) in Abl<sub>5</sub> whereas, the vertical septa in the isolates of *A. brassicae* ranged from 0.3 to 1.4. Maximum number of vertical septa was observed in Ab<sub>11</sub> (1.4). The minimum number (0.3) was recorded in Ab<sub>1</sub> and Ab<sub>7</sub>. In *A. brassicicola* isolates, the maximum number of vertical septa (0.8) was recorded in Abl<sub>5</sub> and minimum in Abl<sub>4</sub> (0.2). Beak length in *A. brassicae* isolates ranged from 68.4 to 88.4 µm. Maximum length was observed in Ab<sub>10</sub> (88.4 µm) followed by Ab<sub>11</sub> (85.6 µm) and Ab<sub>2</sub> (82.2 µm). However, minimum beak length was observed in Ab<sub>1</sub> (68.4 µm). The conidial beak was absent in the isolates of *A. brassicicola*.

Pramila *et al.* (2014) also observed conidial variability in respect of conidial length, width and in number of horizontal septa. Singh *et al.* (2014) observed cultural and morphological variability in respect of radial growth, conidial length, conidial width, beak length and in number of horizontal and vertical septa, which is in agreement with the present findings.

The genotype Varuna was more consistent and showed moderately susceptible to susceptible disease reaction against all the tested isolates. The genotype RSPR-69 showed moderately susceptible disease reaction against the tested isolates except Ab<sub>2</sub>, Abl<sub>4</sub> and Abl<sub>5</sub>. However, the genotype Kranti showed moderately susceptible disease reaction against all the isolates except Abl<sub>5</sub> where moderately resistant reaction was observed (Table 3). Isolates Ab<sub>11</sub> and Ab<sub>10</sub> were found to be most virulent followed by the isolate Ab<sub>7</sub>, Ab<sub>1</sub>, Ab<sub>4</sub> and Ab<sub>2</sub> in *A. brassicae* isolates whereas in *A. brassicicola*, Abl<sub>2</sub> was most virulent and Abl<sub>5</sub> was found least virulent.

**Table 3. Pathogenic variability of *Alternaria* spp. on different genotypes of *Brassica juncea*.**

Isolate	Genotype						Mean
	Varuna		RSPR-69		Kranti		
	Di (%)	Dr	Di (%)	Dr	Di (%)	Dr	
AB <sub>1</sub>	44.71	MS	28.86	MS	36.43	MS	36.66
AB <sub>2</sub>	40.55	MS	24.34	MR	32.96	MS	35.89
AB <sub>4</sub>	40.88	MS	26.57	MS	34.66	MS	34.03
AB <sub>7</sub>	46.42	MS	30.57	MS	38.84	MS	38.61
AB <sub>10</sub>	50.27	S	31.72	MS	40.56	MS	40.85
AB <sub>11</sub>	51.82	S	32.48	MS	41.18	MS	41.82
ABL <sub>2</sub>	42.58	MS	28.38	MS	36.72	MS	35.89
ABL <sub>4</sub>	32.52	MS	22.65	MR	28.34	MS	27.83
ABL <sub>5</sub>	30.88	MS	20.64	MR	24.42	MR	25.31
Mean	42.29		27.36		34.90		
			SE (M)±		LSD = P (0.05)		
Isolate			0.24		0.68		
Genotype			0.14		0.39		
Isolate × Genotype			0.42		1.18		

Di = Disease intensity, Dr = Disease reaction, MR = Moderately resistant (11-25%),

MS = Moderately susceptible (26-50%), S = Susceptible (51-75%), (Source- Conn *et al.*1990).

A major lacuna in resistance breeding against *Alternaria* blight has been the absence of sufficient documentation of pathogenic variability in *Alternaria* blight pathogens, although three pathotypes have been reported in the past (Vishwanath and Kolte 1997). In the present study, isolates of *A. brassicae* and *A. brassicicola* showed variable responses on different *B. juncea* genotypes *viz.* Varuna, RSPR-69 and Kranti. Similar reports on the existence of pathogenic

variability within the isolates of *A. brassicae* were reported by earlier workers (Sharma *et al.* 2013 and Singh *et al.* 2015) The present study also indicates the presence of racial difference prevalent in different geographical regions of Jammu division.

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