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Morphological, cultural and pathogenic variability in *Alternaria brassicae*, the causing agent of black spot of rapeseed and mustard

B. S. Bhatiya*, K. S. Bisht, Pankaj Rautela and R. P. Awasthi

Center of Advance Studies, Department of Plant Pathology, College of Agriculture, G.B.P.U.A.T., Pantnagar, U. S. Nagar- 263145 (Uttarakhand), INDIA

*Corresponding author. E-mail: Bhupendra_Patho@rediffmail.com

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Abstract: The study on pathogenic diversity of twenty isolates of *Alternaria brassicae* collected from different locations of Uttarakhand and Central Uttar Pradesh infecting Brassica species (*Brassica rapa*, *Brassica juncea* and *Eruca sativa*) revealed that there was a distinct difference among isolates in terms of mycelial growth, spore length, width, spore beak length and width. The average spore length varied from 21.23µm to 38.13µm with minimum of isolate AUA-19, AUA-43 i.e 21.23µm and maximum of AUA-47 i.e. 38.13µm . The isolates tested on *Brassica juncea* var.Varuna in green house conditions revealed that all the twenty isolates behaved differently. Among all the isolates, *Brassica juncea* isolates i.e. AUA-25, AUA-39, AUA-41, AUA-47, AUA-19, AUA-24, AUA-22, AUA-21, AUA -31, AUA-43 and AUA-45 from Uttarakhand, and AUP-29 from Central Uttar Pradesh can be grouped into highly pathogenic with range of Alternaria spot size i.e. 5.03-8.30mm in diameter, while isolate of *Eruca sativa* i.e. AUA-38 was found least pathogenic with 1.63mm in dia. and eight isolates AUA-18, AUA-20, AUA-23, AUP-28, AUA-32, AUA-33 and AUA-36 were found moderately pathogenic. This study will be useful in developing integrated management strategies of Alternaria leaf spot and breeding programs of oilseed crops (Brassica sp.).

Keywords: *Alternaria brassicae*, cultural, morphology, pathogenicity, variability

INTRODUCTION

Rapeseed -mustard is important rabi oilseed crops grown all over India with low average productivity for which diseases are major contributing factors. Among the various diseases occurring on rapeseed-mustard, Alternaria black spot incited by *Alternaria brassicae* (Berk.) Sacc. is one of the most important and devastating disease. It has been reported to cause variable losses in yield, depending upon disease severity. Yield loss to the extent of 47 per cent has been reported (Chattopadhyay, 2008; Meena *et al.,* 2010). There are a number of reports on the existence of variability on the basis of mycelial growth, morphology and sporulation among different isolates of *A. brassicae* from different geographical regions of India (Ansari *et al.*,1989; Kaur *et al.,* 2007; Goyal *et al.,*2011) .Since the crop and disease are of paramount importance, the present investigation focused on evaluation of morphological, cultural and pathogenic variability of twenty isolates of *A. brassicae* of Brassica species from Uttarakhand and Central Uttar Pradesh that is essential to design disease management strategies for different places of India by breeding resistant cultivars. It has also been determined whether morphological variations among *A. brassicae* isolates have any relationship with the pathogenic variability.

MATERIALS AND METHODS

Collection of *A. brassicae* isolates:Infected leaves exhibiting different types of typical symptoms of Alternaria leaf spot disease, usually with concentric rings were collected separately from different genotypes of *Brassica juncea, B. rapa* and *Eruca sativa* from Uttarakhand, Central U.P. and the Crop Research Centre, G.B. Pant University of Agriculture and Technology. The leaf samples were brought to the laboratory for microscopic examination and isolation. (Table 1).

Isolation of the fungus: The infected leaves of the Brassica species were thoroughly washed in tap water and separately cut into small pieces about 0.5 cm size. These selected infected spots were washed 3 to 4 times in sterilized distilled water and then surface sterilized by dipping in 0.1% $HgCl_2$ solution for 30 second, followed by washing with sterilized water 3 to 4 times, which were then aseptically transferred between the two fold of sterilized blotting paper to remove excess amount of water to prevent bacterial contamination. After that these pieces were transferred aseptically into Petri dishes containing sterilized potato dextrose agar (PDA) and incubated at 25±2°C for seven days. After seven days growing mycelium was sub-cultured on fresh PDA plates. In this way cultures of different isolates were obtained.

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Identification and purification of the fungus: Temporary slides of the cultures were prepared in lactophenol and examined under compound microscope for the mycelial and spore characteristics. On the basis of their conidiophore and conidial morphology as described by Simmons (2007), the pathogen was identified as *A. brassicae* (Berk.) Sacc. The pathogen *of A. brassicae* was purified by single spore technique. Single spore technique was used for maintaining the purity of each isolate (Gattani., 1954). Thus various isolates collected from different locations were designated as Alternaria Uttarakhand Isolate (AUA) and Alternaria central U.P. isolates (AUP).

Evaluation of morphological variability of different isolates of *A. brassicae*: Morphological variability among the 20 isolates of *A. brassicae* was studied using micrometry technique (Meena et al., 2005). About 20 to 25 spore per slide were examined at 40X magnification by light microscope. The size and shape of conidia was measured by using ocular and stage micrometer. Numbers of transverse septa were also recorded.

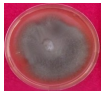
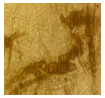
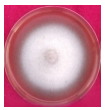
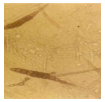
Cultural characteristics of different isolates of *A. brassicae*: The cultural characteristics of each isolates like colony, color, texture, growth, shape, margin and zonation were recorded by direct observation of 15 day old culture-grown on PDA incubated at 25±2°C temperature.

Pathogenic variability of different isolates of *A. brassicae*: In order to confirm the identification of the disease and its causal agent, the pathogenicity test was conducted under polyhouse conditions under pot experiments using *B. juncea* var. Varuna. Seedlings were raised in pots filled with sterilized soil. Pathogenicity test of the fungus was done, on one month old healthy plants of *B. juncea* var. Varuna grown in pots under glasshouse condition. Fifteen days old cultures of *Alternaria brassicae* on PDA was taken, blended

Table 1. Isolates of *Alternaria brassicae* collected from infected leaves of different oilseed Brassica cultivars from Uttarakhand and central Uttar Pradesh.

S. N.	Code	Oilseed *Brassica spp.*	Variety	Plant part	Place of collection	Geographical Data
1	AUA-18	*Brassica rapa*	Yellow Sarson Local	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
2	AUA-19	*B. juncea*	EC-399301	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
3	AUA-20	*B. juncea*	Mustard Local	Leaf	Sitarganj	Latitude : 28°56′N Longitude : 79°42′E
4	AUA-21	*B. juncea*	EC-414317	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
5	AUA-22	*B. juncea*	EC-399296	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
6	AUA-23	*B. juncea*	EC-414322	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
7	AUA-24	*B. juncea*	PWR-9338	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
8	AUA-25	*B. juncea*	Mustard	Leaf	Halduchaud Haldwani	Latitude : 29°13′N Longitude : 79°31′E
9	AUP-28	*B. rapa*	Yellow Sarson	Leaf	Kanpur	Latitude : 27°25′N Longitude : 80°07′E
10	AUP-29	*B. juncea*	Mustard Local	Leaf	Hardoi	Latitude : 27°25′N Longitude : 80°07′E
11	AUA-31	*B. juncea*	Mustard Local	Leaf	Indra Nagar Haldwani	Latitude : 29°13′N Longitude : 79°31′E
12	AUA-32	*B. juncea*	Mustard Local	Leaf	RishiKesh	Latitude : 30°06′12″N Longitud : 78°17′41″E
13	AUA-33	*B. juncea*	PAB-9511	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
14	AUA-36	*B. juncea*	EC-411322	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
15	AUA-38	*Eureca. sativa*	E. sativa	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
16	AUA-39	*B. juncea*	Mustard	Leaf	Kathangri Sitarganj	Latitude : 28°56′N Longitude : 79°42′E
17	AUA-41	*B. juncea*	Mustard	Leaf	Danibangar Haldwani	Latitude : 29°13′N Longitude : 79°31′E
18	AUA-43	*B. juncea*	Mustard	Leaf	Maghera Haldwani	Latitude : 29°13′N Longitude : 79°31′E
19	AUA-45	*B. juncea*	Mustard	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E
20	AUA-47	*B. juncea*	PAB-2002	Leaf	Pantnagar	Latitude : 28°58′N Longitude : 79°25′E

Table 2. Morphological and cultural characteristics of *Alternaria brassicae* isolates in Potato-dextrose Agar media.

S. N.	Isolate Code	Culture						Spore							
		Culture	colour	Tex-ture	Shape	Margin appeared	Concen-tric Rings	Spore with my-celium	Colour	Shape	Length	witdth	Beak length	Beak witdth	Hori-zontalse pta
1	AUA-18		Blackish	Smooth	Circular	Smooth	Present		brown	long ob-pyrifo rm	32.06	5.63	5.20	1.73	8.67
2	AUA-19		Whitish	Smooth , Fluffy	Circular	smooth	Present		brown	long ob-pyrifo rm	21.23	4.33	6.50	2.16	7.00
3	AUA-20		Brownish whitish	Smooth , Fluffy	Circular	Wavy	Present		brown	long ob-pyrifo rm	37.70	4.76	6.06	2.60	6.33
4	AUA-21		Blackish	Smooth	Circular	Wavy	Present		Brown	long ob-pyrifo rm	24.70	3.03	6.50	1.30	6.67
5	AUA-22		Whitish	Smooth , Fluffy	Circular	Wavy	Present		Brown	long ob-pyrifo rm	32.06	3.03	12.13	1.30	7.00
6	AUA-23		Whitish	Smooth Fluffy	circular	Normal	Absent		Brown	long ob-pyrifo rm	27.73	3.46	9.09	1.73	5.67
7	AUA-24		Whitish	Smooth , Fluffy	Circular	smooth	Present		Golden	long ob-pyrifo rm	27.26	4.51	8.42	2.64	7.33
8	AUA-25		Whitish	Smooth , Fluffy	Circular	smooth	Present		brown	long ob-pyrifo rm	25.13	5.20	7.36	1.30	7.67
9	AUP-28		Whitish	smooth, Fluffy	Circular	smooth	Present		Dark brown	long ob-pyrifo rm	27.54	3.26	7.23	1.32	8.33

Contd…..

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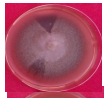
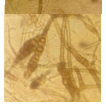
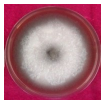
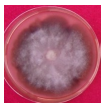
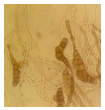
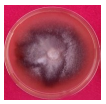
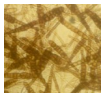
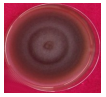
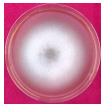
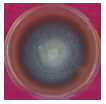
10	AUP-29		Whitish with black-ish periphery	smooth	circular	Normal	Absent		Dark brown	long ob-pyrifo	28.16	3.03	6.06	1.73	4.67
11	AUA-31		Whitish	Smooth flopy	Circular	smooth	Present		brown	long ob-pyrifo rm	26.86	3.90	4.33	1.73	9.00
12	AUA-32		Whitish	Smooth , Fluffy	Circular	normal	Present		Dark brown	long ob-pyrifo rm	24.26	4.76	10.83	2.60	6.67
13	AUA-33		Whitish with black-ish periphery	Rough	Not circular	Wavy	Absent		Dark brown	ob-pyrifo rm	22.10	4.76	10.83	2.16	7.67
14	AUA-36		Whitish with black-ish periphery	Rough	Not circular	Normal	Absent		Dark brown	long ob-pyrifo rm	30.33	3.46	10.83	2.16	8.67
15	AUA-38		Blackish	smooth	Circular	Wavy	Present		Golden	long ob-pyrifo rm	23.83	3.90	8.60	2.60	7.33
16	AUA-39		Whitish	Smooth , Fluffy	Circular	smooth	Present		Golden	long ob-pyrifo rm	24.70	4.76	6.50	2.16	7.67
17	AUA-41		light Whitish	smooth	circular	smooth	Absent		Dark brown	long ob-pyrifo rm	22.10	3.46	11.70	2.16	8.00
18	AUA-43		Whitish with black-ish periphery	Rough	Not circular	Wavy	Absent		brown	ob-pyrifo rm	21.23	3.90	5.63	1.73	6.67
19	AUA-45		Whitish with black-ish periphery	Rough	Not circular	Wavy	Absent		Golden	ob-pyrifo rm	24.26	3.90	9.96	2.16	9.00
20	AUA-47		Blackish	Smooth	Circular	Smooth	Present		brown	long ob-pyrifo rm	38.13	3.90	6.50	1.73	8.67

Table 3. Pathogenicity test of *A. brassicae* isolates on *Brassica juncea* var. Varuna.

S.N.	Different isolates	Average size of Alternaria spots (in mm)	Pathogenicity
1	AUA-18	2.80	++
2	AUA-19	6.17	+++
3	AUA-20	4.77	++
4	AUA-21	5.03	+++
5	AUA-22	5.17	+++
6	AUA-23	4.23	++
7	AUA-24	6.20	+++
8	AUA-25	8.30	+++
9	AUP-28	2.13	++
10	AUP-29	5.60	+++
11	AUA-31	5.67	+++
12	AUA-32	3.63	++
13	AUA-33	2.67	++
14	AUA-36	3.47	++
15	AUA-38	1.63	+
16	AUA-39	6.47	+++
17	AUA-41	7.10	+++
18	AUA-43	5.87	+++
19	AUA-45	6.70	+++
20	AUA-47	6.29	+++

Symptoms observed were ranked as (+) for spots with diameter < 2 mm, (++) for spots with diameter 2-5mm, and (+++) for spots with diameter above 5 mm

with sterilized distilled water, and filtered through cheesecloth. The spore suspensions (15.00 to 20.00 spores/ ml.) were taken in a 100 ml atomizer separately for each isolate. All the leaves of the *Brassica* species were inoculated in triplicate for each isolate of *A. brassicae* and one set was kept as check in which only sterilized water was sprayed. The inoculated plants were separately kept in moist chambers isolate-wise. Symptoms observed were ranked as (-) for no symptom, (+) for spots with diameter < 2 mm, (++) for spots with diameter 2-5mm and (+++) for spots with diameter above 5 mm. Experiments were conducted in completely randomized design (CRD).

RESULTS AND DISCUSSION

Morphological variability among isolates: Significant (P<0.05) morphological variability was observed among all 20 isolates of *A. brassicae* of Brassica species i.e. AUA-18, AUP-28 from rapeseed crop (*Brassica rapa*), AUA-19, AUA-20, AUA-21, AUA-22, AUA-23, AUA-24, AUA-25, AUP-29, AUA-31, AUA-32, AUA-33, AUA-36, AUA-39, AUA-41, AUA -43, AUA-45, AUA-47 from mustard (*Brassica juncea*) AUA-38 from taramira (*Eruca sativa*). (Fig. 1) in respect of spore length, spore width, spore beak length, spore beak width and number of horizontal septa of *A. brassicae*. Maximum spore length of *A. brassicae* was recorded for AUA-47 (38.13µm) and it was significantly different to AUA-19 (21.23µm), AUA-21 (24.70µm), AUA-25 (25.13µm), AUA-32 (24.26µm), AUA-33 (22.10µm), AUA-38 (23.83µm), AUA-39 (24.70µm), AUA-41 (22.10µm), and AUA-43 (21.23µm) and minimum spore length was recorded for the isolate AUA-19 and AUA-43 (21.23µm), followed by AUA-33 and AUA-41(22.10µm). Maximum spore width was recorded for AUA-18 (5.63 µm) and it was significantly higher than AUA-21, AUA-22 and AUP-29 for which minimum spore width (3.03 um) was recorded (Table 2). Minimum spore beak length was recorded for AUA-31 (4.33µm) and it was at par with all isolates except AUA-22 (12.13µm), which recorded maximum length. Maximum spore beak width was recorded for AUA-24 (2.64 µm) and minimum spore beak width was recorded for the isolate, AUA-21, AUA-22 and AUA-25 (1.30µm) followed by AUA-18, AUA-23, AUP-29, AUA-31, AUA-43, and AUA-47 (1.73µm) and these were at par with all other isolates. Highest number of horizontal septa was shown by AUA-31 and AUA-45 (9.0) followed by AUA-18, AUA-36 and AUA-47, whereas AUA-20 (6.33) showed minimum number of horizontal septa (Table 2). These results are in accordance with earlier workers (Awasthi and Kolte, 1989;; Meena *et al.,* 2005; Sharma *et al*., 2013; Pramila *et al*., 2015, who observed morphological variability in *Alternaria brassicae* isolates of difference places of India. Goyal *et al.* (2011) reported variation in conidial morphology, mycelial growth, sporulation of thirteen isolates of *A. brassicae* collected from seven states of India. Similarly, Meena *et al.,* 2005; Sharma *et al*., 2013; Pramila *et al*., 2015 reported variability in the morphological characteristics in *A. brassicae* isolates of different places of India.

Cultural characteristic of *A. brassicae* isolates: Variations in linear growth and color of the colony were observed among all 20 isolates of *A. brassicae.* The color of the colonies and the spore grown on PDA showed little variation. The color of the *A. brassicae* isolates varied from white, brownish white to black, texture of the colony from cottony to fluffy , colony margin from wavy, smooth to rough. Mycelial color varied from brown to golden and spores were golden

and brown in color and were generally long obpyriform in shape with long beak (Table 2). The results are in accordance with Ansari *et al.,* 1989; Patni *et al.,* 2005; Kaur *et al.,* 2007; Sharma *et al*., 2013 who observed cultural variability in *Alternaria* species in respect of mycelial growth and sporulation.

Pathogenic variability among *A. brassicae* isolates: Different isolates of *A. brassicae* of Brassica species (*Brassica rapa, Brassica juncea* and *Eruca sativa*) showed pathogenic variability on host *B. juncea* cultivar Varuna. All the *A. brassicae* isolates from rapeseed (*Brassica rapa*) mustard (*Brassica juncea*) and Rocked salad (*Eruca sativa*) were found to be pathogenic in nature (Table 3). Among them, isolates of *Brassica juncea* i.e. AUA-19, AUA-21, AUA-22, AUA-24, AUA-25, AUA-31, AUA-39, AUA-41, AUA-43, AUA-45, AUA-47 and AUP-29 were found to be highly pathogenic as the spots size produced by them were >5mm in diameter. Isolates AUA-20, AUA-23, AUA-32, AUA-33, & AUA-36 from *Brassica juncea* and AUA-18 & AUP-28 from *Brassica rapa* were moderately pathogenic as spots size produced by them were 2-5mm in diameter, while isolate of *E. sativa* i.e. AUA-38 was least pathogenic as spots size produced < 2 mm in diameter. Similar results were obtained by Michereff, *et al.,* 2003 who studied 38 *Alternaria brassicicola* isolates of cabbage and cauliflower (*Brassica oleracea*) and estimated pathogenic variability based on disease development. Pramila *et al*. (2013) who reported pathogenic variability based on size of spots among 10 isolates of *Alternaria brassicae* of Indian mustard.

Conclusion

The findings of present investigations clearly demonstrated that overall morphological, cultural and pathogenic variability did exist in *A. brassicae* and showed high leval of derangement with no clear grouping of *A. brassicae* and no corelation in studies among the morphological, cultural and pathogenic variability of *A. brassicae* isolates. But in this study 4 isolates of *A. brassicae* from Pantnagar i.e AUA-33, AUA-36, AUA-23 (*B. juncea*), AUA-18 (*B.rapa*), AUA-20 (*B. Juncea*) from Sitargang, AUP-28 (*B. rapa*) from Kanpur and AUA-32 (*B. juncea*) from Rishikesh were found moderately pathogenic with 2.13-4.77mm dia. spot size, whereas isolate AUA-38 (*Eruca sativa*) from Pantnagar was found least pathogenic with 1.63 mm dia. size of Alternaria leaf spot. The determination of pathogenic variability among *A. brassicae* isolates is fundamental to guide the development of appropriate strategies for disease management according to different climatic zones. This result of pathogenic variability will be useful in developing integrated management strategies of Alternaria leaf spot and breeding programs for the crops of Brassica sp.

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