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Variability in *Alternaria brassicae* and characterization of host response in Ethiopian mustard *(Brassica carinata* A. Braun*)*

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Abstract

The present investigation was undertaken to determine variability in virulence among four isolates of *Alternaria brassicae* (Berk.) Sacc., one each from *Brassica juncea*, *B. rapa*, *B. napus* and *B. carinata* on the basis of conidial morphology, cultural characteristics, sporulation intensity and length of incubation period. Results revealed that all parameters significantly affected virulence. The average length and breadth of the most virulent *B. napus* ($A.B_n$) isolate were 171.0 µm and 26.1 µm, compared to only 134.0 and 19.0 µm, respectively, of the least virulent *B. juncea* ($A.B_j$) isolate. Similarly, the horizontal and vertical conidial septations, diameters of both halo formation and concentric rings and sporulation intensity were significantly higher in the most virulent ($A.B_n$) than in the least virulent ($A.B_j$) isolates; incubation period was shortest (2 days) in the ($A.B_n$) isolate. Results of screening of 45 mutants of *B. carinata* against the most virulent $A.B_n$ isolate revealed longest incubation period of 4 to 5 days in the 17 mutants; 10 of the 17 mutants showed very poor sporulation intensity and smaller diameters of both halo formation and concentric rings. These 10 moderately resistant mutants viz; P_7, $(P_{10})_2$, P_{11}, P_{74} in 50 kR, $(P_4)_2$, $(P_5)_2$, P_2 in 70 kR, $(P_4)_2$ in 80 kR, P_{22} in 90 kR, and P_{13} in 100 kR were further field evaluated to confirm their reaction.

Key words: *Ethiopian mustard, Alternaria brassicae, detached-leaf culture technique, virulence*

Rapeseed-mustard are the most important *rabi* oilseed crops grown in India. In general, the crops have low average productivity due to the prevalence of various biotic and abiotic stresses. Diseases are among the major biotic stresses. *Alternaria* blight caused by *Alternaria brassicae* (Berk.) Sacc., is an important and a widespread destructive disease of rapeseed-mustard (Weiss, 1983). Depending on the severity of the disease, yield losses as high as 70 % have been reported in India (Kolte, 1985; Chahal, 1986; Saharan, 1991). Anamorph form of this pathogen shows great variability in morphology, physiology and pathogenicity. Several researchers have reported existence of variability based on morphology, sporulation, and growth and cultural characteristics (Gupta *et al.,* 1972; Saharan & Kadian, 1983; Awasthi and Kolte, 1989; Vishwanath and Kolte, 1997). Although, studies on pathogenic variability are important for the development of pre-breeding populations (Meena *et al.,* 2010), information are lacking regarding differences in aggressiveness/virulence in the pathogenic population isolated from different *Brassica* species from Kangra region in Himachal Pradesh. The present investigation, therefore, was undertaken to determine the differences in virulence among the isolates from *Brassica juncea*, *B. rapa*, *B. napus* and *B. carinata* based on the morphology of conidia. The most virulent isolate discovered from these studies was subsequently used to screen mutants of *B. carinata* for their reaction against *A. brassicae.*

Materials and Methods

Alternaria brassicae-infected leaves, showing characteristic symptoms, were collected from plants of *B. juncea*, *B. rapa, B. napus* and *B. carinata*

grown in different fields of CSKHPKV, SAREC, Kangra, during *rabi,* 2010-11 season (Table 1). The pathogen was isolated, purified by single spore technique (Toussoun and Nelson, 1976), and maintained on PDA/ V_8 slants for further studies. Various isolates collected from different species were designated on the basis of host species *viz.*, *B. juncea* (*A.B*$_j$), *B. rapa* (*A.B*$_r$), *B. napus* (*A.B*$_n$) and *B. carinata* (*A.B*$_{car}$). The morphological characteristics of conidia of each isolate including length and breadth, number of septation (horizontal and transverse), and beak length were recorded in 15 days old cultures.

To determine the pathogenic variability in the laboratory, a detached leaf-culture technique was used (Bansal *et al.,* 1990). The samples of fresh and uniform- sized leaves of *B. carinata* variety 'Jayanti' were collected from the field, washed three times with distilled water and the lower epidermis from both sides of midrib was rubbed gently with the help of wet cotton. About 25 µl spore suspension (2000-2500 conidia/ml) of each isolate was injected on the punctured side of the leaves, and each leaf petiole was swabbed with wet cotton. The leaves were kept inside the wet chambers at 25±1°C for 48-72 hrs. The observations on incubation period, halo formation, concentric ring size, and sporulation were recorded periodically to assess the virulence of four isolates. The most virulent isolate was then used to screen 45 putative mutants of *B. carinata* for their reaction against *A. brassicae.*

Table 1: Name of isolates and their codes

Host species	Isolate code
Brassica juncea	A.B$_j$
B. rapa	A.B$_r$
B. napus	A.B$_n$
B. carinata	*A.B*$_{car}$

Results and Discussion

Conidial characteristics of the isolates:

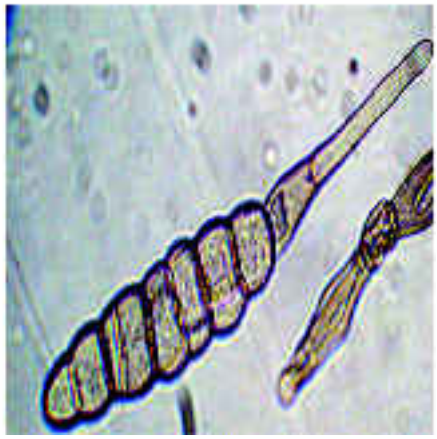
Morphological observations revealed that the isolates differed in their conidial size (Table 2). Amongst the isolates, the average conidial length varied from 134.0 to 171.0 µm. The maximum (358 µm) and minimum (134 µm) length, respectively, were observed in isolate *A.B*$_n$, and *A.Bj*, although, both were statistically at par with each other, the conidial length varied from 80 to 358 µm. The average breadth of conidia varied from 19.0 to 26.1 µm. The isolate *A.B*$_n$ exhibited thickest conidium (26.1 µm) while the isolate *A.B*$_{car}$ was the thinnest (19.0 µm), followed by *A.Bj* (19.1 µm). The isolate *A.B*$_n$ showed maximum range of conidial breadth that varied from 8 to 38 µm. Between *brassica* species isolates did not differ greatly in the average number of septations. Between isolates, the horizontal septations ranged from 4 to 18, and vertical from 0 to 9. Our observations support the report by Kumar *et al.* (2003) where they also observed very little variation in both horizontal (3 to 12) and vertical septation (0 to 6). A significant variation in the beak length between isolates was observed. Although, the average beak length varied from 25.0 to 46.0 µm, it was significantly longer in isolate *A.B*$_n$ and smaller in *A.Bj*. The longest and smallest beak lengths were observed in isolate *A.B*$_{car}$ (188 µm) and isolate *A.Bj* (68 µm), respectively. Similar variations in length and breadth of conidia have also been reported previously (Ellis, 1971; Holliday, 1980; Kumar *et al.* 2003; Singh *et al.* 2009; Meena *et al.* 2012). Kumar *et al.* (2003) also reported variations in the average length (118.6 to 194.5 µm) and breadth (14.5 to 22.7 µm) in *A. brassicae* conidia. Singh *et al.* (2009) also observed variability in conidial length (117.0 to 192.0 µm), breadth (14.0 to 24.0 µm), conidial beak length (42.0 to 116.0 µm), and number of horizontal and longitudinal septa. High level of variability in conidial length, width, beak length, and number of septa *in-vitro* produced conidia of different isolates was also observed by Meena *et al.* (2012). Slight variations in the conidial size between studies may be attributed to the physical conditions of the medium (Plate 1). Variations in the conidial size in the present and several earlier investigations suggest existence of variability in this pathogen.

Plate 1: *Alternaria brassicae* conidia from four *Brassica* species.

(1) $A.B_j$ spores /conidia

(2) $A.B_r$ spores/conidia

(3) $A.B_n$ spores /conidia



(4) $A.B_{car}$ spores/conidia

(1) $A.B_j$ – Spores/conidia of *Alternaria brassicae* isolated from *Brassica juncea*
(2) $A.B_r$ – Spores/conidia of *Alternaria brassicae* isolated from *Brassica rapa*
(3) $A.B_n$ – Spores/conidia of *Alternaria brassicae* isolated from *Brassica napus*
(4) $A.B_{car}$ – Spores/conidia of *Alternaria brassicae* isolated from *Brassica carinata*

Pathogenicity of *Alternaria brassicae* isolates on *Brassica carinata*:

The pathogenic variability of four *Alternaria brassicae* isolates was observed on *Brassica carinata* on the basis of parameters including incubation period, halo formation, concentric ring size and sporulation intensity (Table 3, Plate 2).

It was observed that the length of incubation period, size of concentric ring, halo formation and sporulation intensity were directly related to virulence of the isolates. The virulent isolate, thus, causes higher disease intensity by inducing bigger halo formation, larger concentric rings, higher sporulation, and significantly shorter mean incubation period. The most virulent isolate $A.B_{n,}$ recorded shortest incubation period, largest diameter of concentric ring and halo formation and highest sporulation intensity on *Brassica carinata*. Virulence was also related to the morphology of conidia. The most virulent $A.B_n$ isolate produced significantly larger sized conidia compared to the least virulent $A.B_j$ isolate, thereby indicating a direct relation between virulence and size of conidia. The

Table 2: Variation in conidial size of *Alternaria brassicae* isolates obtained from different *Brassica* species

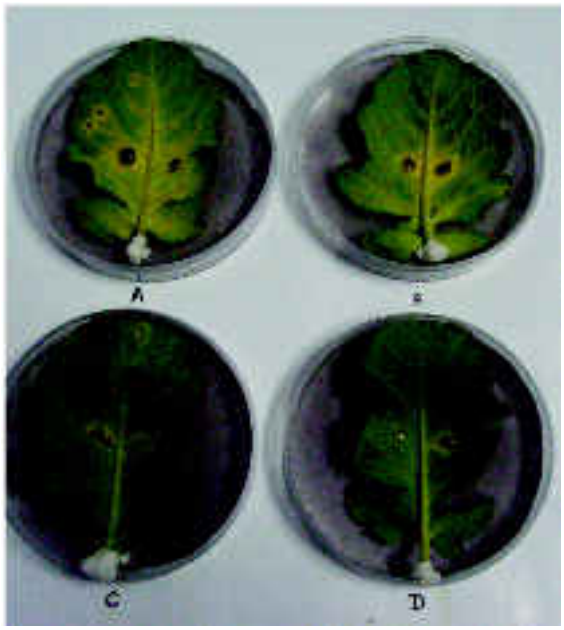
Isolates	Length (µm)		Breadth (µm)		Septation (No.)				Beak length (µm)	
					Horizontal		Vertical			
	A	R	A	R	A	R	A	R	A	R
$A.B_j$	134.0	80-200	19.1	13-32	8	4-14	1	0-4	25.0	10-68
$A.B_r$	154.0	88-288	21.0	16-35	9	4-16	3	0-7	40.0	18-122
$A.B_n$	171.0	105-358	26.1	8-38	10	5-18	2	0-9	46.0	16-150
$A.B_{car}$	142.0	85-250	19.0	13-28	9	4-15	1	0-5	43.0	13-188
Mean	178.6		20.0						43.6	
CD (5%)	41.4		NS						10.3	
CV (%)	15.1		12.4						15.4	

A = Average, R= Range

Table 3: Pathogenicity of four isolates of *Alternaria brassicae* on *Brassica carinata* through detached leaf-culture technique

A. *brassicae* isolates	Mean incubation period	Halo Formation (days)	Concentric ring size (mm)	Sporulation intensity (mm)	Virulence catagories
$A.B_n$	2.0	120	60	much dark	highly virulent
$A.B_r$	3.0	90	56	dark	moderate virulent
$A.B_{car}$	3.7	75	50	medium light	moderate virulent
$A.B_j$	3.3	50	40	very light	least virulent

Plate 2: Detached leaf- culture technique – Test for the pathogenicity of four isolates



Reactions of different *Brassica* species isolates on *Brassica carinata.*
(A) $A.B_n$ (B) $A.B_r$ (C) $A.B_{car}$ (D) $A.B_j$

findings of the present investigation clearly demonstrated existence of pathogenic variability in *Alternaria brassicae* isolated from different *Brassica* species. Wide variation in virulence among *Alternaria brassicae* isolates from rapeseed-mustard have also been observed by Saharan and Kadian (1983). In the present investigation, the most virulent isolate was obtained from *B. napus* thus, partially supporting the observations of Bains and Tewari (1987) where they reported existence of resistance in *Brassica napus, Brassica carinata* and *Brassica rapa* against *Alternaria* blight. This

Table 4: Response of 45 putative mutants of *Brassica carinata* to the most virulent isolate of *A. brassicae* (*A.B*$_n$) through detached leaf-culture technique

B. carinata mutants	Dose	Mean incubation period (days)	Mean halo diameter (mm)	Mean concentric ring size (mm)	Sporulation intensity	Category
P_7	50 kR	4.5	40	38	very less	MR
$(P_{10})_2$	50 kR	4.0	37	37	very less	MR
P_{10}	50 kR	3.0	68	60	dark	HS
P_{46}	50 kR	3.9	64	56	dark	S
$(P_8)_2$	50 kR	4.2	42	41	medium	MS
P_{11}	50 kR	3.7	40	40	very less	MR
P_{74}	50 kR	4.0	45	42	very less	MR
$(P_{11})_2$	50 kR	4.4	48	40	medium	MS
P_{56}	50 kR	3.8	56	49	dark	S
$(P_3)_2$	50 kR	3.6	73	61	dark	S
P_{47}	50 kR	4.2	57	50	dark	S
P_{26}	60 kR	3.8	65	55	medium	MS
P_{39}	60 kR	3.5	56	51	medium	MS
P_{38}	60 kR	3.0	75	68	very dark	HS
$(P_1)_2$	60 kR	3.8	63	58	sparsely	MS
$(P_9)_2$	60 kR	3.9	65	55	dark	S
$(P_4)_2$	70 kR	4.4	45	36	very less	MR
$(P_5)_2$	70 kR	4.5	48	45	very less	MR
P_6	70 kR	3.7	78	70	dark	S
P_{22}	70 kR	4.0	58	50	medium	MS
$(P_2)_2$	70 kR	4.2	50	47	medium	MS
P_2	70 kR	4.0	47	43	very less	MR
$(P_4)_2$	80 kR	4.4	45	36	very less	MR
P_{15}	90 kR	3.0	72	70	dark	HS
P_2	90 kR	4.0	66	50	medium	MS
P_{22}	90 kR	4.5	35	35	very less	MR
$(P_2)_2$	90 kR	4.0	50	38	medium	MS
P_{22}	100 kR	3.9	58	50	medium	MS
P_4	100 kR	3.7	56	49	medium	MS
$(P_1)_2$	100 kR	3.8	60	55	medium	MS
P_{13}	100 kR	4.3	42	40	sparsely	MR
$(P_2)_2$	110 kR	3.5	68	54	medium	S
$(P_2)_2$	0.3% EMS-PS	3.0	75	70	very dark	HS

P_{34}	0.3% EMS-PS	3.0	72	61	very dark	HS
P_{31}	0.3% EMS-PS	3.0	74	60	medium	S
P_{13}	0.3% EMS-PS	3.7	60	52	medium	S
$(P_9)_2$	0.3% EMS-PS	3.1	68	59	dark	S
$(P_{17})_2$	0.3% EMS-PS	4.0	50	45	medium	MS
P_{27}	0.3% EMS-PS	3.0	56	50	medium	S
P_{14}	0.3% EMS-PS	3.5	54	50	very less	MS
P_{18}	0.3% EMS-PS	3.2	58	52	sparsely	MS
P_5	0.3% EMS-PS	3.0	69	65	very dark	S
$(P_{23})_2$	0.3% EMS-PS	3.0	74	70	very dark	HS
$(P_1)_2$	0.4% EMS-PS	3.1	71	67	dark	HS
P_8	0.5% EMS- PS	3.5	62	55	medium	S

MR-Moderately Resistant; MS-Moderately Susceptible; S-Susceptible, HS-Highly susceptible; EMS-PS-Ethyl Methane Sulphonate with pre-soaking

virulent isolate we obtained can now be utilized in screening rapeseed-mustard germplasm and advanced breeding lines for resistance against *Alternaria brassicae.*

Response of *Brassica carinata* mutants to the most virulent *Alternaria brassicae* isolate:

The most virulent *B. napus* isolate $A.B_n$, was used to screen 45 putative mutants of *Brassica carinata.* The longest incubation period of 4-5 days was recorded in 17 mutants, 10 of the 17 mutants showed very poor sporulation intensity and smaller diameters of both halo and concentric rings (Table 4). Because of their moderate resistance to *A. brassicae,* these 10 mutants viz; P_7, $(P_{10})_2$, P_{11} and P_{74} in 50 kR, $(P_4)_2$, $(P_5)_2$ and P_2 in 70 kR, $(P_4)_2$ in 80 kR, P_{22} in 90 kR and P_{13} in 100 kR, were further evaluated under natural field conditions at Kangra.

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