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MORPHOLOGICAL AND PATHOGENIC VARIABILITY OF *ALBUGO CANDIDA* ISOLATES CAUSING WHITE RUST IN RAPESEED-MUSTARD

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ABSTRACT
The present investigation was undertaken to study morphological and pathogenic variability of six *Albugo candida* isolates collected from Pantnagar, Ludhiana, Hisar, Bharatpur, Delhi, Kangra and Jammu. The morphological variability viz. shape, size and sporangial germination in different isolates was slightly different in different *A. candida* isolates . The shape of sporangia in Pantnagar isolate was slightly spherical; while it was globular in all other isolates. The size of sporangia in different isolates was ranged from 16.25 (Pantnagar) to 22.5 µm (Bharatpur). The sporangial germination varied from 74.63(Hisar) to 90.04 per cent (Pantnagar). The pathogenic variability viz. pustule size , shape, incubation period, inoculation period and disease index was also varied in *A. candida* isolates. The small size pustules (0.5-1.5 mm) was observed in New Delhi and Hisar isolates, while large size pustules (1-3 mm) in Pantnagar and Ludhiana isolates. Pinhead raised pustules was observed in Pantnagar, Delhi, Bharatpur, Hisar, Kangra and Jammu isolates, while pinhead scattered pustules in Ludhiana isolates. At cotyledonary stage *A. candida* isolates showed variation in incubation period (IP) ranged from 4-9 days and latent period (LP) from 9-12 days. Maximum mean disease index was in Pantnagar isolate (22.27%), while minimum was observed Delhi isolate (15.87 %).

INTRODUCTION

Albugo candida (*A. cruciferum*), the cause of the white rust/ blister of the rapeseed- mustard, occurs in all parts of the world where cruciferous crops are grown. It is one of the important diseases of rapeseed-mustard in India causing a yield loss of 17-34 per cent (Yadava *et al*., 2011). The disease is characterized by both local and systemic symptom expression. Local infection appears as white or creamy yellow pustules or "blisters" on leaves and stems. Systemic infection results in abnormal growth and distortion of inflorescence and sterility of flowers, commonly called staghead formed as a result of hypertrophy and hyperplasia. The epidemic development of white rust caused by *A. candida* is dependent upon many factors, viz. aggressiveness of race, amount of available initial inoculum, time of first appearance of the disease and prevailing weather conditions**.** *A. candida* isolates from different *Brassica* species/cultivar or from different geographical regions may be different in their incubation period, latent period and production of sporangia and zoospores, pustule size, shape and texture and aggressiveness (Lakra and Saharan, 1988**;** Gupta and Saharan, 2002; Patni *et al*., 2005 and Mishra *et al*., 2009). These studies proved the possibilities of variability within *A. candida* isolates.

Among various management practices breeding for resistant genotypes / varieties is one of the most ecofriendly, economic and effective method for the control of plant diseases. The resistance of any variety may be break down due to the attack of new virulent race of the pathogen. Therefore, study of pathogenic variability among A. *candida* isolates of different geographical regions of India is essential and it should be a continuous process so that different A. *candida* isolates can be used during screening of resistant genotypes / varieties. It has been well documented that *A. candida* also breakdown the resistance against *Hyaloperonospora parasitica* (downy mildew) in rapeseed-mustard (Awasthi *et al*., 1997). Thirteen races of this pathogen have been reported from different *Brassica* species (Verma *et al*., 1999). At least seventeen physiological races of *A. candida* have been described based on the ability to cause disease in different crucifer species (Minchinton *et al*., 2005). Keeping these in view the present investigation was undertaken to study various component under morphological and pathogenic variability leading to aggressiveness of the pathogen.

MATERIALS AND METHODS

Inoculum preparation

White rust infected leaves from different locations of India viz. Pantnagar, Ludhiana, Hisar, Bharatpur and Delhi during 2010-11 and Pantnagar, Ludhiana, Hisar, Bharatpur, Delhi, Kangra and Jammu during 2011-12 were collected. Single rust pustule of each isolate along with a small portion of the leaf were collected and stored at refrigerator temperature (4°C) in glass vials for further studies. Sporangia were obtained from single white rust pustule of each isolate with the help of a needle. Sporangial suspension of each isolate was made in a test tube

containing sterilized distilled water. The suspension was kept at 18°C for 4 hrs for germination of sporangia i.e. release of zoospores. The inoculum of each isolate was purified and multiplied separately on seedlings of susceptible mustard cultivar (Varuna) grown in plastic pots (15 cm in dia.) under glasshouse condition (Singh *et al*., 1999).

Inoculation on cotyledons

The zoospores suspension (2.5×10^5 /ml) of each isolate was adjusted with the help of haemocytometer and sprayed at cotyledonary stage i.e. 7 days after sowing (DAS) using atomizer. The inoculated plants were transferred in plant propagator for 72 hrs to provide 80-90 per cent RH and kept in glasshouse at 18-20°C for infection. After 72 hrs pots were taken out from the propagator. The pure single rust pustules of each isolate was collected 12 DAS and used for the preparation of zoospore suspension as described earlier. The zoospore suspension of each isolate was sprayed on each *Brassica* cultivars viz. EC-399301, NRCDR-513, EC-399296, Rohini, EC-399299, NRCDR-515, BIOYSR, EC-399313, JM-1 (*B. juncea*), EC-414293 (*B. rapa*), GSL-1 (*B. napus*), PBC -9221 (*B. carinata*) and *B. nigra* local at cotyledonary stage i.e. 7 DAS in two consequent years i.e. 2010-11 and 2011-12 and kept in plant propagator for 72 hrs and glasshouse as described earlier. Optimum soil moisture (80-85 %) and temperature (18-20°C) was maintained in glasshouse for the development of symptoms.

Observations

The observations on component of morphological variability i.e. shape and size and sporangial germination of each isolate was recorded after harvesting the sporangia. Shape of the sporangia was recorded under microscope at 40X by measuring 25 sporangia of each isolate. Size of sporangia (µm) was recorded by measuring 10 sporangia of each isolate using micrometer (ocular and stage). Sporangial germination of each isolate was studied by keeping sporangial suspension at 10° C for 7 hrs. The sporangial germination was recorded under microscope at 40X by counting germinated and ungerminated sporangia (per microscopic field) after incubation. The germinated sporangia were represented by release of zoospores i.e. empty sporangia, while ungerminated sporangia were represented by filled zoospores. The per cent germination was calculated by following formula:

$$\% \text{ Sporangial germination} = \frac{\text{No. of sporangia germinated}}{\text{Total no. of sporangia}} \times 100$$

The observations on component of pathogenic variability viz. incubation period was recorded just after the first appearance of the symptoms, latent period after formation of white rust pustules i.e. after complete development of the symptoms and disease index was recorded 13 days after inoculation (DAI) using 0-6 rating scale (Conn *et al*., 1990) and calculated using formula. The shape of white rust pustules produced by each isolate on the leaves of Varuna was recorded based on the visual appearance, while size of the pustule of each isolates (mm) was recorded by taking 25 pustules using measuring scale.

Rating scale (0-6) for disease index (Conn *et al*., 1990)

Rating scale	Leaf area (%) covered by the pustules
0	No symptoms
1	0 – 5
2	5 – 10
3	10 – 20
4	20 – 35
5	35 – 50
6	More than 50

$$\% \text{ disease index} = \frac{\text{Sum of all numerical ratings}}{\text{No. of cytoledons examinatin} \times \text{maximum grade (6)}} \times 100$$

RESULTS AND DISCUSSION

Morphological variability

The results presented (Table 1) revealed that six *A. candida* isolates viz. Delhi, Hisar, Ludhiana, Bharatpur, Kangra and Jammu isolates showed globular shape of sporangia ,while Pantnagar isolate showed slightly spherical sporangia. The present findings are supported by Singh (1993) and Patni *et al*. (2005) who reported that shape of sporangia of different *A. candida* isolates varied from slightly spherical to globular type.

The size of sporangia varied in different isolates and it was in the range of 16.25-22.5 µm. Four isolates viz. Pantnagar, Hisar, Kangra and Jammu have almost same size of sporangia ranged from 16.25-18.00 µm and were different from Bharatpur (19-20 µm) and Ludhiana (20.5-22.5 µm). These findings are in agreement with the work of Kolte (1985), Lakra and Saharan (1988) and Patni *et al*. (2005) who reported difference in sporangial size in different isolates ranged from 12-18 µm, 13.55-21.78 µm and 13.5-20.9 µm respectively (Table 1).

The sporangial germination i.e. releases of zoospores in different isolates also varied and it was in range of 74.63-90.04 per cent after 7 hrs of incubation at 10°C (Table 1). Maximum sporangial germination was observed in Pantnagar isolate (90.04%) followed by Jammu (84.00%), while minimum in Hisar (74.63%).

Pathogenic variability

Shape and size of pustules

All the isolates showed creamy white pustules, however shape and size of pustules were varied in *A. candida* isolates. The size of the pustules in all isolates ranged from 0.5-3.0 mm in dia. The small size pustules (0.5-1.5mm) was observed in New Delhi and Hisar isolates; medium size pustules (1-2mm) in Bharatpur, Kangra and Jammu isolates, while large size pustules (1-3mm) in Pantnagar and Ludhiana isolates.

The shape of rust pustules were also varied in *A. candida* isolates. circularly arranged pustules was observed in Pantnagar, Delhi, Bharatpur, Hisar, and Jammu isolates, while pinhead raised pustules in Kangra and pinhead scattered pustules was in Ludhiana isolates (Table 2).

Incubation (IP) and Latent (LP) period

Experiment conducted during 2010-11 and 2011-12 revealed that different *A. candida* isolates when inoculated on *Brassica* genotypes, showed variation in incubation period (IP) ranged

Table 1: Morphological variability in *Albugo candida* isolates

A. candida isolate	Shape of sporangium	Size of sporangium(μm)	Sporangial germination (%)
Pantnagar	Slightly spherical	16.25-17 x 18-19	90.04
New Delhi	Globular	18.75 – 20.0	80.50
Hisar	Globular	16.5-18.45	74.63
Ludhiana	Globular	20.5-22.5	80
Bharatpur	Globular	19-20	78
Kangra	Globular	17.87- 18.50	80
Jammu	Globular	17.6-18.60	84

Table 2: Pathogenic variability in *Albugo candida* isolates

Brassica Genotype	Shape of pustule	Size of pustule	Colour of pustule
Pantnagar	Circular, pin head, raised	1-3 mm	Creamy white
New Delhi	Pin head, raised, circularly arranged	0.5-1.5 mm	Creamy white
Hisar	Circular, pin head, surrounded by green area, depressed at center	0.5-1.5 mm	Creamy white
Ludhiana	Pin head, scattered	1-3 mm	Creamy white
Bharatpur	Circular , pin head, depressed at center, circularly arranged	1-2 mm	Creamy white
Kangra	Pin head, raised	1-2 mm	Creamy white
Jammu	Circular, pin head	1-2 mm	Creamy white

Table 3: Incubation and latent period (days) of *A. candida* isolates at cotyledonary stage*

Brassica genotype	*Albugo candida* isolate													
	Pantnagar		Bharatpur		Hisar		Delhi		Kangra		Jammu		Ludhiana	
	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP	IP	LP
Rohini	6	9	6	10	6	10	5	10	7	12	6	11	4	9
EC-399299	6	9	6	10	6	10	6	11	6	11	6	11	4	9
EC-414293	7	11	6	11	5	10	7	11	7	11	6	12	5	9
B. nigra local	6	10	6	10	6	10	6	10	6	11	7	11	5	12
EC-399296	6	10	6	11	6	11	6	10	6	12	6	11	5	9
EC-399313	6	9	6	10	6	11	6	10	6	11	6	11	5	12
EC-399301	6	11	6	10	6	11	7	11	6	11	6	12	9	9
JM-1	6	10	6	11	7	11	7	11	9	12	9	12	9	12
PBC-9221	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NRCDR-515	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GSL-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
BIOSYR	0	0	0	0	0	0	5	9	0	0	0	0	0	0
NRCDR513	0	0	0	0	0	0	0	0	0	0	0	0	5	9

* Pooled data of 2010-11 and 2011-12

Table 4: Per cent disease index at cotyledonary stage (13 DAI) on *Brassica* genotypes*

Brassica genotype	*Albugo candida* isolate Per cent disease index						
	Pantnagar	Bharatpur	Hisar	Delhi	Kangra	Jammu	Ludhiana
Rohini	26.83(31.05)	26.5(30.61)	38.15(35.36)	45.96(28.17)	34.00(35.33)	26.66(31.07)	31.30(34.00)
EC-399299	30.00(33.46)	29.63(32.92)	21.50(25.33)	35.00(27.77)	59.00(50.21)	34.00(35.38)	33.60(35.40)
EC-414293	36.6(37.1)	19.33(26.03)	18.31(20.97)	22.33(28.17)	36.33(36.99)	22.33(28.17)	0.00(0.00)
JM-1	38.96(38.58)	24.66(29.49)	41.81(37.52)	19.13(23.67)	32.00(34.22)	40.00(39.13)	35.00(36.20)
GSL-1	0.00(0.00)	0.00(0.00)	0.00(0 .00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)
EC-399301	36.81(37.60)	45.50(42.28)	46.5(42.87)	32.81(34.88)	26.33(30.41)	47.33(43.46)	30.00(33.20)
EC-399296	36.66(37.25)	15.80(19.27)	43.00(39.31)	27.3(31.27)	27.00(31.28)	59.33(50.39)	34.00(35.60)
PBC-9221	0.00(0.00)	0.00(0.00)	0.00(0 .00)	0.00(0 .00	0.00(0.00)	0.00(0.00)	0.00(0.00)
NRCDR-515	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0 .00	0.00(0.00)	0.00(0.00)	0.00(0.00)
B. nigra local	27.98(31.81)	27.98(31.88)	39.96(36.44)	21.16(25.13)	31.66(34.10)	21.66(27.71)	29.30(22.50)
EC-399313	33.31(32.47)	32.30(34.60)	24.65(27.45)	26.65(28.65)	27.33(31.34)	26.66(31.04)	31.30(34.00)
NRCDR-513	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	34.00(35.60)
Mean	22.27(23.05)	17.84(18.27)	18.29(21.30)	15.87(17.8)	22.20(22.56)	20.87(20.69)	19.90(22.82)
		Isolate (a)		Genotype (b)		a × b	
CD (0.05)		2.85(2.6)		4.35(4.00)		10.15(9.30)	

Values in parenthesis are angular transformed, * Pooled data of 2010-11 and 2011-12

from 4-9 days and latent period (LP) from 9-12 days. Minimum IP (4 days) was observed in Ludhiana isolate infecting Rohini and EC 399299. Maximum IP (9days) was found in Ludhiana, Jammu and Kangra isolates infecting EC-399301 and JM-1. Minimum latent period (9 days) was observed in Pantnagar and Ludhiana isolates infecting Rohini and EC 399299. The maximum latent period (12 days) was observed in Ludhiana, Kangra and Jammu isolates infecting JM-1 (Table 3). The present findings are supported by Gupta and Saharan (2002) , who reported that that in different *A. candida* isolates (AC 1-4) the

IP and LP varied from 6-17 and 11-13 days, respectively at cotyledonary stage tested on 16 *Brassica* genotypes**;** Patni *et al*. (2005) who reported IP of 6 and 7 days and LP of 12 and 13 days in *B. juncea* and *B.campestris* isolates, respectively**;** Mishra *et al*. (2009) who reported IP of 10 and 13 days at cotyledonary and true leaf stage, respectively tested on 6 different *Brassica* genotypes (Table 3).

Disease index

Observations recorded during 2010-11 and 2011-12 revealed maximum mean disease index in Pantnagar isolate (22.27%) followed by Kangra (22.20%) and Jammu (20.87%) which were at par with each other, but significantly different from other isolates. However minimum mean disease index was observed in Delhi isolate (15.87 %) which was at par with Bharatpur (17.84%) and Hisar isolate (18.29%). These observations revealed that Pantnagar isolate was found most virulent, while Delhi isolate (15.87%) was least virulent in infecting *Brassica* genotypes (Table 4). None of the isolates was found in infecting GSL 1, PBC 9221, NRCDR 515 genotypes. The NRCDR 513 was only infected by Ludhiana isolate and showed 34 per cent disease index. Maximum disease index (59.33%) was produced by Jammu isolate in EC 399296, while minimum by Bharatpur isolate (15.80%) in EC 399296. No literature was found on disease index produced by different *A. candida* isolates in different *Brassica* genotypes. The *A. candida* isolates collected from different geographical regions in the present studies showed some morphological and pathogenic variability.

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