THE SEARCH FOR RESISTANCE TO MAJOR DISEASES OF RAPESEED AND MUSTARD IN INDIA

S.J. Kolte, D.K. Bordoloi, R.P. Awasthi

G.B. Pant University of Agriculture & Technology
Pantnagar, Uttar Pradesh, 2631455 India

INTRODUCTION

Among the fungal diseases, Alternaria black spot (ABS) caused by Alternaria brassicae (Berk.) Sacc. and white rust (WR) caused by Albugo candida (pers. ex Lev) Ktz are the major disease problems in rapeseed and mustard crops in India (Kolte 1985). The ABS can cause yield losses of over 70 per cent in most susceptible Brassica crops species, while the WR in association with downy mildew (caused by Peronospora parasitica (Pers. ex Fr) is capable of reducing the yield up to 34 per cent (Kolte 1985). In addition to direct yield losses, the AB can lower seed quality by reducing seed size and discolouration and reduction in the oil content (Kaushik et al. 1984) since development of resistant cultivars is the most effective and practical solution for resource-limited farmers, the present studies were carried out with a view to find out (1) variability in A. brassicae and A. candida and (2) to search for sources of resistance to the ABS and WR. The results are presented herein.

MATERIALS AND METHODS

Variability in A. brassicae and A. candida

Variability in A. brassicae and A. candida isolates was studied by obtaining isolates of the respective pathogens from different lesion types and/or from different rapeseed-mustard-growing regions of India. Differences in the culture and growth characteristics of A. brassicae isolates were studied in the laboratory by using standard procedures.

Variability in the pathogenicity of both A. brassicae and A. candida isolates was studied using different Brassica species (Table 3). For this purpose seeds of the respective Brassica species were sown in a soil, sand and compost mixture (2:1:1) in pots in the greenhouse. When the plants were at 3-4 leaf stage, they were inoculated with the isolates of the respective pathogen. As and when required, detached leaf technique was also used to study pathogenic differences among the isolates. For Alternaria infection, a pure culture of A. brassicae isolate(s) was obtained on V-8 juice nutrient agar as the generation of the single spore from a single lesion from the naturally infected leaves. The inoculum of the respective isolate was then prepared from such cultures in distilled sterile water and the spore suspension was adjusted to 10^4 spores per ml of water and sprayed on the test plants with an atomizer or drop inoculated on the detached leaves in the petri dish moist chambers. For WR infection, the chilled sporangia from a single pustule of A. candida isolates were added to 10 ml of double distilled water in a test tube and placed for 4-6 hr. in an incubator at 10-15°C for germination. Following the sporangial germination the zoospores were resuspended in 150 ml double distilled water and sprayed uniformly onto the test plants using the atomizer. The inoculated plants were then kept in a polythene, moist

chamber providing almost 100 per cent relative humidity at the temperature range of 7-25°C under diffused light conditions for 48 hr. Differences in the symptom development, i.e. lesion number and the size of the lesion, incubation period and intensity of sporulation were noted.

Serological Studies

For serological studies, A. brassicae isolate C and A. candida isolate I_4 (from B. juncea) were used for production of antisera. Two white rabbits 3-4 kg in weight were immunized separately with respect to each isolate. For immunization A. brassicae isolate C spores and A. candida isolate I_4 sporangia were sonicated at a frequency of 20 Khz using an ultrasonic processor at 5-10°C, and the cell sonicates (antigens) were preserved by adding 0.1 per cent merthiolate for immunization (Bordoloi 1990). The first subcutanceous infection was given with 4 ml emulsion, i.e. 2:2 mixture of specific antigen and Freund's complete adjuvant. Two booster injections were given consisting each 1 ml of antigen and 1 ml of Freund's incomplete adjuvant at every 7-day interval. Twenty days after the last injection the antisera were collected by cardiac puncture and stored in a refrigerator after adding merthiolate 0.1 per cent as described by Bordoloi (1990).

The serological relationships of six A. brassicae A, C, D isolates (from Pantnagar), K isolate (from Kanpur) and BH_1 and BH_2 isolates (from Bihar) and four A. candida isolates I_1 (from B. campestris var. Yellow Sarson) I_2 (from B. campestris var. Toria), I_3 (from B. campestris var. Brown Sarson) and I_4 (from B. juncea) were studied by slide agglutination and tube agglutination tests as described by Bordoloi (1990).

Screening Techniques for Resistance

Screening for resistance to ABS and WR was carried out using a large collection of cultivated rapeseed-mustard varieties and germplasm and related Brassica species under natural disease pressure in the field during the crop season (October to February) in 1986-87 to 1989-90. The genotypes were grown in two 5-metre rows in two replications. Test lines were separated by a single infector-cum-spreader row of the susceptible B. campestris var. Yellow Sarson cv. Type 151 or B. juncea cv. Varuna to monitor the disease spread. Whenever dry atmosphere prevailed, the disease development was augmented by inoculating the infector rows with A. brassicae spores and/or A. candida zoospore suspension at 60-75 days after sowing. Following inoculation the field was irrigated for successful development of the disease. The screening of the genotypes was also done under greenhouse conditions following the same method of inoculation as described under variability studies. The genotypes were scored for their reaction to ABS and WR infection using 0-9 rating scale. The stem ex-plant culture technique as described by Kolte and Yadav (1991) was also used for screening for resistance of field-grown germplasm.

RESULTS AND DISCUSSION

1. Variability in A. brassicae

Variability in three isolates of A. brassicae was studied and the differences in their characteristics are indicated in Table 1. It was observed that the three isolates showed distinct

Table 1. Differentiating characteristics of the three isolates of Alternaria brassicae

Characteristics	Alternaria brassicae Isolates A	C	D
Mycelial growth	Faster	Slow	Slow
Sporulation on PDA	Poor	Best	Good
Formation of chlamydospores on V-8 juice nutrient agar	Abundant	Poor	Absent
Growth on Sabourd's agar medium	Good	Scanty	Good
Ratio of body length to sporebeak	1:1.90	1:2.86	1:1.28
Spore germination	Usually germinate from the middle cell	Usually germinate from the uppermost cell	The sporebeak itself protrudes to form germ tube
Formation of secondary conidia	Absent	Abundant	Not observed
Growth on solid agar media after 16 days of incubation	Show development of whitish granular texture	Granular growth texture never observed	Granular growth texture never observed
Formation of growth zones on agar media	Absent	Present	Present
Growth on media supplemented with asparagine as a source of nitrogen	Fails to grow	Fails to grow	Fails to grow
Best medium for growth	Radish root extract agar	Brassica alba leaf extract agar	Sabourd's agar
Growth on Sabourd's agar	Whitish cottony growth	White cottony growth	Brownish grey colour
Symptoms on B. carinata	Larger brown concentric spots with dark grey centre and light grey margin	Smaller spots with light grey centre and dark black margin	Black solid dot-like spots without necrosis in the centre

differences in their morphology, growth and culture characteristics. Among the Brassica species, B. carinata showed three distinct types of spots as produced by the three isolates (Table 1). Such differences in development of spots were not clearly visible on leaves of other Brassica species even though the respective Alternaria isolates could be obtained in culture from the infected leaves of these species. This is for the first time from India that such differences among A. brassicae isolates indicating the possible existence of races in A. brassicae is shown. No information is available from other countries regarding existence of the races though preliminary reports on variability in A. brassicae have been made from Holland (Van Schreven 1953) and the United Kingdom (Mridha 1983).

2. Serological Relationship Among A. brassicae Isolates

Agglutinations were observed when A. brassicae antigen isolates A, C, D, BH_1, BH_2 and K were mixed with A. brassicae isolate C antiserum. Fine agglutinations were observed against the antigens C and BH_2. Marked and moderate agglutinations were noticed with the antigens A and BH_1 and D and K respectively (Table 2). Agglutination titer of A. brassicae isolates was determined against its homologous and heterologous antigens. The highest homologous titer of 640 was obtained for the isolates C and BH_2. The Pantnagar isolates A, C and D showed the same titer value for agglutination with respect to isolates BH_1, BH_2 and K respectively (Table 2). The agglutination test thus clearly indicated that the Pantnagar A, C and D resembled the Bihar isolates BH_1, BH_2 and K isolates respectively.

Table 2. Serological relationship among Alternaria brassicae isolates determined by aggulation test.

	Agglutination	Alternaria brassicae Isolates (Antigens) A	B	D	BH_1	BH_2	K
A. brassicae Isolate Antiserum (CFS)	Degree*	+	+++	++	+	+++	++
	Titer**	40	640	80	40	640	80

* Degree of agglutination is indicated by +++ as fine; ++ as moderate and + as marked.

** Titer of agglutination measured as the reciprocal of the highest dilution of antiserum that still aggutinated A. brassicae antigen.

3. Variability in Albugo candida

Ten different isolates of A. candida were collected from different geographical areas of India and the pathogenicity study using 15 different Brassica hosts revealed that A. candida isolates WRT_1, WRT_2 and WRT_3 collected from B. campestris cultivars infected only B. campestris cultivars and not B. juncea or any other host. Similarly WRM_1 to WRM_6 isolates of A. candida collected from mustard (B. juncea) predominantly infected mustard, though WRM_1 isolate was found to produced minute pustules on B. alba and B. campestris var. Toria. The results revealed that isolates infecting B. campestris and

Table 3. Reaction of differential host Brassica species against different isolates of Albugo candida

Species	Albugo candida Isolates									
	B. campestris var. Toria			B. juncea						B. napus
	WRT_1	WRT_2	WRT_3	WRT_1	WRT_2	WRT_3	WRT_4	WRT_5	WRT_6	$WRGS_1$
B. alba	–	–	–	–+	–	–	–	–	–	–
B. campestris var. Toria	+	+	+	–+	–	–	–	–	–	–
B. campestris var. Yellow Sarson	+	+	+	–+	–	–	–	–	–	–
B. campestris cv. Torch	–	–	–	–	–	–	–	–	–	–
B. campestris cv. Tobin	–	–	–	–	–	–	–	–	–	–
B. carinata	–	–	–	–	–	–	–	–	–	–
B. juncea cv. Varuna	–	–	–	+	+	+	+	+	+	+
B. juncea cv. Domo	–	–	–	–	–	–	–	–	–	–
B. napus PPNS	–	–	–	+	–	–	–	–	–	–
B. nigra	–	–	–	–	–	–	–	–	–	–
Campelina sativa	–	–	–	–	–	–	–	–	–	–
Eruca sativa	–	–	–	–	–	–	–	–	–	–
Raphanus sativa cv. Commet	–	–	–	–	–	–	–	–	–	–
R. sativa cv. Cherry Belle	–	–	–	–	–	–	–	–	–	–
R. sativa cv. Local	–	–	–	–	–	–	–	–	–	–

+Indicates good symptom; –+ indicates trace infection; – indicates absence of symptom
WRT_1 = from Toria cv. PT30, WRT_2 = from Toria cv. PT303 and WRT = from Toria cv. T9;
WRM_1 – WRM_6 = from six different locations from B. juncea; WRGS = isolate from B. napus

B. juncea are two distinct races and the isolates from B. juncea are more virulent. The $WRGS_1$ isolate from B. napus was found to be B. juncea pathotype[1] of A. candida infecting B. napus (Table 3). variability in the texture and size of pustules was also noticed and the studies to reveal the differences in such characters are in progress.

4. Serological Relationship among Isolates of A. Candida

Positive agglutination reactions were found against homologous (I_4) and heterologous antigens I_1, I_2 and I_3. Titer of the antiserum was determined by the tube agglutination test against homologous and heterologous antigens. The homologous titer of 320 was found for the antigen I_4 and a titer of 160 was found for the antigens I_1, I_2 and I_3. Thus the results revealed that the isolates I_1, I_2 and I_3 can be placed in one serological group and the isolate I_4 can be grouped in another revealing that A. candida isolates from B. campestris and B. juncea are two distinct physiological races. It seems, therefore, that serological techniques can be useful to identify the races of A. candida.

5. Resistant-tolerant Sources to AB and WR

The resistant and tolerant sources to ABS and WR are indicated in Table 4. Camelina sativa and B. campestris ssp. rapifera showed a very high degree of resistance to both AB and WR diseases.

Table 4. Reaction of Brassica cultures against AB and WR disease

1.	ABS resistant tolerant sources to Alternaria brassicae isolate A	Camelina sativa 'Edmonton Accession' Brassica alba 'Pantnagar collection' B. campestris spp. rapifera cvs. red turnip, B. carinata cv. PPSC-1, B. juncea cv. MKU
2.	WR resistant sources to Albugo candida Race 2	B. campestris spp. rapifera cvs. Candian turnip, red turnip, white turnip, B. caulorapa cv. local, B. campestris cvs. S-67, PPSC-1, B. juncea cvs. MKU, PR8908, 8983, 8987, 86-31, B. juncea exotic cvs. BEC 107, 108, 109 111, 112, 113, 115 127, 129, 132, 135, 138, 141, 143, 144, 149, 152, 164, K-41729, K-41731 K-41732, Canadian, local, B. napus cvs. regent PPNS-1, western, B. oleraca var. capitata cv. local, Camelina sativa cv. Canadian. Eruca sativa cv. Local, Raphanus sativa cvs. cherry belle, local, Rarippa islandica

CONCLUSIONS

Among the causal fungi of major disease of rapeseed-mustard in India, the black spot fungus, A. brassicae is the most variable. Serologically distinct races possessing the characteristic pathogenic differences have been observed to be prevalent in the crop. Failure to recognize such variability partly accounts for the limited success of our efforts to breed

the Alternaria black spot (ABS) resistant varieties. Similarly A. candida, the white rust (WR) fungus, exists in distinct races affecting different Brassica species. The WR race affecting B. juncea is much more virulent. The WR races are found to be serologically distinct from each other besides their common pathogenic characteristics. The number of extensive tests of screening rapeseed-mustard genetic stocks have resulted in identification of some cultures and varieties possessing broad spectrum resistance to ABS as against specific form of resistance to WR.

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