Response of three oilseed *Brassica* species to individual and mixed isolates of *Albugo candida*

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Abstract

The differential response of three genotypes of *Brassica juncea* (RESBJ-830, RESBJ-837 and Varuna), three of *B. rapa* (RESBR-219, RESBR-350 and Pusa-Gold) and one of *B. carinata* (Kiran) was tested against two individual Indian isolates from *B. juncea* and their mixture (AcBjP and AcBjD), one from *B. rapa* (AcBrP) as well as the mixture of the three isolates. *Brassica juncea* Varuna and *B. rapa* Pusa-Gold were compatible with isolates from both host species, but the susceptibility to the isolates derived from the same host species was higher. Other genotypes of *B. juncea* and *B. rapa* expressed various degree of incompatibility with isolates from the opposite host species. *Brassica carinata* expressed incompatibility with all three isolates. The results may suggest the presence of differential specific factors for resistance most pronounced at the species level and to a lesser extent within genotypes from the same species that could be used in breeding for resistance to white rust across *Brassica* species.

Key words: Host specificity, pathogenic variability, white rust

Introduction

Albugo candida (white rust) is an important disease of oilseed *brassica*s in India, particularly on *Brassica juncea* and *B. rapa* while *B. carinata*, which is grown on a limited area, was reported to exhibit high resistance to white rust in some locations (Yadav and Kumar, 2003). The pathogen can infect all above- ground parts of the plant and culminates in systemic stag-head infection of the inflorescence, often in association with *Peronospora parasitica* (downy mildew), which may cause a yield loss of 17-34% (Kolte, 1996).

Pathotypes of *A. candida* have been reported in North America and India based upon their compatibility with different cruciferous species (Verma *et al*., 1999, Rimmer et al., 2000). These isolates may infect *Brassica* species other than those of their origin, especially when the host share a common genome as race specificity is not an absolute adaptation. Reports of variability in virulence of Indian isolates of *A. candida* from *B. juncea* are limited (Verma *et al*., 1999, Townsend *et al*., 2003). Evidence for a gene-for-gene relationship has previously been reported in the *Albugo-Brassica* pathosystem (Adhikari *et al*., 2003)

The aim of this study was to assess the responses of three genotypes of *B. juncea*; Varuna, RESBJ-837 [EC-446033] and RESBJ-830 [EC-446032], three of *B. rapa*; Pusa-Gold, RESBR-219 [EC 446034] and RESBR-350 [EC 446035] and one of *B. carinata* (Kiran) to individual and mixtures of Indian field isolates of *A. candida* from *B. juncea* and *B. rapa*.

Materials and Methods

Varuna and Pusa-Gold were Indian varieties used as a susceptible control to isolates of white rust derived from the same host species. The other genotypes were bred at Rothamsted Research. RESBJ-837 and RESBJ-830, which possess a wide resistance profile to *P. parasitica* were derived from F_4 progenies of a cross involving selected S_3 lines from var. Kranti and var. Krishna (Nashaat *et al*., 2004). RESBR-219 and RESBR-350, which also possess a wide resistance profile to *P. parasitica,* were derived from the S_3 progeny of a line selected from var. PYSLP-2 and YST-15, respectively (Unpublished data).

Seedlings of the above genotypes were grown in separate 8.5 cm diameter pots from untreated seeds sown 1 cm deep in autoclaved compost mixture of soil-less commercial grade agro- peat® (Varsha Enterprises, Bangalore, India). The compost was kept moist by placing the pots in plant growth propagators (30.5×25×10 cm) containing a layer of water 1 cm deep. The plant propagators were kept in a Conviron PGV-36 growth chamber at 22 ± 1 ºC and 16-h photoperiod, with a photon flux of 290 µmol quanta $m^{-2}s^{-1}$.

Three field isolates of A. candida, coded AcBjD, AcBjP and AcBrP, were used in this study. AcBjD was derived from a *B. juncea* genotype Varunza at Delhi near The Energy and Resources Institute (TERI) Experimental Station. Both AcBjP, which was also derived from Varuna and AcBrP, derived from a *B. rapa* genotype Pusa-Gold, were collected at Pantnagar, 250 km from Delhi. The three isolates were tested separately in isolation and as mixtures comprising either 'AcBjD+AcBjP' or 'AcBjD+AcBjP+AcBrP'.

Preparation of spore suspension and inoculation of the plants were performed following techniques described by Singh *et al*. (1999). The inoculum of each isolate was prepared individually in isolation in a laminar flow cabinet. The pustules from

freshly collected infected leaves of *B. juncea* and *B. rapa* were tapped to dislodge the zoosporangia into a sterile glass flask containing 25 ml sterilized distilled water (SDW). Extraneous material was removed from the resulting zoosporangia suspension by filtering twice through three layers of muslin cloth. The concentration of the suspension was adjusted to 5×10^4 zoosporangia per ml for each isolate with a haemocytometer slide and appropriate dilution with SDW. The mixture of isolates was prepared by mixing an equal volume of isolate suspensions. Inoculation was carried out within 20 minutes of preparing the zoosporangia suspension.

Seedlings were inoculated 7 days after sowing. Prior to inoculation, seedlings were sprayed with SDW to remove any agro-peat® debris from the surface of the cotyledonary leaves and allowed to dry for 30 minutes. The zoosporangia suspension of the individual isolates and/or their mixtures was applied by spraying seedlings to run-off with an atomizer. After inoculation, seedlings were incubated in a controlled environment cabinet with the transparent propagator lid placed over them to provide high humidity. Incubation started with 8-h of darkness followed by 16-h photoperiod with a photon flux of 290 μ mol $m^{-2}s^{-1}$ at 18 ± 1 ºC/ 15 ± 1 ºC day/ night temperature.

Results and Discussion

The experiment was arranged in a randomised complete block design with three replicates (propagators); each replicate contained 10 seedlings per genotype. Disease reaction was scored 12 days after inoculation on a 0 (resistance) -7 (Susceptible) scale described in Table 1 (Leckie *et al*., 1996). Disease reactions in the range 0-2, 3-5 and 6-7 were classified as resistant, moderately resistant/ moderately susceptible and susceptible, respectively. The average score of each genotype per propagator was considered as a replicate data value of Disease Index (DI). The DIs of a genotypes inoculated with different isolates were Log transformed and subjected to a two way Analysis of Variance (ANOVA) following Gomez and Gomez, 1984 (Table 1). The results of ANOVA revealed significance of genotype (G), isolates (I) as well as their interaction (G×I) at P= 0.05. However genotype has a major role during G×I interaction indicating host based differential response. Based on the interaction of these isolates with individual genotypes, isolate AcBjD appeared to be more virulent than isolate AcBjP on all *B. juncea* and *B. rapa*. Maximum virulence of the former isolate was observed on *B. juncea* genotype Varuna followed by *B. rapa* genotype Pusa-Gold. Evidence of distinct pathogenic variability among Indian isolates from *B. juncea* and *B. rapa* was reported earlier (Townsend *et al*., 2003). Disease severity was increased significantly in all genotypes against the mixture of the two isolates from *B. juncea* in comparison to individual isolates. Such an increase in disease severity may indicate a form of synergistic effect that might have occurred due to the interaction between the two isolates which may have triggered different host response. In all cases, the mixture of *B. juncea* isolates and that of both *B. juncea* and *B. rapa* were more virulent than their individual isolates. In the case of *B. rapa* genotypes, Pusa-Gold showed a susceptible reaction to the individual and mixture of *B. juncea* isolates, whereas RESBR-219 and RESBR-350 expressed a resistant reaction to the two individual isolates, but a moderate-resistant reaction to the mixture of the two isolates. Similar reactions were reported earlier as a result of interaction between *B. juncea* genotypes and *P. parasitica* isolates (Singh *et al*., 2002).

The results also showed that among *B. juncea* genotypes, only Varuna expressed a susceptible reaction to the *B. rapa* isolate AcBrP; all the three genotypes of this host species exhibited better compatibility with the mixture of the two *B juncea* isolates, AcBjD+AcBjP, than the mixture of all three isolates. The two *B. rapa* genotypes, RESBR-219 and RESBR-350, expressed the highest compatibility with isolate AcBrP, followed by the mixture of three isolates, whereas Pusa-Gold expressed a high compatibility with all isolates. In all cases, Varuna and Pusa-Gold expressed the highest level of compatibility with the individual or mixture of isolates. In the case of *B. juncea* genotypes, the reduced susceptibility to the mixture of three isolates in comparison to the individual isolates AcBjP/ AcBjD, and in comparison to isolate AcBrP in the case of *B. rapa* genotypes, could be attributed to the likely competition for sites of penetration through stomata. However, the possibility of induced host resistance due to infection with isolate(s) from the opposite host species in both cases cannot be ruled out (Singh *et al*., 1999). In general, when plants were inoculated with a mixture of the three isolates, the DIs were higher on *B. juncea* than *B. rapa* genotypes, except in the case of Pusa-Gold (Table 1). This may be attributed to better host-pathogen compatibility and/or the higher proportion of the two *B. juncea* zoosporangia isolates in the mixed inoculum (AcBjD+AcBjP+AcBrP) as compared to *B. rapa* isolate.

B. carinata genotype expressed a high level of resistance to all three individual isolates and their mixtures (Table 1). The higher virulence of the *B. rapa* isolate on the amphidiploid derivative *B. juncea* (AABB) in comparison to that observed on *B. carinata* (BBCC) may be attributed to the shared 'A' genome between *B. juncea* and the diploid species *B. rapa* and an adaptation factor, as *B. juncea* is widely cultivated in India whereas *B. carinata* has a relatively limited cultivation.

The new genotypes of *B. juncea* (RESBJ-837 and RESBJ-830) and *B. rapa* (RESBR-219 and RESBR-350) were reported to be resistant against a wide range of *P. parasitica* isolates (Nashaat *et al*., 2004). Interspecific crosses involving the above genotypes which share partial genome homology may result in the combination of a wide range of factors for resistance to white rust and downy mildew. This is very important since infection with *A. candida* can break down resistance or renders the plants more susceptible to *P. parasitica* (Awasthi *et al*., 1997).

Differentiation of pathogen races in India was mainly based on different *Brassica* species. Characterization of new differential host resistance from individual as well as different *Brassica* species capable of discriminating between pathogen isolates with different factors for virulence would be more appropriate for monitoring the pathogen population and breeding for disease resistance.

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Table 1. Disease indices on the cotyledonary leaves of three *Brassica* genotypes inoculated 7 days after sowing with *A. candida* and scored 12 days after inoculation

Host species	Genotype	Disease index[1, 2]				
		B. juncea isolates			*B. rapa* isolate	
		AcBjP	AcBjD	Mix. (1)[3]	AcBrP	Mixt. (2)[4]
	Varuna	5.97 (0.77)	6.37 (0.80)	6.83 (0.83)	5.83 (0.76)	6.07 (0.78)
B. juncea	RESBJ-837	4.56 (0.68)	5.0 (0.69)	5.3 (0.72)	1.33 (0.11)	4.67 (0.66)
	RESBJ-830	4.8 (0.68)	5.07 (0.70)	5.53 (0.74)	1.30 (0.11)	4.77 (0.67)
	Pusa-Gold	4.93 (0.69)	5.77 (0.76)	5.93 (0.77)	6.27 (0.79)	5.7 (0.75)
B. rapa	RESBR-219	1.53 (0.18)	1.8 (0.25)	2.57 (0.40)	4.77 (0.68)	4.4 (0.64)
	RESBR-350	1.60 (0.20)	1.97 (0.29)	2.6 (0.41)	4.97 (0.69)	4.47 (0.64)
B. carinata	Kiran	0.4 (-0.40)	0.37 (-0.43)	0.43 (-0.38)	0.0 (-2.0)	0.3 (-0.56)
$LSD_{P=0.05, df=70}$				(0.09)		

[1]Disease index was scored on 0-7 Point, where 0= No visible symptoms, 1= Light necrotic flecks, no sporulation, 2= Heavy necrotic flecks, no sporulation, 3= Minute pustules on upper surface of cotyledon, 4= Few pustules on lower surface of cotyledon, 5= Numerous pustules on lower surface of cotyledon, 6= Large scattered pustules on lower surface of cotyledon and 7= Large coalescing pustules on lower surface of cotyledon.

[2]Figure in parenthesis are Log transformed data.

[3]Mix. (1): Mixture of isolates BjPAc+BjDAc.

[4]Mix. (2): Mixture of isolates BjPAc+BjDAc+BrPAc