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Pathological and biochemical changes in *Brassica juncea* (mustard) infected with *Albugo candida* (white rust)

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Components of disease reaction, including incubation period, pustule types, inoculum production and disease index (DI); and contents of protein, phenols, soluble sugars and reducing and non-reducing sugars were investigated in cotyledonary and true leaves of six genotypes of *Brassica juncea*: Varuna, Kranti, EC-399296, EC-399299, EC-399313 and EC-399301, inoculated with *Albugo candida*. Cotyledonary leaves were examined 14 days after inoculation (d.a.i.), whereas true leaves were scored 14 and 21 d.a.i. Disease indices were assessed on a 0% (resistant) to 100% (susceptible) scale. DIs at the cotyledonary leaf stage in the above six genotypes were 67, 65, 32, 31, 31 and 38%, respectively, whereas at the true-leaf stage they were 21, 28, 12, 17, 9 and 4%, respectively at 14 d.a.i., and 35, 45, 17, 19, 20 and 6%, respectively at 21 d.a.i. Protein contents were highest in the genotypes with the highest DIs, such as Varuna at the cotyledonary leaf stage and Kranti at the true-leaf stage, and lowest in the genotypes with the lowest DIs, such as EC-399299 at the cotyledonary stage and EC-399301 at the true-leaf stage. Total phenols, total sugars, reducing sugars and non-reducing sugars were generally negatively correlated with DI, but were not always consistent, particularly when differences in DI were small. The results indicated that factors conditioning the response of host genotypes to *A. candida* may differ or operate in different ways at different growth stages.

Keywords: *Albugo candida*, *Brassica juncea*, biochemical constituents, disease components, disease resistance

Introduction

Brassica juncea (mustard) (AABB = 36) is an important oilseed species, particularly in South Asia. It occupies almost 80% of the 7 million hectares cropped with rapeseed-mustard (oilseed *Brassica*) in India which, during 2005–06 accounted for 29·2% of national oilseed crop outputs (Nashaat *et al*., 2007). *Albugo candida* (white rust) is an economically important oomycete pathogen of *Brassica* spp. and other cruciferous species. Infected plants become covered in white, chalky, blister-like pustules, frequently with hypertrophy and distortion of affected tissues. Systemic infection of meristems and inflorescences gives rise to malformed racemes known as stagheads (Petrie, 1973).

In India, white rust causes annual yield losses of 20–60% in mustard (Saharan *et al*., 1984; Bisht *et al*., 1994; Kolte, 2002; Khunti *et al*., 2003; Sachan *et al*., 2004; Kumar & Kalha, 2005). Thirteen races of this pathogen have been reported from different *Brassica* species (Verma *et al*., 1999).

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Primary infections are by zoospores arising from germinating oospores in soil or plant debris. Thereafter, spread is by airborne zoosporangia. The zoosporangia release zoospores on the surface of the host which encyst and invade the host through stomata (Singh *et al*., 2002).

Isolates of *A. candida* from these species are normally most aggressive on their host genotype or species of origin, but nevertheless may infect and colonize, although generally not as well, some other *Brassica* species (Liu *et al*., 1996). Genotypes of *B. juncea* (Townsend *et al*., 2003) and *B. rapa* (Townsend *et al*., 2008) were identified with differential resistance responses to a range of Indian and Canadian isolates of *A. candida* derived from these two species. Varying levels of host resistance to Australian isolates of *A. candida* were recently identified among genotypes of *B. juncea* from Australia, China and India, with those from India being the most susceptible (Li *et al*., 2008). Pre-infection of *B. juncea* with an incompatible isolate of *A. candida* may induce host resistance against compatible isolates of this pathogen (Singh *et al*., 1999) and the downy mildew pathogen, *Hyaloperonospora parasitica* (Singh *et al*., 2002), whereas pre-infection with a virulent isolate of *A. candida* may induce host susceptibility to downy mildew (Awasthi *et al*., 1997).

Observations at the university field experimental station in Pantnagar (northern India) revealed that four genotypes of *B. juncea*, EC-399296, EC-399299, EC-399313 and EC-399301, which showed moderate visible infection with white rust at the cotyledonary leaf stage, showed relatively little or almost no visible infection with this disease at the true-leaf stage (unpublished). The present study was carried out to investigate some pathological (host reaction, incubation period, pustule types and sporulation potential) and biochemical (contents of proteins, total phenols, total soluble sugars, reducing and non-reducing sugars) aspects of infection with *A. candida* in these four genotypes, in addition to another two susceptible varieties, under controlled environmental conditions at the cotyledonary and true-leaf stages.

Materials and methods

Plant material

Six Indian mustard (*B. juncea*) genotypes were included in this study: two were popular Indian cultivars (Kranti and Varuna) and four related or belonging to accessions classified in three differential resistance groups to downy mildew: EC-399296 [RESBJ-140] was derived from a Chinese accession, Yi Men Feng Wei Zi; EC-399299 [RESBJ-294] and EC-399313 [RESBJ-295] were S_3 lines derived from another Chinese accession, Chang Yang Huang Jie; and EC-399301 [RESBJ-177] was derived from a landrace, BGRC-22527, obtained from Germany (Nashaat *et al.*, 2004). Seedlings of each genotype were raised from untreated seeds in 5-cm-diameter plastic pots placed in a tray (41 × 30 × 7 cm) with each genotype replicated by seven pots. Seeds were sown at approximately 1-cm depth in a mixture of soil + compost + sand (3 : 1 : 1). The trays were kept in a glasshouse compartment at 16/6°C day/night. Emerged seedlings were thinned to two plants per pot. A separate set of plants were raised for inoculation of true leaves, in 30-cm-diameter pots, maintaining eight seedlings per pot with each genotype replicated by seven pots. For each experiment, an uninoculated set of control comparisons was kept where disease did not appear.

Inoculum preparation

A single-pustule isolate of *A. candida* derived from *B. juncea* cv. Varuna from Pantnagar, northeast India, was used for this study. The isolate was maintained on cotyledons of 7-day-old seedlings of the same cultivar (Singh *et al.*, 1999). In order to make inoculum available at any time, sporangia were scraped into gelatin capsules from the surface of the cotyledonary leaves supporting abundant sporulation and stored below –10°C. Whenever needed, 40 mg zoosporangial powder from the gelatin capsule was suspended in 50 mL sterilized distilled water (SDW) by stirring gently with a glass rod in a 100-mL conical flask to disperse the zoosporangia. The concentration of the suspension was adjusted to $1{\cdot}44 \times 10^5$ zoosporangia mL^{-1} with the aid of a haemocytometer slide and appropriate dilution with SDW. The suspension was incubated at 15°C for 4 h and then kept at room temperature (20–25°C) to trigger the release of zoospores. The presence of motile zoospores was confirmed by microscopic examination before inoculation.

Inoculation of cotyledonary and true leaves

Fully expanded cotyledonary leaves of 7-day-old seedlings at growth stage (GS) 1 (Sylvester-Bradley, 1985) were used for inoculation. Seedlings were sprayed with SDW prior to inoculation to remove any compost debris from their surface and left to dry at room temperature for 30 min. Inoculum was carefully applied by pipetting 10-μL droplets onto the adaxial surface of each cotyledonary leaf to avoid any inoculum runoff. After inoculation, plants were incubated in a humid chamber at 80–90% relative humidity inside a controlled-environment (CE) cabinet at 19°C with a 16-h photoperiod and a photosynthetic photon flux (measured at seedling height) of 70 μmol quanta s^{-1} m^{-2} for 3 days. Plants were then transferred to a greenhouse compartment at 16/6°C day/night. A single replica of uninoculated control plants for each accession was maintained separately in isolation under similar conditions. True leaves of plants were inoculated at GS 1 : 1, 10–15 days after sowing, when the first true leaf was fully emerged and a second true leaf was about to emerge. The inoculum was applied by spraying the adaxial surface of each first leaf to runoff with an atomizer. Following initial incubation in a humid chamber, plants were removed to a greenhouse along with the uninoculated plants.

Disease assessment

Disease reaction on the cotyledonary leaves was scored on 0–9 scale, 14 days after inoculation (d.a.i.) (modified from Williams, 1987), where 0 = no symptoms on either leaf surface; 1 = small pinpoint to larger brown necrotic flecks under inoculation point; 3 = very sparse sporulation, one to few pustules on lower surface, no pustules on upper surface; 5 = few to many scattered pustules with good sporulation on lower surface and none to few pustules on upper surface; 7 = many pustules with abundant sporulation on lower surface with none to few pustules on upper surface; and 9 = many large coalescing pustules on lower surface with few to many pustules on upper surface of the cotyledon. Disease index (DI) for cotyledonary leaves was calculated using the following formula:

$$\mathrm{DI} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Maximum grade (9)} \times \text{Number of cotyledons examined}}$$

On the true leaves, disease reaction was assessed on a 0–5 scale, 14 and 21 d.a.i. This scale was based on the percentage of leaf area covered with white rust pustules, where 0 = 0%, 1 = 1–10%, 2 = 11–25%, 3 = 26–50%, 4 = 51–75% and 5 ≥ 75%. The DI for the true-leaf stage was calculated using the following formula:

$$\text{DI} = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Maximum grade } (5) \times \text{Numbers of leaves examined}}$$

The incubation period was considered the time in days between inoculation to the first appearance of white rust symptoms on cotyledons and true leaves. The sizes of 10 pustules on cotyledonary and true leaves were recorded and a total of 10 cotyledonary or true leaves per genotype were observed at random from plants in different pots at each stage. Pustules of 0·5–1·0 mm, 1–3 mm and 3–4 mm diameter were classified as small (S), medium (M) and large (L), respectively. Other pustules were only visible as pinpoints (PP) or necrotic flecks (N). Total leaves were then washed off separately with 25 mL SDW in a glass vial. Zoosporangia in suspensions were counted by haemocytometer. Number of zoosporangia per pustule was calculated using the formula:

$$\frac{\text{Concentration of zoosporangia per mL} \times 25}{\text{Number of pustules per leaf}}$$

Biochemical analysis

Samples of inoculated and uninoculated cotyledonary leaves of the six genotypes were collected 14 d.a.i., whereas the true-leaf samples were collected at 14 and 21 d.a.i. Samples were washed with SDW, air-dried and kept in an oven at 60°C for 48 h, then powdered using a mortar and pestle. The powder was packed in three layered polythene bags and stored in a deep freeze at –10°C to be used later for the estimation of biochemical components.

Protein contents were assessed following the method described by Lowry *et al.* (1951). A sample of 0·5 g from each replicate was ground in phosphate buffer (pH 7·0) in a mortar with the aid of a pestle, then centrifuged at 10 000 r.p.m. for 20 min and the supernatant used for protein estimation after cenrtifugation. The amount of protein in the sample was estimated from a constructed standard graph. Total phenols were estimated colorimetrically with Folin-Ciocalteu reagent (FCR) (Bray & Thorpe, 1954). Total soluble sugars were estimated colorimetrically using the phenol-sulphuric acid method (Dubois *et al.*, 1956). The amount of total soluble sugars present in the sample solution was calculated using the standard graph values and the formula used was as follows:

$$\text{Total soluble sugar in the sample (\%)} = \frac{\text{Sugar value from graph}}{\text{Aliquot sample used}} \times \frac{\text{Total volume of extract}}{\text{Weight of sample} \times 1000}$$

Reducing sugars were estimated following the methodology of Somogyi (1952). A standard stock glucose solution was used for the preparation of a standard curve. Non-reducing sugars were calculated by subtracting the reducing sugars from the total sugars content as follows:

Amount of non-reducing sugar = Amount of total sugars – Amount of reducing sugars

Statistical analyses

Data obtained in the laboratory/glasshouse experiments were analysed using two-factorial completely randomized design (CRD). Protein contents measured as percentage dry-weight were transformed to angular scale to stabilize the variance. Critical differences were calculated at the 5% probability level of significance for comparison of genotype means.

Results

Disease assessment

Incubation period (IP) at the cotyledonary and true-leaf stages ranged between 10 and 13 days, with the average being shortest in Kranti and longest in EC-399301 (Table 1). Disease indices on the cotyledons of cvs Varuna and Kranti were significantly higher than those obtained from the other four genotypes, with EC-399301 showing the highest DI of these four genotypes on cotyledons (Table 1). In the case of true leaves, DIs in Varuna and Kranti were also significantly higher than in the other

Table 1 Reactions of six *Brassica juncea* genotypes to *Albugo candida* at the cotyledonary and true-leaf stages

			Disease index		
	Incubation period (days)		Cotyledonary leaf	True leaf	
B. juncea genotype	Cotyledonary leaf	True leaf	14 d.a.i.	14 d.a.i.	21 d.a.i.
Varuna	11	11	67·0 (54·9)	21·4 (27·5)	35·1 (36·3)
Kranti	10	10	65·1 (53·8)	28·3 (32·1)	45·3 (42·3)
EC-399296	12	11	32·4 (34·7)	11·6 (19·9)	16·6 (24·0)
EC-399299	12	12	30·9 (33·8)	16·6 (24·0)	19·4 (26·1)
EC-399313	11	11	30·7 (33·6)	8·90 (17·3)	20·3 (26·7)
EC-399301	13	12	38·2 (38·1)	3·90 (11·3)	5·50 (13·5)
$LSD_{P=0·05,\ d.f.=125}$			Between different stages	0·30 (0·23)	
			Between genotypes	0·42 (0·32)	
			Interaction	0·74 (0·56)	

d.a.i., days after inoculation.

Figures in parenthesis are angular transformed values.

Table 2 Pustule type of *Albugo candida* and number of zoosporangia per pustule on genotypes of *Brassica juncea* at the cotyledonary and true-leaf stages

	Pustule size			Zoosporangia per pustule (×100)		
	Cotyledonary leaf	True leaf		Cotyledonary leaf	True leaf	
B. juncea genotype	14 d.a.i.	14 d.a.i.	21 d.a.i.	14 d.a.i.	14 d.a.i.	21 d.a.i.
Varuna	L	L	L	270	140	205
Kranti	L	L	L	245	175	215
EC-399296	M	S	S	140	65	85
EC-399299	M	M	M	135	80	105
EC-399313	M	S	S	155	95	140
EC-399301	M	PP/N	PP/N	158	48	65
$LSD_{P=0·05,\ d.f.=179}$				Between stages	2·03	
				Between genotypes	2·87	
				Interaction	4·98	

d.a.i., days after inoculation; L, large (3–4 mm); M, medium (1–3 mm); S, small (0·5–1·0 mm); PP, pinpoint; N, necrotic.

Table 3 Protein contents (percentage dry weight) in the cotyledonary and true leaves of *Brassica juncea* genotypes inoculated or not with *Albugo candida*

	Protein					
	Cotyledonary leaves		True leaves			
	14 d.a.i.		14 d.a.i.		21 d.a.i.	
B. juncea genotype	I	UI	I`	UI	I	UI
Varuna	4·1 (11·6)	4·4 (12·1)	4·5 (12·2)	4·8 (12·6)	4·8 (12·6)	5·1 (13·0)
Kranti	4·0 (11·5)	4·22 (11·8)	4·9 (12·7)	5·1 (13·0)	5·09 (13·0)	5·3 (13·3)
EC-399296	3·5 (10·7)	3·71 (11·1)	3·8 (11·2)	4·2 (11·8)	4·2 (11·8)	4·5 (12·2)
EC-399299	3·1 (10·1)	3·3 (10·4)	4·4 (12·1)	4·6 (12·4)	4·5 (12·2)	4·7 (12·5)
EC-399313	3·2 (10·3)	3·5 (10·7)	3·7 (11·1)	4·1 (11·7)	4·5 (12·2)	4·8 (12·7)
EC-399301	3·6 (10·9)	3·9 (11·3)	3·5 (4·1)	3·89 (11·4)	4·1 (11·7)	4·3 (11·9)
$LSD_{P=0·05,\ d.f.=83}$	(0·02)		(0·05)		(0·06)	

d.a.i., days after inoculation; I, inoculated; UI, uninoculated.
Figures in parenthesis are angular transformed values.

genotypes at 14 and 21 d.a.i., but the corresponding DIs in EC-399301 were the lowest among the remaining four genotypes (Table 1). Furthermore, Varuna and Kranti had larger pustules 14 d.a.i. on cotyledonary leaves and 14 and 21 d.a.i. on true leaves (3–4 mm diameter) than the other four genotypes, all of which had medium sized pustules (1–3 mm) on the cotyledonary leaves and, in the case of EC-399296 and EC-399313, small pustules (0·5–1·0) on the true leaves (Table 2). The true leaves of EC-399301 showed only pinpoint necrosis (PP/N) without producing any visible pustules. The number of zoosporangia produced on the cotyledonary and true leaves of cvs Varuna and Kranti were also significantly higher in all cases than those produced on the other genotypes (Table 2). The number of zoosporangia produced on the true leaves of EC-399301 was significantly lower than that on the true leaves of EC-399296, EC-399299 or EC-399313 (Table 2).

Biochemical components

Protein contents (Table 3) were significantly higher at all three stages in the two most susceptible genotypes, Varuna and Kranti, than in EC-399296, EC-399299 and EC-399313. However, protein content was significantly higher at the cotyledonary stage in EC-399301 than in EC-399296, EC-399299 and EC-399313, whereas at the true-leaf stage, it was significantly lower in EC-399301 than in all other genotypes at 14 and 21 d.a.i. Crude protein contents were generally found to be significantly higher in uninoculated than inoculated cotyledons of all genotypes.

The levels of total phenols (Table 4) were reduced after infection at the three tested stages in all six genotypes, but increased from the earlier to the later plant growth stage. Total phenols at the cotyledonary stage of the uninoculated plants were significantly lower in Kranti and Varuna than in the other four genotypes. Following inoculation of the cotyledonary leaves, EC-399301 showed a significantly higher amount of total phenols than Kranti, Varuna and EC-399299, but less than that of EC-399296 and EC-399313. The highest amounts of total phenols were found in the uninoculated cotyledons of EC-399301 and EC-399296. At the true-leaf stage, EC-399301 had a significantly higher amount of total phenols than other genotypes at 14 and 21 d.a.i. (Table 4).

Table 4 Total phenols and total soluble sugars contents in the cotyledonary and true leaves of *Brassica juncea* genotypes inoculated or not with *Albugo candida*

	Total phenols (μg mg^{-1} dry weight)[a]						Total soluble sugars (μg mg dry weight^{-1})[a]					
	Cotyledonary leaf		True leaf				Cotyledonary leaf		True leaf			
	14 d.a.i		14 d.a.i.		21 d.a.i.		14 d.a.i.		14 d.a.i.		21 d.a.i.	
B. juncea genotype	I	UI	I	UI	I	UN	I	UI	I	UI	I	UI
Varuna	1·61	1·90	1·81	2·0	2·1	2·28	35·5	41·7	40·5	42·0	38·2	41·8
Kranti	1·38	1·50	1·50	1·80	2·0	2·08	31·0	39·5	34·4	43·0	32·5	40·3
EC-399296	1·90	2·40	2·0	2·4	2·05	2·45	42·3	45·5	45·6	49·2	43·0	47·5
EC-399299	1·52	2·10	1·8	2·3	2·30	2·60	41·6	47·5	43·5	51·0	42·2	49·2
EC-399313	2·0	2·25	2·05	2·30	2·10	2·30	43·0	45·0	44·03	49·0	43·5	49·0
EC-399301	1·81	2·40	2·23	2·50	2·31	2·75	39·0	46·0	47·5	54·5	46·4	51·6
$LSD_{P=0·05, d.f.=83}$	0·14		0·21		0·14		2·36		0·69		0·62	

[a]d.a.i., days after inoculation; I, inoculated; UI, uninoculated.

Table 5 Reducing and non-reducing sugar contents in cotyledons and true leaves of *Brassica juncea* genotypes inoculated or not with *Albugo candida*

	Reducing sugar (μg mg^{-1} dry weight)[a]						Non-reducing sugar (μg mg^{-1} dry weight)[a]					
	Cotyledonary leaf		True leaf				Cotyledonary leaf		True leaf			
	14 d.a.i.		14 d.a.i.		21 d.a.i.		14 d.a.i.		14 d.a.i.		21 d.a.i.	
B. juncea genotype	I	UI	I	UI	I	UI	I	UI	I	UI	I	UI
Varuna	12·3	16·2	14·9	16·4	12·8	14·1	23·1	25·5	25·6	25·6	25·4	27·7
Kranti	10·8	14·9	12·9	18·2	11·8	15·2	20·1	24·6	21·5	24·8	20·7	25·1
EC-399296	16·1	16·9	18·5	18·9	16·8	17·9	26·2	28·6	27·1	30·3	26·2	29·6
EC-399299	16·4	18·1	16·7	19·2	16·5	18·6	25·2	29·4	26·8	31·8	25·7	30·8
EC-399313	16·3	18·2	19·0	19·8	18·0	18·8	26·7	28·6	24·9	29·2	25·3	28·2
EC-399301	14·5	17·11	19·3	20·5	18·6	21·5	24·5	27·8	28·1	34·0	27·8	30·1
$LSD_{P=0·05, d.f.=83}$	0·44		0·44		0·38		2·21		0·77		0·85	

[a]d.a.i., days after inoculation; I, inoculated; UI, uninoculated.

Total soluble sugars (Table 4) were generally found to be significantly higher in uninoculated than inoculated cotyledons. Both Varuna and Kranti, which expressed the best compatible relationship with *A. candida*, had significantly lower levels of sugars than other genotypes. The total soluble sugar content was also found to be significantly lower in the inoculated cotyledonary leaves of EC-399301 (39·0 μg mg^{-1} dry weight [DW]), which expressed the least compatible relationship with the pathogen, than in EC-399296 (42·3 μg mg^{-1} DW), EC-399299 (41·6 μg mg^{-1} DW) and EC-399313 (43·0 μg mg^{-1} DW). However, in all cases the total soluble sugars content of true leaves was highest in EC-399301 and lowest in cv. Kranti.

Reducing sugar contents (Table 5) were significantly higher in uninoculated than inoculated cotyledonary and true leaves in all genotypes. Reducing sugars were also found to be significantly lower in inoculated and uninoculated cotyledonary and true leaves of cvs Varuna and Kranti than in the other four genotypes. However, reducing sugar content of the inoculated cotyledonary leaves was found to be significantly lower in EC-399301 (14·5 μg mg^{-1} DW) than in EC-399296 (16·1 μg mg^{-1} DW), EC-399299 (16·4 μg mg^{-1} DW) and EC-399313 (16·3 μg mg^{-1} DW), whereas at the true-leaf stage it was higher at both 14 and 21 d.a.i.

Non-reducing sugar contents (Table 5) were found to be lower in Varuna and Kranti than in the other four genotypes, but were generally significantly reduced in all infected genotypes. Among the infected genotypes, non-reducing sugar contents in the cotyledonary leaf were highest in EC-399313, which expressed moderate compatibility with *A. candida*, and lowest in Kranti, whereas in the true leaves, non-reducing sugars were highest in EC-399301 at 14 and 21 d.a.i. and lowest in Kranti.

Discussion

Both Varuna and Kranti, which were shown to be the most susceptible genotypes in this study, were also among the genotypes that expressed the highest degree of susceptibility among a range of Australian, Chinese and Indian genotypes when tested against Australian isolates of *A. candida* (Li *et al.*, 2008). The differences observed in

the reaction to *A. candida* between the cotyledonary and true leaves in all genotypes may be attributed to the same or different factors conditioning host-pathogen interactions (Liu & Rimmer, 1990; Singh *et al*., 2003; Yadav & Sharma, 2004). The relationship observed between host-pathogen compatibility and higher protein content in the host tissues substantiated earlier findings in *B. juncea* inoculated with *A. candida* (Pahuja & Sangwan, 2002) and *Alternaria* sp. (Gupta *et al*., 1984). Phenolic compounds and related oxidative enzymes are considered to be the most important biochemical parameters for disease resistance – the accumulation of total phenols is usually higher in resistant than susceptible genotypes (Arora & Wagle, 1985; Bashan, 1986). The general positive correlation shown in the present study between increasing level of phenols in host tissues and pathogen incompatibility followed almost the same trends previously reported elsewhere (Yadav *et al*., 1996; Singh, 2000; Pruthi *et al*., 2001; Kumar *et al*., 2002).This was particularly well demonstrated in this study when comparisons were made between the different growth stages and among the six genotypes at each stage.

There appeared to be a general trend towards increasing resistance in host tissues with increasing total sugars content, which was clearly demonstrated, for example, by the cotyledonary leaves of EC-399313 at 14 d.a.i. and true leaves of EC-399301 at 14 and 21 d.a.i. A decline in the level of total sugars observed after inoculation was also reported by others (Kumar *et al*., 2002; Guleria & Kumar, 2003). Post-infection decrease in sugar levels may be caused by rapid hydrolysis of sugars during pathogenesis through enzymes secreted by the pathogen (Jaypal & Mahadevan, 1968). The co-existence of free-sugars and phenols results in glycosylation of phenols by sugars, forming phenolic glycosides, which are more soluble in cell sap, leading to more efficient expression of resistance (Walker, 1975).

The increased reducing sugars contents found in this study in association with higher resistance, and the decrease in this content after infection, are in agreement with earlier work on other genotypes of *B. juncea* infected with *A. candida* (Yadav *et al*., 1994). Higher levels of non-reducing sugars in resistant genotypes were also reported in *B. napus* infected with downy mildew and white rust (Singh, 2000). It was interesting to note that these observations differed between the cotyledonary and true-leaf stages. EC-399296, EC-399299 and EC-399313 were less resistant than EC-399301 at the cotyledonary leaf stage, whereas at the true-leaf stage this was reversed, as the latter genotype expressed a higher level of resistance than the former three (Tables 1 and 5). This is another indication that factors for resistance vary at different growth stages. These observations also reinforce the importance of testing plants at both growth stages when screening for disease resistance. The resistance and virulence in this study require further characterization using a wider range of pathogen isolates and host genotypes, with a view to future utilization in studies of host-pathogen genetics and breeding for disease resistance.

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