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BIOCHEMICAL STUDIES ON SOME GENOTYPES OF *BRASSICA JUNCEA* (L.) INFECTED BY *ALBUGO CANDIDA*.

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ABSTRACT
Biochemical studies on resistance and susceptible genotypes of *Brassica juncea* (L.) infected with *Albugo candida* were observed at 14, 56 and 84 days after sowing (DAS). Estimated by Kjeldahl method , nitrogen and protein content at per cent dry weight were observed low in genotypes EC-399296 and EC-399301 in comparison to susceptible genotype Kranti in both healthy as well as infected leaf samples. Total phenol estimation was carried out with Folin-Ciocalteu reagent (FCR). Phenol content (¼g mg^{-1} dry weight) was found maximum in resistance genotype EC-399301 as compared to susceptible genotype of Kranti.

INTRODUCTION

Indian mustard (*Brassica juncea* (L.) Czern and Coss) is an important oil seed crop, grown both in tropical and subtropical regions of the world. It occupy the second position in oil seed crop after groundnut with 6 million hectares, with production about 5 to 6 million tonnes of seed annually (Anonymous, 2008). In Asia, India stands first both in acreage and production of rapeseed and mustard. The crops are cultivated in an area of 70 lakh ha with a production of 81 lakh tonnes and with an average yield of 1149 kg/ha (Anonymous, 2006). 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 (DM) caused by *Peronospora parasitica* (Pers. Ex Fr) is capable of reducing the yield up to 34 per cent (Kolte, 1985).

Yield losses caused by WR or a mixture of WR and DM, range between 17% to 60%, (Harper and Pittman, 1974; and Kolte *et al.*, 1981). 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). Protein content were significantly higher at all three stages in the two most susceptible genotype Varuna and Kranti than in EC-399296, EC-399299 and EC-399313. Total phenol at the cotyledonary stage of the uninoculated plants were significantly lower in Kranti and Varuna than in the other four genotypes (Mishra *et al.*,2009). Also related work observed in rapeseed-mustard by (Bhatt, 2012).

Keeping the above facts in view, the present investigation was carried out. Biochemical changes in different genotypes of *Brassica juncea* (L.) infected samples with *Albugo candida* in resistance and susceptible genotype.

MATERIALS AND METHODS

The eight Indian mustard *B. juncea* (L.) genotype namely EC-399313, EC-399299, EC-399296, EC-399301, EC-399302, Kranti , Varuna and Divya samples have collected from field at NEB-Crop Research Centre, Pantnagar experiment site and observed the biochemical estimation under laboratory condition, resistance and susceptible accessions at assessed the different period at 14, 56 and 84 days after sowing (DAS).

The amount of nitrogen was estimated by Kjeldahl (1883) method. A weighed quantity of the substrate (0.5g) was placed in a special long- necked 'Kjeldahl flask. Nitrogen estimated using the following

formula:

$$\text{Nitrogen in \%} = \frac{\text{TV} \times 0.1\text{N titration} \times 1.4007}{\text{W}}$$

TV = Sample titration value - blank titration value

Where,

TV = Titration value

N = Normality of H_2SO_4

W = Weight of sample

The total nitrogen content estimated by the micro-Kjeldahl method was multiplied with the conversion factor to get the

value of crude protein content. Total proteins were estimated on the basis of following expression: Total proteins = Total nitrogen × 6.25

Where, 6.25 is conversion factor.

Total phenol estimation was carried out with Folin-Ciocalteu reagent (FCR). Measured at 650 nm colorimetrically (Bray and Thorpe,1954). The sample (0.5 g) was weighed and ground using mortar and pestle in 10 times volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes. Catechol was taken as standard and absorbance at 650 nm. Statistical analysis, data obtained in laboratory condition were analysed using two-factorial completely randomized design (CRD).

RESULTS AND DISCUSSION

The mean nitrogen content in healthy leaves were observed more as compared to infected leaves in each entries (Table 1).Within individual genotype nitrogen content was found maximum in 84 DAS sample followed 56 DAS and lowest in 14 DAS within healthy sample and within infected samples. It revealed that with the growth of the plant nitrogen content also increased accordingly. It also revealed that as plant became infected by *Albugo candida* nitrogen content decreased in all the stages respectively. Nitrogen content within eight *B. juncea* genotypes was observed maximum in Kranti followed by Varuna where as it was present minimum in EC-399296 followed by EC-399301. Mishra *et al*., 2009 reported that disease indices on cotyledons and on true leaves of cultivar Varuna and Kranti significantly higher than those obtained from the other four genotypes with EC-399301 showing the highest DI of these four genotypes on cotyledon. Genotype EC-399301 was resistant in comparison to Kranti and Varuna. Nitrogen content level may be correlated with ability of resistance. Similar results are obtained by Gupta *et al*. (1984 and 1992) and Sindhan and Parashar (1996) who reported higher content of nitrogen in susceptible genotype which decreased after infection. Higher content of nitrogen in resistant varieties as compared to susceptible ones has been observed in downy mildew infected Lucerne leaves (Luthra *et al.,* 1988). Genotypic variation in *B. juncea* (L.) Czern. cultivars in growth, nitrate assimilation, antioxidant responses and phytoremediation potential during cadmium stress (Sharma *et al.,*2010). Related work also found by (Bhatt, 2012).

The mean protein contents (Per cent dry weight) were obtained significantly higher in genotype Kranti followed by Varuna and Divya in comparision to all the exotic genotypes tested *i.e.* EC-399301, EC-399296, EC-399299, EC-399313 and EC-399302 in both the cases healthy leaves and infected leaf samples (Table-2). Mean proteins level were found higher in healthy leaf samples in comparison to their corresponding infected leaf samples. It indicates that as plant became infected by *A. candida* proteins content declined significantly. Maximum protein content were observed at leaf samples collected after 84 DAS followed by 56 DAS and least protein content were observed in the sample collected after 14 DAS. It showed that as plant grows protein content increases significantly. It was observed that at reproductive stages, where the disease severity was more; there was increase in protein content in all the genotypes as compared to what they contained at vegetative stage (Yadav *et al.*, 1996). Boller (1985) was of the opinion that proteins are associated with defence

Tabel 1: Nitrogen content (percentage dry weight) at different growth stages in *B. juncea*

Genotype	Nitrogen content (per cent dry weight)							
	Healthy leaves				Infected leaves			
	*14 DAS	*56 DAS	*84 DAS	Mean	*14 DAS	*56 DAS	*84 DAS	Mean
EC-399313	0.51	0.6	0.61	0.58	0.43	0.52	0.57	0.51
	-4.1	-4.45	-4.49	-4.35	-3.77	-4.14	-4.34	-4.08
EC-399299	0.48	0.57	0.61	0.56	0.43	0.51	0.56	0.5
	-3.99	-4.33	-4.49	-4.27	-3.76	-4.1	-4.28	-4.04
EC-399296	0.46	0.54	0.56	0.52	0.41	0.5	0.52	0.48
	-3.89	-4.21	-4.29	-4.13	-3.67	-4.04	-4.15	-3.95
EC-399301	0.46	0.55	0.6	0.54	0.42	0.5	0.52	0.48
	-3.89	-4.25	-4.44	-4.19	-3.72	-4.05	-4.12	-3.96
EC-399302	0.54	0.62	0.63	0.59	0.44	0.54	0.57	0.52
	-4.21	-4.5	-4.54	-4.42	-3.8	-4.2	-4.33	-4.11
Kranti	0.69	0.9	1	0.86	0.62	0.82	0.91	0.79
	-4.75	-5.44	-5.74	-5.31	-4.53	-5.21	-5.47	-5.07
Varuna	0.68	0.8	0.93	0.8	0.6	0.74	0.86	0.73
	-4.72	-5.13	-5.52	-5.12	-4.44	-4.95	-5.31	-4.9
Divya	0.66	0.78	0.9	0.78	0.59	0.72	0.84	0.72
	-4.66	-5.08	-5.43	-5.06	-4.41	-4.87	-5.26	-4.84
Mean	0.56	0.67	0.73	0.65	0.49	0.61	0.67	0.59
	-4.28	-4.68	-4.87	-4.61	-4.01	-4.44	-4.66	-4.37
CD 5 %						0.83		
Genotype				0.81		(0.32)		
				-0.3		0 .51		
Days.				0.49		-0.19		
				-0.18		0.14		
Genotype × days				0 .14		-0.55		
				-0.52				

* Mean of three replications;( ) values in parenthesis are angular transformed

Tabel 2: Protein content (percentage dry weight) at different growth stages in *B. juncea*

Genotype	Protein content (per cent dry weight)							
	Healthy leaves				Infected leaves			
	*14 DAS	*56 DAS	*84 DAS	Mean	*14 DAS	*56 DAS	*84 DAS	Mean
EC-399313	3.19	3.77	3.83	3.6	2.71	3.25	3.58	3.18
	-10.29	-11.2	-11.29	-10.92	-9.47	-10.39	-10.91	-10.26
EC-399299	3.02	3.56	3.83	3.47	2.69	3.19	3.48	3.12
	-10.01	-10.88	-11.29	-10.73	-9.44	-10.29	-10.75	-10.16
EC-399296	2.88	3.38	3.5	3.25	2.56	3.11	3.27	2.98
	-9.76	-10.59	-10.78	-10.38	-9.21	-10.15	-10.42	-9.93
EC-399301	2.88	3.44	3.75	3.36	2.63	3.13	3.23	3
	-9.76	-10.69	-11.17	-10.54	-9.33	-10.19	-10.35	-9.96
EC-399302	3.38	3.86	3.92	3.72	2.75	3.36	3.56	3.22
	-10.59	-11.33	-11.42	-11.11	-9.55	-10.56	-10.88	-10.33
Kranti	4.29	5.63	6.25	5.39	3.9	5.15	5.69	4.91
	-11.95	-13.72	-14.48	-13.38	-11.39	-13.12	-13.8	-12.77
Varuna	4.23	5	5.79	5.01	3.75	4.65	5.36	4.58
	-11.87	-12.92	-13.92	-12.9	-11.17	-12.45	-13.38	-12.33
Divya	4.13	4.9	5.61	4.88	3.69	4.5	5.25	4.48
	-11.73	-12.79	-13.7	-12.74	-11.07	-12.25	-13.25	-12.19
Mean	3.5	4.19	4.56	4.08	3.09	3.79	4.18	3.68
	-10.75	-11.76	-12.26	-11.59	-10.08	-11.17	-11.72	-10.99
CD 5 %					0 .51			
Genotype				0.51				-0.8
				-0.77				0 .31
Days.				0.31				-0.49
				-0.47				0 .89
Genotype × days				0.88				-0.13
				-0.13				

* Mean of three replications;( ) values in parenthesis are angular transformed

Tabel 3: Phenol content (*µ*g mg $^{-1}$dry weight) at different growth stages in *B. juncea*

Genotype	Total Phenol content (*µ*g mg $^{-1}$dry weight)							
	Healthy leaves				Infected leaves			
	*14 DAS	*56 DAS	*84 DAS	Mean	*14 DAS	*56 DAS	*84 DAS	Mean
EC-399313	3.13	5.27	4.95	4.45	2.7	4.97	4.8	4.16
EC-399299	2.72	5.12	4.8	4.21	2.53	4.87	4.69	4.03
EC-399296	4.1	5.3	4.97	4.79	3.98	5.12	4.57	4.56
EC-399301	4.48	5.6	4.6	4.89	4.37	5.17	4.43	4.66
EC-399302	3.03	4.97	4.57	4.19	2.47	4.63	3.71	3.6
Kranti	2.42	3.7	2.23	2.78	2.25	3.38	2.1	2.58
Varuna	2.43	3.68	2.25	2.79	2.2	3.43	2.13	2.59
Divya	2.49	3.62	2.3	2.8	2.39	3.33	2.16	2.63
Mean	3.1	4.66	3.83	3.86	2.86	4.36	3.58	3.6
CD 5 %								
Genotype				0 .86				0 .85
Days.				0 .53				0 .52
Genotype × days				0.15				0 .14

* Mean of three replications

of plants by their action on the cell wall invading pathogens. Singh (2000) found that the resistant (*B. napus* cv. NDBN-1) and moderately resistant (*B. carinata* cv. HC-9-1) cultivars contain lower amount of total protein than the susceptible (*B. juncea* cv. Varuna) cultivar at all three stages *i.e.* cotyledonary, leaves and inflorescence**.** Gupta *et al*. (1984, 1992); Sindhan and Parashar (1996) and also reported related results Mishra *et al.,*2009; also observed by(Bhatt,2012).

The mean phenol content (¼g mg^{-1} dry weight) were found significantly higher in the samples collected at 14 DAS, 56 DAS followed by 84 DAS (Table-3) in both healthy as well as infected leaf sample. It revealed that phenol content increase rapidly and observed maximum at flowering stage *i.e.* 56 DAS then after it decreases slowly *i.e.* 84 DAS. As whole, phenol content higher in healthy leaf sample in comparison to infected leaf sample. Within genotype, Phenol content observed

maximum and significantly higher in genotype EC-399301 and EC-399296 as compared to Kranti,Varuna, Divya. EC-399299 and EC-399313 as a whole all the exotic genotypes produced higher phenol content in comparison to Kranti, Varuna and Divya. Phenol content in healthy leaf sample were recorded higher in comparison to their corresponding infected leaf sample. Resistant genotypes contained higher level of phenols and orthodihydroxy phenols and exhibited increased deposition of leaf surface waxes compared to controls (Singh *et al.,* 1998). Related result have found by (Mishra *et al.,* 2009) *A. candida* inoculated and Uninoculated leaves of *B. juncea* genotypes**.** (Kumar *et al*., 2010). obtained that Phenolic compound was higher in resistant cultivar, resistant genotype RH 8113(R) at vegetative stage 35 DAS at early sown 56.57 (mg/g) and late sown 22.63(mg/g). In other of susceptible genotype Varuna at vegetative stage at early sown 34.56(mg/g) and late sown 28.26(mg/g). (Kulkarni and Benagi, 2013) phenol content was found increased due to infection and the rate of increase was higher in resistant genotypes. Comparatively lesser sugar and higher phenol content were observed in resistant and moderately resistant genotypes

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