Research Paper :

Biochemical alterations caused by *Alternaria alternata* in *Raphanus sativus* L. var. (Mino Early)

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SUMMARY

The alterations in total soluble sugar, reducing sugar, non-reducing sugar and total phenol contents caused by *Alternaria alternata* in *Raphanus sativus* L. var. Mino Early were studied. The quantitative estimations were done at 40 days after sowing (DAS) *i.e.* before inoculation, 20 days after inoculation (DAI) and 40 DAI. It was observed that total sugars and non-reducing sugars decreased with age and infection. In case of reducing sugars a decline was recorded in all the tissues with increasing plant age but the amount increased in all the infected tissues as compared to healthy tissues. Total phenols however, were reduced with both, plant age and infection.

Key words : *Raphanus sativus* L., *Alternaria alternata*, Sugars, Phenols

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Radish (*Raphanus sativus* L.) is one of the most ancient brassicaceous root vegetables grown in both tropical and temperate regions. It is a good source of vitamin A, thiamine, riboflavin, nicotinic acid and vitamin C. Yield of radish is affected by several factors, among these, diseases are the major constraints in successful cultivation of this crop. Among diseases, infection caused by *Alternaria alternata* lead to severe foliage and pod blight. Suhag *et al.* (1983) reported that *A. alternata* infection in radish reduced seed yield by as much as 18%.

Various metabolites of host tissues suffer modifications in their composition due to infection by a pathogen. Alteration in the levels of sugars and phenols in many plants due to *Alternaria* infection have been investigated by many workers (Chandramohan *et al*., 1967; Gupta *et al.*, 1987; Saharan *et al.*, 2000; Joshi *et al*., 2004; Saharan and Saharan, 2004 and Kushwaha and Narain, 2005). The present investigations were therefore, made to study the effect of *A. alternata* infection on sugar and phenol contents of *Raphanus sativus* L.

MATERIALS AND METHODS

The locally grown variety of radish (*R. sativus* L. var. Mino Early) was selected as host for the study. The pathogen, *Alternaria alternata* isolated by single spore culture technique from naturally infected radish leaves growing in Rai Bareilly district was maintained on PDA slants. 15-days old cultures growing on PDA medium were used for inoculation of experimental plants.

The surface sterilized seeds were sown in 30cm pots and 5 plants/pot were maintained. There were 15 replicates of such pots. 40 days after sowing (DAS) the plants were inoculated with spore-cum-mycelial suspension of *A. alternata* and were kept covered by plastic bags to maintain humid micro-environment for 48hrs. A set of control plants was also raised by sowing surface sterilized seeds in sterilized soil and sprayed with distilled water only.

For biochemical estimations the leaves were collected forty days after sowing (DAS) just before inoculation and thereafter 20 and 40 days after inoculation (DAI). The respective tissues were taken from control plants. From inoculated plant leaves, diseased (necrotic + chlorotic portions), pre-halo and intervening tissues (apparent green tissues between pre-halo tissues of adjacent spots), were collected. The samples were oven dried at 60^0C for 48hrs. One gram of powdered leaf tissue samples was used for all the estimations.

The total soluble sugars were estimated by the Anthrone method of Dubois *et al.* (1951) and reducing sugars by Nelson - Somogyi's method (Nelson, 1944).Standard curves were prepared with glucose. Non-reducing sugars were calculated by subtracting reducing sugar values from total sugars content values. Total phenols were estimated by Folin-ciocalteu

method given by Bray and Thorpe, 1954. The statistical analyses were made by MS- Excel programme of Windows XP (service pack-2).

RESULTS AND DISCUSSION

From the data given in Table 1 it is evident that all the four constituents decreased with increase in plant age. The amounts of total sugars in healthy tissues were 28.9 mg/g at 40 DAS before inoculation which become 23.52 and 22.05 mg/g at 20 and 40 DAI. The reducing sugars were 16.73mg/g at 40 DAS and, 14 and 12.70 mg/g at 20 and 40 DAI in healthy tissues. Similar trends of reduction was also recorded in non-reducing sugars as it was 12.17mg/g at 40DAS and 9.52 and 9.35 mg/g at 20 and 40 DAI in healthy tissues, respectively. The reduction in sugar contents in different diseased tissues with increasing plant age was also reported. The present results are in conformity with those recorded by Chahal, 1986; Gupta *et al.*, 1987 and Sugha *et al.*, 1992. Saharan and Saharan, 2004 also recorded decrease in sugar contents with increasing plant age in both susceptible and highly susceptible varieties of clusterbean infected with *Alternaria cucumerina* var. *cyamopsidis*.

Total sugars and non-reducing sugars decreased in all the tissues from inoculated leaves as compared to healthy tissues on both 20 and 40 DAI. The per cent decrease was higher in case of non-reducing sugars. The reducing sugars on the other hand increased in all the tissues of inoculated leaves on both days of observations. At 20 DAI reducing sugars were 14 mg/g in healthy tissues but in diseased, pre-halo and intervening tissues an increase of 9.29%, 5.71% and 3.57% was noticed. At 40DAI it was 12.7 mg/g in healthy tissues but it increases by 14.96%, 10% and 5.51% in diseased, pre- halo and intervening tissues. The reduction in total sugars due to infection was observed by Singh and Bedi, 1976; and Bhardwaj *et al.*, 1985. According to Chopra and Jhooty, 1974 the change in sugar contents may be due to altered rates of synthetic and respiratory activity due to infection by pathogen. Nema (1983) suggested that reduction is due to degradation of metabolism in diseased tissues. But Mc Combs and Winstead, 1964 attributed this reduction to rapid utilization of sugars by developing fungus.

Chaudhary *et.al.*, 1980 recorded reduction in total sugars and increase in reducing sugar contents in apple due to *Pestalotia anonicola*, *Stachybotrys* sp. and *Trichoderma viride* infection. They suggested that reduction in total sugars would have resulted due to its hydrolysis by the pathogen. The resulting reducing sugars due to hydrolysis of total sugars would have been

Table 1 : Effect of *Alternaria alternata* on the biochemical constituents of *Raphanus sativus* L. var. Mino Early

Days / Type of tissue	Total soluble sugars (mg/g dry weight)			Reducing sugars (mg/g dry weight)			Non-reducing sugars (mg/g dry weight)			Total phenols (mg/g dry weight)		
	40 DAS#	20 DAI##	40 DAI	40 DAS	20 DAI	40 DAI	40 DAS	20 DAI	40 DAI	40 DAS	20 DAI	40 DAI
Healthy (H)	28.90	23.52	22.05	16.73	14.00	12.70	12.17	9.52	9.35	6.23	5.22	4.8
Diseased		16.38	15.60		15.30	14.6		1.08	1.00		3.75	3.4
(D)		(-30.36)*	(-29.25)		(+9.29)*	(+14.96)		(-88.66)*	(-89.3)		(-28.2)	(-29.17)
Pre-halo		17.22	15.90		14.80	13.97		2.39	2.03		4.35	4.0
(Ph)		(-26.79)	(-27.9)		(+5.71)	(+10.0)		(-74.89)	(-78.29)		(-16.67)	(-16.67)
Intervening		17.64	16.05		14.50	13.4		3.11	2.65		4.68	4.31
tissues (IT)		(-25.0)	(-27.21)		(+3.57)	(+5.51)		(-67.33)	(-71.66)		(-10.34)	(-10.21)
SE		0.24	0.09		0.13	0.11		0.28	0.19		0.034	0.04
CD		0.48	0.19		0.26	0.21		0.56	0.38		0.067	0.08

* Figures in parenthesis are the per cent decrease (-) or increase (+) over healthy

	Total soluble sugars				Reducing sugars				Non-reducing sugars				Total phenols			
	20 DAI		40 DAI		20 DAI		40 DAI		20 DAI		40 DAI		20 DAI		40 DAI	
	SE	CD	SE	CD	SE	CD	SE	CD	SE	CD	SE	CD	SE	CD	SE	CD
H vs D	0.24	0.48	0.09	0.17	0.12	0.23	0.11	0.23	0.13	0.25	0.20	0.40	0.02	0.04	0.06	0.13
H vs Ph	0.08	0.16	0.09	0.17	0.20	0.40	0.12	0.24	0.25	0.50	0.20	0.41	0.02	0.03	0.02	0.04
H vs IT	0.16	0.32	0.15	0.30	0.09	0.18	0.17	0.35	0.25	0.50	0.31	0.62	0.05	0.10	0.04	0.73
D vs Ph	0.29	0.58	0.00	0.00	0.09	0.18	0.33	0.07	0.37	0.75	0.03	0.07	0.02	0.04	0.05	0.95
D vs IT	0.35	0.70	0.09	0.17	0.09	0.18	0.06	0.12	0.34	0.67	0.13	0.25	0.05	0.10	0.04	0.07
Ph vs IT	0.21	0.43	0.09	0.17	0.15	0.30	0.07	0.13	0.28	0.56	0.15	0.30	0.32	0.06	0.17	0.03

\# - Days after sowing

\## - Days after inoculation

consumed by the pathogen leaving a lowered increased magnitude of it. Similar findings were also recorded by Thind *et al*., 1977 and Saharan and Saharan, 2004 in *Alternaria* infected clusterbean variety HG-365.

The amount of total phenols was 6.23 mg/g dry weight of tissue in healthy tissues at 40 DAS which reduced to 5.22 and 4.8 mg/g at 20 and 40 DAI. The data also reveals that total phenols reduced gradually in all the diseased, pre-halo and intervening tissues. Singh, 2004 also observed reduction in total phenols in *Brassica* spp. due to *Peronospora parasitica* infection. Jayapal and Mahadevan, 1968; Kushwaha and Narain, 2005 and Bhardwaj *et al*., 1985 also reported similar results.

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