Research Paper :

Quantitative changes in sugar and phenolic contents of *Brassica* leaves induced by *Alternaria brassicae* infection

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SUMMARY

Quantitative changes in biochemical constituents in leaves of *Brassica campestris* L. var. Varuna type-59 infected with *Alternaria brassicae* causing leaf and pod blight were studied. The total soluble sugars, reducing sugars, non-reducing sugars and total phenols were estimated in healthy and different diseased tissues of infected leaves 20 and 40 days after inoculation. The amount of all four constituents were also recorded and compared with those at 40 Days after sowing (*i.e.* before inoculation). All the four chemicals decreased with increase in plant age in both diseased and healthy tissues. The reduction was also recorded in total phenol contents in diseased leaf tissues.

Key words : *Alternaria brassicae*, *Brassica campestris*, Sugars, Phenols

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Brassica campestris L. is one of the important oil-yielding crops in India. It is a rich source of vitamins and minerals and also contains many medicinal properties. *Brassica* crops are infected by a number of pathogens culminating in huge losses in seed yield. Among various diseases, *Alternaria* leaf and pod blight caused by *Alternaria brassicae* is highly destructive leading to 10-70 per cent yield losses (Kolte *et al*., 1987). This fungus also deteriorates the quality of the produce (Kadian and Saharan, 1983).

Alternaria infection causes considerable changes in the sugar and phenolic contents of the plant (Chopra and Jhooty, 1974; Nema, 1983; Chahal, 1986; Gupta *et al*., 1987; Kumar and Singh, 1996; Saharan and Saharan, 2004; Joshi *et al*., 2004; Kushwaha and Narain, 2005). Therefore, the present study was undertaken to know the effect of *Alternaria brassicae* infection on different biochemical constituents of *Brassica* leaves.

MATERIALS AND METHODS

Alternaria brassicae was isolated from diseased leaves of *Brassica campestris* from fields in Rae Bareli district, purified by single spore technique and maintained on PDA slants. The 15 day old cultures grown on PDA medium were used for inoculation.

The locally grown variety of *Brassica campestris* (Varuna Type-59) was taken for the study. The surface sterilized seeds were sown in 30cm pots and 5 plants/pot were maintained. After attaining the age of 40 days the plants were inoculated with spore-cum-mycelial suspension of the pathogen and were kept covered for 48 hrs in humid plastic bags. A set of control plants were also raised in sterilized soil and sprayed with distilled water only.

The leaves for biochemical estimations were collected at 40 days after sowing (before inoculation) and 20 and 40 days after inoculation (DAI). From infected leaves the diseased portions (consisting of necrotic and chlorotic *i.e.* halo-region tissues), pre-halo tissues and intervening tissues between 2 spots (apparently healthy green tissues) were collected separately. The leaves from uninoculated healthy plants were also collected. All the samples were oven dried at 60^0C for 24 hrs and powdered.

One gram leaf samples were used for the estimations of total soluble sugars, reducing sugars, non-reducing sugars and total phenols. The total soluble sugars were estimated by the Anthrone method of Dubois *et al*. (1951) and reducing sugars by Nelson's method, 1944 (Nelson's modification of Somogyi's method). Standard curves were prepared with glucose to calculate the total sugars and reducing sugars. Non-reducing sugars were calculated by subtracting reducing sugars from total sugars. The total phenols were estimated by Folin-Ciocalteu method given by Bray and

Thorpe (1954) and their concentrations were calculated from the catechol standard curve. For estimation of sugars, cell-free extract of tissues were made in K-PO_4 buffer (pH 6.6; 1:10:W/V) whereas for phenols alcoholic extract were prepared (1:10 W/V). 1ml of these extracts were used.

The statistical analyses were made by Ms- Excel programme of Windows XP (service pack-2).

RESULTS AND DISCUSSION

Total soluble sugars, reducing sugars, non-reducing sugars and total phenols (mg/g dry weight of tissues) in healthy, diseased, pre-halo and intervening tissues have been shown in Table 1.

It is apparent from the data given in Table that all the four biochemical constituents of *Brassica* leaves decreases gradually in all the tissues with increase in plant age. In healthy tissues the total sugars were 24.36mg/g at 40 DAS which reduced to 20.53 and 14.93mg/g at 20DAI and 40 DAI. In diseased, pre-halo and intervening tissue it was 16.81, 18.2 and 19.46 mg/g at 20DAI and decreased to11.05, 12.74 and 13.6mg/g at 40 DAI. The reducing sugars were 19.20 mg/g at 40DAS in healthy tissues which reduced to 15.87 and 11.27 mg/g at 20 and 40DAI. The reducing sugars were 13.27, 14.50 and 15.6mg/g at 20DAI and at 40DAI it reduced to 9.07, 9.83 and 10.43mg/g in diseased, pre-halo and intervening tissues. Similar trend of reduction was also reported in non-reducing sugars. The total phenol content of healthy tissues was 5.16 mg/g at 40DAS but it reduced to 4.67 and 3.67 mg/g at 20 and 40DAI. The amount was 3.48, 3.7 and 3.86 mg/g in diseased, pre-halo and intervening tissues at 20DAI and decreased upto 1.98, 2.91 and 3.17 mg/g at 40DAI, respectively. Chahal (1986) and Lodha *et al*. (1993) also reported reduction in total sugars, reducing sugars and total phenols with the increase in age of plants in healthy tissues of clusterbean.

On both 20 and 40 DAI the total soluble sugars, reducing sugars and non-reducing sugars decreased in the diseased, pre-halo and intervening tissues as compared to healthy ones. Similar results were observed by Dayal and Joshi (1968); Chopra and Jhooty (1974); Mandokhot *et al*. (1979), Saharan and Saharan (2004) and Kushwaha and Narain (2005). According to Mc Combs and Winstead (1964) this reduction is due to rapid utilization of sugars by developing fungus but Padmanabhan *et al.*, 1974, suggested that this reduction may be due to the impairment of photosynthetic activity due to infection.

The total phenols also decreased in diseased, pre-halo and intervening tissues. The per cent decrease was higher in diseased tissues in comparison to others. Gupta *et al*., 1985, reported decrease in total phenols in both susceptible and resistant varieties of groundnut due to *Cercosporidium personatum* infection.Jayapal and

Table 1 : Effect of *Alternaria alternata* on the biochemical constituents of *Raphanus sativus* L. var. Varuna Type-59

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)	24.36	20.53	14.93	19.2	15.87	11.27	5.16	4.67	3.67	3.65	3.11	2.56
Diseased		16.81	11.05		13.27	9.07		3.48	1.98		2.55	1.94
(D)		(-18.12)*	(-25.79)		(-16.38)	(-19.52)		(-25.48)	(-46.05)		(-18.01)*	(-24.22)
Pre-halo		18.2	12.74		14.5	9.83		3.7	2.91		2.88	2.34
(Ph)		(-11.35)	(-14.67)		(-8.63)	(-12.78)		(-20.77)	(-20.71)		(-7.4)	(-8.59)
Intervening		19.46	13.6		15.6	10.43		3.86	3.17		2.99	2.46
tissues (IT)		(-5.21)	(-8.91)		(-1.7)	(-7.45)		(-17.34)	(-13.62)		(-3.86)	(-3.13)
SE		0.24	0.13		0.1	0.12		0.24	0.05		0.02	0.03
CD		0.49	0.26		0.2	0.24		0.48	0.1		0.05	0.06

* 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	0.13	0.26	0.12	0.24	0.12	0.23	0.06	0.12	0.20	0.40	0.07	0.14	0.04	0.08	0.01	0.02
H vs Ph	0.36	0.73	0.04	0.07	0.15	0.29	0.07	0.13	0.16	0.32	0.03	0.06	0.01	0.03	0.05	0.10
H vs IT	0.26	0.52	0.16	0.32	0.13	0.27	0.12	0.24	0.33	0.66	0.05	0.11	0.02	0.05	0.02	0.05
D vs Ph	0.20	0.40	0.04	0.09	0.07	0.13	0.07	0.13	0.31	0.63	0.06	0.12	0.02	0.05	0.01	0.02
D vs IT	0.24	0.48	0.15	0.30	0.03	0.07	0.12	0.24	0.06	0.12	0.05	0.10	0.03	0.05	0.04	0.08
Ph vs IT	0.21	0.43	0.19	0.38	0.06	0.12	0.17	0.34	0.27	0.54	0.03	0.05	0.01	0.03	0.04	0.07

\# - Days after sowing

\## - Days after inoculation

Mahadevan (1968) and Kushwaha and Narain (2005), also reported such decline in total phenols due to infection.

Gupta *et al*., 1992 observed reduction in total phenols due to infection in both susceptible (MH-1, JL-24) and tolerant (MH-2, C-333) varieties of groundnut leaf infected by leaf spot pathogens. Similar results were also observed by Mitter *et al*. (1997) in case of chickpea infected with *Botrytis cinerea* and they attributed the decline in sugar content to amylolytic activity of the pathogen but Sharma *et al*. (1992) found that total phenols decreased in susceptible variety but increased in resistant ones in response to the infection of *Exserohilum turcicum*. They attributed that reduction it due to synthesis of post infectionally formed phenolics at lower pace.

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