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ALTERATIONS IN ENZYME ACTIVITY DURING INDUCED ANTIVIRAL STATE BY LEAF EXTRACT[1]

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ABSTRACT

Partially clarified aqueous leaf extract of *Clerodendrum aculeatum* induced an antiviral state against tobacco mosaic virus infection in *Nicotiana tabacum* L. var. Samsun NN when administered onto the leaf surface. Antiviral activity was witnessed both at treated as well as at untreated (remote) sites, attaining an absolute value of 100% 4h subsequent to extract application and maintained this level upto 24h. Spectrophotometric analyses of activity patterns of catalase, polyphenoloxidase and peroxidase enzymes from resistant and non-resistant tissues revealed marked alterations in resistant ones. Samples in all cases were prepared from leaves harvested at intervals of 30 seconds, 2h, 4h, 8h, 16h and 24h after extract application. Catalase displayed a peak in activity at 2h after treatment at site which shifted to 4h after treatment at remote site. Polyphenoloxidase elicited a peak elevation in activity at 4h after extract application both at site as well as at remote site. The 4h peaks were commensurate with the 100% antiviral state displayed at that time. Peroxidase, however, behaved variously at the two sites showing a slightly increased activity at site of treatment while the activity pattern at remote site exhibited a marked depression. Since induction of antiviral state is a host mediated phenomenon, the alterations in activity profiles of catalase, polyphenoloxidase and peroxidase may suggest their involvement.

INTRODUCTION

Clerodendrum aculeatum L. leaf extract (CA inducer) is known to induce systemic resistance against several viruses in susceptible plants (Verma *et al.*, 1983). This resistance is effective against both spherical and tubular viruses, albeit to varying degrees, on the one hand while on the other, is able to check hypersensitive and systemic symptom expression at sites of treatments as well as at remote sites. Use of antimetabolites such as actinomycin-D in the early stages reverses resistance induction indicating host mediation (Verma *et al.*, 1983).

Nicotiana tabacum var. Samsun NN was tested for resistance induction by CA inducer against tobacco mosaic virus (TMV) infection and resistance associated enzymological studies.

MATERIALS AND METHODS

Test host, *Nicotiana tabacum* var. Samsun NN, was raised in earthen pots using sterilized soil in an insect free glass house and was used at a 6-leaf stage. The three basal leaves of test plants were administered by a 1:5 (w/v) aqueous leaf extract of *Clerodendrum aculeatum* after being partially clarified by centrifugation at 8,000 rpm for 30 min. The resultant superna-

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tant served as the CA inducer. Treated basal as well as non-treated upper leaves were harvested at intervals of 30 seconds, 2h, 4h, 8h, 16h and 24h after CA inducer application and tested separately for enzyme activity. Control plants were similarly treated with distilled water and leaves harvested alongwith those of treated set. A third treated set was challenge inoculated with TMV at the said time intervals for testing antiviral resistance induction.

TMV culture was maintained on *N. tabacum* var. NP 31. For testing antiviral activity, a 1:100 (w/v) TMV suspension was challenge inoculated on both treated as well as non-treated remote areas. The percent antiviral activity was calculated separately for treated and non-treated sites using the formula :

$$\frac{C-T}{C} \times 100$$

where C=Number of lesions on control plants/leaves

T=Number of lesions on treated plants/leaves

A 10% enzyme extract was prepared by homogenizing leaf discs in chilled 0.1M Phosphate buffer pH 6.5, squeezing through two folds of muslin and centrifuging at 8,000 rpm for 30 min. at 4°C. The supernatant was passed through a column of Sephadex G-25 and eluted with the extraction buffer in cold. The eluate was used as enzyme source. Catalase, polyphenoloxidase and peroxidase were all assayed spectrophotometrically as detailed by Mahadevan and Sridhar (1982). Catalase activity was determined by recording change in absorbance at 230 nm with hydrogen peroxide as substrate. Catechol was used as substrate for polyphenoloxidase which was scanned at 495 nm while peroxidase was assayed at 420 nm in the presence of hydrogen peroxide and pyragallol.

Three replicates were maintained for each set and the data presented is the mean from them.

RESULTS

On treatment with CA inducer, *N. tabacum* var. Samsun NN displayed a 100% antiviral state 4h onwards upto 24h.

At site of treatment, catalase exhibited a couple of peaks in leaves harvested 2 & 24h after CA inducer application with an increase of 142 and 150% respectively over control values. A similar pattern was observed in remote site samples where the enhancement was greater in 4h sample being 200% but was 118% in 24h samples. The 2h peak at site of treatment had shifted to 4h at remote sites (Table I, Fig. 1).

Polyphenoloxidase displayed a higher activity in all samples as compared to catalase. A 90% increase at 2h rose to 114% at 4h, decreased slightly at 8h with 71% increase and peaked again at 16h with a 125% increase dropping down to normal control level at 24h at site. However, the enhancement was almost phenomenal in remote site samples which at 2h displayed a 172% increase, at 4h a 650%, at 8h a 160%, at 16h a 20% and at 24h the second peak with a 322% increase over control rate (Table I, Fig. 2).

Peroxidase was variable in its response exhibiting minor peaks in activity at 2h and 24h with 55% and 51% enhancement respectively at site over control values but decreased consistently at remote site upto 8h displaying a 37, 55 and a 61% decrease at 2, 4 and 8h respectively after which it climbed steadily approaching control value at 16h and crossing over to a 35% increase at 24h (Table I, Fig. 3).

TABLE I

ALTERATIONS IN ACTIVITY PROFILES OF CATALASE, POLYPHENOLOXIDASE AND PEROXIDASE IN *CLERODENDRUM ACULEATUM* INDUCED RESISTANCE IN *NICOTIANA TABACUM* VAR. SAMSUN NN

Time Interval	At Site				At Remote Site			
	% AVA	Percent Increase			% AVA	Percent Increase		
		CAT	PPO	PER		CAT	PPO	PER
30s	1	50	33	7	000	000	40	—12
2h	47	142	90	55	39	20	172	—37
4h	100	63	114	35	99	200	650	—55
8h	100	23	71	17	100	000	160	—61
16h	100	33	125	32	100	100	20	— 4
24h	100	150	000	51	100	118	322	35

AVA — Antiviral activity; CAT — Catalase; PPO — Polyphenoloxidase; PER — Peroxidase

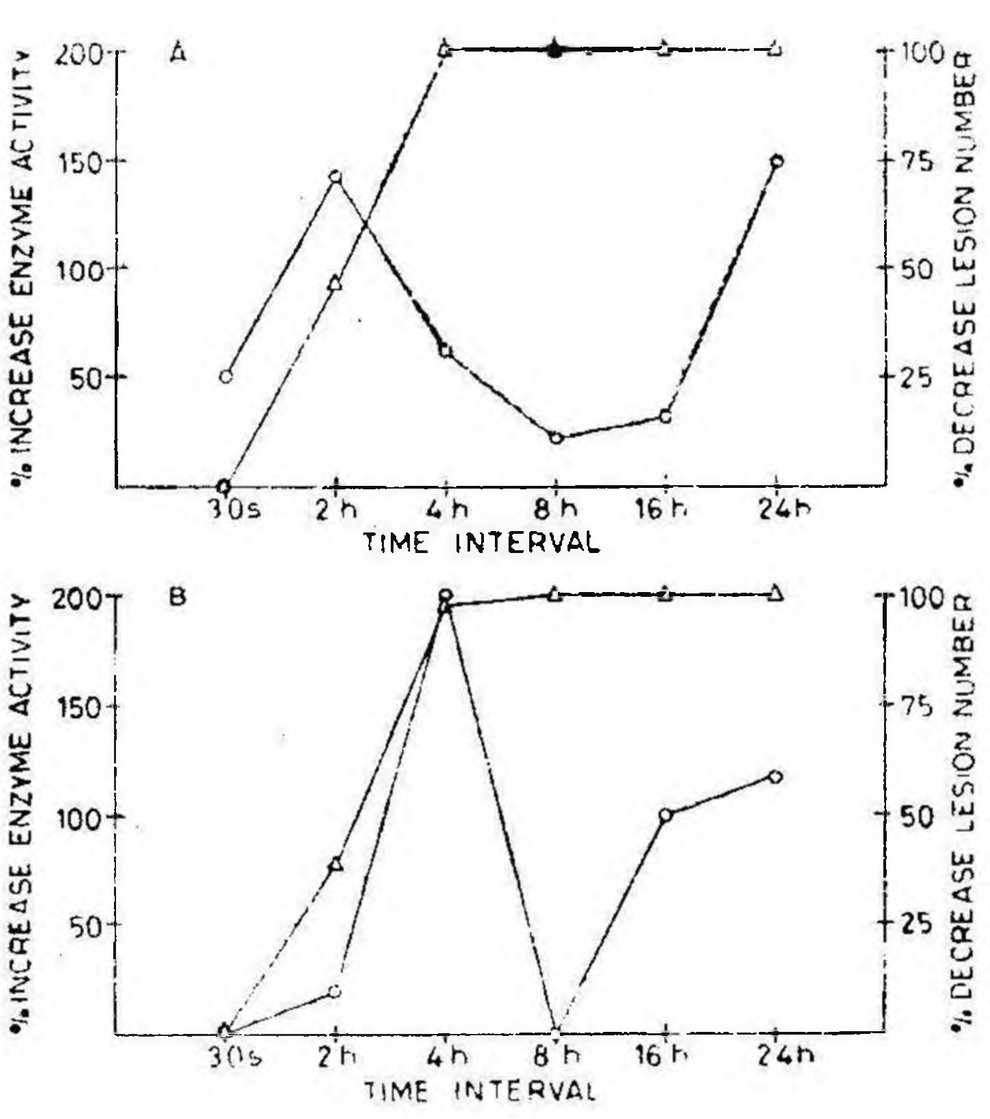

Fig. 1. Resistance induction (△-△) and catalase activity (O-O) in *N. tabacum* var. Samsun NN by CA inducer at site (A) and at remote site (B).

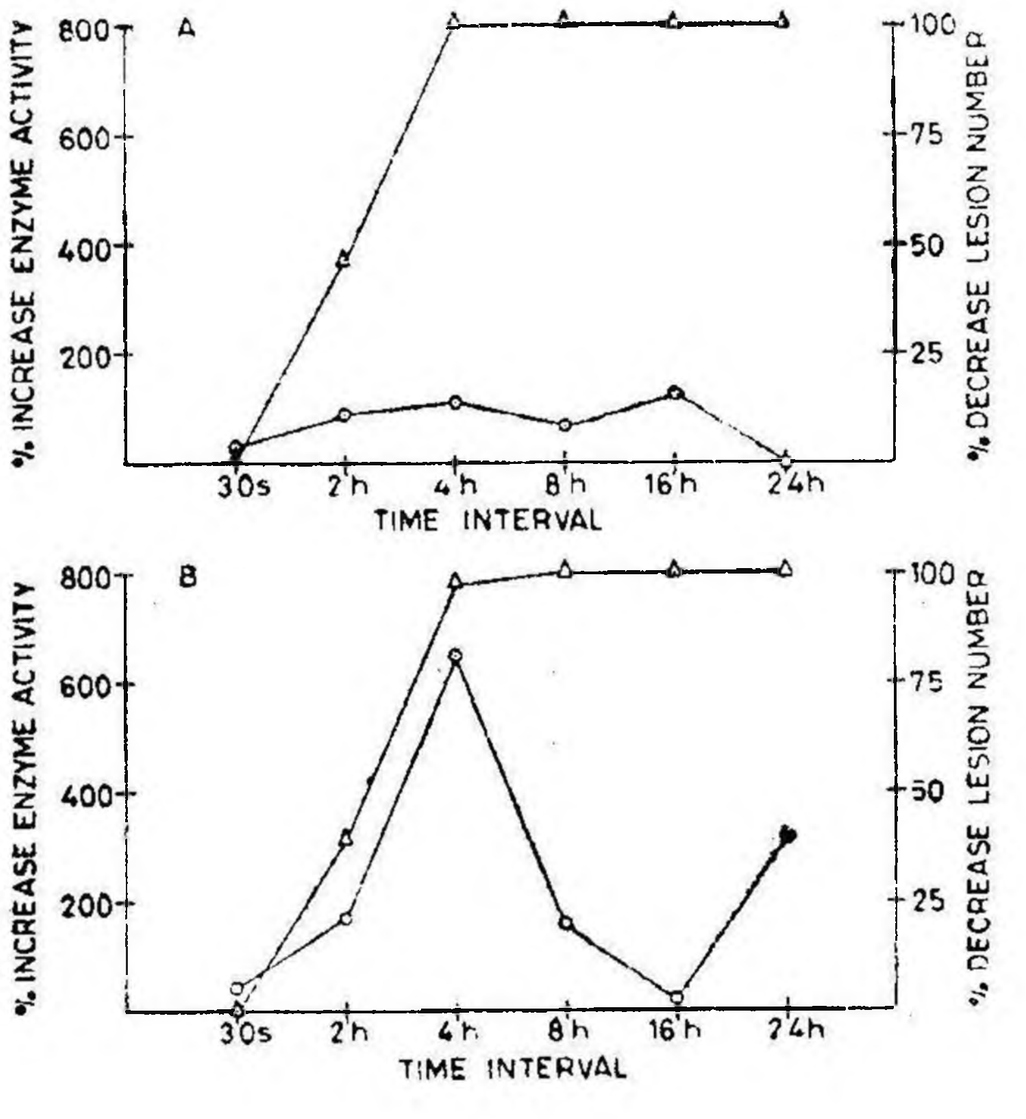

Fig. 2. Resistance induction (△-△) and polyphenoloxidase activity (O-O) in *N. tabacum* var. Samsun NN by CA inducer at site (A) and at remote site (B).

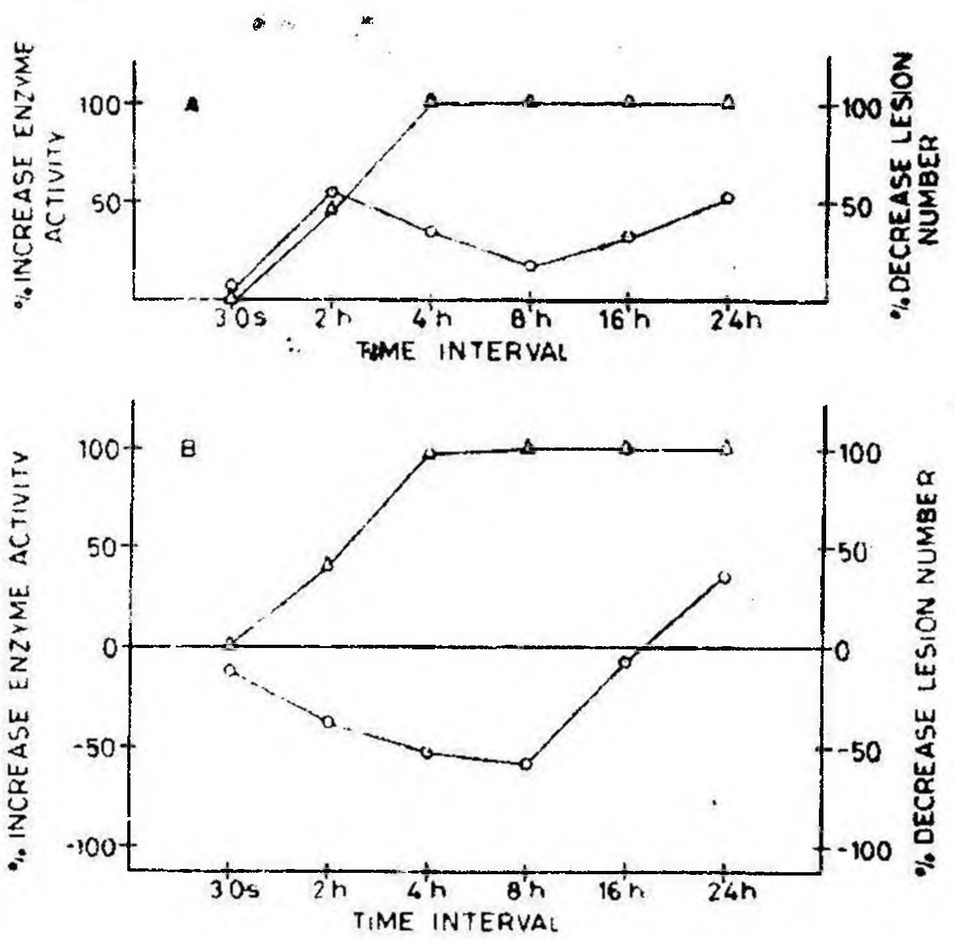

Fig. 3. Resistance induction (△-△) and peroxidase activity (O-O) in *N. tabacum* var. Samsun NN by CA inducer at site (A) and at remote site (B).

DISCUSSION

CA inducer conferred resistance on *N. tabacum* var. Samsun NN when applied onto the leaves. This resistance was evident both at site of treatment as well as at remote sites, attaining its absolute value of 100% 4h subsequent to CA inducer administration exhibiting a time reaction and translocation of the resistance conferring factor.

Biochemical studies carried out in case of resistance induction by higher plant estracts, chemicals and viral, bacterial and fungal infections have disclosed host mediation (Fraser, 1982; Gianinazzi, 1982; Verma and Prasad, 1983). The enhancement in activity profiles of enzymes tested also supports the idea of induced resistance being a host mediated response. The first peak in most samples at 4h is commensurate with the antiviral state developed at that time after CA inducer application. The initial peak could be a consequence of altered host metabolism in response to the inducer applied which is followed by a depression even though the antiviral state offers no such dip. The secondary peaks can be attributed to the synthesis of the active agent directly responsible for antiviral activity, the two overlapping in a fashion so as to maintain the antiviral state at its utmost.

Catalase exhibits elevated activity in cases of natural senescence and hypersensitive necrosis whereas peroxidase is increased during artificial ageing as well, as is polyphenoloxidase (van Loon, 1983; van Loon and Callow, 1983). This indicates a bringing forward in young plants a metabolism resembling that of mature ones which would certainly not be favourable for viral multiplication and spread. *O*-quinones, which are the oxidative products of phenols catalysed by polyphenoloxidase, have been reported to inactivate a few viruses (Hampton and Fulton, 1961; Mink and Saksena, 1971). A higher polyphenoloxidase activity could lead to increased production of *o*-quinones which may bear upon viral infections.

The oxidized form of the substrates could, in some way, be altering host metabolism sufficiently enough to interfere with viral replication. Elevated enzyme activity forms a link between the inducer action and the synthesis of an active antiviral agent which is translocated from sites of treatment to untreated remote sites. Whatever the mainspring of resistance induction, changes in these enzymes could well be an important sequence of events.

This is the first report of resistance induction and alterations in activity profiles of catalase, polyphenoloxidase and peroxidase in CA inducer treated *N. tabacum* var. Samsun NN.

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