ACQUIRED LOCAL AND SYSTEMIC ANTIVIRAL (TMV) RESISTANCE INDUCED BY TREATMENT WITH T-POLY (TRICHOTHECIUM POLYSACCHARIDE) IN NON-HYPERSENSITIVE HOST PLANT *NICOTIANA TABACUM* CV, NP-31

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ABSTRACT

Effect of Trichothecium polysaccharide (T-poly) treatment on virus multiplication rate in *N. tabacum* CV. NP.-31 plants infected systemically with tobacco mosaic virus (TMV) was assessed. T-poly was shown to retard initially but did not completely inhibit TMV multiplication as could be judged by local lesion assay.

INTRODUCTION

IT has been reported earlier[2,4,5] that the fungus *Trichothecium roseum* L. produces in culture a potent non-toxic broad spectrum *antiviral* (tobacco mosaic, southern bean mosaic and tobacco necrosis viruses) substance (a complex polysaccharide) effective in plants. It was also the first report of an antiviral substance of microbial origin shown to exert effect against viruses in a dose-dependent manner through the action directed primarily against the *host* while having no virucidal property or ability to combine with viruses *in vitro* (see Bawden,[1] for review). Subsequently, several other substances were shown to exert antiviral (TMV) effect *via* host, namely, in *Nicotiana glutinosa* by foreign RNA[3] in *N. tabacum* var. Samsun NN by yeast RNA[7] or by poly I : C and synthetic polyanions[8,9,10]. Recent work[4] with Trichothecium polysaccharide (T-poly) showed that the antiviral (TMV) resistance induced in *N. glutinosa* plants locally at treated site spreads to remote parts as well. Simultaneous application of actinomycin-D at treated site caused 50% reversal of the virus inhibition due to T-poly both at site or away from it. Further, the age of the host plant influences the local and systemic resistance to TMV induced by the inhibitor[12].

The aforesaid reports on inhibition of plant virus multiplication have been mainly concerned with effects produced on virus hypersensitive hosts, namely, plants and their cultivars that produce local lesions in contact with virus. In this paper evidence is presented to show that T-poly induces antiviral resistance in nonhypersensitive host plants as well (*N. tabacum* CV.NP.—31).

MATERIALS AND METHODS

T-poly

The fungus *T. roseum* ex Fries, Himachal strain, was maintained and produced T-poly in a medium containing sucrose 30·0 gm, magnesium sulphate 0·5 g, ferrous sulphate 0·01 g, sodium nitrate 3·0 g, potassium dihydrogen phosphate 1·0 g, peptone 1·0 g and yeast extract 10·0 g[4]. For studies reported here, *T. roseum* was grown in the above medium at 26° C ± 1° C for 28 days under stationary condition and toxin-free T-poly was extracted[2] by the use of ethanol and trichloroacetic acid with the modification in that the starting material for extraction was the mycelial mat homogenised in its own fungal culture filtrate[4].

Virus

Tobacco mosaic virus was maintained on *N. tabacum* var. NP-31 plants raised in glasshouse at 23° C with 8 hr sunlight ; under these conditions the virus produced nonhypersensitive symptoms (systemic mosaic). Whenever needed the infected leaves (1·0 g) were crushed to pulp in 10 ml of distilled water. The crude lysate was centrifuged at 3,000 r.p.m. for 30 minutes. The supernatant was taken as the stock virus. Whenever required, dilutions in distilled water were prepared in proportion of 1 : 250.

Treatment of Plants, Virus Challenge and Assay of Virus Inhibitory Activity

T-poly (2·5 mg/ml) was rubbed with forefinger onto the basal leaves of all test plants. Unless stated, 48 hr later the residual T-poly was washed off from the surface by spraying with distilled water (pH 6·5) and all leaves of the test (T-poly treated) and control (water treated) plants were challenged (inoculated) by rubbing with TMV.

For *N. glutinosa* and the two other hypersensitive host plants the mean number of local lesions formed per leaf (6 to 8 leaves per plant for *N. glutinosa* or *N. rustica*, and 8–10 for *C. amaranticolor*) was computed on the 4th day after virus challenge. Unless stated, the virus inhibitory effect of T-poly was assessed on the basis of differences between the two mean

TABLE I

*Mean local lesions per leaf per plant obtained by pre-inoculation treatment of basal leaves with T-poly in different species of host plants**

	Host species	Two basal leaves treated with	Lesions/ leaf ± S.E. mean (whole plant)	Inhibition of virus (% reduction)†
1.	*N. glutinosa*	H_2O (C)	113±2·0	90·0
		Poly (T)	11± 2·4	
2.	*N. tabacum***	H_2O	241± 4·5	80·0
	cv. NP. 31	Poly	47± 5·3	
3.	*N. rustica*	H_2O	291±16·3	55·0
		Poly	131±13·7	
4.	*C. amaranti-*	H_2O	556±33·5	2·0
	color	Poly	545±92·0	

* Total number of plants for each host was 4.

** Samples of leaf discs punched off on the 4th day after virus challenge (*i.e.*, 6th day after T-poly) and assayed in test plants (*N. glutinosa*). Age of the first three host plants was between 35–40 days, and for *C. amaranticolor*, 30–35 days.

$$\dagger = \frac{C - T}{C} \times 100.$$

counts obtained in the test and controls. Where needed this difference in lesion counts was converted into per cent of the control.

In the case of *N. tabacum* CV. NP-31, following T-poly treatment and virus challenge as with hypersensitive hosts, leaf disc samples (five per leaf) were punched off from all leaves (6 to 8 leaves per plant) of the treated and untreated (control) plants at specified time intervals. Samples from treated and untreated plants were pooled separately, weighed and triturated in distilled water at the rate of 1·0 g/10 ml. The two solutions so obtained were centrifuged (3,000 r.p.m. for 30 min), diluted 1 : 250 and assayed on leaves of 45 day old *N. glutinosa* for virus activity. The difference in the mean lesion counts obtained between the control and treated solutions formed the basis for expressing virus inhibitory action of T-poly.

RESULTS

(1) *Effect of a Single Preinoculation Treatment with T-poly on Different Host Plants*

Four different species of host plants (*N. glutinosa*, *N. tabacum*, CV. NP-31, *N. rustica* and *C. amaranticolor*) were treated. For *N. tabacum*, samples of leaf discs were punched off after virus challenge (see Methods) and the reduction in virus concentration in treated leaf was assessed by tests on *N. glutinosa*. For the hypersensitive hosts lesion counts were taken directly on them four days after virus challenge (see Methods).

Results (Table I) show that the maximum virus inhibition was achieved with *N. glutinosa*, followed by *N. tabacum* NP-31, *N. rustica* and *C. amaranticolor*, in that order. Thus the virus inhibitory activity of T-poly varied with the host species, the three hypersensitive hosts—*N. glutinosa*, *N. rustica*, *C. amaranticolor*—also differ to some extent amongst themselves.

(2) *Effect of a Single Preinoculation Treatment with T-poly on the Virus Multiplication Rate of TMV in NP–31 Plant*

The experiment (Table II) was done to find out the duration of the virus inhibitory activity of T-poly in *N. tabacum* CV. NP–31 plant. For this, virus multiplication rate in treated and control plants were compared. Samples of leaf discs were collected both from *upper untreated* (remote site) and basal treated leaves of the test and control plants on the 4th, 8th and 12th day following virus challenge (see Methods). Leaf disc samples (of control and test plants) were homogenized and assayed for virus activity on *N. glutinosa* plants, and per cent virus (local lesion) reduction was computed from the difference in mean lesion counts.

TABLE II

Virus inhibition in plants caused by T-poly (2·50 mg/ml) in contact with two basal leaves of N. tabacum .NP.CV-31 *for 2 days prior to challenge with TMV determined by local lesion assay at intervals*

Assay interval days	Lesions/leaf (±S.E. mean)				Per cent reduction*
	H_2O (Control)		T-poly (Test)		
	Basal (Treated site)	Upper (Remote site)	Basal (Treated site)	Upper (Remote site)	
4	72 ± 16·0	119 ± 18·8	37 ± 5·5	21 ± 1·6	70·0
8	349 ± 35·3	275 ± 49·1	309 ± 14·5	223 ± 55·6	14·1
12	495 ± 17·9	484 ± 36·0	556 ± 08·8	510 ± 25·5	8·9

$$* = \frac{\text{Col (3)} - \text{Col (6)}}{\text{Col (3)}} \times 100.$$

The results (Table II) indicate that if T-poly remained in contact with host plants for two days, virus multiplication was reduced in test plants to the extent of 70%. However, the virus inhibitory activity of T-poly declined with time. On the 12th day of virus challenge, assay indicated that the concentration of virus in treated and control plants were about the same.

Discussion

Our investigations show that pre-inoculation treatment of nonhypersensitive *N. tabacum* CV. NP–31 plants by rubbing with a single dose (2·5 mg/ml) of T-poly retarded TMV multiplication in such host plants, as judged by local lesion assay, down to 70% of the control figure (Table II) on the 4th day of virus inoculation. Acquired antiviral resistance induced locally at treated site (basal leaf) spread to the remotest part (upper leaves) as well. Both effects weared off with time ; the concentration of virus (local lesion forming units), as high as in the untreated control, was recovered from treated plants on the 12th day (Table II). Results obtained with the nonhypersensitive host plants here (NP-31) are in agreement with those of VanLoon[11] who reported that *N. tabacum* (Samsun) plants, like its hypersensitive counterpart, have the capacity to elaborate new soluble leaf proteins upon infection with TMV, the appearance of which is associated with induced acquired systemic resistance.

Results of tests showed that resistance to virus in treated *N. tabacum* CV. NP-31 plants develop systemically at remote site (upper untreated leaves) in parallel with virus inhibited locally at the site of treatment (basal leaves). Both local (treated site) as well as systemic resistance (untreated site) induced by a single dose pretreatment were transient in nature.

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ON THE OCCURRENCE OF ARGINASE IN THE MUSCLE AND ITS ROLE IN VARIOUS TISSUES OF FROG, *RANA HEXADACTYLA*, DURING DENERVATION ATROPHY AND CHRONIC AMMONIA TOXICITY

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Abstract

Arginse activity was increased in the gastrocnemius muscle, brain, liver and kidney tissues following unilateral sciatectomy and induced ammonia intoxication. The increase in enzyme activity was more pronounced in brain and muscle tissues of both the normal and denervated frogs as compared to liver and kidney during induced ammonia stress. The similarity in the pattern of arginase response to surgical denervation and imposed ammonia toxicity has been discussed.

Introduction

It is well documented that sciatectomy results in the elevation of ammonia levels in various tissues of frog[1–3]. Animal tissues are extremely sensitive even to low concentration of ammonia in their environment, since it is highly toxic[4]. Hence, an efficient means of nitrogen disposal will be required in order to keep the ammonia content at a low level. The major mechanisms involved in the detoxification of ammonia constitute the synthesis of glutamine and urea[5]. Very scant attention has been paid to the ammonia detoxification mechanisms in denervation atrophy. Earlier report have shown that the administration of ammonium salts into animals result in the elevation of tissue ammonia levels which warrants rapid detoxification[2,6]. Since

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