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SHORT COMMUNICATION

THE EFFECT OF TOBACCO MOSAIC VIRUS AND POTATO VIRUS X ON PEROXIDASE ACTIVITY AND PEROXIDASE ISOZYMES IN *NICOTIANA GLUTINOSA*

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Abstract—Peroxidase activity and peroxidase isozymes were determined at intervals over a period of 14 days and 28 days respectively in *Nicotiana glutinosa* leaf tissue infected by tobacco mosaic virus (TMV) or potato virus X (PVX). Peroxidase activity was higher in extracts from leaves showing necrosis, than in healthy leaves or from leaves showing chlorosis. Increase in peroxidase activity was accompanied by alterations in isozyme pattern. Five isozymes were found in extracts from healthy leaves and an additional one in virus-infected leaves. This isozyme and one of the others which was apparently associated with senescence, appeared earlier in extracts from necrotic than from extracts from chlorotic leaves.

INTRODUCTION

INCREASES IN the peroxidase activity in leaves have been recorded in plants reacting with local necrotic[1] or local and systemic chlorotic[2,3] symptoms to virus infection. A new peroxidase isozyme has been recorded in leaves of *Nicotiana glutinosa* L. infected with tobacco mosaic virus (TMV). On the other hand, infection of French bean or Cowpea by TMV or tobacco necrosis virus (TNV) merely induced the earlier appearance of certain isozymes, possibly due to accelerated ageing of the infected leaves.[5]

The present investigation was undertaken to determine the effect of TMV and potato virus X (PVX), on the peroxidase activity and isozyme patterns of *N. glutinosa* during the period of symptom development. TMV produces local necrotic lesions while PVX produces local and systemic chlorosis on the leaves of this host.

RESULTS

Peroxidase Activity

TMV-inoculated leaves of *Nicotiana glutinosa* showed distinct local lesions 3–4 days after inoculation. Peroxidase activity began to rise at this time and reached maximum levels within 7–11 days of inoculation and declined rapidly thereafter (Fig. 1). The highest relative activity was found in the leaves with most lesions 7 days after inoculation.

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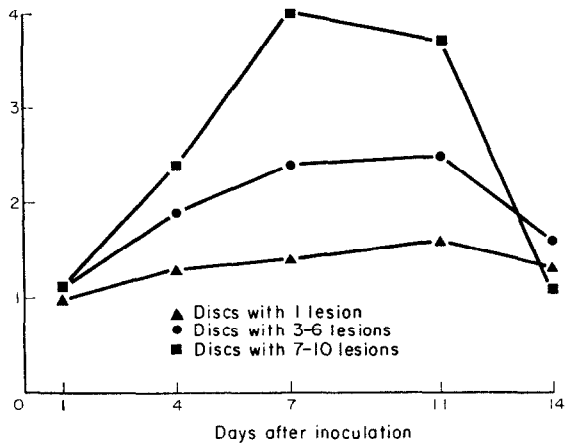

FIG. 1.

With PVX infection, peroxidase activity did not increase significantly until the symptoms appeared after 7 days in inoculated, and 14 days in the non-inoculated, systemically infected leaves. Thereafter activity increased, reaching a maximum at 21 days for systemically infected non-inoculated leaves (Table 1).

TABLE 1. PEROXIDASE ACTIVITY IN LEAF DISCS OF *Nicotiana glutinosa* INFECTED WITH POTATO VIRUS X

Time of sampling after inoculation (days)	Inoculated (lower) leaves			Non-inoculated (upper) leaves		
	Peroxidase activity		Relative activity	Peroxidase activity		Relative activity
	Healthy	Infected		Healthy	Infected	
1	0·32*	0·35	1·09†	0·39	0·40	1·02
4	0·43	0·50	1·16	0·58	0·60	1·04
7	0·48	0·78	1·63	0·83	0·83	1·00
11	0·48	0·78	1·63	0·63	0·73	1·16
14	0·43	1·10	2·58	0·67	0·95	1·39
21	—	—	—	0·53	1·10	2·08
28	—	—	—	0 47	0·83	1·76

* Results expressed as fresh weight (g fr. wt. sec)$^{-1}$ using 0·5 ml enzyme extract mixed with 5 ml 0·05 M pyrogallol in phosphate buffer (pH 6·0) and 0·5 ml 1% H_2O_2.

† Relative activity = peroxidase activity, infected leaves/peroxidase activity, healthy leaves.

Isozyme Patterns

Five isozyme bands were obtained from extracts from healthy leaves and one of these (band 1) appeared only in the 14-day sample (Fig. 2). Six isozyme bands appeared in extracts from *N. glutinosa* infected with either TMV or PVX. The second band (2) of relatively low electrophoretic mobility, found in virus-infected leaves, was not present in extracts from comparable healthy control leaves. TMV-infection induced earlier synthesis of bands 1 and 2 than did infection with PVX.

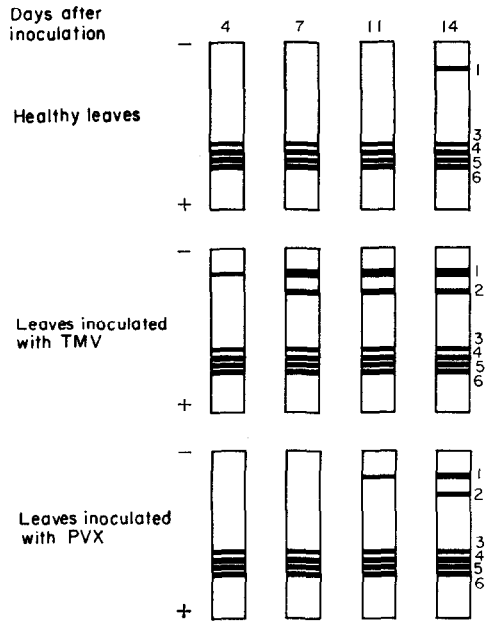

FIG. 2.

DISCUSSION

It is well established that peroxidase activity does not increase in virus-infected plants until symptoms appear.[1,3] It is possible that oxidized phenolic compounds accumulate as a result of increased peroxidase activity and this may account, in part, for the production of symptoms. Large accumulations of polyphenol oxidation products may well produce necrotic rather than chlorotic symptoms. While we have not demonstrated such an accumulation, our data show that there is a greater stimulation of peroxidase activity associated with necrosis than with chlorosis and there is a direct correlation between the amount of necrosis and peroxidase activity in *Nicotiana glutinosa*. We found that infection with either TMV or PVX produced up to 10% decrease in dry wt. in *N. glutinosa* leaves. If the results had been expressed on a dry wt. basis the differences in peroxidase activity between extracts from healthy and infected leaves would have been correspondingly greater. Our results are in agreement with those of previous reports which have shown a relationship between severity of mosaic symptoms and increased peroxidase activity in TMV-infected tobacco (*N. tabacum*)[3] and the amount of necrosis and increased peroxidase activity in TNV-infected French bean (*Phaseolus vulgaris*).[1]

The same four peroxidase isozymes, represented by bands 3–6, were obtained in all samples from healthy and virus-infected leaves. We can offer no explanation for the fact that we have found one more isozyme in uninfected leaves than has been previously recorded.[4] Increase in peroxidase activity in infected tissues is accompanied by the early appearance of a fifth band (1) of low electrophoretic mobility and by a further band (2) which is unique to extracts from infected tissues. Our results, therefore, are in agreement with those of Solymosy *et al.*[4] in that there is one isozyme which appears only in extracts from virus-infected *N. glutinosa* leaves. It seems probable that the earlier appearance of band 1 in virus-infected

tissues is associated with premature senescence. Our experiments show that the production of two isozymes is stimulated earlier by necrosis than by chlorosis of tissues.

EXPERIMENTAL

Nicotiana glutinosa L. plants were grown in John Innes Compost No. 2 in 5 in. plastic pots in a greenhouse with mean temp. 25°. They were maintained in a growth room for the duration of the experiment, at 23 ± 1°, with illumination for 16 hr in every 24 hr provided by Osram "de luxe warm white" 80 W fluorescent lamps. When they reached the nine-leaf stage the three middle leaves were inoculated with TMV (legume strain) by rubbing a purified suspension on Kieselguhr-dusted leaves with a small poster brush. The three middle leaves of *N. glutinosa* plants at the nine-leaf stage were inoculated in a similar way with PVX. The leaves of the control plants were rubbed with distilled water instead of inoculum.

For measuring peroxidase activity, discs 1 cm dia. were punched from inoculated leaves 1, 4, 7, 11 and 14 days after inoculation, and in the case of PVX-infected plants and their controls, the three upper leaves were sampled at the same times and at 21 and 28 days. In the TMV experiment samples were selected with discs showing either 1, 3–6 or 7–10 lesions/disc corresponding approximately to 1·3, 4·9 and 10·1 lesions/cm². Extracts were made by grinding 0·75 g of discs in 5 ml ice-cold 0·066 M phosphate buffer (pH 6·0) for 3 min in a chilled pestle and mortar. Sap was expressed through cheese-cloth and centrifuged at 4° for 5 min at 5000 *g* and the supernatant stored at 4° before assay. Peroxidase activity was determined by the method previously described[1] and the values for each sample expressed as $(\text{sec/g fr. wt.})^{-1}$; relative activity is defined as activity, infected leaves/activity, healthy leaves. Three replicate samples were used for the assays. Peroxidase isozymes were separated from soluble protein extracts made by grinding 1 g samples of leaf discs in a chilled pestle and mortar in 1 ml Tris buffer (pH 8·0) containing 17% sucrose, 0·1% ascorbic acid and 0·1% cysteine hydrochloride. The liquid was expressed through cheese-cloth, centrifuged at 10,000 *g* for 15 min at 4°, and the supernatant fluid centrifuged at 100,000 *g* at 4°. The final supernatant was subjected to polyacrylamide gel electrophoresis as described previously.[5]