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BRIEF COMMUNICATION

Virus-induced Virus Inhibitors from Systemically Virus-infected Plants

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Abstract. After infection of *Nicotiana tabacum* cv. Samsun with tobacco mosaic virus (TMV) crude extracts from dark-green spots of upper leaves had a more strongly marked inhibitory effect upon TMV added *in vitro* than crude extracts from the surrounding light-green tissue. Likewise, crude extracts from leaves of *Nicotiana tabacum* cv. Samsun showing recovery after infection with tobacco ringspot virus (TRV) were seen to have a marked inhibitory effect on TMV added *in vitro*. The results obtained suggest that virus inhibitors are produced after virus infections not only in hypersensitive hosts but also in systemic hosts. Necrotizing processes are not an indispensable prerequisite of the production of virus-induced virus inhibitors.

During the last decade knowledge has been obtained as to systemically transportable antiviral principles effective *in vitro* and *in vivo*, which are induced after virus infection of hypersensitive hosts, *e.g.* after infection of *Nicotiana glutinosa* L. with tobacco mosaic virus (TMV; for literature see SCHUSTER and WETZLER 1982). But there is little knowledge as to virus-induced virus inhibitors from systemically virus-infected plants. Thus we tried to prove the presence of antiviral factors in the dark-green tissues of upper leaves of TMV-infected plants of *Nicotiana tabacum* L. cv. Samsun which contain less infectious virus than the surrounding yellow-green areas (ATKINSON and MATTHEWS 1967). Moreover, dark-green tissues do not appear to be able to support the virus replication (MURAKISHI and CARLSON 1976).

Besides TMV, which does not form any necrosis on the leaves of the host plant, we included into our trials tobacco ringspot virus (TRV). This virus forms necrotic spots on leaves of *Nicotiana tabacum* L. cv. Samsun but is not restricted to the region of the necrotic lesions as is the case with TMV on leaves of *Nicotiana glutinosa* L. and other hypersensitive hosts. On younger

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TABLE 1

Virus contents and inhibitory activities of crude extracts from yellow-green and dark-green tissues of TMV-infected leaves of *Nicotiana tabacum* L. cv. Samsun (average lesions per leaf of the test plant *Nicotiana glutinosa* L.)

	Crude extracts from			Variation in lesion numbers (yellow-green tissue = 100%)
	healthy plants	TMV-infected plants yellow-green tissue	TMV-infected plants dark-green tissue	
a In crude extracts	0	155.4 ± 12.8	93.9 ± 9.7	60.4^{++}
b TMV added	78.1 ± 8.5	231.2 ± 14.1	144.3 ± 8.7	62.5^{++}
c Difference between a and b, abs.	78.1 ± 8.5	75.8 ± 11.5	50.4 ± 11.4	
d Difference between a and b in % (78.1 = 100%)	100	97	64.5	
e Inhibition in %	0	3	35.5^{+}	

leaves of *Nicotiana tabacum* cv. Samsun the TRV necroses are suppressed 2 to 3 weeks after virus infection. We tried to prove whether and to which extent this recovery from infections with TRV (see KÖHLER 1964, pp. 73 ff) is connected with, or probably caused by, the formation of antiviral principles.

A green strain of TMV maintained for more than 10 years in the laboratory of the second author and a strain of TRV obtained from the Institute of Phytopathology, Aschersleben, were included in our experiments. *Nicotiana tabacum* cv. Samsun was used as a host plant of these viruses. The virus contents were assayed on the hypersensitive hosts *Nicotiana glutinosa* and *Vigna sinensis* L., respectively, using leaf-half comparisons.

We separated dark-green tissues of secondarily TMV-infected leaves from yellow-green ones and prepared crude extracts by means of a slightly modified method described by KIMMINS (1969). Then, virus contents were assayed. At the same time a fixed solution of purified TMV was added to aliquots of the crude extracts both from the dark- and yellow-green spots and from healthy plants. Three hours later all samples were bioassayed for inhibitory activity as mentioned above.

The TMV necessary for these experiments was purified using a polyethylene glycol method described by BYHAN *et al.* (1978). The significance of the changes observed, which are mostly expressed in the form of percentage values, was tested by a single tail *t*-test (see, *e.g.* MUDRA 1958).

The test results are characterized using the following symbols:

$$^{+}: P \leqq 5\%; ^{++}: P \leqq 1\%; ^{+++}: P \leqq 0.1\%, ^{*}: P > 5\%.$$

The results confirmed that dark-green tissues contain lower virus concentrations than yellow-green ones (Table 1, line a). TMV added to crude extracts from yellow-green tissues increased the number of local lesions to nearly the same extent as in crude extracts from tissues of healthy leaves. But the increase of local lesion numbers was less, when equal quantities of TMV were added to crude extracts from dark-green tissues (Table 1, lines b to e). Thus there is evidence that dark-green but not yellow-green tissues

TABLE 2

Inhibitory activities of crude extracts from recovery leaves of TRV-infected plants of *Nicotiana tabacum* L. cv. Samsun and healthy leaves, respectively, when mixed with TMV and inoculated onto *Nicotiana glutinosa* L.

Experiment	Average lesions per leaf of the test plant when TMV was added to crude extracts from		Inhibition in % (healthy = 100%)
	healthy leaves	recovery leaves	
I	40.8 ± 3.5	28.7 ± 2.4	29.7++
II	27.9 ± 2.9	19.8 ± 1.8	29.0+

contain an additional virus inhibitor active against TMV *in vitro*. A complete repetition of the whole experiment confirmed the results demonstrated in Table 1.

In order to prove whether and to what extent recovery is connected with, or probably caused by, the formation of antiviral factors, we inoculated lower leaves of *Nicotiana tabacum* cv. Samsun with TRV. When recovery was observed in younger (upper) leaves of infected plants we inoculated every third leaf of plants of the same age uninfected up to this time. When obvious symptoms appeared in all leaves of these plants, crude extracts from leaves of the same age of plants showing (a) obvious TRV symptoms or (b) recovery were bioassayed for TRV-contents on *Vigna sinensis*. Crude extracts from leaves with TRV symptoms (a) caused in average of 25.0 ± 1.8 lesions per leaf of the test plant, whereas crude extracts from recovery leaves (b) caused 8.9 ± 0.7 lesions per leaf. This decrease of 64.4% (a = 100%) is of high significance ($P \leqq 0.1\%$). These results confirm that in leaves with recovery TRV-contents are reduced considerably in comparison with leaves of the same age and position showing obvious TRV symptoms.

In the following experiments equal quantities of a purified solution of TMV were added to aliquots of the crude extracts from leaves showing recovery and to crude extracts from healthy leaves, respectively. Three hours later all these samples were bioassayed for TMV on *Nicotiana glutinosa*. As shown in Table 2, the activity of TMV added to crude extracts from recovery leaves is reduced by about 30% compared with the activity of TMV added to crude extracts from healthy leaves. Thus there is further evidence for a virus inhibitor in leaves showing TRV recovery.

The results obtained suggest that virus inhibitors are induced after virus infection not only in local lesion hosts but also in systemically infectible hosts. As the inhibitory principles demonstrated in the dark-green spots of TMV-infected plants have been produced independently of necroses, it is evident that necrosis-induced alterations of the metabolism need not necessarily play a key role in the production of antiphytoviral principles, as may be obvious from the results of studies of thermally stable virus-induced virus inhibitors in hypersensitive virus-host combinations (SCHUSTER and WETZLER 1982).

MICZYŃSKI and MACNEILL (1976) isolated, besides stimulators of virus infection, an antiviral principle from tomato plants systemically infected with a tomato strain of TMV. This principle was also induced independently

of necrotization processes. Finally AMEMIYA and MISAWA (1977) report that the resistance of cucumbers to CMV is due to a virus-induced antiviral principle whose production may be stopped by actinomycin D and α-amanitine, suggesting that virus-induced antiviral principles may promote the development of virus resistance. Therefore virus-induced virus inhibitors may be of importance as models for the development of antiphytoviral substances including resistance inducers. On that account further studies are necessary in the near future. They should include inhibitors arising in connection with the development of recovery the knowledge of which is extremely small.

REFERENCES

AMEMIYA, Y., MISAWA, T.: Studies on the resistance of cucumber to cucumber mosaic virus. II. Induction of resistance by infection. — Tohoku J. Agr. Res. **28** : 18—25, 1977.

ATKINSON, P. H., MATTHEWS, R.E.F.: Distribution of tobacco mosaic virus in systemically infected tobacco leaves. — Virology **32** : 171—173, 1967.

BYHAN, O., KLUGE, S., PUSTOWOIT, B., PUSTOWOIT, W., SCHUSTER, G.: Einfluss von 1-β-D-Ribofuranosyl-1,2,4-triazol-3-carboxamid (Virazol) auf die Bildung und Konzentration von Tabakmosaikvirus- und Wirts-RNA sowie auf Wirtsproteine. — Biochem. Physiol. Pflanzen **173** : 521—535, 1978.

KIMMINS, C. W.: Isolation of a virus inhibitor from plants with localized infections. — Can. J. Bot. **47** : 1879—1886, 1969.

KÖHLER, E.: Allgemeine Viruspathologie der Pflanzen. — Paul Parey Publ., Berlin—Hamburg 1964.

MICZYŃSKI, K. A., MACNEILL, B. H.: Further studies on the antiviral principle from tomatoes infected systemically with tobacco mosaic virus. — Phytopathol. Z. **85** : 251—279, 1976.

MUDRA, A.: Statistische Methoden für landwirtschaftliche Versuche. — Paul Parey Publ., Berlin—Hamburg 1958.

MURAKISHI, H. H., CARLSON, P. S.: Regeneration of virus-free plants from dark-green islands of tobacco mosaic virus-infected tobacco leaves. — Phytopathology **66** : 931—932, 1976.

SCHUSTER, G., WETZLER, C.: On virus-induced virus inhibitors from locally TMV-infected plants of *Nicotiana glutinosa* L. — Phytopathol. Z., **104** : 46—59, 1982.