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Virus-specific expression of systemic acquired resistance in tobacco mosaic virus- and tobacco necrosis virus-infected 'Samsun NN' and 'Samsun' tobacco

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Abstract

Systemic acquired resistance induced in intact 'Samsun NN' tobacco plants by either tobacco mosaic virus (TMV) or tobacco necrosis virus (TNV) was more effective against challenge inoculation with the same than with the other of the two viruses. However, in trimmed 'Samsun NN' plants resistance induced by either of these viruses was stronger against TMV than against TNV. In intact 'Samsun' tobacco plants TNV induced a systemic resistance against itself identical to the one expressed in 'Samsun NN'. Moreover, it induced systemic resistance against TMV as based on a decrease in TMV content in challenge-inoculated leaves. These observations indicate that systemic acquired resistance is not limited to combinations in which both inducing and challenging virus give rise to a hypersensitive reaction, and further point to virus-specific factors regulating the extent of resistance expressed.

Introduction

In several virus-host plant combinations in which the inoculated leaves react with the formation of necrotic local lesions, a resistance is induced in non-inoculated, symptomless, non-virus containing plant parts. After challenge inoculation, this systemic acquired resistance is expressed by the development of smaller and occasionally fewer lesions than result from a similar inoculation of comparable leaves on plants not previously infected (Ross, 1966). Little or no indication of virus specificity has been obtained: induced resistance is evident after challenge inoculation with either the same virus or any other virus that induces similar symptoms. So, almost any virus that induces local lesions on *Nicotiana tabacum* 'Samsun NN' gives rise to a systemic resistance nearly indistinguishable from the one induced by TMV. Quantitatively, the resistance induced by either TMV or TNV was reported to be less effective against TNV than against TMV (Ross, 1961). This suggests that the extent of resistance expressed is a function of the challenging rather than the inducing virus. Different extents of resistance due to different inducing viruses were, however, noticeable in previous studies (Van Loon, 1972, 1975). Therefore, a quantitative reinvestigation of the effects of TMV and TNV as both inducing and challenging virus seemed desirable, in order to assess how far virus-specific factors regulate the extent of resistance expressed. On the one hand, 'Samsun NN' tobaco was used as the plant host, while on the other hand the study was extended to the interaction of both

viruses in 'Samsun' tobacco, which reacts similarly to TNV, but develops systemic mosaic symptoms after infection with TMV.

Materials and methods

The viruses TMV and TNV were the same strains as used previously and purified accordingly (Van Loon, 1972, 1975). For inoculation, purified preparations were diluted with distilled water. Other virus inocula were prepared by grinding leaves of infected 'Samsun' or 'White Burley' tobacco plants with a pestle in a mortar and squeezing the sap through cheesecloth. Inoculations were made by rubbing leaves previously dusted with carborundum with a gauze pad.

Tobacco plants 'Samsun NN' and 'Samsun' were obtained by propagation through selfing at the Laboratory of Virology, Wageningen. They were grown for 9–11 weeks, either in a greenhouse at 22–25°C (for half-leaf tests) or in a growth chamber at 18–20°C (for experiments with intact plants) (Van Loon, 1976). Half-leaf tests were performed as described by Ross (1961). Plants were decapitated and all leaves, except two successive leaves about 8 cm wide, were removed. For the induction of systemic resistance, the left leaf-halves were inoculated with either a 10^4 dilution of sap from leaves of 'Samsun' plants systemically infected with TMV, or a 10-fold dilution of sap from TNV-infected leaves from 'White Burley' plants. Left leaf-halves of control plants were inoculated with similarly diluted sap from non-infected tobacco plants. After 7 days, the right leaf-halves of all plants were challenge inoculated with either TMV- or TNV-containing sap.

For experiments with intact plants, all green leaves were inoculated with a purified virus suspension of either TMV or TNV at 100 μg per ml. Control plants were inoculated with distilled water. Fourteen days later, the two successive leaves that had first developed after inoculation, and comparable leaves from control plants, were challenge inoculated with TMV or TNV at 10 and 100 μg per ml, respectively.

Seven days after challenge inoculation the diameters of 10–30 local lesions per right leaf-half or upper leaf were measured and lesions were occasionally counted. Measurements were made under a dissecting microscope equipped with an ocular micrometer at $\times$ 10. For each lesion, two diameters, in directions perpendicular to each other were determined and then averaged. The differences between test and control plants were statistically analyzed using either the Wilcoxon Signed Rank test (Table 1), or Student's *t*-test (Table 2). Differences between treatments were evaluated by analysis of variance.

To study interference in mosaic-diseased plants, 'Samsun' plants were inoculated with TMV 7–8 weeks after sowing. The effect of local infection was studied in plants inoculated with TNV at the 10–11-week stage. The content of TMV in 'Samsun' plants was determined by assaying crude extracts on leaf-halves of *N. glutinosa*. In one experiment. these extracts were further purified and the amount of virus determined spectrophotometrically (Van Loon, 1972).

Results

Systemic resistance in leaf-halves of trimmed 'Samsun NN' tobacco plants induced with either TMV or TNV was expressed consistently through a smaller lesion size

Table 1. Extent of systemic resistance[1] attained in leaf-halves of trimmed 'Samsun NN' tobacco plants inoculated with TMV or TNV.

First inoculation[2]	Second inoculation[3]	Median number of lesions as percent of control[4, 5]	Number of experiments	Median lesion diameter (mm) as percent of control[4, 5]	Number of experiments
TMV	TMV	54**	14	50** (A)	18
TMV	TNV	79*	14	86 (B)	18
TNV	TMV	110	11	78** (A, B)	19
TNV	TNV	78	10	85* (B)	14
				$s^2 = 1004.5$; df	30

[1] Systemic resistance expressed as reduction in lesion number and/or diameter with regard to control.

[2] Left leaf-halves inoculated with either TMV- or TNV-containing leaf sap, diluted 10^4 and 10 times, respectively.

[3] Right leaf-halves similarly inoculated 7 days after the first inoculation.

[4] Number and diameter of lesions on right-halves of leaves inoculated with virus on the left-halves previously, as percentage of those on right-halves of control leaves inoculated earlier on the left-halves with diluted sap from non-infected plants.

[5] One and two asterisks indicate values significantly different from controls (100) at the 5 and 1 % level, respectively. Different letterings (A), (B) indicate statistically significant differences between treatments.

Tabel 1. Mate van verworven systemische resistentie in bladhelften van 'Samsun NN'-tabak waarvan de toppen en bladeren op twee na verwijderd waren, na inoculatie met TMV of TNV.

only (Table 1). A decrease in lesion number was evident only when resistance had been induced with TMV and, in this connection, no differences were noted when either TMV or TNV were used as the challenging virus.

The effects on lesion size corroborate the observations reported by Ross (1961): irrespective of whether TMV or TNV was used to induce resistance, the reduction in lesion size appeared larger when TMV, than when TNV was used as the challenging virus. When TMV was used for challenge inoculation, the resistance induced by TMV itself tended to be more effective than the resistance induced by TNV. These effects were verified by factorial analysis. Significantly stronger resistance was expressed 1) when TMV rather than TNV was used as the challenging virus, 2) when the second inoculation was performed with the same virus as used for inducing resistance instead of the other one, but 3) also when TMV, as opposed to TNV, was the inducing virus.

When in intact plants resistance was induced by inoculation of all green leaves with TMV or TNV, upon challenge inoculation of two newly emerged leaves 14 days later, lesions remained consistently smaller in the homologous than in the heterologous combination. However, in intact 'Samsun NN' plants both TMV and TNV were equally effective in inducing resistance against themselves (Table 2). Moreover, both viruses were equally less effective in inducing resistance against the other one. Hence, under these conditions the extent of resistance depended mainly on whether the same or another virus was used for the challenge inoculation as for inducing the resistance.

In 'Samsun' plants TNV induced a systemic resistance to itself that was both qualitatively and quantitatively identical to the one in 'Samsun NN' (Table 2).

Table 2. Extent of systemic resistance[1] or interference in leaves of intact 'Samsun NN' and 'Samsun' tobacco plants inoculated with TMV or TNV.

Tobacco cultivar	First inoculation[2]	Second inoculation[3]	Average lesion diameter (mm) after second inoculation on		Percent of control[5]
			control leaves[4]	test leaves	
Samsun NN	TMV	TMV	0.57 ± 0.16	0.24 ± 0.09	43** (A)
Samsun NN	TMV	TNV	0.35 ± 0.15	0.25 ± 0.09	70** (B)
Samsun NN	TNV	TMV	0.57 ± 0.16	0.38 ± 0.16	67** (B)
Samsun NN	TNV	TNV	0.35 ± 0.15	0.18 ± 0.10	51** (A)
Samsun	TMV	TNV	0.51 ± 0.18	0.78 ± 0.33	155** (C)
Samsun	TNV	TNV	0.51 ± 0.18	0.25 ± 0.07	49** (A)

[1] Systemic resistance expressed as reduction in lesion diameter with regard to control.
[2] All green leaves inoculated with either TMV or TNV at 100 μg per ml.
[3] Two young, successive leaves of 'Samsun NN' plants inoculated 14 days after the first inoculation; similar leaves of 'Samsun' plants 28 days after the first inoculation.
[4] First inoculation with water only.
[5] Double asterisks indicate values significantly different from controls at the 1% level. Different letterings (A, B, C) indicate statistically significant differences between treatments.

Tabel 2. Mate van systemische resistentie of interferentie in bladeren van intacte 'Samsun NN'- en 'Samsun'-tabak na inoculatie met TMV of TNV.

Systemic infection of 'Samsun' plants with TMV also interfered with lesion enlargement upon challenge inoculation with TNV, but in the opposite way: substantially larger lesions were formed than on control plants previously inoculated with water.

It was further investigated whether the systemic resistance induced by TNV in 'Samsun' plants would also affect infection with TMV, that does not induce local

Table 3. Influence of previous infection with TNV on synthesis of TMV in 'Samsun' tobacco plants[1].

Experiment number	Infectivity[2]: Number of lesions per leaf-half on		Percent of control	Amount of TMV (μg/g leaf)[4]		Percent of control
	control leaves[3]	test leaves		control leaves[3]	test leaves	
I	182	42	23	–[5]	–	–
II	143	91	64	24	17	71

[1] Three 'Samsun' plants were inoculated with either water or TNV (100 μg/ml) 17 days previous to extraction. Fourteen days later two young, subsequently developed leaves were inoculated with TMV (100 μg/ml).
[2] Three days after inoculation with TMV, centrifuged extracts from the inoculated leaves were assayed on 12 leaf-halves of *N. glutinosa*.
[3] Leaves from plants originally inoculated with water.
[4] The amount of virus was determined spectrophotometrically after purification.
[5] Not investigated.

Tabel 3. De invloed van een eerdere infectie met TNV op de synthese van TMV in 'Samsun'-tabak.

lesions in this cultivar. Interference was estimated from the extent of multiplication of TMV in young leaves of plants that had been inoculated with TNV 14 days earlier. Three days after inoculation of these leaves with TMV, the amount of virus synthesized in the inoculated leaves was determined. A substantially lower infectivity was recovered in extracts from plants originally inoculated with TNV than in those inoculated previously with water (Table 3). Although the extent of this reduction differed considerably between the two experiments, both point to a decrease in TMV synthesis. In one experiment, the amounts of virus present were also determined quantitatively by spectrophotometry of purified suspensions. As shown in Table 3, the results obtained were found in close agreement with the local lesion assay of crude extracts. Thus, previous infection of 'Samsun' tobacco plants with TNV reduced multiplication of TMV upon challenge inoculation.

Discussion

The virus-specificity in the extent of systemic acquired resistance expressed in 'Samsun NN' tobacco differed between leaf-halves on trimmed plants and whole leaves of intact plants. The results obtained with leaf-halves on trimmed plants under the same conditions as employed by Ross (1961) confirm this author's conclusion that systemic resistance is less effective against TNV than against TMV. However, in newly developed leaves of intact plants, a markedly stronger effect was found in the homologous combinations (TMV-TMV; TNV-TNV) than in the heterologous ones (TMV-TNV; TNV-TMV). The mechanism of systemic acquired resistance operating in uninoculated halves of leaves of which the other half had been inoculated, and in newly developed leaves of plants of which all leaves previously present had been inoculated, is considered to be identical (Bozarth and Ross, 1964; Ross, 1966). The main difference between the results obtained under the two experimental conditions thus appears to reside in the less effective resistance against TNV in the leaf-halves of trimmed plants. This difference might be due to physiological and biochemical alterations in the leaf-halves induced by trimming (Van Loon, 1976; cf. Ross, 1961). Thus, virus-specific factors influence the extent of systemic resistance attained.

The resistance expressed in intact plants of both 'Samsun NN' and 'Samsun' upon inoculation with the combination TNV-TNV was identical, indicating that the same mechanism can operate in both cultivars. In 'Samsun' plants this type of resistance appeared to be effective also against TMV, although this virus does not induce local lesions and eventually invades the plant systemically. As the difference in the amount of virus determined in systemic resistant and non-resistant leaves was maintained through virus purification, the difference in infectivity is not due to different amounts of inhibitors in the original extracts. Thus, the actual effect appears to be on virus synthesis, as with systemic acquired resistance in hypersensitively reacting leaves (Ross, 1966). Hence, systemic acquired resistance evidently is not expressed exclusively when a virus giving rise to a hypersensitive reaction is used for challenge inoculation, but also, at least initially, when the second inoculation is carried out with a virus infecting the plant systemically.

Whether, in reverse, a similar acquired resistance can be induced by a systemic infection is difficult to assess, because competition between the inducing and challenging virus cannot be avoided. Clearly, no such resistance was induced in 'Samsun'

plants by TMV when TNV was used as the challenging virus, TNV lesion enlargement being stimulated by previous infection with TMV. Similarly, TMV lesion enlargement in 'Samsun NN' plants is stimulated in the presence of potato virus X (Ross, 1961, 1966; Ross and Israel, 1970). However, both potato virus Y and cucumber mosaic virus induce resistance towards TMV in 'Samsun NN' (Davis and Ross, 1968; Van Loon, 1975). These latter forms of resistance resemble systemic acquired resistance in hypersensitively reacting combinations in that the same specific proteins are present in the resistant plant parts (Van Loon and Van Kammen, 1970; Van Loon, 1975). Therefore, acquired resistance appears to be more general than has been assumed thus far.

Samenvatting

Virus-specifieke expressie van verworven systemische resistentie in 'Samsun NN'- en 'Samsun'-tabak geïnfecteerd met tabaksmozaïekvirus en tabaksnecrosevirus

Verworven systemische resistentie geïnduceerd in *Nicotiana tabacum* 'Samsun NN' door inoculatie met tabaksmozaïekvirus (TMV) of tabaksnecrosevirus (TNV) kwam bij herinoculatie met een van beide virussen steeds tot uiting in een verminderde lesiegrootte. In nieuw gevormde bladeren van intacte planten was deze vermindering sterker wanneer herinoculatie plaatsvond met hetzelfde virus als waarmee resistentie was geïnduceerd, dan wanneer herinoculatie met het andere virus werd verricht (Tabel 2). Daarentegen was in bladhelften van getrimde planten de resistentie, geïnduceerd door elk van beide virussen, sterker tegen TMV dan tegen TNV (Tabel 1). Virus-specifieke factoren moeten dus van invloed zijn op de mate waarin systemische resistentie tot uiting komt.

In intacte 'Samsun'-tabak induceerde TNV systemische resistentie tegen zichzelf identiek aan die in 'Samsun NN'-tabak (Tabel 2). Eerstgenoemde resistentie bleek tevens werkzaam tegen TMV, op grond van een verminderd TMV-gehalte in met dit virus geïnoculeerde jonge bladeren (Tabel 3). Daarentegen bleek systemische infectie van 'Samsun'-tabak met TMV de uitbreiding van vlekken ontstaan door inoculatie met TNV te bevorderen (Tabel 2). Eerder is gebleken dat echter in sommige andere virus-waardplant combinaties waarin systemische symptomen optreden, wel resistentie wordt geïnduceerd. Deze resistentie is evenals verworven resistentie gecorreleerd met overeenkomstige veranderingen in oplosbaar-eiwitpatronen. Verworven systemische resistentie lijkt daarom niet beperkt te zijn tot virus-waardplant combinaties waarin zowel het resistentie inducerende als het voor herinoculatie gebruikte virus aanleiding geven tot een overgevoeligheidsreactie.

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