# Characterization of *Solanum dulcamara* Yellow Fleck-Ob: A Tobamovirus that Overcomes the N Resistance Gene

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We wish to dedicate this paper to the late Laslo Beczner who originally started the study of SDYFV and SDYFV-Ob in Hungary and during his stay in Vancouver in 1986.

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## ABSTRACT

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A tobamo-like virus, Solanum dulcamara yellow fleck-Ob (SDYFV-Ob), originally isolated from green pepper (*Capsicum annuum* L.), possessing morphological and capsid protein properties characteristic of tobamoviruses, spread systemically in inoculated *Nicotiana tabacum* L. 'Xanthi nc' (NN) and *Nicotiana glutinosa* L., both of which contain the N gene for resistance to tobacco mosaic virus. The virus, previously considered a strain of tomato mosaic virus (ToMV), showed strong homology in dot blot hybridization with Solanum dulcamara yellow fleck tobamovirus (SDYFV), but not to several others, including ToMV. SDYFV and SDYFV-Ob induced similar symptoms in a variety of hosts; however, SDYFV did not spread systemically in hosts containing the N resistance gene. The level of accumulation of SDYFV-Ob in tobacco protoplasts was comparable to that of another tobamovirus (ToMV-$LS_1$). In *N. tabacum* 'Xanthi nc' (NN), SDYFV-Ob did not complement systemic movement of other tobamoviruses. Furthermore, in 40% of the plants, systemic spread of SDYFV-Ob was prevented by coinoculation with other tobamoviruses. These results are discussed in relation to possible resistance mechanisms.

Several resistance genes to tobamoviruses have been identified. In tomato, the Tm-1 gene inhibits replication of tomato mosaic virus (ToMV) both in plants and in protoplasts (24); mutations in the replicase gene of ToMV confer the ability to overcome the Tm-1 gene (21). In contrast, the Tm-2 resistance gene in tomato inhibits viral infection of whole plants but not of isolated protoplasts (23,24). Tm-2 gene resistance can be eliminated by mutations in the 30-kDa protein (22), which is involved in cell-to-cell movement of the virus (3,16). These observations suggest that Tm-1 acts at the level of viral replication and Tm-2 at the level of viral movement. The modes of action of the N (origin *N. glutinosa*) and N′ (origin *Nicotiana sylvestris* L.) resistance genes are much less understood. Mutations in the coat protein of tobacco mosaic virus (TMV) were shown to modify the hypersensitive response of plants containing the N′ gene (14,29,30). However, the exact mode of action of the N′ gene is not known. In tobacco species containing the N gene, host response is temperature sensitive. At temperatures below 25 C, a hypersensitive reaction occurs, limiting virus infection to the inoculated leaf. At elevated temperatures (above 28 C), hypersensitivity is suppressed and the virus spreads systemically (32). Considerable effort has been directed toward characterizing the response of N gene hosts to infection with tobamoviruses. Although various anti-viral proteins have been isolated (5,18,34), none have been conclusively associated with N gene activity. Furthermore, no viral gene has been shown to facilitate recognition by the N gene product.

To further study the mechanism of action of the N gene, tobamovirus SDYFV-Ob from pepper is of great interest because it systemically infects not only pepper (*Capsicum annuum* L.) containing the $L_1$ allele of the TMV resistance gene, but also *N. tabacum* 'Xanthi nc' (NN) at temperatures below 25 C (1) (temperatures at which the N gene product is normally activated by tobamovirus infection). This is the only reported case of a tobamovirus with the ability to overcome the N resistance gene. Although tobamovirus SDYFV-Ob was originally described as a pepper strain of ToMV (1), it was later suggested that SDYFV-Ob and other viruses isolated from pepper, such as Solanum dulcamara yellow fleck tobamovirus (SDYFV), are only distantly related serologically to ToMV and should be considered as a distinct group of tobamoviruses (13,31). This study presents the characterization of some biological properties of SDYFV-Ob and of its relationship to other tobamoviruses.

## MATERIALS AND METHODS

**Viruses.** The original isolate of SDYFV-Ob was a kind gift from A. Gerwitz, AFRC Institute of Horticultural Research, Wellesbourne, Warwick, U.K. After a series of single local-lesion transfers in *N. glutinosa*, the virus was purified as described (10). Size of the coat protein was determined by electrophoresis of the virus preparation (denatured by boiling in sodium dodecyl sulfate [SDS]) on 10% SDS-polyacrylamide gel electrophoresis (PAGE). The L, $LS_1$, and $T_{346}$ strains of ToMV (12,20,25), the VRS strain of TMV-$U_1$ (33), the Ca10 strain of pepper mild mottle virus (PMMV; 37), the cowpea strain of sunnhemp mosaic virus (SHMV-$C_p$; 17), cucumber green mottle mosaic virus (CGMMV), kyuri green mottle mosaic virus (KGMMV; 8,15), and the *Hesperis matronalis* L. (6), T-281, LTV2 (28), and the TH2 strains of ribgrass mosaic virus (RMV; 11), were obtained from the virus collection at the Agriculture Canada Research Station, Vancouver, British Columbia. TMV-$U_1$, the L-D/H strain of ToMV, SDYFV (13,31), the $U_2$ strain of tobacco mild green mosaic (33), RMV, and the Tcs strain of RMV were brought by L. Beczner to the Vancouver Research Station from the Plant Protection Institute, Hungarian Academy of Sciences, Budapest. Samsun latent virus (SLV) was obtained from the American Type Culture Collection.

**Hybridization analyses.** All tobamoviruses used in the hybridization studies were purified by isopycnic centrifugation through cesium chloride gradients. RNA was extracted from virions using phenol/chloroform/octanol (25:24:1) in the presence of 0.1 M Tris-HCl, pH 8.9, and 1% SDS. RNA obtained in this manner was largely intact and contained little or no low molecular-weight (degraded) material, as determined by denaturing agarose gel electrophoresis. Size of SDYFV-Ob RNA was also measured by denaturing agarose gel electrophoresis. RNA concentrations were determined spectrophotometrically. For hybridization analyses,

nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, NH) were soaked in deionized water for 5 min, then in 20× SSC (1× SSC is 0.15 M NaCl/0.015 M trisodium citrate) and allowed to air dry. Purified RNA (100 ng in 20 μl of sterile water) was applied to the dry membranes. Alternatively, 50 ng of RNA in 2.5 μl of sterile water was applied directly to nylon membranes (Zeta-probe, Bio-Rad Laboratories, Richmond, CA). Filters were air-dried and then baked for 2 h at 80 C in a vacuum oven. Prehybridization was for 5–30 min at 42 C in hybridization buffer. Hybridizations were carried out in 50% deionized formamide, 10% sodium dextran sulfate, 1 M NaCl, 20 mM Tris-HCl, pH 7.7, 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.1% sodium pyrophosphate, and 250 μg/ml of sheared denatured salmon sperm DNA. Random-primed $^{32}$P-labeled cDNA (specific activity = approximately $2 \times 10^6$ cpm/μg) prepared to purified virion RNA was added at a final concentration of 1–3 μg probe/ml hybridization solution and incubated with filter-bound RNA for 16–20 h at 42 C. Filters were washed for 20 min in 2× SSC/0.1% SDS at 60 C, then in successively lower concentrations to a final concentration of 0.1× SSC.

**Production of antisera.** Rabbits and chickens were given intramuscular injections of 1.87 mg of whole purified virus in Freund's complete adjuvant and boosted twice at 2-wk intervals with the same concentration of virus in Freund's incomplete adjuvant. Animals were bled 2–4 wk after the final injection.

**Replication of viruses in protoplasts.** Protoplasts were prepared from *N. tabacum* 'Samsun' (nn) and 'Xanthi nc' (NN), essentially as described for *Nicotiana plumbaginifoliae* L. (9). Isolated protoplasts ($6 \times 10^5$ per 300 μl) were transfected with 10 μg of virus in the presence of 40% polyethylene glycol 4000. Transfected protoplasts were incubated in the dark at 26 C and aliquots taken at 0, 4, 20, 43, and 56 h postinoculation. Samples were diluted with an equal volume of W5 medium (9), centrifuged at 1,000 *g* for 10 min, washed 4× with W5 medium, and resuspended in 1 ml of 0.1 M Tris-HCl, pH 7.4. Virus was released by 4 cycles of freeze-thawing. Virus concentration was estimated by quantitative enzyme-linked immunosorbent assay (ELISA) (19). The results shown in Figure 2 represent an average of two independent transfection experiments that differed by no more than 2%.

**Systemic movement of SDYFV-Ob.** *N. tabacum* 'Xanthi nc' (NN) and 'Xanthi Turk' (nn) plants were grown in a greenhouse to the four-leaf stage. In single infections, plants were manually inoculated on Carborundum-dusted leaves 2 and 3 using 2 μg/ml of purified virus preparation in 10 mM sodium phosphate buffer, pH 7.2. In mixed infections, 2 μg/ml of each virus was used as an inoculum. Plants were incubated in a growth chamber at 22 C (day), 20 C (night) with fluorescent/incandescent lamps, for 16 h/day at an irradiance of 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 3 wk, individual leaves were removed, weighed, and ground in blocking buffer (10% BSA, 10% polyvinylpyrrolidone 40,000 in phosphate-buffered saline) using beveled rollers. Virus was precipitated from the extracts with 4% polyethylene glycol 8000, 0.08 M NaCl, and concentration was determined by quantitative ELISA (19). Purified virus was used as a standard. Data were pooled as follows: starting immediately above the inoculated leaves, systemic leaves 1–3 (bottom); leaves 4–9 (middle), and leaves 10–15 (top). Results were obtained from two independent experiments. Plants (three to four per treatment and per experiment) were analyzed. The data were subjected to analysis of variance using the general linear model method (SAS Institute Inc, Cary, NC) and LSD was calculated at $P \leq 0.05$.

## RESULTS

**Serological and hybridization analysis of SDYFV-Ob.** A previous investigation (1) suggested that SDYFV-Ob was closely related serologically to ToMV. To examine this possible relationship in greater detail, both serological and hybridization analyses were conducted. SDYFV-Ob did not react in ELISA tests with polyclonal antisera generated against ToMV-$LS_1$ or TMV-$U_1$ (*data not shown*). Similarly, ToMV-$LS_1$, TMV-$U_1$, and PMMV did not react with a polyclonal antiserum generated against SDYFV-Ob. However, SDYFV cross-reacted strongly with a polyclonal antiserum against SDYFV-Ob. The coat protein of SDYFV-Ob had a molecular weight of 18 kDa, and an RNA molecular weight of $6.7 \times 10^6$. Particle morphology and length were also typical of tobamoviruses when examined in the electron microscope using negative staining. Purified preparations of SDYFV-Ob and TMV-$U_1$ were compared. For both viruses, the majority of particles were rigid rods approximately 310–315 nm long and 20–22 nm wide. Shorter particles that may be associated with subgenomic RNAs were also apparent in both preparations.

Dot blot hybridization studies were also conducted in which $^{32}$P-labeled cDNA probes prepared to SDYFV-Ob were hybridized to equal amounts of virion RNA extracted from three different isolates of ToMV ($LS_1$, L-D/H, and $T_{346}$; see Materials and Methods). No detectable hybridization occurred in this test or in reciprocal hybridization tests in which ToMV-$LS_1$ was used as a probe (Fig. 1A and B), suggesting that SDYFV-Ob is not a strain of ToMV, as previously proposed (1). Further dot blot hybridization analyses were therefore conducted using virion RNAs extracted from five other distinct tobamoviruses (see Materials and Methods): TMV-$U_1$, tobacco mild green mosaic (TMGMV-$U_2$ strain), PMMV (Ca10 strain), SHMV-$C_p$, and RMV. Also included in the analysis was purified virion RNA from SDYFV, a tobamovirus originally isolated from diseased pepper and which has been proposed to be a distinct tobamovirus member (13). Figure 1A shows that the SDYFV-Ob probe hybridized to SDYFV but not detectably to any other tobamovirus tested. Since it has been demonstrated that cross hybridization occurs between tobamovirus strains but does not occur or occurs only weakly between different distinct members (27,36), the data in Figure 1A suggest that SDYFV-Ob and SDYFV constitute strains of a distinct tobamovirus member. To further test this

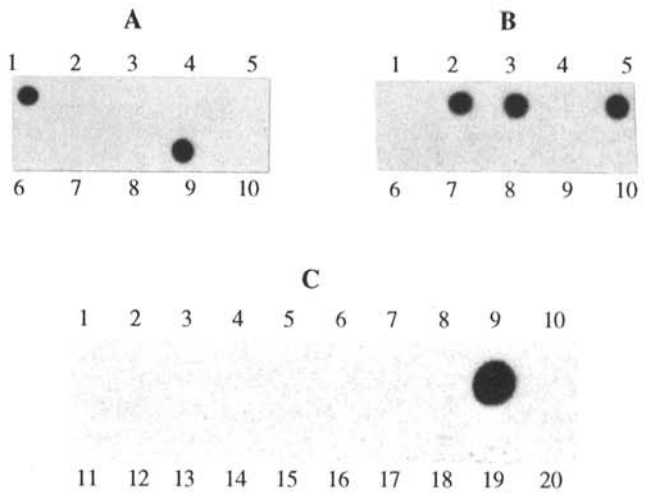


**Fig 1.** Dot blot hybridization analysis of several tobamovirus RNAs. **A,** Equal concentrations (50 ng) of intact RNA extracted from virions of SDYFV-Ob (spot 1), ToMV-$LS_1$ (spot 2), ToMV-L-D/H (spot 3), TMV-$U_1$ (spot 4), ToMV-$T_{346}$ (spot 5), TMGMV-$U_2$ (spot 6), SHMV-Cp (spot 7), RMV-type (spot 8) , SDYFV (spot 9), and PMMV-Ca10 (spot 10) were dotted onto a nylon membrane and hybridized to a $^{32}$P-labeled random primed cDNA probe of SDYFV-Ob virion RNA; **B,** same as (A) except the probe was to ToMV-L-D/H RNA; **C,** Equal concentrations (100 ng) of intact RNA extracted from virions of TMV-$U_1$ (spot 1), TMV-$U_1$-VRS (spot 2), ToMV-L-D/H (spot 3), ToMV-$T_{346}$ (spot 4), PMMV-type (spot 5), PMMV-Ca10 (spot 6), PMMV-SLV (spot 7), TMGMV-$U_2$ (spot 8), SDYFV (spot 9), RMV-$T_{281}$ (spot 11), RMV-type (spot 12), RMV-Hesp (spot 13), RMV-LTV2 (spot 14), RMV-Tcs (spot 15), RMV-TH2 (spot 16), SHMV-Cp (spot 17), CGMMV (spot 18), and KGMMV (spot 19) were dotted onto nitrocellulose and hybridized to a $^{32}$P-labeled random-primed cDNA probe of SDYFV RNA. Non-tobamovirus heterologous RNA controls were barley stripe mosaic virus RNA (100 ng; spot 10) and tobacco leaf RNA (1 μg; spot 20).

possibility, additional dot blot hybridization studies were conducted in which $^{32}$P-labeled cDNA synthesized from SDYFV-virion RNA was hybridized to the same group of tobamoviruses and also to CGMMV, KGMMV, two additional isolates of PMMV, and 5 strains of RMV (Fig. 1C). The lack of hybridization between SDYFV and any other tobamovirus tested (with the exception of SDYFV-Ob, see reciprocal hybridization in panel A) further suggests that the isolates designated SDYFV and SDYFV-Ob together constitute a separate tobamovirus. Finally, it should be mentioned that several reciprocal hybridizations were conducted using the type member of each tobamovirus shown in Figure 1C and all of the tobamoviruses mentioned in this study as probes. At high stringency, strong hybridization occurred only between strains of tobamoviruses and not between members of distinct tobamoviruses (*data not shown*) as classified by the International Committee on the Taxonomy of Viruses (7).

**Symptom development.** Preliminary experiments at 22 C confirmed previous findings (1) that SDYFV-Ob spreads systemically in *Nicotiana* species containing the N gene for resistance. In cultivar Xanthi-nc, local necrotic lesions were observed 4–5 days after inoculation and expanded gradually to form large lesions after 7–10 days. Typically, systemic symptoms were apparent 7 days after inoculation and consisted of a mild systemic necrosis and chlorosis. Eventually, younger, systemically infected leaves were severely stunted. In *N. glutinosa* at 22 C, local necrotic symptoms were visible 4 days after inoculation. Systemic symptoms occurred 10–12 days after inoculation and consisted of discrete necrotic spots similar to local symptoms on the inoculated leaves. Eventually, severe stunting or death of the stem apex occurred.

Based on our hybridization studies, SDYFV-Ob was found to share sequence homology with SDYFV. We therefore compared the symptoms induced by these viruses on a variety of hosts (Table 1). Both viruses caused identical symptoms in most hosts tested, with the exception of *Nicotiana* species containing the N resistance gene where SDYFV-Ob, but not SDYFV, induced systemic symptoms. Although *N. rustica* L. plants appeared to be slighty stunted with mild distortion of systemically infected leaves, neither SDYFV-Ob nor SDYFV could be detected by ELISA or electron microscopy.

TABLE 1. Host reaction[a] to infection with Solanum dulcamara yellow fleck virus (SDYFV) and SDYFV-Ob

| | SDYFV | | SDYFV-Ob | |
|---|---|---|---|---|
| Host species | Inoc. leaf[b] | Syst. leaves[c] | Inoc. leaf | Syst. leaves |
| *N. rustica* | Nec. les. | St. | Chl. les | St. |
| *N. glutinosa* | Nec. les. | NS | Nec. les. | Nec. les. + St. |
| *N. tabacum* 'Samsun' | NS | Mos. | NS | Mos. |
| *N. tabacum* 'Harrownova' | NS | Mos. | NS | Mos. |
| *N. clevelandii* | NS | Mos. | NS | Mos. |
| *N. tabacum* 'Xanthi nc' | Nec. les. | NS | Nec. les. | Chl. + Nec. + St. |
| *N. benthamiana* | NS | Chl. | NS | Chl. |
| *N. sylvestris* | Nec. les. | NS | Nec. les. | NS |
| *D. stramonium* | Nec. les. | NS | Nec. les. | NS |
| *L. esculentum* 'Starfire' | NS | Mos. | NS | Mos. |
| *C. annuum* 'California Wonder' | Chl. | Chl. | Chl. | Chl. + Nec. |
| *C. amaranticolor* | Chl. les | NS | Chl. les. | NS |

[a] No symptoms (NS), mosaic (Mos.), necrosis (Nec.), chlorosis (Chl.), necrotic lesions (Nec. les.), chlorotic lesions (Chl. les.), and stunting (St.).
[b] Symptoms observed in the inoculated leaf (Inoc. leaf).
[c] Symptoms observed in the systemic leaves (Syst. leaves).

**Systemic spread of SDYFV-Ob in single and mixed infections of *Nicotiana* species with and without the N gene.** Spread of SDYFV-Ob in *Nicotiana* species with (*N. tabacum* 'Xanthi nc') or without (*N. tabacum* 'Xanthi Turk') the N gene was followed by measuring virus concentration in systemically infected leaves from plants grown at 22 C. Although SDYFV-Ob was detected in both genotypes, the virus concentration was much lower in systemically infected leaves from plants containing the N gene (Table 2). SDYFV-Ob accumulation could be compared to ToMV-$LS_1$ in infected plants because antibodies against ToMV-$LS_1$ did not cross-react with SDYFV-Ob. This allowed us to measure the accumulation of each virus in plants infected with a mixture of both viruses. As expected, single infection with ToMV-$LS_1$ gave rise to systemic infection of cultivar Xanthi Turk but not of Xanthi-nc. Single inoculation with SDYFV-Ob always resulted in systemic infection (21 of 21 plants tested). To test whether SDYFV-Ob could complement the systemic spread of ToMV-$LS_1$ in plants with the N gene, equal concentrations of the two viruses were coinoculated. Of six plants tested, none showed accumulation of ToMV-$LS_1$ in systemically infected leaves, indicating that complementation did not occur (Table 2). In four of six plants, the accumulation of SDYFV-Ob in systemically infected leaves was the same as that found in a single infection with SDYFV-Ob (Table 2). However, in the remaining two plants, neither SDYFV-Ob nor $LS_1$ spread systemically (Table 2). In a further experiment, SDYFV-Ob failed to spread systemically in two of three plants in a mixed infection with $LS_1$. Therefore in four of nine plants tested, systemic movement of SDYFV-Ob was completely prevented. Interestingly, similar results were obtained in double infection of cultivar Xanthi-nc with SDYFV-Ob and either ToMV-L or TMV-$U_1$ (*data not shown*). In cultivar Xanthi Turk, mixed infections with SDYFV-Ob and ToMV-$LS_1$ resulted in systemic spread of both viruses in all eight plants tested (Table 2). SDYFV-Ob significantly reduced the accumulation of $LS_1$ in doubly infected cultivar Xanthi Turk, whereas $LS_1$ did not affect the accumulation of SDYFV-Ob. Finally, it is noteworthy that the movement of ToMV-$LS_1$ (containing a temperature-sensitive movement protein; 25) was not complemented by SDYFV-Ob at 32 C (*data not shown*). In fact, the systemic spread of Ob was generally far less efficient at 32 C than at 22 C.

**Multiplication of SDYFV-Ob in protoplasts.** Multiplication of viruses in whole leaves is dependent on both rate of replication and cell-to-cell movement. In order to measure replication per se, the multiplication of SDYFV-Ob, ToMV-$LS_1$, and a mixture of both viruses was compared in protoplasts prepared from leaves of *N. glutinosa* (NN) and *N. tabacum* 'Samsun' (nn) (Fig. 2).

TABLE 2. Systemic spread of ToMV-$LS_1$ and SDYFV-Ob in leaves of *Nicotiana tabacum* 'Xanthi nc' (NN) and 'Xanthi Turk' (nn) at 22 C in single and mixed infections

| | | ToMV-$LS_1$ content[a] | | SDYFB-Ob content[b] | |
|---|---|---|---|---|---|
| | | Inoculum | | | |
| Cultivar | Leaf position | $LS_1$ | $LS_1$ + Ob | Ob | $LS_1$ + Ob |
| 'Xanthi nc' (NN) | bottom[c] | 0 | 0 | 10 | 14[d] |
| | middle | 0 | 0 | 380 | 420 |
| | top | 0 | 0 | 2,970 | 2,850 |
| 'Xanthi Turk' (nn) | bottom | 1,740 | 910 | 2,780 | 2,740 |
| | middle | 2,740 | 1,430 | 7,360 | 8,230 |
| | top | 650 | 300 | 9,370 | 8,840 |

[a] ToMV-$LS_1$ content in μg of virus per g of fresh weight in single and mixed infections. LSD = 260 ($P = 0.05$).
[b] SDYFB-Ob content in μg of virus per g of fresh weight in single and mixed infections. LSD = 1050 ($P = 0.05$).
[c] Bottom: leaves 1–3 immediately above inoculated leaf; middle: leaves 4–9 above inoculated leaf; top: leaves 10–15 above inoculated leaf.
[d] Values represent average of four of six plants tested. In the remaining two plants, SDYFV-Ob could not be detected in the systemically infected leaves.

Overall, there was little difference in the accumulation of either virus in *N. glutinosa* or cultivar Samsun, in agreement with previous observations (26). Furthermore, SDYFV-Ob and ToMV-$LS_1$ replicated at a very similar rate. When protoplasts were transfected with a mixture of the two viruses, the rate of accumulation of each virus equaled those found in single infections with either virus. Therefore, the observed systemic spread of SDYFV-Ob in N gene hosts cannot be explained on the basis of a greatly enhanced replication rate.

## DISCUSSION

SDYFV-Ob is the only tobamovirus known to spread systemically in tobacco containing the N resistance gene (1). However, the mode of action of the N gene and the mechanism by which SDYFV-Ob can overcome this action are not known. Our results suggest that SDYFV-Ob only partially overcomes resistance induced by the N gene. Interestingly, infection of cultivar Xanthi-nc by SDYFV-Ob gave rise both to a local and systemic necrosis. This suggests at least two components in the defense mechanism induced by the N gene: an inhibition of viral spread which can be overcome only by SDYFV-Ob and a necrosis which is activated by infection with all tobamoviruses, including SDYFV-Ob. One possible explanation is that the N resistance gene is composed of at least two closely linked genes; one of which might control viral systemic spread but would not recognize SDYFV-Ob, and the other which would induce the necrosis reaction and would recognize SDYFV-Ob. Another explanation is that SDYFV-Ob is recognized by the products of the N gene but that the virus multiplies and reaches the vascular system before the defense response that limits virus systemic spread is operative. This would imply that SDYFV-Ob replicates and/or undergoes movement more rapidly than other tobamoviruses. The replication rate of SDYFV-Ob in protoplasts from *Nicotiana* species with or without the N gene was similar to the replication of ToMV-$LS_1$. This suggests that SDYFV-Ob does not have an intrinsic ability to replicate much faster than other tobamoviruses. Although SDYFV-Ob could spread systemically in *N. tabacum* 'Xanthi nc', the virus accumulated to much lower concentrations than in *Nicotiana* species without the N gene. We do not know whether this is due to an indirect effect of the necrosis reaction (e.g., a self-induced systemic resistance) or to a partial restriction of virus multiplication and systemic spread.

Our results show that in about half of the N-gene-containing plants coinoculated with other tobamoviruses, SDYFV-Ob did not spread systemically. This implies that in those plants the N gene was activated by the coinoculated tobamovirus before SDYFV-Ob could spread in the vascular system. In the remaining cases, SDYFV-Ob did spread systemically but was unable to complement the spread of other tobamoviruses. This suggests that in those plants, SDYFV-Ob could escape to the vascular system before the N gene was activated. One could imagine that the two viruses are competing for their multiplication and/or movement to the vascular system. A series of experiments with sequential inoculation of SDYFV-Ob and other tobamoviruses might supply more information on potential competition in mixed infections. Taken together, our results suggest that SDYFV-Ob does not block the action of the N gene, rather that the virus is not efficiently recognized.

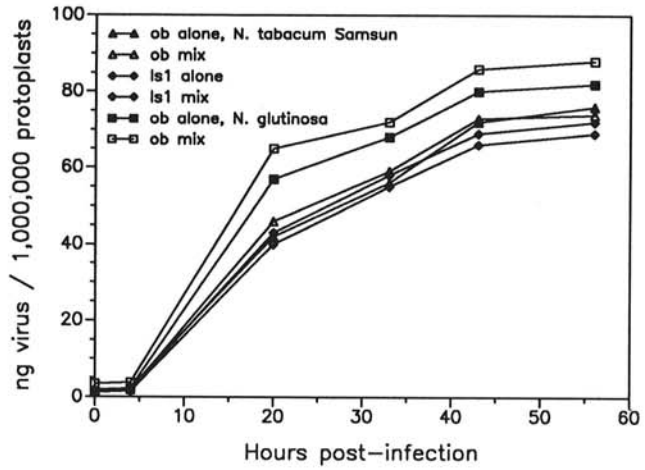


**Fig 2.** Multiplication of SDYFV-Ob and ToMV-$LS_1$ in protoplasts from *Nicotiana glutinosa* (squares and circles) and *N. tabacum* 'Samsun' (triangles and diamonds). Concentration of each virus at different times postinfection is shown in single infection (closed symbols) and in double infections (open symbols). In double infections with both SDYFV-Ob and ToMV-$LS_1$, concentration of SDYFV-Ob was measured with SDYFV-Ob antibodies (Ob mix) and concentration of ToMV-$LS_1$ with ToMV-$LS_1$ antibodies ($LS_1$ mix).

Several modes of action of the N resistance gene have been proposed. Although a protein (IVR) isolated from infected protoplasts and from the intercellular fluid of infected tobacco plants has been shown to inhibit virus replication (18,34), the replication rate of tobamoviruses is similar in protoplasts produced from tobacco plants with or without the N gene (26, our results). As an alternative, Deom et al (4) showed that the N gene product is directly or indirectly preventing the modification of the plasmodesmata by the 30-kDa protein, thereby inhibiting cell-to-cell movement. However, expression of the 30-kDa protein alone is not sufficient to induce the necrotic response in transgenic cultivar Xanthi-nc, suggesting that another viral gene might be recognized by the products of the N gene (4). The coat protein is not required for induction of the N gene because mutant viral genomes that did not contain the coat protein were still recognized (2,35). It must be assumed that other viral proteins, perhaps in combination, are necessary to induce the N resistance.

In this study we have shown strong homology between the genomes of SDYFV-Ob and SDYFV. Interestingly, these two viruses are only distantly related to other tobamoviruses, including PMMV and ToMV, and should be considered as a new member of the tobamovirus group as suggested by Horvath and Beczner (13). These two viruses induced similar symptoms on most hosts tested, except for their ability to spread systemically in hosts containing the N gene. The cloning and sequencing of the SDYFV-Ob genome, its comparison to the SDYFV genome, and domain exchange experiments between the SDYFV and SDYFV-Ob genomes will give important clues as to which viral gene or which combination of viral genes is interacting with the N gene resistance mechanism.

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