

# Modification of Tomato Aspermy Virus Symptom by Cucumber Mosaic Virus-Associated Satellite RNA.

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## Cucumber Mosaic Virus-associated Satellite RNA에 의한 Tomato Aspermy Virus의 병징변화.

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### ABSTRACT

A tomato Aspermy Virus (TAV-B) served as a helper virus for multiplication and encapsidation of satellite RNAs which were isolated from two different CMV isolates, D and K. These two satellite RNAs induced remarkable attenuation of TAV symptoms in infected tobacco, which was correlated with a reduction of virus content in the plant. The CMV satellite RNAs also caused lethal necrosis in TAV-infected tomato as in the case of CMV system.

*Key words:* tomato aspermy virus, CMV, satellite RNA.

### 要 約

Tomato aspermy virus (TAV-B)는 두 개의 다른 CMV 균주 D 및 K에서 분리한 satellite RNA의 증식 및 encapsidation에 대하여 helper virus의 역할을 하였다. 이 두 satellite RNA는 역시 CMV system에 있어서는 바와 같이 TAV가 감염된 담배에서 TAV 병징을 크게 감소시켰으며, 이는 감염된 식물내의 Virus 함량의 감소와 관계가 있었다. 또한 CMV가 감염된 토마토에서 lethal necrosis를 유발시킴을 볼 수 있었다.

### INTRODUCTION

Kaper and Waterworth (1977) demonstrated that satellite RNA (CARNAS; CMV-associated RNA5; a small replicating RNA encapsidated with and dependent upon, but not part of the viral

genome) from a South African strain of CMV (CMV-S) was responsible for inducing lethal necrosis in tomato plants. Since this work, the presence of different satellite RNAs in infected tomato was reported. Some of them caused lethal necrosis (7,8,9,18,20) or white leaf symptom (4), while others did not induce a particular symptom or even

cause to attenuate symptoms (5,7,8,12,14,15).

In contrast to symptom expression in tomato, addition of satellite RNA generally caused remarkable attenuation of symptoms induced by CMV in most hosts (4,7,10,15,18). However, a satellite RNA from CMV-Y has been reported to be responsible for a unique yellow symptom in tobacco (18).

This paper reports that CMV satellite RNAs can be encapsidated with and multiplied upon a tomato aspermy virus (TAV-B), and that these CMV satellite RNAs can modify TAV symptoms in infected plants.

## MATERIAL AND METHODS

**Virus and plants.** The cucumovirus which had been isolated from chrysanthemum in Belgium in 1953 by Dr. G. Roland and identified as a strain of tomato aspermy virus (17) was used as recipient virus in this study. This virus which induces severe deformations and filiform leaves in *Nicotiana glutinosa* L., was devoid of satellite RNA, even after multiple passages in tobacco. Because its RNAs showed the typical electrophoretic pattern of tomato aspermy virus (TAV), the isolate was referred to as TAV-B.

Donor strains of satellite RNA were two cucumber mosaic virus (CMV) isolates, D and K, from France and Korea: D strain isolated from tomato and received from Dr. Marrou(1), and K strain isolated from tobacco and denoted by Dr. Park (16).

Viral strains were propagated either in *Nicotiana tabacum* L. c.v. "Xanthi" or *N. glutinosa* L. grown in a greenhouse at 20-25°C, under 14 hours of artificial light a day.

Ten to thirteen days after inoculation, the virus was purified from infected leaves as described by Lot et al. (1972). Level of purification was ascertained by polyacrylamide gel electrophoresis(PAGE) and virus yield was estimated by measuring optical density at 260nm.

**Extraction of genomic and satellite RNAs.** Viral RNAs were extracted from purified virus by the

phenol-SDS bentonite method (6). RNA preparations were checked by PAGE, using 2.4% polyacrylamide. Electrophoresis was performed for 3 hours at 5mA per gel in 6mm tubes; gels were subsequently scanned at 260nm. The fractions corresponding to satellite RNA were cut out from frozen gel and RNA was eluted by maceration of the gel pieces in 0.02M glycine buffer (pH9.0). Satellite RNA was then purified and concentrated according to the method of Dolja et al. (1977). The final preparations were again controlled by PAGE.

The phenol-SDS-bentonite method was also used to prepare genomic RNAs of TAV. The final preparation was controlled by UV scanning from 220nm to 300nm.

**Inoculation of genomic RNAs and satellite RNA**  
The preparations of TAV genomic RNAs (200µg/ml) and satellite RNA (40µg/ml) in TNE buffer (0.01M Tris-HCl (pH 7.4), 0.5M NaCl and 0.01M EDTA) were inoculated either separately or together on 3 leaves of young "Xanthi" tobacco plants. The virus offspring (0.2mg/ml) in 0.07M NaH<sub>2</sub>PO<sub>4</sub> buffer was inoculated to tomato plants (*Lycopersicon esculentum* L.) cultivar "Money maker".

## RESULTS

The RNA PAGE pattern of the original TAV-B strain used is presented in Figure 1. After successive transfers to tobacco plants, the RNA contents of progeny viruses of all combinations between TAV-B and one of the 2 satellite RNAs were analysed by electrophoresis in 2.4% PAGE in the presence of SDS.

Satellite RNA alone failed to infect either tobacco or tomato plants. TAV-B itself induced severe symptoms in tobacco while all combination of TAV-B with satellite RNA from CMV strains gave attenuated symptoms in these species corresponding to a great (60-70%) reduction in virus yield (30-40mg virus per 200g of infected leaves compared with 100mg virus yield of TAV-B alone) from infected leaves (Fig. 2A).

The TAV-B recipient strain by itself induced

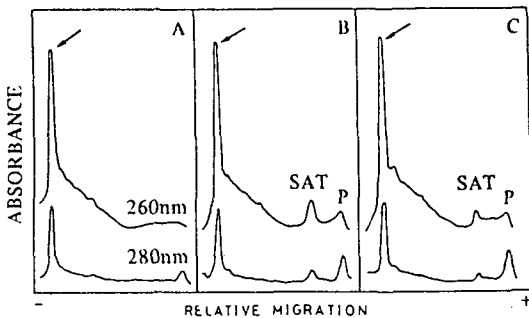


Fig. 1. SDS-polyacrylamide gel electrophoregrams of viral RNAs: RNAs contained in the virions purified from tobacco plants with A, the original TAV-B isolates; B, TAV-B plus satellite RNA of CMV-D; C, TAV-B plus satellite RNA of CMV-K. Gels were scanned at 260 and 280nm. SAT = satellite RNA, P = coat protein.  $\nearrow$  = TAV-B genomic RNAs.

severe leaf malformations and stunting in tomato, but no apical necrosis developed in this plant. However, all mixed inoculation with TAV-B plus any satellite RNA obtained from either of these CMV strains induced lethal necrosis in tomato (Fig. 2B).

## DISCUSSION

Although cucumber mosaic virus (CMV) and tomato aspermy virus (TAV) do not show any detectable serological relationship (1) or nucleotide base sequence homology (3), TAV has been known to act as helper virus for acceptance, multiplication and encapsidation of satellite RNAs associated to several CMV strains (15). The same authors reported that the relatively severe symptoms produced by the TAV strains (N and V) were not attenuated in the presence of satellite RNA. In my study TAV-B also acted as a helper for the multiplication and encapsidation of satellite RNAs from CMV-D and CMV-K. The inoculums, constructed by combining the TAV-B genomic RNAs and the satellite RNA preparations from 2 different CMV isolates, induced a remarkable attenuation of symptom expression in infected tobacco, which was correlated with a

reduction of virus content in the plant. This "attenuation" phenomenon has also been reported by many workers to be correlated with a significant reduction in the production and specific infectivity of purified CMV, together with a striking increase in the ratio of encapsidated satellite RNA to viral RNAs, sometimes reducing genomic RNA1 or RNA1+2 to insignificant proportions in the virions (7,8,11,15,18,19). The high proportions of a satellite RNA in virions, however, did not always attenuate CMV symptom expression (15).

Kaper and Tousignant (1977) indicated that tomato necrosis of CMV-D was associated with the presence of satellite RNA in CMV-D inoculum. This satellite RNA from CMV-D also caused tomato necrosis when mixed with helper CMV strains (1 F, K and M) devoid of satellite RNA. In my case of the combination between TAV-B genomic RNAs and satellite RNA preparations from each of 2 different CMV isolates, all induced lethal necrosis in tomato as in CMV systems. It was also suggested that TAV-B like CMV strains induce tomato necrosis when acting as a helper virus of CMV satellite RNA.

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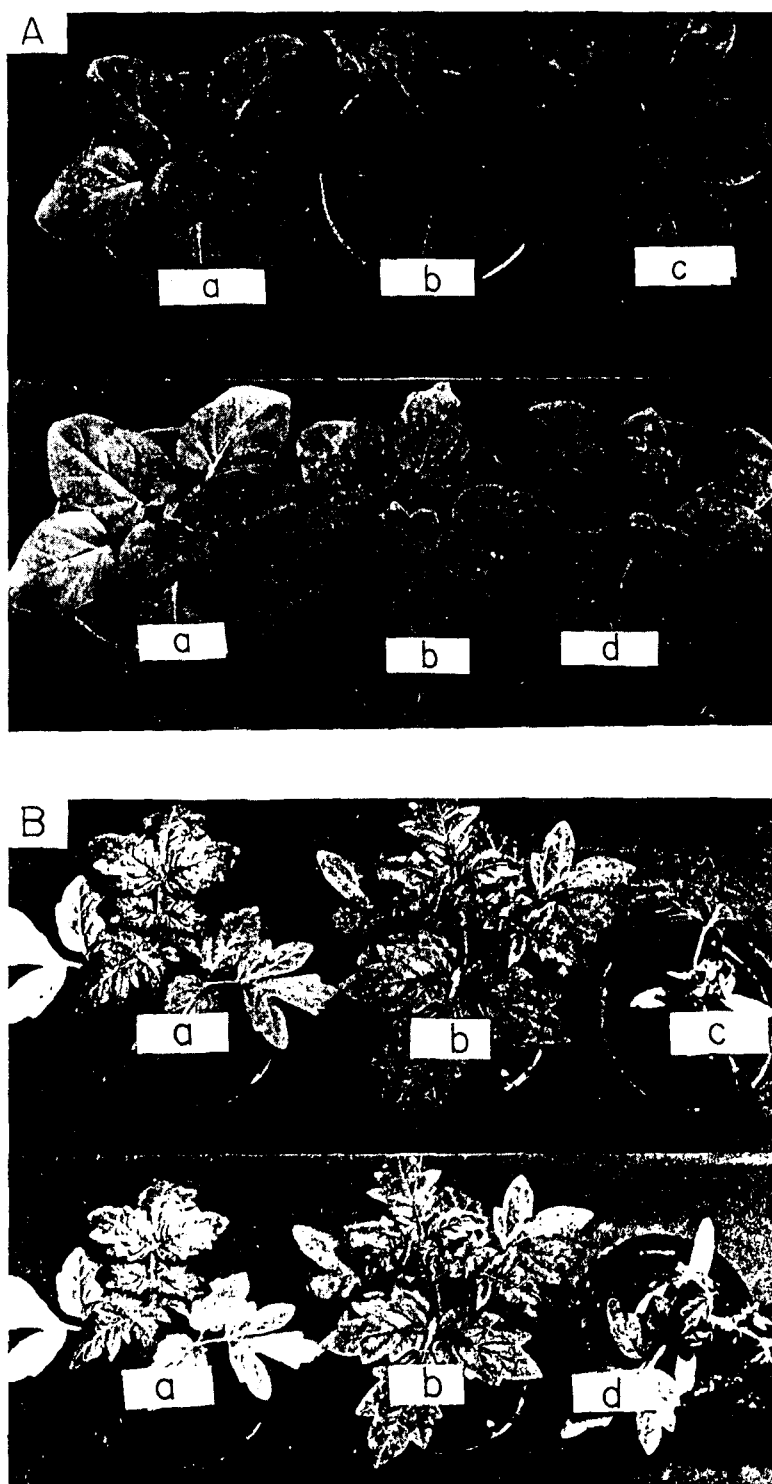


Fig. 2. Systemic symptoms induced on *Nicotiana glutinosa* (A) and on tomato cultivar "Money maker" (B) : a, healthy control; b, plant inoculated with the genomic RNAs of TAV-B; c, plant inoculated with a mixture of TAV-B genomic RNAs plus satellite RNA of CMV-D; d, plant inoculated with a mixture of TAV-B genomic RNAs plus satellite RNA of CMV-K.