Studies Involving Two Tomato Virus Strains of Tobacco Mosaic Virus (TMV) in Tomato Lycopersicum esculentum L. 'Revermun'

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The following experiments have been conducted during 1966-1967 in the virology department at the State Plant Pathology Institute in Lyngby.

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Introduction

Denmark's commonly grown variety of tomato *Lycopersicum esculentum L.* 'Revermun' was used as the host plant in a series of studies involving two tomato strains of tobacco mosaic virus (TMV), one an attenuated form of the other, and a tobacco strain of TMV. Infected tomato plants were tested for percent of infection of the fruit and seed as well as movement of virus from the seed to primary leaves, stem, and roots following germination. This study is an extension of a series of experiments involving these two strains of tomato mosaic virus which have been conducted at this research center.

Physical and Serological Properties of the Strain

The tomato strain of tobacco mosaic virus used in this test is one, that is commonly found in Denmark. The attenuated strain is the former strain, which was subjected to 38° C for 150 days. They will be refered to throughout this paper as the normal and attenuated tomato strains of TMV. The tobacco strain of TMV derived from tobacco plants. All three strains were supplied to the author by Mr. Niels Paludan. Before the studies were initiated the three strains were characterized by dilution end point, range 1/10 to $1/10^6$ (table 1), thermal death point, range 50 to 95° C (table 2), longevity in vitro (table 3), and by serological reactions. All inoculations were made by the carborundum method onto 3 detached leaves of *Nicotiana glutinosa* L. in a temperature control room. The infectivity of each strain was tested on different aged leaves of *Nicotiana glutinosa* as well as different areas of the 10, 18, and 25 day old leaves to determine more accurately the number of local lesions.

Four to six hundred grams of two week old infected *Nicotiana tabacum* 'Samsun' leaves were harvested and Wetter's (5) purification procedure followed to produce a 2 ml virus suspension that was homogenized with an equal volumn of Freund's incomplete adjuvant and injected intramuscularly into male white rabbits. All three strains of the virus were purified in a similar manner. Weekly purifications were made of each virus and injections given until a desired titer was reached at which time 50 to 70 ml of blood was drawn from the marginal ear vein. The whole blood was stored at room temperature for five hours and refrigerated overnight. The following day the sera was separated by centrifugations, stored in small vials and placed in a freezer. Standard precipitin tests (1) were conducted to determine the titer of the sera as well as cross reactions between the strains (table 4). In conducting the precipitin test, the antigen was diluted 1/12 in saline and the antisera was diluted two fold to 1/16,000. The tubes were placed in a 37° C water bath and the results read after 1 hour. Controls using healthy plant extract (HPE) and normal rabbit sera (NRS) were normally included in each test.

Seed Investigations

One-hundred eighty 'Revermun' plants were set out in a greenhouse May 1,1966. When the plants were 15 cm high, 36 were inoculated with the normal tomato virus strain (group A), and 36 with the attenuated tomato virus strain (group B). In late June, with the fifth truss flowering, 36 more plants were inoculated with the normal tomato virus strain (group C), and 36 with the attenuated tomato virus strain (group D). An additional 36 uninoculated plants served as the control (group E), which became initially infected in late July. In September 100 fruit were harvested from each of the 5 test groups. The seed was extracted from 40 fruit of each group, subjected to fermentation for 5 days, washed and allowed to dry. Fifty seeds were selected from each fruit, ground and used as inoculum on detached leaves of *Nicotiana glutinosa.* In totalling the number of fruit which showed infectivity, the percent of virus infection for each group was determined (table 5).

The percent of seed infection within each group was obtained by randomly selecting 320 seeds from fruit within each group. Single seed inoculations were made until the percent of infection reached a plateau and remained at that level for at least three additional inoculations (table 6).

Economically it now became interesting to investigate the possible presence of virus in the upper portions of the seedling following germination of infected seed. One hundred seeds randomly selected from fruit which exhibited at least 75% infection were plated out on filter paper in plastic trays containing distilled water. To avoid contact between seedlings, no more than nine seeds were placed in a tray. Five to seven days later those plants, which had expelled the seed coat from the primary leaves, were removed and tested for infectivity. The seed coat, stem, primary leaves and the roots were dissected with alcohol dipped and flamed tweezers and knives. The inoculum was ground and applied to detached leaves of *Nicotiana glutinosa.* The results were read 72 hours later (table 7).

Results

Table 1. Dilution end point of the 3 strains of tobacco mosaic virus

Virus strains of TMV	10^y	10^2	10^3	10^4	5×10^4	10^5	10^6
Tobacco	50[z]	26	10	1	2	2	—
Tomato (normal)	50	400	7	5	4	—	—
Tomato (attenuated)	45	40	25	2	—	—	—

y = reciprocal values
z = ave.number of lesions for 3 leaves

Table 2. Thermal death point of the 3 strains of tobacco mosaic virus

		°C					
Virus strains of TMV	UN[x]	60	70	80	85	90	95
Tobacco	150	150	100	50	50	12	—
Tomato (normal)	150	150	100	50	31	—	—
Tomato (attenuated)	150	150	100	30	—	—	—

x = undiluted sap was subject to each temperature for 10 minutes before being used as inoculum for detached leaves of *Nicotiana glutinosa.*

Table 3. Longevity in vitro of the 3 strains of tobacco mosaic virus stored at room temperature, September 8, 1966

Testing date	Tobacco	Tomato normal	Tomato attenuated
October 6, 1966.....	150*	150	150
November 1, 1966....	150	100	100
December 1, 1966....	150	100	60
January 1, 1967.....	100	50	25
March 1, 1967.....	100	60	6
April 1, 1967.....	100	50	4
May 1, 1967.....	100	45	—

* = average number of lesions for 3 detached leaves of *Nicotiana glutinosa*.

Table 4. Titers of antisera as determinded by standard precipitin test

Antisera to strains of TMV:	Antigens tob.	tom N.	tom A.	H.P.E.
Tobacco.....	8.192*	8	4	—
Tomato (normal)...	32	4.096	1.024	—
Tomato (attenuated)	16	4.096	1.024	—
Normal rabbit sera..	—	—	—	—

tob. = tobacco.strain
tom N. = tomato strain (normal)
tom A. = tomato strain (attenuated)
H.P.E. = healthy plant extract
* = reciprocal values

The infectivity of the strains did not flucuate when different aged *Nicotiana glutinosa* leaves or areas of these leaves were inoculated.

Table 5. The percent of virus infected fruit as caused by the 3 strains of tobacco mosaic virus

Groups:	A	B	C	D	E
Fruit infected/tested.....	36/40	20/40	40/30	39/40	21/40
% of infection.....	90	50	100	98	53
Average number of lesions on *Nicotiana glutinosa* .	49	6,2	62	59	10

Table 6. The percent of virus infected seed within each group (A to E)

Groups:	A	B	C	D	E
Number of infected seed/tested...	81/320	61/316	274/320	228/320	86/320
% of infection.....	25,3	19,3	85,6	70,0	27,4

Table 7. The percent of virus located in seedlings parts following germination of virus infected seed (group C)

	Seeds	Leaves	Stem	Roots
Number infected/tested.....	79/100	23/100	22/100	5/25
% of infection.....	79	23	22	20
Average number of lesions of *Nicotiana glutinosa*..	11,4	3,2	1,5	1,5

Discussion

Fruit from the 5th truss inoculated plants showed as high as 100% infection (table 5) for the normal virus strain and 98% for the attenuated virus strain. Fruit from plants which were inoculated at an early stage with the normal virus strain proved to be 90% infected as compared to 50% for the attenuated virus strain. This is in contrast to Broadbent's (2) report that the percent of infected fruit did not vary according to the inoculation date.

The seed that was selected for the determination of percent infection within each fruit (table 6) had been stored in paper envelopes 4 months before being tested. It is interesting to note the decrease in percent of infected seed from that seed, which was directly extracted from freshly harvested fruit (table 5). Late infection (C and D)

caused a much higher percent of seed infection than the plants that were infected at an earlier stage of growth (A and B).

Seed from group C was selected for the determination of virus in leaves, stems, and roots. This seed had been stored in paper envelopes for 8 months at room temperature. The percent of infected seed (table 7) had not decreased notably during the 4 months of storage that existed between the tests represented by tables 6 and 7. Other workers have shown decreases in infected seed following extraction by formentation and varies lengths of storage. Chamberlain and Frey (3) report that seed extracted by fermentation inhibits the percent of seed transmission while varied lengths of storage had little affect on the percent of transmission. John and Sova (4) reported that no reduction in TMV transmission occurred in 3 year old seed held in common storage, but complete loss of infectivity did occur in small seed samples stored for 1 month in paper envelopes in the laboratory. Using extreme care to avoid contamination 23%, 22% and 20% of the leaves, stems and roots respectively showed presence of infection (table 7). The virus was found to occur in both the leaves and stems of eight seedlings while the leaves and stems were found to be infected singularly in 15 and 16 seedlings respectively. In one seedling the roots and cotyledons were both infected. Although the number of local lesions essayed on *Nicotiana glutinosa* was low for all plant parts tested infection was evident. Throughout the study a low percent (3-4) of necrotic seed was found, while germination ranged from 90 to 95%.

Under commercial conditions the percent of infected seeds and seedlings would presumably be less than that found in this study, primarily due to varied methods of extraction and storage. It is nevertheless evident that a small percent of infected seed could be the cause of initial infection.

An electron microscopy study has been initiated at this laboratory to investigate the presence of virus particles in the testa, endosperm and embryo. At this point it is difficult to say if the virus is merely a contaminant or is truly seed transmitted in 'Revermun', for few experiments have so far been conducted with this variety.

Summary

Using tomato *Lycopersicum esculentum* L. 'Revermun' as the host plant, two tomato strains of tobacco mosaic virus (TMV), one an attenuated form of the former (150 days at 38°C) and a tobacco strain of TMV were studied for percent of virus infected fruit, seed, and presence of virus in cotyledons, stems, and roots following germination of infected seed (75% infected).

Plants which were inoculated with the normal tomato strain of TMV at a height of 15 cm held fruit that was 90% infected, as compared to 50% with the attenuated strain of TMV. Plants that were inoculated when the fifth truss was flowering held fruit which was 100% infected with the normal tomato strain of TMV, while those plants that were inoculated with the attenuated virus strain were 98% infected (table 5).

In investigating the percent of seed infection, in individual fruit is was found that 25% of the seed from early inoculated plants were infected (normal tomato virus strain) while 19% of the seed from those plants inoculated with the attenuated virus strain were infected. With the late inoculated plants, 85% of the seed was found to be infected (normal virus strain), and 70% with the attenuated virus strain (table 6).

Studies concerning the presence of virus in cotyledons, stems, and roots showed 23%, 22%, and 20% infection, respectively (table 7). For this study seed was selected that proved to be at least 75% infected.

Sammendrag

Tomat *Lycopersicum esculentum* L 'Revermun' er blevet anvendt som værtplante ved en række forsøg, hvor to tomatlinier og en tobaklinie af tobakmosaik-virus (TMV) blev anvendt. Tomat-viruslinierne bestod af en normal forekommende linie samt dens svækkede form (150 døgn ved 38°C.).

Undersøgelserne omfattede pct. virusinficerede frugter og frø samt tilstedeværelsen af virus i kimblade, stilke og rødder ved spiring af 75 pct. inficeret frø.

Ved tidlig smitte (plantehøjde 15 cm) blev, ved inokulation med den normale tomat-viruslinie, 90 pct. af frugterne inficeret sammenlignet med

50 pct., hvor den svækkede tomat-viruslinie var anvendt.

Ved sen smitte (5. klase i blomst) udvikledes, ved inokulation med den normale tomat-viruslinie, 100 pct. inficerede frugter mod 98 pct. inficerede, hvor den svækkede tomat-viruslinie anvendtes (tabel 5).

Frøinfektionen i de enkelte frugter var ved tidlig smitte 25 pct., hvor normal tomat-viruslinie var anvendt, mod tilsvarende 19 pct. ved den svækkede tomat-viruslinie. Ved sen smitte blev 85 pct. af frøene inficeret (normal linie) mod 70 pct., hvor den svækkede linie blev anvendt (tabel 6).

Undersøgelser vedrørende tilstedeværelsen af virus i kimblade, stilke og rødder ved spiring af 75 pct. inficeret frø, viste en infektionsprocent på henholdsvis 23,22 og 20 (tabel 7).

Literature Cited

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