# Factors Affecting the Incidence of Systemic Necrosis in $F_1$ Hybrid Tomato Plants Resistant to Tobacco Mosaic Virus

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**ABSTRACT**

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In tomato plants heterozygous for the *Tm*-$2^a$ gene for tobacco mosaic virus localization, the incidence of systemic necrosis at high temperature (30–31 C) decreased with increase in plant age at the time of inoculation with an isolate of TMV strain 0. Similarly, fewer plants were necrotic when the length of exposure to high temperature was reduced and the time interval from inoculation to the heat treatment was extended. The presence of a certain amount of virus at the primary site of infection may be necessary for systemic necrosis to develop at high temperature.

Additional key words: *Lycopersicon esculentum*, tobacco mosaic virus infectivity

In tomatoes, the *Tm*-$2^a$ gene confers resistance to tobacco mosaic virus (TMV) based on hypersensitivity (5–7,9,10). However, the local reaction of heterozygous (*Tm*-$2^a$/+) plants to TMV inoculation frequently changes to systemic necrosis when plants are grown at temperatures above 26–28 C (3,5,6,10). Laterrot (6) reported 19–87% systemic necrosis in different $F_1$ hybrids heterozygous for *Tm*-$2^a$. Working with *Tm*-$2^a$/+ plants of the same $F_1$ line, I found that the incidence of systemic necrosis in different tests ranges from 62 to 89% (10). Cirulli and Alexander (3) indicated that *Tm*-$2^a$/+ plants become necrotic when inoculated with Ohio strains I, II, III, and V but not when inoculated with strain IV. Pelham (9) observed that the occurrence of systemic necrosis in plants of the genotype *Tm*-$2^a$/+ varies, depending on the specificity of the TMV strain.

The aim of the present work was to evaluate the effects of plant age at the time of inoculation, the length of exposure of inoculated plants to high temperature, and the time interval from inoculation to heat treatment on the incidence of systemic necrosis in plants heterozygous for the *Tm*-$2^a$ gene.

## MATERIALS AND METHODS

Crosses were made in the greenhouse between *Lycopersicon esculentum* 'Marmande' (TMV-susceptible) and line 825 (TMV-resistant, genotype *Tm*-$2^a$/*Tm*-$2^a$). The cultivar Marmande was used as the female parent. The TMV resistance was derived from line 680818 furnished by L. J. Alexander, Wooster, OH. Line 825 was obtained after six backcrosses to Marmande, followed by three generations of selfing.

Seeds of the $F_1$ hybrid and the parental lines (controls) were sown in a greenhouse chamber at 22–23 C under natural light in asbestos flats (44.0 × 23.0 × 6.5 cm) filled with sand, peat, and vermiculite (1:1:1, v/v). Each line was sown in a separate flat. Two or three seeds were planted in 50 sites per flat, arranged in five rows of 10 sites, with 4 cm between sites. Seedlings in all flats were thinned to one plant per site 7 days after sowing.

Treatments were arranged in a completely randomized design with five to eight replications (flats) per treatment. Tests were done in two growth chambers at 30–31 C (high temperature) and 20–21 C (low temperature), with a 14-hr photoperiod. Light intensity at the top of the plants (10 days old) was between 6,400 and 6,800 lux. Generally, 8 days after planting, the flats were transferred from the greenhouse chamber to the growth chamber set at 20–21 C.

An isolate of TMV strain 0 (8), maintained in the greenhouse at 22–23 C in *Nicotiana tabacum* L. 'Samsun' plants, served as the inoculum. TMV cultures were renewed by manual inoculations every 6–8 wk. Inoculum was prepared by grinding fresh, infected Samsun leaves and diluting the expressed sap 1:10 in distilled water. Inoculum was rubbed onto Carborundum-dusted cotyledons of 10-day-old seedlings at the first leaf stage, unless stated otherwise.

Symptoms were recorded daily as local reaction, systemic necrosis (necrotic reaction), and mosaic (typical systemic mottling of foliage on the TMV susceptible control). The final record was taken 8 wk after inoculation. Plants of the homozygous resistant control line reacted only locally to TMV. Plants of the $F_1$ hybrid and the two control lines inoculated with healthy sap and maintained at identical conditions remained healthy. All experiments were performed two or three times with similar results. Statistical analysis was based on arcsin transformed values (12).

Samples of infected cotyledons were collected at intervals after inoculation and stored at −15 C until assayed for virus infectivity by inoculating half-leaves of *Datura stramonium* L. with sap diluted 1:2 in distilled water (2). Paired comparisons were made separately in 10 tests (not performed simultaneously). In each test, 16 leaves of *D. stramonium*, previously dusted with Carborundum, were inoculated by using a soft artist's brush. Inoculum from one sample was applied to one half-leaf and that from the second sample was applied to the other half of the same leaf. Inoculated plants were placed in the greenhouse at 24–26 C for 3–4 days, after which local lesions were counted. Results were analyzed by the sign test (11).

## RESULTS

The effect of plant age at time of inoculation on the incidence of systemic necrosis at high temperature was tested by sowing the $F_1$ line at three different dates (10-day intervals) and inoculating the plants simultaneously. Plants 10, 20, and 30 days old were inoculated on the first, third, and fifth leaf, respectively, and then incubated at 30–31 C.

Susceptibility to systemic necrosis was greater in the younger plants at time of inoculation (Table 1). Although the total incidence of systemic necrosis in plants inoculated 10 and 20 days after sowing was similar, necrotic plants were first recorded in the youngest group. The increase in the incidence of necrosis after inoculation was faster in this group than in the older groups. Thus, 50% of maximum necrosis incidence in plants inoculated 10, 20, and 30 days after sowing was reached 6, 13, and 16 days after inoculation, respectively. No further development of systemic necrosis was recorded in the youngest group after the 20th day and in the older groups after the 26th day.

**Heat treatment.** Seedlings were inoculated with TMV, immediately

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incubated at 30–31 C for 3, 6, 10, and 20 days, and then transferred to 20–21 C until 8 wk after inoculation. The incidence of systemic necrosis in plants incubated at 30–31 C for 6, 10, and 20 days was similar and was significantly higher than that in plants incubated for only 3 days (Table 2). The progress of the incidence of necrosis after inoculation was similar for plants incubated for 10 and 20 days. The cumulative percentage of necrotic plants 14 days after inoculation was significantly higher in those incubated for 10 and 20 days than in those incubated for 6 days

**Time from inoculation to heat treatment.** Four groups of 250 plants were incubated at 30–31 C at 1, 2, 5, and 10 days after inoculation. The incidence of systemic necrosis decreased markedly when the interval between inoculation and the heat treatment was extended from 2 to 5 days (Table 3). Furthermore, when the interval was extended from 5 to 10 days, none of the $F_1$ plants became necrotic, even though they had been incubated at 30–31 C for 46 days.

The decrease in the incidence of systemic necrosis, as the time from inoculation to the heat treatment was increased, could be related to the process of virus inactivation by the host's resistance mechanism before heat treatment. To test this possibility, the TMV content of cotyledons was compared 2, 5, and 10 days after inoculation. At each interval, 40 infected cotyledons were harvested from plants maintained at 20–21 C. Bioassays on *D. stramonium* indicated that infectivity of TMV was highest in cotyledons collected 2 days after inoculation, followed in order by those collected at 5 and 10 days (Table 4).

**Table 1.** Effect of plant age at the time of inoculation with tobacco mosaic virus on the incidence of systemic necrosis in *Tm*-$2^a$/+ tomato plants incubated at 30–31 C

| Plant age at inoculation (days after sowing) | Systemic necrosis[z] (days after inoculation) | | | | |
|---|---|---|---|---|---|
| | 5 | 7 | 14 | 20 | 26 |
| 10 | 19 a | 47 a | 79 a | 83 a | 83 a |
| 20 | 0 b | 4 b | 39 b | 64 b | 74 a |
| 30 | 0 b | 2 b | 18 c | 40 c | 50 b |

[z]Cumulative percentage; means of eight replicates (50 plants per replicate). Numbers within columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

**Table 2.** Effect of the length of exposure to high temperature (30–31 C) on the incidence of systemic necrosis in *Tm*-$2^a$/+ plants inoculated with tobacco mosaic virus[y]

| Duration of heat treatment (days) | Systemic necrosis[z] (days after inoculation) | | | | |
|---|---|---|---|---|---|
| | 7 | 10 | 14 | 21 | 28 |
| 3 | 1 a | 4 a | 11 a | 24 a | 35 a |
| 6 | 18 b | 62 b | 64 b | 78 b | 86 b |
| 10 | 21 b | 75 b | 87 c | 90 b | 90 b |
| 20 | 19 b | 78 b | 89 c | 91 b | 91 b |

[y]Ten-day-old tomato seedlings growing at 20–21 C were inoculated on both cotyledons and immediately transferred to 30–31 C. After heat treatment, the plants were moved back to 20–21 C.

[z]Cumulative percentage; means of six replicates (50 plants per replicate). Numbers within columns followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

**Table 3.** Effect of time interval from inoculation with tobacco mosaic virus to heat treatment at 30–31 C on the incidence of systemic necrosis in *Tm*-$2^a$/+ tomato plants

| Days from inoculation to heat treatment[y] | Systemic necrosis[z] (days after inoculation) | | | |
|---|---|---|---|---|
| | 7 | 14 | 21 | 28 |
| 1 | 51 a | 85 a | 92 a | 92 a |
| 2 | 46 a | 78 a | 89 a | 89 a |
| 5 | 0 b | 21 b | 38 b | 47 b |
| 10 | 0 b | 0 c | 0 c | 0 c |

[y]Ten-day-old seedlings growing at 20–21 C were inoculated on both cotyledons and were transferred to 30–31 C at the indicated intervals after inoculation. The trial ended 56 days after inoculation.

[z]Cumulative percentage; means of five replicates (50 plants per replicate). Numbers within columns followed by the same letter are not significantly different ($P$=0.05) according to Duncan's multiple range test.

## DISCUSSION

The *Tm*-$2^a$ gene confers resistance to TMV strains 0, 1, 2, and 1·2 (9). The defense mechanism against TMV spread in tomato plants heterozygous for *Tm*-$2^a$, which results in virus localization, is fully expressed at temperatures below 26–28 C. However, higher temperatures markedly affect the ability of these plants to suppress virus multiplication and movement, and systemic necrosis may occur (3,5,6,9,10). In this study, each external factor tested influenced the incidence of systemic necrosis. Susceptibility to systemic necrosis decreased substantially with increase in seedling age at the time of inoculation (Table 1).

The results indicate that the decrease in incidence of systemic necrosis in heterozygous (*Tm*-$2^a$/+) plants, as the time interval between inoculation and heat treatment was increased, was associated with a reduction in virus concentration in the inoculated cotyledons before the heat treatment (Table 4). These results and others (10) support the hypothesis that a certain amount of virus at the primary site of infection is required for the development of systemic necrosis at high temperature.

Other work (1,4,10,13) has suggested that the reaction of hypersensitive hosts that the local or systemic reaction of hypersensitive hosts to virus infection depends on a certain quantitative equilibrium between the host's resistance mechanism and the virus titer at the primary site of infection. High temperature may affect such an equilibrium by increasing the rate of virus multiplication. An increase in the length of exposure to high temperature (30–31 C) from 3 to 6 days resulted in more than a twofold increase in the percentage of necrotic plants (Table 2).

The present study suggests that the decrease in TMV infectivity at 20–21 C in plants of genotype *Tm*-$2^a$/+ below the minimum required for systemic symptoms to develop is relatively slow. Indeed, about 50% incidence of systemic necrosis was recorded among plants that were transferred to the high temperature regime 5 days after inoculation. Only when the heat treatment was delayed longer did none of the plants become necrotic (Table 3). The resistance based on the *Tm*-$2^a$ gene is not caused by the inability of TMV to multiply within the plant cells (10). Hence, the decrease in virus infectivity in *Tm*-$2^a$/+ plants, as the time from inoculation to the heat treatment was extended, may be the result of virus inactivation, suppression of virus multiplication, or prevention of cell-to-cell movement of virus.

In contrast to the relatively high incidence (47–92%) of systemic necrosis in *Tm*-$2^a$/+ plants that were transferred from 20–21 C to 30–31 C between 1 and 5 days after inoculation (Table 3), none of the plants of the homozygous (*Tm*-$2^a$/*Tm*-$2^a$) resistant control became necrotic when treated similarly. Therefore, it appears logical to assume that the different reactions of genotypes *Tm*-$2^a$/*Tm*-$2^a$ and *TM*-$2^a$/+ at high temperature are due to greater capacity of plants of genotype *Tm*-$2^a$/*Tm*-$2^a$ to suppress virus replication and/or movement in the inoculated cotyledons under both low and high tempera-

**Table 4.** Half-leaf infectivity assay of tobacco mosaic virus from cotyledons of *Tm*-$2^a$/+ tomato plants harvested at intervals after inoculation[a]

| Comparison | Sap[b] collected from cotyledons (days after inoculation) | Local lesions[c] |
|---|---|---|
| 1 | 2 | 424.5[d] |
| | 5 | 208.6 |
| 2 | 2 | 367.0[e] |
| | 10 | 113.6 |
| 3 | 5 | 223.1[e] |
| | 10 | 96.4 |

[a]Ten-day-old seedlings kept at 20–21 C were inoculated on both cotyledons.

[b]Diluted 1:2 with distilled water.

[c]Mean number of local lesions on 16 half-leaves of *Datura stramonium* in 10 tests.

[d]Difference in paired comparison was significant ($P = 0.05$; sign test) in seven of 10 tests.

[e]Difference in paired comparison was significant ($P = 0.05$; sign test) in 10 tests.

ture regimes.

The results reported here were obtained with TMV strain 0. This study will be continued to determine whether these reactions are specific to strain 0 or also occur with other strains that may cause systemic necrosis at high temperature on plants heterozygous for the *Tm*-$2^a$ gene.

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