Evaluation of Transgenic Tomato Plants Expressing the Coat Protein Gene of Cucumber Mosaic Virus Strain WL under Field Conditions

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ABSTRACT

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Transgenic tomato plants expressing the coat protein (CP) gene of cucumber mosaic virus (CMV) strain WL, a member of CMV subgroup II, were evaluated for resistance to CMV infections under field conditions for 2 years. Three transgenic inbred lines, two hemizygous and one homozygous, and one transgenic hybrid were field tested. CMV subgroup I strain Fny was used as challenge inoculum. Disease incidence was assessed by monitoring symptom development, enzyme-linked immunosorbent assays (ELISA), and bioassays on indicator hosts. The four transgenic tomato lines exhibited a high level of resistance to CMV infections, since all 747 transgenic plants remained symptomless throughout the crop cycle. Moreover, CMV could not be detected by ELISA nor recovered from uninoculated leaves of transgenic plants. These developed to normal height, and showed a 17-fold increase in productivity along with a 44% increase in fruit weight compared with nontransformed control plants. Since our transgenic CMV-resistant homozygous tomato line also has resistance to TMV that is conferred by the *Tm-2*^{2} gene in the parental cultivar, it can be used as a breeding germ plasm to develop commercial hybrids resistant to both CMV and TMV, two important viruses that affect tomato crops.

Cucumber mosaic virus (CMV) occurs worldwide and causes severe damage in many vegetable crops (24). In tomato, economic losses due to CMV infections have been reported in many countries. Due to the severity of CMV epidemics, tomato production has been abandoned in some areas.

CMV is a cucumovirus consisting of isometric particles with a diameter of about 29 nm, three single-stranded positive sense genomic RNA species, and a fourth subgenomic RNA, which acts as the messenger RNA for the coat protein (CP), of about 24 kDa (24). In addition, some CMV isolates often support the replication, encapsidation, and spread of additional single-stranded RNA species of 330 to 391 nucleotides (nt) designated satellite RNA (24) that are involved in modulation of symptoms (18). CMV isolates carrying satellite RNA have been found associated with severe necrosis (18). However, most satellite RNA attenuate the symptoms induced by CMV on tomato (5,12,17). Based on serological relationships and nucleotide homologies, CMV isolates can be divided into two subgroups, designated I and II. Amino acid sequence homologies among CMV CP genes are 94 to 99% within isolates of the same subgroup, and 80 to 83% among isolates from different subgroups (24).

Resistance to CMV has been described in several wild tomato species; however, no commercial tomato variety with CMV resistance has been developed (32). Cross-protection with the mild CMV-S and -SR strains was investigated as a practical means to protect tomato plants against CMV infections (7,14). Benign satellite RNAs have been extensively used to control CMV (15,31). Deliberate inoculation of tomato with two mild CMV strains, CMV-S and -KO2, carrying a non-necrogenic satellite RNA, have been successful in protecting tomato plants against severe CMV strains (8,21). Disease incidence in protected tomato plants was remarkably reduced, and fruit production significantly increased. This strategy has been widely applied in The People's Republic of China (31). However, concerns about possible mutations of the satellite RNA and mild strains have restricted a broader application of this strategy.

Parasite-derived resistance (30) is an effective strategy recently applied to develop resistance against numerous plant viruses in transgenic crops. Several genetic engineering approaches have been attempted to control CMV. Transgenic tobacco (13,16, 19,25,34) and tomato (20,29) plants engineered to express constructs of CMV satellite RNA show some protection to CMV infections. Transgenic tobacco plants expressing a truncated CMV replicase gene display a high degree of resistance (1), but the resistance is serogroup and strain specific (35). Transgenic tobacco (6,22,23, 27), cucumber (11), and melon (10) plants expressing CMV CP genes or anti-sense CP gene constructs (6,28) show variable degrees of resistance against infections by CMV strains.

We reported on the development of transgenic tomato plants expressing the CP gene of the CMV subgroup II white leaf (WL) strain (33). Under greenhouse conditions, R_1 progeny of self-pollinated transgenic R_0 plants showed a high level of resistance to WL and Chi strain of subgroup I (33). The WL CP gene also conferred resistance against nine other CMV subgroup I isolates and two other CMV subgroup II isolates (26). Inoculated transgenic plants remained asymptomatic and virions could not be recovered from non-inoculated leaves (26,33). Resistance displayed by the transgenic plants was high in that protection was independent of virus inoculum titer (26). Here, we report on field evaluation of several of these transgenic tomato lines expressing the WL CP gene.

MATERIALS AND METHODS

Tomato genotypes and identification of transgenic seedlings. The transgenic hemizygous inbred lines TT5-007 and TT5-004 used in this study were described previously (33). Transgenic hemizygous line TT5-007 was self-pollinated to obtain a transgenic homozygous line designated TT5-007-11 (26). Line TT5-007-11 was further crossed with the nontransformed cultivar Solarset to develop the transgenic hybrid TT5-007-11 × Solarset. All transgenic genotypes expressed the WL CP gene, the selectable marker gene from the transposon *Tn5* coding for the enzyme neomycin phosphotransferase II (NPT II), and the screenable marker gene *UidA* coding for β-glucuronidase (22). In 1992, tomato seeds were germinated on wet filter papers and then transferred to the greenhouse to peat pots containing Cornell Mix

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(a mixture of peat, vermiculite, ground limestone, and Uni-mix 10-20-5). In 1993, tomato seeds were germinated in graded quartzite (Q-Rok, Pennsylvania Glass Sand Corp., Berkeley Springs, WV), and subsequently transferred to soil.

Transgenic seedlings of the two hemizygous lines were identified by enzyme-linked immunosorbent assay (ELISA) for expression of the NPT II protein using cotyledon or leaf samples, and commercial NPT II γ-globulins (5 Prime → 3 Primer, Boulder, CO). No NPT II ELISA was needed to identify transgenic seedlings of the homozygous and hybrid lines since the transgenes were known to be present in all seedlings of these two genotypes. Expression of the WL CP gene in transgenic plants was evaluated by ELISA using γ-globulins to CMV-WL developed in our laboratory.

Inoculations with CMV. Mechanical inoculations were performed on 14-day-old tomato seedlings 2 weeks before plants were transplanted to the field. Half of the seedlings were mechanically inoculated with CMV-Fny, a subgroup I endemic strain of New York. Corundum-dusted cotyledons and the first two leaves were rubbed with a 1:20 dilution of crude sap extracts of Fny-infected tomato cultivar G-80 in 1992, and Fny-infected summer squash cv. President in 1993. Inoculations were repeated 3 to 5 days later on the third and fourth leaves. The inoculum titer was estimated on *Chenopodium quinoa* Willd., a local lesion host for CMV. Plants were held for 1 week in a screenhouse before transplanting to the field.

Field site and layout. An isolated site was chosen on a Cornell University farm at Geneva, NY. The area was surrounded by woods and apple orchards. Field trials were conducted under USDA permits.

In 1992, squash and melon plants were also tested for CMV resistance in plots adjacent to the tomatoes. The tomato plot consisted of 24 rows 1.80 m apart and 24 plants spaced 0.60 m apart within each row. Two hemizygous transgenic inbred lines, TT5-004 and TT5-007, and the nontransformed parent line G-80 were tested. Each tomato genotype consisted of two treatments (mechanically inoculated and noninoculated plants) in a complete block design. For each treatment/genotype combination, eight replicates of 12 plants each were randomly allocated among four blocks (two replicates per block). In addition, inoculated nontransformed plants were planted at predetermined locations (6 plants per replicate, 4 plants per row every two rows) to ensure an even distribution of the CMV inoculum throughout the plot. These additional inoculated control plants constituted 7% of the total plants tested.

In 1993, the field trial was conducted at the same site but without cucurbits. The tomato plot consisted of 16 rows 1.80 m apart and 55 plants spaced 0.60 m apart within each row. The homozygous transgenic inbred line TT5-007-11, the trans-

Fig. 1. Reaction of tomato plants inoculated with cucumber mosaic virus. **(A)** Transgenic homozygous TT5-007-11 is resistant (left), while nontransformed parent G-80 is stunted with mosaic and fern leaf symptoms (right). **(B)** Transgenic hybrid TT5-007-11 × Solarset is resistant (right), and nontransformed Solarset is susceptible (left). **(C)** Fruit production of transgenic homozygous TT5-007-11 (top) versus that of nontransformed G-80 (bottom).

genic hybrid TT5-007-11 × Solarset, and the nontransformed parent lines G-80 and Solarset were tested. For the homozygous line and its parent line G-80, two treatments (mechanically inoculated and noninoculated plants) were selected in a complete block design. For each treatment/genotype combination, 9 replicates consisting of 20 plants each were randomly allocated among 2 blocks (2 or 3 replicates per block). In an adjacent field plot, the transgenic hybrid and its nontransformed parent cultivar Solarset were analyzed in a completely randomized design with 4 replicates of each treatment and 5 plants per replicate. To ensure good distribution of the CMV-Fny inoculum, additional inoculated nontransformed plants (2 plants per replicate, 5 plants per row) were planted at predetermined locations throughout the plot. These inoculated controls constituted 9% of the total plants.

Test plants were transplanted to the field on 6 July 1992 and 18 June 1993, respectively, to match the crop cycle with the time when the population of indigenous alate aphids is high in New York. No vector control measures were used because we wanted to evaluate the resistance of the transgenic plants to natural spread of CMV by aphids. Sencor, a pre-emergence herbicide, was applied and hand-weeding was done. At the end of the growing seasons, fruits were collected at the field site, analyzed for horticultural traits, and destroyed by autoclaving or burying them directly at the field site. To terminate the trials, Roundup herbicide was sprayed and the plant debris incorporated into the ground.

Resistance evaluation. Infection rates were estimated by monitoring symptom development, serological assays, and infectivity tests. Plants were scored weekly for CMV symptoms. Leaves in positions 1 to 3 on apical shoots of each test plant were sampled three times throughout the growing season and examined for CMV by ELISA (4) using antisera developed to CMV subgroup I. Such ELISAs allowed us to identify Fny-infected plants because subgroup I γ-globulins do not strongly cross-react with the WL (subgroup II) CP expressed in transgenic plants. Field samples were ground in 1 ml of extraction buffer (150 mM NaCl, 1.5 mM K_2HPO_4, 10 mM Na_2HPO_4, 2 mM KCl, 2% polyvinylpyrrolidone [PVP], pH 7.4). Samples were regarded as positive if the OD_{405} value was at least three times the average reading of the healthy controls. Control samples were healthy and Fny-infected nontransformed plants as negative and positive references, respectively, and noninoculated transgenic plants to estimate the constitutive WL CP expression level. If ELISAs were ambiguous, bioassays were performed by inoculating *Nicotiana benthamiana* Domin. or summer squash cv. President.

Horticultural performance. Plant height was measured as indicative of plant growth and vigor. Yield was evaluated by recording the total of all fruits produced per plant. In 1992, tomatoes were harvested once, and in 1993 tomatoes were harvested from each plant at three separate dates. Average fruit weight was also calculated.

Statistical analysis. Factorial field experiments were conducted to evaluate the combined effect of the two main factors (genotype × treatment) on CMV resistance. During 1992, a 3 × 2 factorial experiment was conducted to test three genotypes (two transgenic hemizygous lines and one control line) with two treatments (CMV inoculated versus noninoculated). During 1993, two 2 × 2 factorial experiments were conducted to test two genotypes (one transgenic homozygous line and one control line, or one transgenic hybrid and one control) versus two treatments (CMV inoculated versus noninoculated). Treatments were assigned to experimental units at random. Data collected on fruit number, fruit weight, and plant height was subjected to analysis of variance and regression analysis using SAS (SAS Institute, Cary, NC) to evaluate relationships among the variables.

RESULTS

Resistance evaluation. *1992 field trial.* Transgenic seedlings of the two hemizygous lines were identified by ELISA for the expression of the marker gene NPT II. A 3:1 ratio was obtained for transgenic line TT5-007 with 296 seedlings tested; 222 seedlings were NPT II positive, and 74 were NPT II negative, indicating a Mendelian segregation for the NPT II gene. For transgenic line TT5-004, 242 seedlings were tested; 151 were NPT II positive and 91 were NPT II negative, indicating a 1.6:1 segregation ratio for a single dominant gene. Expression of the WL CP gene was very high in the two transgenic lines with an average ELISA reading of 0.585 OD_{405} after 1 h substrate hydrolysis.

Transgenic tomato plants of the two hemizygous inbred lines TT5-004 (Fig. 1A) and TT5-007 showed high levels of resistance in that none of the 348 transgenic plants became symptomatic. Transgenic plants remained uninfected throughout the growing season, regardless of whether they were inoculated or not. In addition, none of the transgenic plants reacted positively in ELISA (Table 1). ELISA readings (OD_{405} values using subgroup I γ-globulins) were 0.022 for the buffer, 0.038 for uninfected nontransformed plants, 0.080 for transgenic plants, and 0.450 to 0.836 for infected nontransgenic plants after 1 h substrate hydrolysis. Moreover, CMV could not be recovered from leaves of transgenic plants to indicator host plants. Some of these symptomless indicator plants were tested for CMV infection by ELISA, but all reacted negatively. In contrast, mechanically inoculated nontransformed control plants were infected by CMV early in the season. Their infection rate increased from 55 to 78% 34 and 87 days post-planting, respectively. Infected plants showed reduced leaf laminae, mosaic, and severe stunting. There was good correlation between visual scoring and ELISAs since 80% of the control plants showed positive reactions for CMV infection at the end of the growing season (Table 1). Due to low aphid transmission, only a few noninoculated control plants became infected; 5% were symptomatic and 10% showed positive reactions in ELISAs by the end of the trial period (Table 1).

To identify possible host reservoirs for CMV, the weed population surrounding the experimental plot was randomly surveyed for the presence of CMV, which was detected in several dandelions (*Taraxacum officinale* Wigg.) showing viruslike symptoms.

1993 field trial. Expression of the WL CP gene was high in both the transgenic homozygous TT5-007-11 and hybrid TT5-007-11 × Solarset plants with an average ELISA reading of 0.465 OD_{405} after 1 h substrate hydrolysis.

All transgenic plants remained asymptomatic during the trial period and did not react for CMV in ELISAs (Table 1). The 355 homozygous (Fig. 1A,C) and 44 hy-

Table 1. Resistance evaluation of transgenic tomato plants against cucumber mosaic virus infections under field conditions

Year/line	Inoculated Plants		Noninoculated Plants	
	Infected/tested[a]	Infection (%)	Infected/tested[a]	Infection (%)
1992				
Transgenic hemizygous TT5-004	0/48	0	0/79	0
Transgenic hemizygous TT5-007	0/117	0	0/104	0
Control G-80	119/148	80	10/104	10
1993				
Transgenic homozygous TT5-007-11	0/176	0	0/179	0
Control G-80	65/176	37	16/175	9
Transgenic hybrid TT5-007-11 × Solarset	0/22	0	0/22	0
Control Solarset	17/22	77	3/22	14

[a] Number of plants positive in enzyme-linked immunosorbent assay about 3 months post-planting / number of plants analyzed.

brid (Fig. 1B) transgenic plants remained virus-free, regardless of whether they were inoculated prior to transplanting (Table 1). ELISA readings were similar to those for the 1992 field test. Indicator hosts inoculated with sap from transgenic plants remained symptomless. Conversely, disease incidence was significantly higher for the nontransformed control plants: 35% of the inoculated plants were symptomatic, and 37% reacted positively for CMV in ELISAs by the end of the season. Aphid vectors infected 9 and 14% of the uninoculated nontransformed G-80 and Solarset plants, respectively, as verified by ELISA at the end of the trial period (Table 1).

Yield data. *1992 field trial.* In 1992, not all fruits matured because plants were killed by early frost in September. Nevertheless, we measured yield of fruits with diameter >2.5 cm, and differences in yield were observed among treatments. Only 10% of the infected, compared with 39% of the noninfected, nontransformed plants produced fruits, while 24 to 40% of the transgenic plants produced fruits (Table 2). Infected nontransformed plants had significantly ($P < 0.01$) reduced production (16 versus 100% in fruit weight index) compared with noninfected counterparts (Table 2). In contrast, inoculated transgenic plants yielded significantly ($P < 0.01$) more (88 to 100 versus 16% in fruit weight index) than the inoculated controls, and performed as well as the noninoculated transgenic and the uninfected nontransformed plants (Table 2).

There were no significant differences in height between the two transgenic hemizygous lines (average value 61 ± 14 cm), regardless of whether the plants were inoculated (Table 2). Moreover, the heights of the transgenic plants and the noninfected controls were not significantly different ($P < 0.01$, Table 2). In contrast, infected control plants had 35% reduction in height ($P < 0.01$, 40 versus 62 cm), while the transgenic plants mechanically inoculated with CMV-Fny were not stunted (Table 2).

Analysis of variance was calculated only on plant height since fruits did not mature. In addition, only plants in block no. 1 were analyzed because differences in the results among the four blocks were significant ($P < 0.01$), likely due to poor drainage of three of the blocks. Data showed that the interaction genotype × treatment was significant ($P < 0.01$), and that the differences among transgenic and control lines were significant due only to treatment interactions. Basically, transgenic and nontransgenic tomato plants showed similar behavior, except after infection when controls became stunted.

1993 field trial. More than 90% of the transgenic and noninfected control plants produced mature fruits, while only 56% of the infected nontransformed plants produced fruits (Table 3). CMV caused a dramatic reduction ($P < 0.01$) in the number of fruit in control plants (2 versus 21) while transgenic homozygous plants showed number of fruits similar to that of noninfected control plants ($P < 0.01$, 16 to 19 versus 21, Table 3). CMV reduced fruit set by 96% (108 versus 2,440 g), and fruit weight by 53% (Table 3, Fig. 1C). No reduction in numbers or fruit weight was observed among treatments for the transgenic homozygous and the transgenic hybrid lines. Therefore, transgenic plants allowed a 17-fold increase in production (1,840 versus 108 g) with a 44% increase in fruit weight (54 versus 96 g). When performance was analyzed over time, the data showed differences among genotypes. Fruit number and yield increased over time for all genotypes but not for inoculated nontransformed plants. Inoculated and noninoculated transgenic plants performed similarly to uninfected nontransformed controls, but showed a dramatic increase in production compared with similarly inoculated controls (Fig. 2). In this study, the transgenic hybrids were not considered for yield, since the appropriate control, G-80 × Solarset, was not included in our trial.

Plant height was also used as a measure of vigor and growth. The average heights of the transgenic homozygous and the noninfected nontransformed plants were not significantly different ($P < 0.01$, 62 versus 65 cm, Table 3). The height of inoculated transgenic homozygous plants was similar to that of uninoculated transgenic counterparts. In contrast, CMV caused severe stunting of the infected nontransformed plants with a significant ($P < 0.05$) reduction in height (47 versus 65 cm, Table 3).

Analysis of variance showed that the interaction treatment × genotype was significant (Table 3), indicating that differences among genotypes developed only when plants became infected and stunted. Differences among blocks were not significant ($P < 0.01$).

DISCUSSION

Field evaluation showed that transgenic tomato plants expressing the CP gene of CMV-WL were highly resistant to CMV infections and had good yield perfor-

Table 2. Plant height and fruit yield of transgenic hemizygous tomato lines TT5-004 and TT5-007, and nontransformed tomato G-80 in a 1992 field trial

Line	Treatment	No. plants tested	Plant height (cm)	Plant with fruits (%)[a]	Fruit weight per plant[b]
Transgenic TT-004	Noninoculated	79	61 ± 16	24	91
	Inoculated[c]	48	62 ± 15	40	88
Transgenic TT-007	Noninoculated	104	62 ± 13	29	100
	Inoculated	117	60 ± 14	24	89
Nontransformed G-80	Noninoculated	104	62 ± 13	39	100
	Inoculated	119	40 ± 10	10	16

[a] Fruits with diameter >2.5 cm. None of the fruits were mature.
[b] Index relative to the fruit weight of noninfected nontransformed plants (100%).
[c] Plants inoculated in the greenhouse with CMV-Fny on 24 June, and transplanted to the field on 6 July.

Table 3. Plant height, fruit yield, and summary of analysis of variance for transgenic homozygous tomato line TT5-007-11, and nontransformed tomato G-80 in a 1993 field trial

Line	Treatment	No. plants tested	Plant height	Plants with fruits (%)	Fruits no./ plant[b]	Yield (g)/ plant	Fruit weight (g)
Transgenic TT5-007-11	Noninoculated	179	60 ± 11	91	16 ± 9	1,520	95
	Inoculated[a]	176	64 ± 12	92	19 ± 11	1,840	97
Nontransformed G-80	Noninoculated	175	65 ± 10	92	21 ± 13	2,440	116
	Inoculated	65	47 ± 11	56	2 ± 3	108	54

		Plant height		Fruit number		Yield	
Source of variation	df	Mean square[c]	*F* value	Mean square	*F* value	Mean square	*F* value
Model	4	759	40.13	1,256	56.59	18,317	54.57
Block	1	406	21.49	744	33.54	6,756	20.13
Genotype (G)	1	589	31.14[d]	1,964	88.53[d]	18,059	53.80[d]
Treatment (T)	1	928	49.08[d]	2,008	90.50[d]	33,972	101.21[d]
G × T	1	2,213	117.07[d]	2,614	117.81[d]	45,930	136.83[d]
Error	519	19		22		336	

[a] Cumulative values on 2, 8, and 29 September 1993.
[b] Plants were inoculated in the greenhouse with CMV-Fny on June 6 and transplanted to the field on 18 June.
[c] Mean squares were derided from type III sums of squares for the general linear model.
[d] Significant at the 0.01 probability level

mance. The resistance was very high at either a homozygous, hemizygous, or a hybrid form, thus indicating a resistance of potential commercial value. Additionally, these transgenic tomato plants are resistant to mechanical inoculations of 13 CMV isolates belonging to subgroups I and II that originated from different geographic regions (26). This type of broad resistance was initially observed with transgenic tobacco expressing the WL CP gene (22). So far, only a single variant of the CMV-WL satellite RNA has been reported to overcome the resistance of the transgenic tomato plants (26), and this was observed under greenhouse conditions. This is the first report of a tomato line exhibiting such a high level of resistance to CMV.

Numerous efforts have been devoted to control CMV in tomato. Natural genes for resistance have not been incorporated into commercial cultivars (32). Cross protection strategies that are based on use of attenuated CMV strains (7,14) or benign satellite RNA (8,15,21,31) have shown effectiveness, but they are labor intensive and require regular certification of the inoculum. Transgenic plants expressing CMV satellite RNA constructs have shown a narrow spectrum of protection (20,25,29). High natural variability of satellite RNA might be another limitation to their use (2). It is clear from this work and recent results (26, 33) that the broad resistance of transgenic tomato plants expressing the CMV-WL CP gene offers a potentially effective and convenient means to control CMV in tomato plants on a worldwide basis.

Our results suggest that the transgenic homozygous inbred line can be used as breeding germ plasm to develop hybrids that are resistant to CMV and have commercial value. Although the transgenic homozygous line is highly resistant to CMV infections, horticultural characteristics can be improved. Therefore, selected fruits of the transgenic homozygous plants were harvested, and their progenies will be improved for horticultural traits by classical breeding.

One of the objectives of our field trial was to evaluate the resistance of the transgenic plants to natural transmission of CMV by aphids. However, only 9 to 14% of the noninoculated control plants became infected by aphids even though 10 to 20% of the total number of plants were used as primary inoculum. In contrast, CMV-Fny spread rapidly in cucurbit plots that were located close to the tomato fields. The poor aphid transmission obtained in our field trials with tomato plants may be attributed to host preference by aphids or to probing inhibition by glandular tomato trichomes (3). Since natural transmission of CMV was limited in Geneva, NY, our transgenic tomato plants need to be field tested at other locations where CMV spread occurs readily. Nevertheless, our conditions allowed us to compare the fruit yield of transgenic versus noninfected control plants within the same field test plots.

The infection rate of the nontransformed plants, which was 80% with infected tomato plants as inoculum, dropped to 37% with infected squash plants as inoculum. This poor infection rate is due primarily to the use of squash as inoculum. Similar observations have been made previously (R. Provvidenti, unpublished).

The transgenic homozygous line TT5-007-11 is resistant to two important viral pathogens of tomato, CMV and tobacco mosaic virus (TMV). The resistance to CMV is conferred by the WL CP gene and the resistance to TMV is conferred by the *Tm-2*^{2} gene, which is present in the chromosomal background of the parent line G-80 (33). The breadth of resistance of the homozygous transgenic line can be further improved by incorporating resistance genes to other viruses. We recently crossed our homozygous CMV-resistant line with a transgenic G-80 line expressing the nucleocapsid gene of tomato spotted wilt virus (TSWV). Transgenic progeny showed resistance to both CMV and TSWV (9).

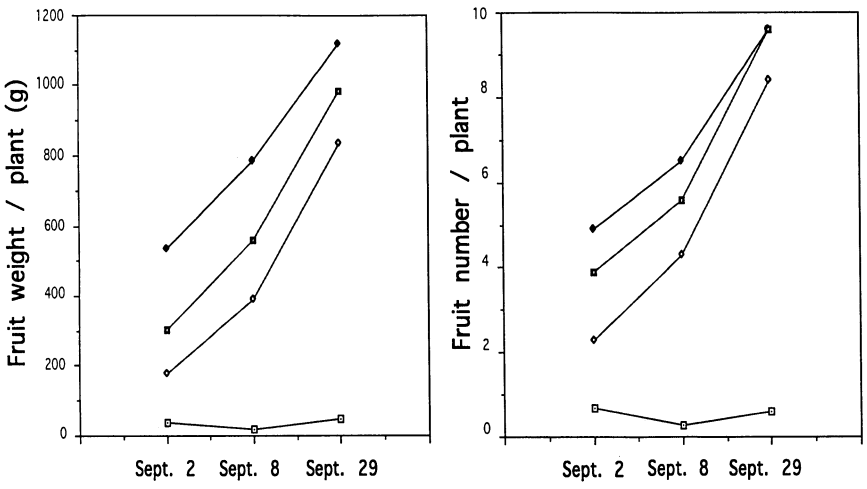

Fig. 2. Average fruit weight and number of fruits per plant on three different dates for uninoculated (open diamond) and inoculated (open rectangle) transgenic homozygous TT5-007-11, and uninoculated (solid diamond) and infected (open rectangle with dot) nontransformed G-80.

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