REACTION OF SUSCEPTIBLE AND RESISTANT TOMATO GENOTYPES TO TOBACCO MOSAIC VIRUS IN SOUTHWESTERN ONTARIO

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Abstract

Tomato genotypes with resistance or tolerance to tobacco mosaic virus (TMV) were tested with 27 TMV isolates from Essex County, Ontario. Genotypes containing genes Tm-2^2 or Tm-2 resisted all the isolates. Those containing Tm-2^2 were agronomically better than those with Tm-2, and the performances of some cultivars with Tm-2^2 that have been released for commercial use were assessed. Although the resistance of these cultivars to TMV was usually satisfactory, small plants developed systemic necrosis when inoculated and kept in unusually hot greenhouse conditions.

Introduction

Because of the trend toward monoculture, greenhouse tomatoes (*Lycopersicon esculentum* Mill.) are usually infected with tobacco mosaic virus (TMV) in southwestern Ontario. Even after steaming or fumigating the soil, the reservoir of virus is not completely eliminated in root debris, so that the next crop becomes infected about 1 month after transplanting. Breeding for resistance has made progress in recent years and this work has been reviewed by Pelham (10). The gene Tm-1 gives a tolerance to the virus. The genes Tm-2 and Tm-2^2 cause a hypersensitive reaction to the virus, resulting in a form of resistance.

Selections of *Lycopersicon* species were used by McRitchie and Alexander (8) to group isolates of TMV found in Ohio into 4 classes or strains. Genotypes with Tm-2^2 resisted all four strains. More recently, a further TMV strain, V, was found to overcome the resistance of plants heterozygous for Tm-2^2 at 80-85°F, (27-29C), but not at 60° F(16C) (4).

Since several tomato genotypes with tolerance or resistance to TMV are being grown to a limited extent in Essex County, it was necessary to determine their reaction to a spectrum of local isolates. Genotypes containing Tm-1 and Tm-2 were kindly supplied by Dr. E.A. Kerr, Horticultural Research Institute of Ontario, Vineland Station, Ontario, and genotypes with Tm-2^2 by Dr. L.J. Alexander, Ohio Agricultural Research and Development Center, Wooster, Ohio. The Tm-1 used by Dr. Kerr was from J.M. Waltz's material and appears to be the same as Tm-1 of Holmes and other workers (6). Tm-2^2 was derived by Alexander from *Lycopersicon peruvianum* (L.) Mill. P. I. 128650 (1). We are reporting the behavior of these genotypes when grown under normal spring and fall greenhouse cropping conditions.

Methods

Virus isolates

Twenty-two isolates were obtained from greenhouse tomato crops in Essex County and five from locally available seed. Isolates from seed were multiplied in burley tobacco; for other isolates the original tomato leaves were frozen until required. To inoculate plants, infected leaf tissue was ground in 0.1 M phosphate buffer of pH 7 and rubbed onto leaves powdered with carborundum dust. All soils, pots, and other containers for growing plants were steamed before use.

Preliminary tests with young potted plants

Sets of five seedlings of about 5 cm diameter, planted around the edges of pots 13 cm in diameter, were inoculated on the cotyledons and first true leaves. Symptomless plants were tested for virus by inoculation, as above, of leaves of *Nicotiana glutinosa* L., or *N. tabacum* L. cv. Samsun NN.

Tests under normal greenhouse crop conditions

Seeds were sown in flats and seedlings transplanted into pots 10 cm in diameter. Seedlings were transplanted into the greenhouse when 7 weeks old and about 20 cm tall. Plants were 45 cm apart in double rows 55 cm apart, with 75 cm between the pairs of rows. Normal greenhouse procedures were followed in growing the crops.

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Results

A. PRELIMINARY TESTS

The 27 TMV isolates were inoculated, usually individually, to 10 seedlings of each host genotype. All TMV isolates infected all plants of the susceptible cultivar Michigan-Ohio Hybrid, so that symptomless plants of other genotypes were unlikely to have escaped being exposed to infection.

One isolate induced very marked narrowing of the leaflets of Michigan–Ohio Hybrid, but the isolates differed more in symptom severity than in types of symptoms. The degree of multiplication in the plant cells seemed to be involved because isolates to which Lycopersicon peruvianum (L.) Mill. and Lycopersicon peruvianum var. humifusum C. H. Mull. reacted with mosaics tended to vroduce more necrosis on hypersensitive -tomato genotypes than did isolates for which these plants were symptomless carriers.

(i) Reaction of genotypes containing Tm-2.

The three genotypes of Tm-2^2 used (Table 1, entries 1, 4, and 5) were homozygous or nearly so for this gene. They resisted all the virus isolates. Five hundred and sixty-seven seedlings gave resistant (hypersensitive) reactions, evidenced by lack of visible symptoms, by occasional local lesions without further symptoms, or, in eight seedlings only, by systemic necrosis. Eighteen plants of one genotype gave normal mosaics and presumably were segregates lacking the resistance gene. Symptomless plants, however, occasionally contained small amounts of TMV.

(ii) Reactions of genotypes containing Tm-2

Two genotypes of Tm-2 (Table 1) were tested. Plants showing the netted virescent character, which would be homozygous for Tm-2, were discarded. Seedlings groups consisted therefore of plants heterozygous for Tm-2 and plants lacking the resistance gene.

For all the virus isolates, each genotype contained some plants which gave resistant (hypersensitive) reactions. In all, 336 seedlings gave resistant reactions and 150 gave normal mosaics. Of the 336 hypersensitive plants, 67 showed systemic necrosis. Eighteen of the 27 virus isolates produced this symptom on occasion. Resistant symptomless plants occasionally contained small amounts of TMV.

(iii) Reactions of genotypes containing Tm-1

Eleven genotypes were tested with groups of virus isolates. All genotypes contained some plants that developed mottle or mild mosaic. Some genotypes reacted only in this way; others contained some plants that were symptomless carriers of the virus.

B. TESTS OF EXPERIMENTAL RESISTANT GENOTYPES UNDER NORMAL GREENHOUSE TOMATO CROP CONDITIONS

The genotypes listed in Table 1 were grown under fall and spring greenhouse conditions (see Methods and Table 1). The fall experiment was planted in the greenhouse on Aug. 21 and plants were inoculated with TMV on Aug. 23 with a typical local TMV isolate. Fruits were harvested between Oct. 29 and Dec. 5. In the spring experiment, plants were set in the greenhouse on Jan. 30 and inoculated on Feb. 20 with a mixture of the 27 local isolates of TMV used in the preliminary tests. Plants were harvested between April 29 and June 25. Plants in the check plots were not inoculated but in both experiments became naturally infected about 1 month after planting.

(i) Genotypes containing Tm-2^2

All except 3 of the 72 plants inoculated remained free from typical TMV symptoms and appeared essentially healthy. However, traces of TMV were occasionally detected in leaves, suckers, and fruit, and occasional fruit lesions and slight leaf mottle were noted. Yields were good (Table 1), but the fruit had somewhat greenish, ribbed shoulders.

Three inoculated plants developed systemic necrosis. The results of later experiments suggested that young plants inoculated during a period of hot weather are more likely to become systemically necrotic than young or older plants grown under cool conditions following inoculation.

(ii) Genotypes containing Tm–2

Fifteen of 24 plants were resistant (hypersensitive) and two more reacted with systemic necrosis. The other seven shaved normal mosaics and presumably did not contain the resistance gene. Later all plants showed mild mosaic symptoms. Resistant plants had lesions on the fruits, which were hollow and of poor shape.

(iii) Genotypes containing Tm-1

Plants of 'Vendor', like the susceptible cultivars used as checks, showed mild to bright mosaics on the leaves and produced fruit which was of good appearance but contained much TMV.

C. TESTS OF COMMERCIALLY AVAILABLE RESISTANT AND SUSCEPTIBLE GENOTYPES

Plants of M-R9, M-R12, W-R25, Michigan–Ohio Hybrid, and Vendor were grown under normal fall and spring greenhouse conditions (see Methods and Table 2). M–R9 and M-R12 are cultivars containing Tm-2^2 released by Alexander (2, 3). Vendor contains Tm-1 (9).

Table 1. Yields of tomato genotypes with genes for resistance to tobacco mosaic virus when grown under normal greenhouse crop conditions* in fall and spring and inoculated with isolates of TMV from Essex County, Ontario

Resistance gene and genotypes		Total yield (g/plant)			
		Fall		Spring	
		Not Inoc.†	TMV**	Not Inoc.†	TMV††
Tm-2^2	66.2144	1630	1745	4897	4114
	64.2144-1^u	2170	1807	4658	5266
	64.2119-B2+	1934	1828	5207	5292
	66.2156	1577	2067	5045	4856
	64.2132-1^u	2014	1979	4411	3524
	66.2161	1852	1609	5198	4913
Mean		1863	1839	4903	4661
Tm-2	153-5	1466	1592	4814	3119
	167-7-10	1453	1616	3457	2607
Mean		1460	1604	4136	2863
Tm-1	Vendor	1424	1383	3547	3411
Susceptible checks					
	Vinequeen	1640	1597	3826	3721
	Michigan-Ohio Hybrid	1795	1733	4486	4717
Mean		1718	1665	4156	4219

* Temperatures (°C):

Fall			Spring			
Weeks of	Night	Day high (2-5 hr)	Weeks of	Night	Day high (2-5 hr)	Occasionally
Aug. 16	21-24	38	Feb. 5-Feb. 26	20	24-27	32
Aug. 23-Sep. 27	16-21	30-35	Mar. 5-Apr. 16	16-17	21-24	30
Oct. 4-Oct. 11	19	24-27	Apr. 23-June 18	16-18	24-30	32
Oct. 18-Dec. 6	19	19-21	June 25	19	35-38	

† Susceptible genotypes became naturally infected about 1 month after transplanting.

** Inoculated 2 days after transplanting with a typical local isolate.

†† Inoculated 3 weeks after transplanting with a mixture of 27 local isolates.

(i) Fall crop 1970

Plants were transplanted into the greenhouse on August 4 and inoculated on August 6 with a mixture of the 27 local isolates used previously. Eleven days later, inoculated plants of Vendor and the two susceptible varieties, Michigan–Ohio Hybrid and W-R25, showed mosaic symptoms, and 7 of the 40 plants of M-R12 had developed areas of systemic necrosis.

Unexpectedly by Sept. 3, 21 of the 40 inoculated plants of M–R9 and 29 of those of M-R12 had developed severe systemic necrotic reactions. These plants made little further growth and produced only unmarketable fruit with brown necrotic areas. This reaction seems to occur when several days of hot greenhouse conditions follow the inoculation of small plants. Noninoculated plants of susceptible cultivars showed clear mosaic from natural infection on Sept. 3, but noninoculated plants of M–R9 and M-R12 showed only local areas of necrosis and by October, except for one plant, appeared to be in good condition.

Yields of M–R9 and M-R12 in the noninoculated but naturally infected plots were similar to those of W-R25 and Michigan-Ohio Hybrid (Table 2). Inoculated plants of M–R9 and M-R12 that resisted systemic necrosis yielded well under reduced competition from the systemically necrotic plants. However, their fruits, especially those of M-R12, on occasion developed necrotic blotches. One truss on a naturally infected plant of M–R9 showed these blotches.

Young and old leaves of inoculated plants of M-R9 and M-R12 that resisted systemic necrosis contained traces of virus. Top shoots of the severely necrotic plants contained much virus.

Table 2. Yields of commercially available tomato cultivars with genes for resistance to tobacco mosaic virus when grown under normal greenhouse crop conditions* and inoculated with isolates of TMV from Essex County, Ontario

Cultivar	Total yield marketable fruit (g/plant)					
	Fall 1970		Spring 1971			Fall 1971
	Not Inoc.†	TMV§	Not Inoc.†	TMV**	TMV††	TMV§§
Resistant						
M-R9	2053	2513	5132	4992	4849	1283
M-R12	2553	2874	5242	5064	4918	1194
Non-resistant						
W-R25	2105	2171	4510	4014	4231	1595
Michigan-Ohio Hybrid	2219	2031	4841			1733
Vendor	1952	1924				2365

* Temperatures (°C):

Fall 1970

Weeks of	Night	Day high (2-5 hr)
Aug. 4-Aug. 26	16-21	35-38
Sept. 2-Sept. 9	16-21	30-32
Sept. 16-Sept. 23	19-21	27-30
Sept. 30-Oct. 15	17-19	24-27
Oct. 23-Dec. 4	17-18	19-21

Spring 1971

weeks of	Night	Day high (2-5 hr)	Occasionally
Jan. 25-Feb. 25	19-21	24-27	30
Mar. 4-May 7	17-18	24-27	30
May 14-June 23	19-21	27-32	35

Fall 1971

Weeks of	Night	Day high (2-5 hr)
July 30-Aug. 20	18-22	38
Aug. 30-Sept. 29	18-22	27-35
Oct. 6-Oct. 27	17-20	22-24
Nov. 3-Dec. 1	18	18-21

† Susceptible cultivars became naturally infected about 1 month after transplanting.

§ Inoculated 2 days after transplanting with a mixture of 27 local isolates. Yields from plants without systemic necrosis.

** Inoculated 2 weeks after transplanting with the mixture of 27 local isolates or with sap from plants with systemic necrosis.

†† Inoculated 3 weeks after transplanting with the mixture of 27 local isolates or with sap from plants with systemic necrosis.

§§ Inoculated 4 weeks after transplanting with sap from plants with systemic necrosis.

(ii) Spring crop 1971

In the spring crop, cultivars **W–R25, M-R9, M–R12,** and Michigan–Ohio Hybrid were transplanted to the greenhouse on Jan. 25–27. Plots to receive virus were inoculated 2 weeks after transplanting, when about 30 *cm* high, or a week later when about 50 cm high. Some plots were inoculated with a mixture of the 27 isolates used previously; others with sap from severely necrotic shoots of **M-R9** and **M–R12** from the previous fall crop.

Inoculation of young plants of **M-R9** and **M-R12** with either virus inoculum had caused no systemic necrosis 1 month later, except in two plants of **M–R12** inoculated 2 weeks after transplanting with sap from necrotic plants of **M-R9** and **M-R12.** Apart from these two plants, the resistant cultivars showed no virus symptoms during the season, whereas **W-R25** inoculated 2 weeks after transplanting showed good symptoms and retarded growth. Occasional fruit lesions were seen on **M-R12** in April–June, and more developed on both resistant varieties when the temperatures rose later in the season.

Until April 20, **W–R25** showed little yield loss from TMV infection, but its yield was eventually reduced, especially in plants inoculated early (Table 2).

(iii) Fall crop 1971

Plants were transplanted on July 30 and were inoculated with ***TMV*** on an upper leaflet on Sept. 1, using sap from necrotic leaves from the fall crop of 1970. Daytime high temperatures in September were 27–35" C compared with 38° C in August. Symptoms appeared 8 days after inoculation in the susceptible cultivars, but the resistant cultivars under these conditions showed no sign of the systemic necrosis that was so frequent in the fall crop of 1970.

Yields of marketable fruit of M-R9 and M-R12 were less than those of W-R25 and Michigan-Ohio Hybrid, and about half the yield of Vendor (Table 2). Total yields (marketable and unmarketable) of M-R9 and M-R12 were 2202 and 2452 g/plant respectively, compared with 2942 from Vendor, but in this experiment much fruit of the two resistant varieties was discarded because of blotchy ripening, hollowness, and blossom-end rot. Michigan-Ohio Hybrid and W-R25, with total yields of 2794 and 2523 g/plant respectively, suffered less from these disorders.

Discussion

The term "resistant" is used here to describe plants which remain as a whole free of nearly free from TMV after inoculation, although the cells whose hypersensitive reaction localizes the virus and prevents its spread are themselves susceptible. Plants which show mild or no symptoms, but which contain much virus, are considered "tolerant".

None of the 27 isolated from Essex County failed to elicit the resistance reaction of Tm-2 and Tm-2^2. Hence TMV strain IV of Alexander — was not among these isolates. Local isolates would come into Alexander's classes I - 111, corresponding to Pelham's 0 and 1 (0 = I + II) (11). Separation of our isolates into classes I, II and III is not possible since they were only tested in groups on genotypes with Tm-1, but the presence of symptomless carriers and of plants with mosaics suggests the presence of strains II and 111. Two hundred and nine isolated collected by Fletcher and MacNeill (5) from tomato crops in southern Ontario came into Pelham's classes 0 and 1, mostly into class 0.

The gene Tm-2^2 showed most promise for TMV resistance and crop quality. However, the development of systemic necrosis in plants homozygous for this gene was a feature of the fall experiment of 1970. In this experiment plants about 20 cm high were inoculated and exposed for several days to temperatures of 35-38 C during the middle of the day. Cirulli and Alexander (4) suggested that the occasional appearance of systemically necrotic plants might be related to inoculum dose and incubation temperature. They did not specify that only their strain V is involved, though this strain was mentioned, and their high temperatures were 27-28°C. At 24-27°C and 27-35° C and with older plants, this necrosis did not develop in our experiments. The virus in our systemically necrotic plants merits study as a possible strain with type V characters, but it may well be that at 35° C the hypersensitive reaction fails to restrict TMV strains in general, as is the case in Nicotiana glutinosa.

The virus that occurs in low levels on occasion in nearly resistant plants homozygous for Tm-2^2 also merits study. Cirulli and Alexander report a similar finding (4). It may be that the hypersensitive reaction is modified in older plants, permitting some virus development. The presence of some TMV in these plants increases the likelihood of development of strains unaffected by the resistance mechanism. Strain selections or strain changes in resistant tomatoes have been shown by MacNeill and Fletcher (7).

In Essex County the two commercial resistant varieties M-R9 and M-R12 if protected from early inoculation and high temperatures appear to resist TMV satisfactorily. To date, M-R12 has been found slightly more susceptible to systemic necrosis and fruit necrosis than M-R9. However, Vendor, Michigan-Ohio Hybrid, and Vinequeen give fruit of good quality even though foliage and fruit contain much TMV. Further study of resistant genotypes meeting the precise nutritional requirements of these cultivars is necessary to determine their usefulness to the greenhouse industry.

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