Extreme Resistance to Tomato Yellow Top Virus and Potato Leaf Roll Virus in *Lycopersicon peruvianum* and Some of its Tomato Hybrids

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ABSTRACT

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Plants in populations of *Lycopersicon peruvianum*, U.S. Department of Agriculture Plant Introduction (P. I.) 128655, and two, selected F_5 hybrid progenies of P. I. 128655 × *L. esculentum* were graft inoculated with specific isolates of tomato yellow top virus and potato leaf roll virus. Both viruses could be recovered from some, but not all, of the plants of both the parent and the hybrid populations 9 wk later by graft or aphid transmission. Virus isolates that could not be recovered from a plant would not pass through stem sections of the same plant that were grafted between infected stocks and healthy, susceptible scions in 16 wk, but limited movement into one stem section was detected at 16 wk. Plants that were not invaded by one virus isolate often were invaded by other isolates of the same virus when challenged by graft inoculation. Invasion required extremely long periods of union between test plants and infected graft scions. Virus isolates that could not be recovered from plants 8 wk after graft inoculation often were recovered from the same plants after 24 wk. Virus isolates recovered from plants after graft inoculation frequently were not retained in new growth on cuttings of the same plants after severance from the scion source of virus. The longer the time required for virus to invade a test plant, the less likely it was that the virus would be retained after separation from the scion. Virus concentration in plants that retained virus was below that detectable by enzyme-linked immunosorbent assay. All 16 P. I. 128655 plants tested against 10 virus isolates contained at least one isolate 24 wk after graft inoculation, but five of the plants would not retain any of the isolates independently of the infected scion.

Tomato yellow top disease was first reported as a serious disease of tomatoes (*Lycopersicon esculentum* Mill.) in Brazil (7). It occurs in New Zealand (1) and has devastated tomato crops in Australia (5). Since its first detection in the United States in 1973 (18), it has remained prevalent in the Yakima Valley of eastern Washington, where it seriously threatens the economic potential of cultivated tomatoes. More recently the disease appeared in major tomato-growing regions of Florida (19), and we have isolated the virus from tomato plants with typical symptoms collected in California (Hassan and Thomas, *unpublished*).

We have determined the host range, symptomatology, and transmission properties (10) of the tomato yellow top virus (TYTV) from Washington and have pointed out etiological distinctions between TYTV and the related viruses: potato leaf roll virus (PLRV) and beet western yellows virus (BWYV) (9). Although TYTV is serologically indistinguishable with polyclonal antisera from most virus isolates that cause potato leaf roll disease (Hassan and Thomas, *unpublished*), it causes a severe disease of tomato that is not caused by isolates of PLRV (10), and its epidemiology is not associated with that of potato leaf roll disease (9). The TYTV of Washington is composed of a complex of variants and is serologically related to the TYTV of Australia (14). It is more distantly related to BWYV (Hassan and Thomas, *unpublished*).

Adequate resistance to control tomato yellow top disease has not been reported in domestic tomatoes. Among hundreds of tomato breeding lines screened in eastern Washington primarily for resistance to beet curly top virus (BCTV), no strong resistance to tomato yellow top was noted. (M. W. Martin, *personal communication*).

In a search for resistance to TYTV, we examined a number of U.S. Department of Agriculture Plant Introduction (P.I.) collections of *L. peruvianum* (L.) Mill., *L. peruvianum* var. *dentatum* (L.) Mill., *L. hirsutum* (L.) Mill., and *L. glandulosum* (L) Mill. (13). Several collections contained resistant plants. *L. peruvianum* P.I. 128655, which contained both resistant and susceptible plants, was of particular interest because it also had contained resistance to BCTV (15,17) and because we had already hybridized BCTV-resistant selections with the cultivated tomato cultivar Bonnie Best (Hassan and Thomas, *unpublished*).

In another study published as a companion paper (16), we found that neither TYTV nor PLRV could be recovered by graft or aphid (*Myzus persicae* (Sulz.) transmission from plants of P. I. 128655 or two of its hybrid progenies after they were aphid inoculated.

After the same plants were graft inoculated, aphids recovered both viruses from some plants but not others. The companion paper (16) deals with the resistance of plants that apparently could not be infected by aphid inoculation but could be infected by graft inoculation. This study deals with the resistance of the apparently immune plants that could not be infected by graft inoculation

MATERIALS AND METHODS

Relative concentrations of virus in susceptible and resistant tissues were compared using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) as described by Clark and Adams (6). Gilford EIA plates (50-well, polystyrene) were precoated with purified gamma globulins and incubated overnight at 4 C with 200 μl of sample in each well. After washing, they were incubated for 4 hr at 37 C with 200 μl of conjugate in each well. Plates then were washed with PBS-Tween buffer and incubated at 23 C with 200 μl of *p*-nitrophenol phosphate substrate added to each well. Optical density was read after 1 hr at 405 nm using a Gilford model EIA Manual Reader. Gamma globulins and conjugates were used at concentrations of 1 μg/ml in all assays.

All other materials and methods were identical to those described in the companion paper (16).

RESULTS

Transport through stem sections of resistant plants. The fact that neither TYTV nor PLRV could be recovered from some plants of P. I. 128655 and two of its hybrid progeny populations that were graft inoculated with infected tissue suggested that the viruses either could not translocate through the test tissues or were inactivated when they did. To test this hypothesis, we determined whether virus would pass through 15-cm-long stem sections of resistant plants grafted between systemically infected *Datura tatula* stocks and healthy, susceptible *D. tatula* scions. Stem sections were selected from six P. I. 128655 plants and three plants of each of two F_5 hybrid progenies (16). The stem sections were taken from plants that had indexed free of virus 12 wk after graft inoculation with isolates of TYTV or PLRV and grafted to terminal shoots of *D. tatula* infected with the same virus isolates. As controls, healthy *D. tatula* stem sections were grafted between infected stocks and healthy shoot tips of the same species. The healthy *D. tatula* terminal shoots and the axillary shoots that grew from the control and the *L. peruvianum* and hybrid stem sections were indexed by aphid transmission twice, at 8 and 16 wk after grafting.

After 8 wk, virus was recovered by aphid transmission from all of the terminal shoots on control plants, all of which had clear symptoms. Clearly, both PLRV and TYTV passed from the infected stocks through the *D. tatula* stem sections and infected the terminal *D. tatula* shoots of control plants. However, all of the terminal shoots grafted on the test plant stem sections remained symptomless, and virus was not recovered from any of them at 8 or 16 wk after grafting. Virus was not recovered from any of the axillary branches from the test plant stem sections after 8 wk. However, TYTV was recovered from the first axillary branch above the graft union of one stem section after 16 wk. The branch arose about 1 cm above the virus source.

Virus isolate specific response. Experiments were conducted to determine whether the apparent graft immunity to one isolate of virus was effective against other isolates of the same virus. To test this, eight groups of P. I. 128655 plants were graft inoculated with a different isolate of TYTV or PLRV. After 9 wk, the plants that indexed positively by aphid transmission to indicator hosts were eliminated. Two plants from which virus could not be recovered after 9 wk were selected from each of the eight groups, and 10 rooted cuttings were propagated from each of these 16 plants. The 10 clonal propagants of each plant were each challenged against a different virus isolate by graft inoculation with infected *D. tatula* scions. Before inoculation, each propagant had been pruned to produce three basal shoots. When the shoots were 30- to 40-cm tall, an infected scion was grafted at the apex of each shoot. All of the propagants were indexed after 8 and 24 wk by aphid transmission to *P. floridana* seedlings from new axillary shoots on each inoculated shoot.

All plants remained symptomless. After 8 wk, virus was recovered from 27 of the 160 clone-isolate combinations (Table 1). No virus isolates were found in any propagants of four clones, but one or more isolates were recovered from each of the remaining 12 clones. After 24 wk, virus was recovered from 51 additional clone-virus combinations. Although virus still could not be recovered from more than half of the clone-virus combinations, at least two virus isolates were recovered from every clone.

To determine whether the propagants from which virus was recovered would retain the virus if grown independently of the infected graft scions, six subcuttings were propagated from each propagant that contained virus. New growth on the subcuttings was indexed by aphid transmission 8 wk later. The subcuttings of only 26 of the 78 propagants that contained virus 24 wk after graft inoculation retained it when severed from the scion source of virus (Table 1). Nearly half (13 of 27) of the propagants that contained virus after 8 wk retained the virus in new growth after the infected scions were severed. In contrast, only 13 of the 51 additional propagants that acquired virus between 8 and 24 wk after inoculation retained the virus in new growth after the infected scions were severed. Three of the six subcuttings from each propagant were incubated in a fiberglass-covered house, and three were incubated in a glasshouse. Virus was retained in all three

TABLE 1. Recovery of tomato yellow top virus (TYTV) and potato leaf roll virus (PLRV) isolates 8 and 24 wk after scion graft inoculation of *Lycopersicon peruvianum* P. I.[a] 128655 plants that were previously not infected after graft inoculation with other isolates of the same viruses[b]

Virus isolate	Plants immune to individual virus isolates															
	TYTV-1[c]		TYTV-17		TYTV-79		TYTV-84		TYTV-85		TYTV-87		TYTV-106		PLRV-1	
	2	3	3	4	1	4	2	4	1	2	9	10	5	6	2	3
TYTV-1	−+[d]	−−	−+	++	−+	+−+	−−+	−−	−−+	−−	−−+	−−	−−	−−	+−+	+−+
TYTV-17	−−	−−	−−	−−	−−+	−−	−−	−−+	−−+	−−	−+	−+	+,+	−−	−+	+,+
TYTV-79	−−	−−+	−−+	−−+	−−	−−+	−−+	−−	−−	−−+	−−+	+−+	+,+	−−	−−	−−
TYTV-84	−−	−−	−−	−−	−−	−−+	−−	−+	−−	−+	−−+	−+	−−	−−	−−	−−
TYTV-84A	−−	−−	−−	−−	+−+	−−	−−	+,+	+−+	−−	−−	+−+	+,+	−−	−−	−−
TYTV-85	−−	−−	−−	+,+	−+	−−	−−	−−+	−−	−−	−−	−−+	+−+	−+	−−	−−+
TYTV-86A	−−+	−−+	−−+	−−	−−	−−	−−	−−	−−	−−+	−+	−−+	+−+	−−	−−+	+,+
TYTV-87	−−+	−−	−−	−−	−−	−−	−−	−−+	−−	+−+	+−+	−−	+,+	−−	−−	−−
TYTV-106	−−	−−	−−	+−+	+,+	−−	+,+	−+	−−	−−	−−+	−+	+−+	−−+	−−+	−−+
PLRV-1	−−	−−	−−	−−+	−+	−−+	+,+	−−	−−+	−−+	−+	+,+	+−+	−−	−−+	−−

[a] P. I. = U.S. Department of Agriculture Plant Introduction.

[b] Two plants presumed to be immune to invasion by each of eight virus isolates were selected. Ten cuttings of each immune plant were each graft inoculated with one of 10 virus isolates by grafting a virus-infected *Datura tatula* scion on each of three shoots of each plant.

[c] Designation for virus isolate used in selection of immune plants (above) and the numerical designation of the specific immune plants (below).

[d] Above: recovery of virus from graft-inoculated plants, first 8 wk, then 24 wk after inoculation. Below: survival of virus in plants grown independently of infected scions.

subcuttings of a propagant in the fiberglass house but in none of those in the glasshouse in five instances. In the remaining 73 propagants tested, virus was retained in some but not all subcuttings in seven instances. In no instance was virus retained in subcuttings of a propagant in the glasshouse when it was not also retained in the fiberglass house.

Five clones (TYTV-1, No. 3; TYTV-17, No. 3; TYTV-79, No. 4; TYTV-85, No. 1, and TYTV-106, No. 6) appeared particularly resistant. Few virus isolates were recovered from propagants of these clones and none of the isolates was retained when plants were severed from the infected graft scions. Three clones (TYTV-87, No. 9 and No. 10; TYTV-106, No. 5) were particularly susceptible. Many virus isolates were recovered from propagants of these clones, and many isolates were retained when plants were severed from the infected graft scions.

Some virus isolates may have been somewhat more invasive than others. TYTV-106 was recovered from 10 of 16 clones, whereas TYTV-84 and TYTV-87 were recovered from only 5 of 16. In some instances, the two least invasive isolates were recovered from clones from which the most invasive isolate was not recovered.

Virus concentration in infected plants. The plants from which virus was recovered by aphid transmission 8 wk after severance from the infected scions retained virus at concentrations below the level of detection by ELISA. The A_{405} readings for plants that retained recoverable virus (range = −0.002–0.023, mean = 0.007, standard deviation = 0 006) were the same as those for plants that did not retain virus (range = −0.007–0.015, mean = 0.005, standard deviation = 0.005). The mean A_{405} for plants that retained virus was about 1/15 as high as that of three infected VF 145 tomato plants (mean = 0.111, standard deviation = 0.036) and 1/210 that of three infected *Physalis floridana* plants (mean = 1.54, standard deviation = 0.354) used as controls.

DISCUSSION

These studies were performed with plants selected as being potentially immune to TYTV and PLRV on the basis that virus could not be recovered from them 9 wk after an infected scion was grafted into the growing point of each plant. After clonal propagants of these plants were graft inoculated (infected scions grafted into growing points of test plants) with other isolates of the same viruses, some of the isolates could be recovered from some of the plants. However, it was not always possible to distinguish whether virus recovered from plants was actually produced in infected cells of the test plant or whether it was derived from the infected graft scion and occurred in the test plant as a passive transient of the phloem. The fact that virus could be recovered from new growth on cuttings of some plants long after separation from the infected graft scions is strong evidence that such plants were actually infected. In contrast, it is conceivable that the virus recovered from plants that did not retain recoverable virus in new growth after severance from the infected scion was actually derived from the infected scion. If this were true, then five of the 16 plants originally selected as being potentially immune (TYTV-1, No 3; TYTV-17, No. 3; TYTV-79, No. 4; TYTV-85, No.1; and TYTV-106, No 6) were not infected by any of the virus isolates and could be classified as immune.

The fact that plants selected as potentially immune differed in their apparent susceptibility to the various virus isolates suggested that the resistance of these plants may be virus strain specific. The long period of time required for infection to occur suggests that plants did not become infected until virus mutants or selections arose in the graft scion that were able to infect the resistant tissue. However, the extreme resistance of most of these plants to the virus isolates suggests that the establishment of infection in any plant by any virus isolate may have been a matter of chance. Clearly, the chances for systemic presence of virus in a plant increased as contact period between infected scion and the test plant increased beyond 8 wk. More than half of the clonal propagants of the resistant plants still did not contain recoverable virus after 24 wk. Perhaps all virus isolates eventually could have been recovered from all the plants if the contact period between graft and scion had been extended. The fact that clonal propagants of some plants were sometimes invaded by the same virus originally used to select the plant as being potentially immune supports this hypothesis.

The potato seedling USDA 41956 is immune to potato virus X, but the virus readily moves through stem sections of this seedling from an infected stock to a susceptible scion (12). In contrast, virus did not pass upward through stem sections of the resistant plants grafted between an infected stock and a susceptible scion in these studies, but it readily passed through similar stem sections of a susceptible species. Furthermore, a very long contact period between scion and resistant test plant was required for the virus to pass downward through shoots and into new axillary shoots. Based on these data, it is logical to hypothesize that the basis for resistance of the P. I. 128655 plants is resistance to transport of virus in the phloem. However, this hypothesis is not consistent with convincing evidence (3) that plant viruses are carried passively through sieve tubes of the phloem in the mass flow of photosynthate. Only one previous report (11) associates resistance of a phloem-limited virus (barley yellow dwarf) with slow movement in the phloem.

In their review of plant virus transport (2), Atabekov and Dorokhov accept the hypothesis that viruses are transported passively through sieve tubes. They propose two conceivable mechanisms to account for resistance to virus transport. Neither is completely compatible with the resistance we have observed in P. I. 128655. The first is that a virus-specific transport protein is essential to effect the release of virus from initially infected cells. The released virus might move directly into adjoining cells or it could move first into the sieve tubes for long-distance, passive transport and then into new cells at some distance from the initially infected cell. The suppression of this transport function (TF) would confine infection to the few cells that might be initially infected following natural transmission. However, graft transmission should completely overcome this type of resistance. Because TF would not be inhibited in the susceptible graft scion, TF would effect the release of a sufficient supply of virus in the scion to cause systemic infection of the resistant stock. Only if inhibition of TF originating from the resistant stock moved into and suppressed the release of virus from cells of the graft scion could this hypothesis account for the resistance we observed in P. I. 128655.

The second conceivable mechanism advanced (2) to account for resistance to virus transport is that the initial infection might induce acquired resistance to secondary infection throughout the plant. Virus would move freely in a plant with this type of resistance but new infections would be suppressed or completely prevented. This mechanism could account for the long contact period between scion and test plant required for infection to occur in the resistant plants. However, it could account for the failure of virus to move through stem sections of the resistant plants grafted between an infected stock and a healthy, susceptible scion only if it also induced resistance in the susceptible scion.

We propose a third hypothetical mechanism. A virus-specific protein produced during initial infection might induce systemic susceptibility when it accumulates to a critical concentration. Bennett (4) first observed a phenomenon that may be explicable on this basis. He found that the probability of systemic infection of *Chenopodium capitatum* L. plants with sugar beet yellows virus depended upon the mass of cells infected by initial inoculation. Only about 10% of the plants became systemically infected when inoculation produced 25 lesions per plant or less. About 200 lesions were required to ensure systemic infection in every plant. In our studies, the initial selection for immunity was based on graft inoculation with a single scion in each plant, whereas subsequent challenge inoculations involved three scions grafted into each plant. Furthermore, the mass of virus in the infected scions was further increased due to growth of the scions during the 24-wk contact period, and there was a corresponding gradual increase in number of plants infected.

The very low concentration of virus in plants that retained virus after severance from the scion suggests either that few cells were infected or that virus replication was slow in infected cells. If few

cells were infected, one could expect erratically distributed infection centers, as observed for taterleaf virus in citrange plants (8). However, the fact that all cuttings of infected plants usually contained virus suggests that virus infection was uniformly distributed.

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