Loss of Aphid Transmissibility of Turnip Mosaic Virus

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ABSTRACT

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Turnip mosaic virus (TuMV) isolate 31, which previously had been aphid-transmissible, was not transmitted by five species of aphids, to five species of test plants, or from four species of source plants whereas isolate 1 was transmitted at high frequencies. Isolate 1 was transmitted by *Myzus persicae* that had fed through artificial membranes on extracts from infected turnip leaves, while aphids were unable to transmit isolate 31 from extracts of infected turnip leaves. Addition of a soluble fraction from turnip infected with isolate 1 to partially purified virus of the same isolate resulted in aphid transmission of isolate 1, but no transmission resulted when mixtures of either partially purified virus of isolate 1 plus the soluble fraction of isolate 31 or the reverse combination were used for acquisition. The evidence suggests that a helper component required for aphid transmission of TuMV occurred in turnip leaves infected with the aphid-transmissible isolate but not in leaves infected with the aphid-nontransmissible isolate. Possible mechanisms for the loss of aphid transmissibility of TuMV are discussed.

Additional key words: acquisition factor, mutation.

Partial or complete loss of aphid transmissibility has been reported for styletborne viruses including cucumber mosaic virus (1), bean yellow mosaic virus (3,9,15,16), pea seedborne mosaic virus (5), and tomato aspermy virus (8). It has been generally postulated that these viruses lost transmissibility because of successive mechanical inoculations to host plants for virus propagation.

Turnip mosaic virus (TuMV) belongs to the potyvirus group (4) and is easily transmitted in the styletborne, nonpersistent manner by several aphid vectors.

This report described the loss of aphid-transmissibility of an isolate of TuMV following its maintenance in turnip by repeated mechanical inoculations with crude sap. An attempt is made to clarify the mechanism of the loss of aphid transmissibility.

MATERIALS AND METHODS

Virus isolates. Two isolates of TuMV were used in this study: Isolate 31 was originally obtained in 1956 by the late H. Yoshii (19) from a field-infected Japanese radish plant (*Raphanus sativus* L. var. *acanthiformis* Makino) and had been transferred both by aphids and mechanical inoculations until 1969. At that time, several experiments on aphid transmission of TuMV using this isolate had been completed (17,18) and subsequently it was maintained in Japanese turnip plants (*Brassica rapa* L. var. *glabra* Kitamura) by serial mechanical inoculations with crude sap only. Isolate 1 was collected from a naturally infected Japanese radish plant with mosaic symptoms in 1977 at Saga City. The isolates were transferred either by aphids or mechanical inoculations and propagated in Japanese turnip plants which were used as sources of virus unless otherwise stated.

Vectors and test plants. The green peach aphid, *Myzus persicae* (Sulzer) reared on Japanese turnip was used in most experiments. *Brevicoryne brassicae* L. reared on cauliflower (*Brassica oleracea* L. var. *botrytis* L.), *Lipaphis erysimi* Kaltenbach reared on Japanese radish, *Aphis craccivora* Koch reared on broadbean (*Vicia faba* L.), *Dactynotus gobonis* Matsumura reared on great burdock (*Arctium lappa* L.), also were used in some tests. Test plants were seeded and grown in pots filled with steamed field soil and maintained at 22–28 C in an insect-free glasshouse.

Aphid transmission tests. After a 2–3 hr starving period in glass vials, the aphids were given a 1–2 min acquisition access period and then groups of five aphids were placed on each test plant. After 12–16 hr in a growth room at 24–26 C the inoculated plants were sprayed with an insecticide. Then they were placed in the greenhouse and observed for symptom development for at least 4 wk.

Artificial membrane feeding. Chambers for artificially feeding aphids on test solutions were made from glass tubing (1 cm in height and 2 cm in diameter). The open end of the tubing, covered on the outside with black nylon tape, was placed on a flat stage and the other was enclosed with a stretched Parafilm M membrane. Either 0.4 or 0.6 ml of the test solutions containing a final concentration of 20% (w/v) sucrose, was placed on the upper surface of the membrane and the aphids fed on the solution from the lower surface of the membrane which was illuminated from above with a light bulb.

RESULTS

Symptom expression in test plants. Isolates 1 and 31 of TuMV were mechanically inoculated to test plants by using crude sap from infected leaves of Japanese turnip plants. Both isolates readily infected the test plants and induced systemic mosaic or mottle and leaf distortion in Japanese turnip, systemic mosaic in zinnia (*Zinnia elegans* L.), systemic yellowish spotting and leaf distortion in spinach (*Spinacia oleracea* L.), systemic yellowish or chlorotic spotting in *Chyrsanthemum coronarium* L., and systemic mosaic or yellowish spotting in *Physalis floridana* Rydb. No marked differences in symptom expression were observed between the two isolates in the above test plants, except that it was extremely difficult to infect Japanese radish with isolate 31 even though this isolate had initially produced a systemic mosaic symptom. Isolate 1 caused a typical mosaic symptom in the radish. Isolate 1 caused no symptom expression in *Nicotiana glutinosa* L. and the virus was localized only within the inoculated leaves whereas isolate 31 induced a systemic chlorotic or necrotic spotting as shown in the description of Yoshii et al (19). The reaction on *N. glutinosa* could be used to distinguish the two isolates.

Aphid transmission experiments. In a preliminary test, isolate 31 was examined for transmissibility by *M. persicae* by using the single-probe method. No transmission was observed in 55 Japanese turnip test plants.

The transmissibility of the two isolates by *M. persicae* and other species reported to be good vectors for TuMV (12) was tested with

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Japanese turnip plants as the source and test plants. In all trials, the five species of aphids tested (*M. persicae, B. brassicae, L. erysimi, A. craccivora, D. gobonis*) could transmit isolate 1 at high frequencies as shown in the previous report (12) whereas these aphids did not transmit isolate 31 (Table 1).

The possibility that *M. persicae* could transmit isolate 31 to host plants other than turnip also was investigated. Five species of test plants (*R. sativus, Z. elegans, S. oleracea, P. floridana,* and *C. coronarium*) were thus used in attempts to transmit with *M. persicae* as the vector and Japanese turnip plants as the source plant. *M. persicae* transmitted isolate 1 to all of the species of test plants, but failed to transmit isolate 31 (Table 2).

Experiments were also conducted to test the ability of *M. persicae* to transmit the two isolates from the four species of source plants other than Japanese radish which had been infected by mechanical inoculation. Again, *M. persicae* failed to transmit isolate 31 from any of the four source plants to Japanese turnip in contrast with isolate 1, which was transmitted from all the source plants.

Membrane feeding experiments. Aphid acquisition and transmission of nonpersistent plant viruses can be demonstrated by a membrane feeding technique (10,13). But Kassanis and Govier (10) failed to transmit potato virus Y with aphids fed through membranes on infective sap prepared in inappropriate extracting media. Leaf extracts from infected plants, prepared in several extracting media were tested for the capacity of aphids to transmit isolate 1 following its acquisition from such extracts by membrane feeding. Turnip leaves (5 g) infected with isolate 1 were homogenized in 10 ml of each extracting medium for 5 min at 2–4 C. The homogenate was squeezed through two layers of gauze, the filtrate was centrifuged at 8,000 *g* for 15 min, and the resulting supernatant fluid was used as the source of inoculum. *M. persicae*, which probed into fresh crude extracts except those prepared in distilled water, transmitted isolate 1 of TuMV to Japanese turnip plants. Of several extracting media shown in Table 3, the highest frequencies of transmission were consistently obtained when 0.5 M potassium phosphate buffer, pH 8.5 containing no additives was used as an extracting medium. The same buffer containing Na-DIECA and Na-EDTA yielded crude extracts with higher frequencies of transmission than did buffers containing Na-DIECA and thioglycolic acid or Triton X-100 (Table 3). In contrast, the results of all attempts to transmit isolate 31 through a parafilm membrane were negative even though 0.5 M potassium phosphate buffer, pH 8.5, was used as an extracting medium.

Following the demonstration that *M. persicae* could transmit isolate 1 from crude extracts, tests were made to find out whether the aphids could acquire TuMV from a soluble fraction or from partially purified virus which was prepared from crude extracts from Japanese turnip leaves infected with the two isolates. After extraction in 0.5 M potassium phosphate buffer (pH 8.5) as above, the resulting crude extracts were centrifuged at 123,000 *g* for 90 min and the supernatant fluids were used as the soluble fraction. The pellets were resuspended in the same volume of 0.5 M potassium phosphate buffer (pH 7.5) containing 0.01 M $MgCl_2$ (2). The resuspended material was clarified by centrifugation at 6,000 *g* for 10 min and the pellets were discarded. This clarified solution, which was highly infective when assayed by mechanical inoculation to *Chenopodium amaranticolor,* was used as the partially purified virus preparation.

M. persicae was allowed to feed through membranes on the following preparations: the soluble fraction from leaves infected with either isolates 1 or 31; partially purified virus preparations of isolates 1 or 31; a partially purified virus preparation of isolate 1 mixed with the soluble fraction of the same isolate; a partially purified virus preparation of isolate 1 mixed with the soluble fraction of isolate 31; a partially purified virus preparation of isolate 31 mixed with the soluble fraction of isolate 1.

The aphids acquired and transmitted isolate 1 only from the mixture containing the soluble fraction of isolate 1 and the partially

TABLE 1. Transmission of two turnip mosaic virus isolates by different aphid species[a]

Aphid species	Transmission frequency			
	Isolate 31		Isolate 1	
Myzus persicae	0/10[b]	0/10	8/10	10/10
Brevicoryne brassicae	0/10	0/10	9/10	10/10
Lipaphis erysimi	0/10	0/10	7/10	9/9
Aphis craccivora	0/10	0/10	8/12	10/10
Dactynotus gobonis	0/10	0/10	7/10	10/10

[a] Japanese turnip was used as source and test plant.
[b] Numerator equals number of plants infected and denominator equals number of test plants inoculated. Five aphids were placed on each test plant.

TABLE 2. Transmission of two turnip mosaic virus isolates from turnip plant by *Myzus persicae* to different test plants

Test plant	Transmission frequency			
	Isolate 31		Isolate 1	
Raphanus sativus	0/10[a]	0/12	9/12	11/12
Zinnia elegans	0/8	0/10	4/12	5/11
Spinacia oleracea	0/10	0/10	9/10	10/10
Physalis floridana	0/10	0/10	9/10	10/10
Chrysanthemum coronarium	0/10	0/10	4/10	7/10

[a] Numerator equals number of plants infected, denominator equals number of test plants inoculated. Five aphids were placed on each test plant.

TABLE 3. Transmission of turnip mosaic virus (isolate 1) by *Myzus persicae* following its acquisition from crude extracts of infected turnip leaves by membrane feeding

Extracting medium	Transmission frequency		
Distilled water[a]	0/10[b]	0/8	0/9
0.5 M potassium phosphate buffer, pH 7.5 plus 0.01 M Na-DIECA, 0.1 % thioglycolic acid	2/12	2/10	1/10
0.5 M potassium phosphate buffer, pH 8.5	9/11	8/10	9/10
0.5 M potassium phosphate buffer, pH 8.5 plus 0.01 M Na-DIECA, Na-EDTA	4/10	2/10	4/11
0.5 M potassium phosphate buffer, pH 8.5 plus 0.1 % Triton X-100	0/12	5/11	1/12

[a] In all test extracts, sucrose was added to make a final concentration of 20 % (w/v).
[b] Numerator equals number of turnip plants infected, denominator equals number of test turnip plants inoculated. Five aphids were placed on each test plant.

TABLE 4. Effect of soluble fractions from turnip leaves infected with two turnip mosaic virus isolates on aphid transmission of TuMV acquired through a parafilm membrane

Inoculum source[a]	Transmission frequency		
Virus of isolate 1	0/10[b]	0/10	0/10
Soluble fraction[c] of isolate 1	0/10	0/10	0/10
Virus of isolate 31	0/10	0/10	0/10
Soluble fraction of isolate 31	0/10	0/10	0/10
Virus of isolate 1 plus soluble fraction of isolate 1	8/10	5/10	4/7
Virus of isolate 1 plus soluble fraction of isolate 31	0/10	0/10	0/10
Virus of isolate 31 plus soluble fraction of isolate 1	0/10	0/10	0/10

[a] Mixtures were made by mixing equal volumes of the two solutions, and sucrose was added to all test solutions to make a final concentration of 20 % (w/v).
[b] Numerator equals number of turnip plants infected, denominator equals number of test turnip plants inoculated. Five aphids were placed on each test plant.
[c] Crude extract was prepared by grinding infected leaves in 0.5 M potassium phosphate buffer, pH 8.5 followed by centrifugation at 8,000 *g* for 15 min. The clarified fluid was centrifuge at 123,000 *g* for 90 min and the supernatant fluid was used as the soluble fraction.

purified virus preparation of the same isolate (Table 4). It is interesting that when the partially purified virus preparation of isolate 31 was added to the soluble fraction of isolate 1, no aphid transmission of isolate 31 occurred. Although this was unexpected, additional experiments confirmed these results.

A similar series of aphid transmission experiments with isolate 1 was made using virus purified by the method of Choi et al (2). In these experiments (data not shown), the aphids transmitted isolate 1 when purified virus of isolate 1 was mixed with the soluble fraction of infected leaves of the same isolate, but did not transmit purified virus of isolate 1 alone.

DISCUSSION

It is commonly inferred that the loss of aphid transmissibility of nonpersistent viruses might be attributed to the selection of an aphid-nontransmissible type from a virus population or to the selection of an induced mutation (1,3,5,9,15,16). The results obtained in the present study show that aphid transmissibility of isolate 31 of TuMV was completely lost, presumably as the result of repeated mechanical inoculations.

The data presented here using the membrane feeding technique show that aphid transmission of isolate 1 was dependent on the presence of the soluble fraction prepared from the infected leaves because the virus was never transmitted in the absence of this fraction. The failure of aphids to transmit TuMV from purified preparations confirms the earlier work of Pirone and Megahed (13). It is reasonable to assume that the soluble fraction contains a key factor which affects the aphid transmissibility of TuMV. Kassanis and Govier (10) and Lung and Pirone (11) have proposed that a helper factor necessary for aphid transmission is present in leaves of host plants infected with potato virus Y or cauliflower mosaic virus. Govier and Kassanis (6) also reported that helper component is needed for aphid transmission of potato virus Y from a purified virus source. The most likely explanation for my data is that the key factor in the soluble fraction from leaves infected with isolate 1 is a helper component essential for aphid transmission of TuMV.

According to the hypothesis of Govier et al (6,7) the helper component has two specific sites, one for the vector and the other for the virus and it acts as a connecting bridge between vector and virus. This explanation for the phenomenon seems most plausible at this time.

On the other hand, little is known about the mechanism by which a previously aphid-transmissible virus becomes nontransmissible. Simons (14) showed that an aphid-nontransmissible strain of tobacco etch virus failed to produce a necessary assistor material in the leaves of host plants and that an aphid-transmissible isolate of potato virus Y could serve as a source of assistor material for the nontransmissible strain of tobacco etch virus.

Two possible mechanisms can be proposed to explain the loss of aphid transmissibility of isolate 31 of TuMV. The results obtained in these experiments provide evidence that an active helper component was not present in the soluble fraction from crude extracts of leaves infected with the nontransmissible isolate 31. Alternatively, the fact that the active helper component in the soluble fraction from leaves infected with isolate 1 did not promote aphid transmission of isolate 31 suggests that the specific site for the helper component on the virus particle of isolate 31 changed in such a way that the site did not interact with the helper component. This mutation could be ascribed to either a structural or a comformational change in the coat protein of the virus particle. In conclusion, it is tempting to speculate that mutation of the aphid-nontransmissible isolate 31 might occur through both mechanisms described above. It remains to be determined if the helper component from TuMV-infected plants is similar to, or different from, that of potato virus Y-infected plants.

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