Aphid Transmission of a Nonaphid-Transmissible Strain of Tobacco Etch Virus

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ABSTRACT

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Aphid transmission of a previously nonaphid-transmissible strain of tobacco etch virus TEV(NAT) was accomplished by first allowing nonviruliferous green peach aphids, *Myzus persicae,* to probe on a pepper plant infected with an aphid-transmissible strain of potato virus Y PVY(AT) prior to transferring it to a TEV(NAT)-infected plant for acquisition of TEV. Tobacco etch virus transmitted in this manner was not aphid-transmissible to additional susceptible plants. When mixed infections of TEV(NAT) plus PVY(AT) were used as sources of virus, only TEV(NAT) was recovered. Squash plants infected with aphid-transmitted watermelon mosaic virus did not serve as a donor of assistor material for TEV(NAT). Efforts to transmit TEV(NAT) derived from a mixed infection of PVY(AT) plus TEV(NAT) using a low temperature (14 C) for acquisition of virus and a higher temperature (26 C) for inoculation were not successful. When plants infected with TEV(NAT) were challenged with an aphid-transmitted strain of TEV TEV(AT) cross protection against the challenge strain was found. When TEV(AT) infected plants were challenged with TEV(NAT), the TEV(AT) was readily recovered by aphids, again demonstrating cross protection. It would appear that the assistor material necessary for aphid transmission of TEV is intimately associated with the virus particle.

Additional key words: acquisition factor, potato virus Y.

Reduction in or loss of aphid transmissibility of stylet-borne viruses has been reported by several workers (1, 2, 3, 8, 11). This phenomenon is often associated with repeated mechanical inoculation of virus, but Swenson et al. (9) have shown this is not necessarily always the case.

About a year ago, an isolate of tobacco etch virus (TEV) which had been mechanically transferred for some 50 passages was tested for transmissibility by the green peach aphid and no transmissions were obtained. In view of the recent work by Kassanis and Govier (4) on the role of potato virus Y (PVY) as a source of assistor material for either potato virus C or potato aucuba mosaic virus, it seemed worthwhile to attempt to obtain aphid transmissibility of TEV through the use of other aphid-transmitted viruses as sources of assistor material.

MATERIALS AND METHODS

Viruses.—Tobacco etch virus (aphid- and nonaphid-transmitted strains) and aphid-transmitted PVY and watermelon mosaic virus (WMV) were used. Potato virus Y and TEV were maintained in pepper, *Capsicum annuum* 'California Wonder' (CW). Watermelon mosaic virus was maintained in squash, *Cucurbita pepo* var. *melopepo* 'Early Bush Scallop'. Except for the nonaphid-transmitted TEV, all viruses were maintained in source plants by aphid inoculation.

Plants.—Test plants were either CW pepper or a PVY-immune selection of pepper cultivar Italian E1 (IE). Plants were grown in 5.1 cm^2 diameter plastic pots. Test plants were either in the cotyledon or two-leaf stage at the time of inoculation and were grown in an air-conditioned greenhouse programmed for about 21 C night and 27 C daytime temperatures.

Ioculations.—Viruses were mechanically inoculated by means of cotton swabs dipped in freshly prepared plant sap that had been diluted about 1:50 in distilled water. Celite was used as an abrasive.

Aphid transmissions of virus were made by allowing green peach aphids a 1- to 3-minute access period on leaves of inoculum source plants that usually had been infected for 14 days followed by inoculation access feeding times of >1 hour on healthy test plants. Plants were sprayed with an insecticide following inoculation feedings.

Serology.—Serological tests performed by T. W. Zitter of the University of Florida, Belle Glade, were made by using the agar gel diffusion technique. Antisera had been prepared by D. E. Purcifull, University of Florida, Gainesville, Florida.

Aphids.—Mature apterous green peach aphids, *Myzus persicae* (Sulz.), reared on CW pepper were used in all aphid transmissions of virus.

Terms used.—(AT) = aphid-transmissible; (NAT) = nonaphid-transmissible.

RESULTS

The first trials utilized either PVY(AT)- or WMV(AT)-infected plants as sources of assistor material. Aphids were first allowed access probes of 1-3 minutes on these plants and then they were transferred to a source of TEV(NAT) for an access probing period of 1-3 minutes before being moved to healthy test plants for inoculation

TABLE 1. Use of aphid-transmitted PVY or WMV as sources of assistor material for nonaphid-transmissible TEV. Two aphids were used on each test plant

Sequence of use of viruses (plants)	Infections[a]	Serological results
TEV(NAT)[b] →PVY(AT)[c] →IE pepper	0/50	...
PVY(AT) →TEV(NAT) →IE pepper	10/50	+TEV
TEV(NAT) →IE pepper	0/50	...
WMV(AT) →TEV(NAT) →CW pepper	0/50	...
TEV(NAT) → IE pepper	0/50	+TEV[e]
TEV(AT) → IE pepper	24/25[d]	+TEV
WMV(AT) →CW pepper	0/25[d]	...
WMV(AT) →squash	48/50	...
PVY(AT) →IE pepper	0/50	...
PVY(AT) →CW pepper	47/50	+PVY
Healthy CW and IE pepper		Neg.

[a]Numerator equals number of plants infected; denominator equals number of plants inoculated.
[b]NAT = nonaphid-transmitted.
[c]AT = aphid-transmitted.
[d]Only tested in the second trial.
[e]The inoculum sources were tested.

feeding. In addition, the reverse order of procedure was used with aphids first probing on the TEV(NAT) source, before they were moved to PVY(AT) or WMV(AT) plants, and then to healthy test plants. Two kinds of test plants (pepper and squash) were used: IE pepper which is susceptible to TEV but not to WMV; and as controls, CW peppers inoculated with TEV(NAT), WMV(AT), and PVY(AT) and squash plants were inoculated with PVY(AT) and WMV(AT). In addition, an aphid inoculated strain of TEV was included to verify that green peach aphids can transmit TEV, and PVY(AT) was inoculated by aphids to both CW and IE peppers to show that IE pepper was immune to PVY. In general, 25 plants were used in each treatment and two aphids were used per plant. Two trials were carried out. Combined results are shown in Table 1.

PVY(AT) →TEV(NAT) →IE pepper was the only combination tried that gave transmission of TEV(NAT). Watermelon mosaic virus did not serve as a source of assistor material.

Verification of the identity of TEV was made by serology and by mechanically inoculating IE and CW plants using plants from the PVY(AT)→TEV(NAT)→IE series into IE and CW plants. Symptoms of TEV infection were produced in each of the pepper cultivars. Serological tests were positive for TEV and negative for PVY.

Attempts were made to transmit TEV from the IE plants by aphids. They were unsuccessful. This failure to obtain continued aphid transmission of the TEV(NAT) was interpreted to mean that the assistor material had not multiplied in the IE plants. Since the assistor material is intimately associated with PVY; and, since IE pepper does not support multiplication of PVY, it seemed possible that use of the IE pepper might have mitigated against establishment of an aphid-transmitted type of TEV. Thus, it was decided to repeat the work using CW plants doubly infected with PVY(AT) and TEV(NAT).

Sources of doubly infected CW plants were prepared by inoculating five plants with PVY(AT) (six aphids per plant) followed 5 hours later by mechanical inoculation of TEV(NAT) to one leaf of each of the five plants.

Two weeks later, recovery of virus was made by aphids to IE plants using 25 plants for each virus source and two aphids per test plant. As a control, a plant infected with TEV(NAT) was used. TEV(NAT) was recovered from each of the doubly infected plants with transmission ranging from 36-72% ($\bar{x}$ = 49.6%), one infection (4%) occurred among the 25 plants inoculated with TEV(NAT). The use of the mixed infection was an effective way to accomplish aphid transmission of TEV(NAT).

Fourteen days later (12 September), a TEV-infected IE plant was selected for further aphid recovery work. CW pepper was used as a test plant and access feedings were carried out at two temperature regimes 14 C and 27 C. Twenty-five plants (three aphids per plant) were used for each access temperature. Inoculation feedings were made at 27 C for all plants. Two of 25 plants became infected when the 14 C access probing temperature was used; none for 25 occurred at the 27 C access probing temperature.

Sixteen days later (28 September), the two plants infected at the 14 C access probing temperature were tested for aphid transmission of virus, again using the 14 C and 27 C access probing temperature and 27 C inoculation feeding temperature. No transmissions occurred among the 50 test plants used.

Also on 12 September, the IE source plant used for the aphid recoveries was used to prepare a mixed infection of PVY plus TEV(NAT) in CW pepper. Five CW plants were mechanically inoculated with TEV(NAT) about 5 hours after they had been inoculated with PVY by means of 10 aphids per plant. Aphid transmission of TEV(NAT) to IE pepper was accomplished easily on 28 September (ten infections of 25 IE plants inoculated), but subsequent attempts to aphid-transmit TEV(NAT) from these IE plants proved negative (0 infections in 50 plants inoculated).

Cross protection studies.—Since I was not successful in reestablishing the aphid transmissibility of TEV(NAT) using PVY as a source of assistor material, it seemed worthwhile to look at the possibility of determining whether cross protection existed between the TEV(NAT)

and TEV(AT) strains of virus. Aphid-transmissible tobacco etch virus is easily transmitted by the green peach aphid; 80-90% transmission routinely is obtained using two aphids per plant (Table 1). Likewise, I knew that TEV(NAT) is more easily transmitted by mechanical means than TEV(AT). [In a duplicate test done in early October using a 1:250 dilution of viruses from 2-week-old sources, TEV(NAT) infected 22/25 and 23/25 CW pepper plants inoculated, respectively; whereas, TEV(AT) infected 13/25 and 9/25 test plants, respectively.] In addition, symptoms of TEV(NAT) are more severe in CW pepper than are those of TEV(AT).

In the first cross-protection trials, I challenged five CW pepper plants that had been infected for 2 weeks with TEV(NAT) by inoculating with TEV(AT) using 10 aphids per plant. Two weeks later, these five plants were assayed for aphid-transmissible virus using 25 CW pepper plants per inoculum source and two aphids per test plant. Controls were CW pepper plants infected for 2 weeks with either TEV(NAT) or TEV(AT). Twenty-five test plants (two aphids per plant) were used for each control. For the doubly inoculated plants, 3/125 transmissions occurred indicating a high degree of protection afforded by the prior presence of TEV(NAT). Control results were 0/25 for TEV(NAT) and 16/25 for TEV(AT). A repeat test gave similar results.

In the reciprocal trial, I challenged two CW pepper plants inoculated by aphids with TEV(AT) (10 aphids per plant) with mechanically inoculated TEV(NAT). Two days elapsed between the TEV(AT) and challenge TEV(NAT) inoculations. Fourteen days after the challenge inoculation, the doubly inoculated plants were assayed by aphids to CW pepper plants (two aphids per plant for 25 test palnts for each of the two inoculum sources). Controls consisted of CW pepper plants infected for 14 days with either TEV(AT) or TEV(NAT). Results were as follows: (i) for doubly inoculated plants 33/50 became infected, (ii) for plants infected only with TEV(AT) 33/50 became infected, and (iii) for plants infected only with TEV(NAT) only 1/50 became infected. Again, a high degree of cross protection was demonstrated.

To determine if the virus transmitted from the doubly inoculated plants was aphid-transmissible, I assayed 10 of the 33 plants using 15 test plants per virus source, and two aphids per test plant. Transmission ranged from 10/15 to 14/15 ($\bar{x}$ = 11.4/15) infections. Cross protection was again demonstrated. A repeat trial confirmed these results.

DISCUSSION

These results extend the finding of Kassanis and Govier (4) by showing that PVY can serve as a source of assistor material for another virus, TEV. Failure to obtain similar results with WMV as a source of assistor only indicates that more than one assistor exists rather than that assistor material is not needed for aphid transmission of WMV.

The cross-protection studies provide evidence that the assistor material is closely associated with the presence of the aphid-transmitted strain of TEV. Since the assistor material failed to reach a sufficiently high titer in plants first infected with TEV(NAT) to allow for a significant amount of aphid transmission after a challenge inoculation with TEV(AT), it seems likely that: (i) the TEV(AT) is the source of the assistor material, and (ii) TEV(AT) must multiply appreciably in the doubly infected plant for production of sufficient assistor material for efficient aphid transmission of virus.

Conversion of TEV(NAT) back to TEV(AT) did not occur under the conditions prevailing in this work. However, there was no reason that this could not be accomplished if suitable circumstances for the recombination of virus and assistor material were provided. In vitro combination of purified virus and assistor material coupled with membrane feeding of aphids is a likely possibility.

We were not successful in increasing transmission of TEV derived from mixed infections of TEV(AT) and TEV(NAT) by low-temperature access feedings. However, it is likely that effects of temperature on aphid transmission of stylet-borne viruses (6, 10) and differences in the transmission of closely related strains of PVY [Simons (7)] are related to the influence of a two-component system.

Kassanis and Govier (5) proposed a specific adsorption hypothesis to account for the action of the assistor material. An hypothesis that the assistor material has two specific sites of activity, one for aphid stylets and the other for the virus seems as simple and likely an explanation as any at this time.

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