Transmission and Preliminary Characterization of Citrus Tristeza Virus Strain K

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ABSTRACT. C. Bove *et al.* (4) have discovered a strain of citrus tristeza virus (CTV) in kumquat, strain K, which does not induce symptoms on Mexican lime. CTV strain K (CTV-K) was shown to multiply in graft-inoculated seedlings of Mexican lime, sour orange and sweet orange by ELISA. Surprisingly, graft-inoculated budlings of sweet orange on sour orange remained ELISA negative. No transmission of CTV-K by *Aphis gossypii* could be demonstrated. CTV-K in Mexican lime offered no cross-protection against CTV strains T1 and T4 from Florida. The molecular weight of capsid protein of CTV-K was found to be 25,000, slightly larger than strain T4 capsid protein analyzed in parallel. The three dsRNAs typical of CTV were detected in CTV-K infected Mexican lime seedlings. A fourth dsRNA was detected and was similar in size (0.5 kd) to the dsRNA associated with seedling yellows.

Index words. graft transmission, cross-protection, aphid transmission, capsid protein, dsRNAs.

A strain of citrus tristeza virus (CTV), strain CTV-K, was discovered in two of three kumquat trees of line K123 (4). This strain was able to multiply to normal titers in Mexican lime seedlings, but did not induce vein clearing, stem pitting or stunting and the seedlings remained symptomless. Strain CTV-K seemed to be a good candidate for cross-protection studies and it was therefore used for transmission experiments to seedlings and budlings of various citrus species. CTV-K multiplied and invaded seedlings of sour orange and sweet orange but did not become systemic in sweet orange on sour orange rootstock. It did not cross-protect against pathogenic strains of CTV. Some properties of strain CTV-K are presented here.

MATERIALS AND METHODS

The origin of strain CTV-K has been described (4). The T1 and T4 Florida strains of CTV (9) were used for comparison and kindly made available to us by Dr. S. M. Garnsey, USDA-ARS, Orlando, Florida.

For transmission of CTV-K to citrus, two pieces of infected bark 1 x 3 cm, were inoculated by the usual T-graft technique. In the case of budded seedlings, the graft inoculation was carried out either at time of budding with one bark inoculum being grafted above and the other below the bud, or later, when the bark pieces were grafted either on the rootstock or on the scion.

Attempts to transmit CTV-K by aphids followed the method of Bar-Joseph and Loebenstein (2).

The presence of CTV-K in citrus seedlings or budlings was determined by ELISA (3) with goat IgGs against the Reunion strain of CTV and alkaline phosphatase as the enzyme. The IgG concentration for coating was 1 μg/ml. The conjugate was used at a 400:1 dilution.

CTV-K was purified by the method of Bar-Joseph *et al.* (1). One dimensional polyacrylamide slab gel electrophoresis for CTV-K capsid protein analysis was according to Laemmli (11). Blot transfer of proteins was according to Bowen *et al.* (5) as modified by Candresse *et al.* (6), and immunodetection of blot transferred proteins was according to Hawkes *et al.* (10).

Double stranded (ds) RNAs in CTV-K-infected Mexican lime seedlings were analyzed by the method of Dodds and Bar-Joseph (7).

RESULTS

Graft-transmission assays of CTV-K and other CTV strains to various citrus seedlings and budlings. CTV-K was discovered in two

of three line K123 kumquat trees grafted on Rough lemon, imported to Corsica from Morocco in 1959 (4). The infected trees (K123-T22 and K123-T24) and their progeny trees served as sources of inoculum for the initial CTV-K transmissions. The healthy tree (K123-T23) and its progeny served as sources of buds for the production of healthy K123 kumquat trees on various rootstocks.

CTV-K from trees K123-T22 and K123-T24, could be easily graft-transmitted to healthy line K123 kumquat on trifoliate orange, citron 861-51 on Volkamer lemon, and to seedlings of the following species: Mexican lime, Orlando tangelo and Hamlin, Madam Vinous, Valencia Late and Washington Navel sweet oranges. The presence of CTV-K in these plants was determined by ELISA. Table 1 shows that CTV-K-infected bark inoculum of the above scions or seedlings was able, when assayed, to transmit CTV-K to line K123 kumquat on trifoliate orange, citron 861-51 on Volkamer lemon, Mexican lime, Orlando tangelo, Madam Vinous and Hamlin sweet oranges, and sour orange seedlings.

In these experiments the total number of inoculated plants of each category, was at least 10. Table 1 indicates that the percentage of CTV-K positive plants to the total number of inoculated plants was high in the case of kumquat on trifoliate orange, citron on Volkamer lemon and Mexican lime, but lower for Orlando tangelo, sweet orange and sour orange seedlings. Also the percentages of transmission were lower with kumquat as the CTV-infected bark donor species than with the other species.

As shown by Table 1, CTV-K could not be transmitted from any source of CTV-K-infected bark to the following citrus species or combinations of species: trifoliate orange, Troyer citrange, Valencia or Washington Navel sweet orange on sour orange, Clementine on sour orange and mandarin on Volkamer lemon. In these experiments ELISA assays were carried out 5 months and 2 yr after inoculation. The ELISA results, whether positive or negative, were the same after 5 months or 2 yrs.

The finding that CTV-K could not be detected in the combination of sweet orange on sour orange was sur-

TABLE 1
GRAFT TRANSMISSION EXPERIMENTS OF CITRUS TRISTEZA VIRUS, STRAIN K (CTV-K) TO VARIOUS CITRUS SPECIES

Species or combination inoculated	CTV-K-Infected inoculum bark					
	Kumquat K123[z]	Citron 861-51[y]	Mexican lime	Orlando tangelo	Mme Vinous sweet orange	Sour orange
Kumquat/trifoliate orange	90[x]	ND[w]	90	ND	ND	ND
Citron/Volkamer lemon	70	100	100	ND	ND	ND
Mexican lime seedling	80	100	99	100	100	ND
Orlando Tangelo seedling	20	60	ND	ND	ND	ND
Mme Vinous sweet orange seedling	20	60	60	ND	90	0
Hamlin sweet orange seedling	20	ND	40	ND	80	ND
Sour orange seedling	40	50	ND	ND	0	ND
Valencia sweet orange/sour orange	0	0	0	ND	0	0
Washington Navel/sour orange	0	0	0	ND	0	0
Clementine mandarin/sour orange	0	0	0	ND	0	0
Trifoliate orange seedling	0	0	0	ND	ND	ND
Troyer citrange seedling	0	ND	0	ND	ND	ND

[z]grafted on trifoliate orange
[y]grafted on Volkamer lemon
[x]percentage of the CTV plants that were ELISA-positive
[w]ND = not done

prising since sweet orange and sour orange, as seedlings, could become infected with CTV-K. Therefore this experiment was repeated on a large scale. Four yrs after inoculation of CTV-K-infected Mexican lime bark into 36 plants of Valencia or Washington Navel sweet orange on sour orange, none of these plants had become CTV-K positive in spite of the fact that some of the plants had received up to 20 pieces of bark inoculum. Interestingly, CTV-K could be detected, even 4 yrs after inoculation, in the grafted pieces of bark used to inoculate the sweet orange on sour orange plants. In the same experiment, CTV strain T1 and T4, were easily be detected respectively in five of five and 15 of 15 plants of sweet orange on sour orange. However, no significant differences were found between the three strains as to their ability to invade Mexican lime and sour orange seedlings, even though strains T1 and T4 induced tristeza symptoms, but strain K did not.

To further investigate the inability of CTV-K to become systemic in sweet orange on sour orange plants, additional experiments were carried-out. Both partners of sweet orange on sour orange plants graft-inoculated with CTV-K-infected bark remained CTV-K negative by ELISA (but the grafted inoculum remained CTV-K positive) whether inoculum was placed on the sour orange stock or the sweet orange scion, whether the bark inoculum was grafted on sour orange at the same time as the sweet orange bud or later, and in spite of high numbers of bark pieces used as inoculum. CTV-K could not be detected in sweet orange seedlings when the bark inoculum was sour orange and conversely, sour orange seedlings remained CTV-K negative when sweet orange bark was the inoculum. However, sweet orange seedlings became CTV-K positive when they were inoculated with sweet orange bark. The results for infection of sour orange seedlings with sour orange bark are not yet available. When a healthy sweet orange (or clementine) bud was grafted on a CTV-K-infected sour orange seedling, the developing sweet orange (or clementine) shoot remained CTV-K negative. Conversely, when a CTV-K-infected sweet orange bud was grafted on a healthy sour orange seedling, the sour orange stock remained CTV-K negative even though the sweet orange shoot grown from the bud was CTV-K positive.

All these experiments indicate that there is no transmission of CTV-K from sour orange to sweet orange and vice-versa: CTV-K seems to be unable to cross the graft union.

Finally, symptomless CTV-K-infected (ELISA positive) Mexican lime seedlings or budlings on Volkamer lemon did not show cross-protection when they were challenge-inoculated with the pathogenic T1 or T4 strains of CTV. The symptoms induced by T1 and T4 in the CTV-K-infected plants developed as quickly and severely as those in the CTV-K-free control plants.

Transmission of CTV-K by *Aphis gossypii* has been attempted. The aphids were reared on melon or cotton. After acquisition feeding for 24 hr on infected Mexican lime seedlings the insects were transferred to healthy, 4-month-old, Mexican lime seedlings (100 aphids per plant). Eighty plants in three experiments have been exposed in this way to infection by *A. gossypii*. All plants were negative for CTV-K even when ELISA was carried out as late as 12 months after exposure to aphids. These negative results agree with the fact that no evidence for natural spread of CTV-K has ever been found in Corsica.

Properties of CTV-K. As indicated previously (4), there were no significant morphological or spectrophotometrical differences between CTV-K and the pathogenic Reunion strain of CTV.

In one-dimensional polyacrylamide gel electrophoresis (PAGE),

the capsid protein of CTV-K was found to have a molecular mass of approximately 25,000 d, very slightly higher than that of CTV-T4. After Western blotting, the capsid proteins of CTV-K and CTV-T4 reacted equally well to IgGs directed against the Reunion strain of CTV.

Mexican lime seedlings infected respectively with CTV-K, CTV-T4 and a Brazilian strain of CTV were analyzed by PAGE for the presence of dsRNAs (7). The three dsRNAs characteristic of CTV infection (13.3, 1.9 and 0.9 kd) were detected on the gels of all infected lime seedlings including those infected with CTV-K. In addition, the limes respectively infected with CTV-K and CTV-Brazil, but not CTV-T4, carried a fourth dsRNA (0.5 kd). This small dsRNA is often associated with strains of CTV inducing seedling yellows (7, 8). CTV-K does not induce seedling yellows nor, for that matter, any other pathological manifestations, yet it results in the presence of the small dsRNA.

DISCUSSION AND CONCLUSION

The data presented here and in the accompanying paper (4) clearly indicate that the virus from kumquat line K123 is a strain of CTV. This strain (CTV-K) is unusual for several reasons. Mexican lime seedlings infected with CTV-K remain fully symptomless, even though they contain amounts of virus similar to those present in CTV-T4-infected limes. Strain CTV-K does not seem to pass through graft unions involving sweet orange and sour orange tissues, which explains the results in where CTV-K did not become systemic in sweet orange when sour orange was the inoculum and vice versa. Even more surprisingly, the combination of sweet orange on sour orange seems to be resistant to infection by CTV-K since, as reported above, none of 36 such budlings became infected with CTV-K when inoculated, in the scion or the stock with one or more pieces of CTV-K-infected Mexican lime bark. Infected bark from kumquat K123, citron 861-51, sour orange and sweet orange was similarly unable to induce systemic infection of sweet orange on sour orange. The determining factor seems to be the association of sweet orange and sour orange in combination since sweet orange and sour orange seedlings alone can easily become infected with CTV-K by bark inoculation from Mexican lime, kumquat and citron. Mexican lime seedlings infected with CTV-K contained, in addition to the three large dsRNAs characteristic of CTV infection, a small 0.5 kd dsRNA associated with CTV strains carrying seedling yellows (7). The association of this dsRNA with CTV-K, a strain which is symptomless also in sour orange, an indicator plant of seedling yellows, shows that the 0.5 kd dsRNA is not specific of the seedling yellows reaction.

It is well known that most strains of CTV induce decline of sweet orange on sour orange due to the necrosis of phloem tissue of sour orange below the budunion line. However, the mechanism of this specific pathological reaction is not yet known. The unusual transmission pattern of CTV-K involves also sweet orange and sour orange when these two species are associated through a graft union. It is tempting to speculate that the cytopathic effect of tristeza disease of sweet orange (and other citrus species) on sour orange, and the unusual transmission pattern of CTV-K both reflect some basic properties of CTV, yet to be discovered.

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