Separation of Citrus Tristeza Virus (CTV) Serotypes Through Aphid Transmission

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ABSTRACT. Twenty different subcultures of citrus tristeza virus (CTV) were obtained from 15 different original field sources by transmission with the aphid *Toxoptera citricidus* (Kirk). The 20 aphid-transmitted subcultures (ATS), and 15 field sources were tested by double sandwich indiret ELISA using two monoclonal antibodies (MAb), MCA13 and 3DF1, and by double sandwich ELISA using polyclonal antibodies (PAb). Four ATS isolates derived from two field source (FS) isolates showed reactions different from their parent FS. For example, a mild CTV from one FS, M27, reacted strongly to MCA13, 3DF1 and PAb, while three ATS isolates from M27 reacted with PAb, but not with either MAb. These results indicate that at least two different serotypes can infect one citrus plant, and that separation of certain serotypes can occur through aphid transmission.

Index words: ELISA, monoclonal antibody.

In the accompanying paper (6), the serological diversity of citrus tristeza virus (CTV) from different field sources (FS) in Japan was identified using monoclonal antibodies (MAb) which are specific to different epitopes in the CTV coat protein (3). CTV is known to exist as a complex or mixture of different isolates in the field, and separation of components from these complexes has been reported by several workers. For example, separation of mild strains has been accomplished by tissue grafting after heat treatment of infeced plants (1), by vector transmission (9), and by tissue grafting from trees which showed recovery from seedling yellows (SY) (14).

In this paper, we compared the reactions of parent FS cultures and aphid-transmitted subcultures (ATS) with two MAbs, MCA13 (10) and 3DF1 (13), and a polyclonal antibody (PAb) to determine whether their serological reactions change after aphid transmission. Data are presented indicating that separation of a specific CTV component from a mixture of components in the same plant can be achieved by aphids and detected by use of differential MAb.

MATERIALS AND METHODS

Virus sources. The virus source plants and all other plants for experiments were maintained in vector-free screenhouses. The CTV FS tested are shown in Table 1 and have been previously described (4, 5, 6, 7).

Aphid vectors. *Toxoptera citricidus* (Kirk.) was collected from a citrus grove at the Okitsu Branch, Fruit Tree Research Station (FTRS) and reared on synthetic diet (8) for at least 8 days to obtain a colony of non-viruliferous aphids. The diet (enclosed in laboratory film sachets on a glass cup) was changed every 2 days. The aphids were transferred to healthy trifoliate orange seedlings, which are resistant to CTV.

Virus transmission. Donor plants were graft-inoculated rough lemon or Madam Vinous sweet orange seedlings or the FS cultivar grafted to rough lemon seedlings. Aphids were reared on new flushes of the acquisition host for at least 7 days at 20-25 C in an air-conditioned chamber. More than 20 viruliferous aphids were transferred to each of 3-6 receptor plants. The receptor plants were healthy Madam Vinous sweet orange or rough lemon seedlings 6 to 12 months old. The inoculation feeding period was 48 hr or more at 20-25 C in an air-conditioned chamber. After each inoculation, plants were sprayed with insecticide to eliminate the vectors.

Enzyme-linked immunosorbent assay (ELISA). Double antibody sandwich (DAS) ELISA with polyclonal antibody (PAb) was used to verify CTV infection in the receptor plants 2

Contribution Fruit Trees Res. Sta. B-168.

TABLE 1
REACTION OF CTV FIELD SOURCES (FS) AND THEIR APHID-TRANSMITTED SUBCULTURES (ATS) TO THE CTV MONOCLONAL ANTIBODIES (MCA13 AND 3DF1) AND TO POLYCLONAL ANTIBODY (PAb)

CTV source	Plant code	Variety[z]	Total evaluation[y]			Symptomatology[x]
			MCA13	3DF1	PAb	
1215	0703	RL	+	+	+	SY
1215A[wv]	0705	RL	+	+	+	SY
1417	0707	RL	+	+	+	T
1417A	0708	RL	+?	+	+	SP[u]
1513	0709	RL	+	+	+	SY
1513A[v]	0712	RL	+	+	+	SY
1595	0714	RL	+	+	+	SP
1595[v]	0719	RL	+	+	+	SP
BOUQ	0935	RL	+	+	+	?
BOUQA	0924	MV	–	+	+	?
KS3	0770	RL	+	+	+	SY
KS3A	0771	RL	+	+	+	SY
M12	0787	ML	–	+	+	M[u]
M12A	2010	YZ	–	+	+	?
M16	0791	ML	–	–	+	M[u]
M16A	0793	YZ	–	–	+	M[u]
M22	0796	ML	+	+	+	M[u]
M22A[v]	0977	ML	+	+	+	M[u]
M23	0797	ML	+	+	+	M[u]
M23A	0873	YZ	+	+	+	M[u]
M27	0802	ML	+	+	+	M[u]
M27A[v]	0963	ML	–	–	+	M[u]
PM8	0783	ML	+	+	+	INT[u]
PM8A	0910	RL	+	+	+	?
PM25	0800	ML	+	+	+	INT[u]
PM25A[v]	0992	ML	+	+	+	?
S5	0807	RL	+	+	+	SY
S5A	0808	RL	+	+	+	SY
SIY	2025	MV	+	+	+	SY
SIYA	0811	MV	+	+	+	SY

[z]RL: rough lemon, MV: Madam Vinous sweet orange.
ML: Mexican lime, YZ: Yuzu.
[y]Evaluation based on at least 3 assays + = positive, and – = netative.
[x]SY = seedling yellows strain of CTV; SP = stem pitting strain of CTV; INT = intermediate strain of CTV; M = mild strian of CTV; ? = pathogenicity of CTV not determined; T = tristeza strain of CTV.
[w]'A' indicates isolate is aphid-transmitted subculture of the designated field source.
[v]Test plant was graft-inoculated subpropagation of the original receptor plant.
[u]Reaction of sweet orange grafted on sour orange rootstock not yet tested.

to 8 months after the inoculation feeding. Double antibody sandwich indirect (DAS-I)-ELISA with MAbs, MCA13 and 3DF1 was used to check the serological characteristics of ATS. Details of the ELISA procedures used were as shown in the companion paper (6). The rate of ELISA reaction was calculated

as the change in OD_{415} per minute. The ratio of reaction for each sample in relation to positive controls placed in each plate was calculated. Samples were considered negative if the ratio was less than 1:10, questionable if between 1:10 and 1:5 and positive when above 1:5. Reactions of each isolate to each antibody are described in the sequence (MCA13/3DF1/Polyclonal). For example, if the reaction to all is positive, it is described as (+/+/+).

Biological indexing. Several FS isolates and all of the ATS isolates were indexed using Mexican lime and sour orange or Eureka lemon seedlings. Other FSs were indexed previously by Koizumi (7), and by Ieki and Yamaguchi (4, 5).

RESULTS AND DISCUSSION

T. citricidus transmitted almost all of the severe FS isolates to at least one receptor plant, but some of the mild sources were not transmitted. Some FSs and their ATSs shared the same pattern of reaction to each antibody (Table 1). Two different ATS isolates from each of the three parent FS isolates, KS3, M16, and S5, showed the same serological reaction as their parent FS. However, ATS isolates of BOUQ (BOUQA) and M27 (M27A) showed a serological reaction different from their parent FS. Three different ATSs of M27 showed the reaction (–/–/+) in contrast to the parent reaction (+/+/+). The change presumably resulted from separation or segregation by the aphid of a specific serotype component from a mixture of serotypes, and not a serological modification by aphid passage (15). For example, M27 (FS) would contain at least two serotypes, designated as $M27X_n$ (n = 1, 2 · · · ·) and M27A. The serological character of M27A is (–/–/+) and $M27X_n$ would be (+/+/+), or a mixture of serotypes including (+/–/+), (–/+/+), and (+/+/+).

Based on these results, we conclude that at least two different serotypes can infect one citrus plant, and that separation of a specific serotype can occur through aphid transmission.

CTV has been regarded as a complex of different biological strains, and different strains can propagate simultaneously, even in one tree. Previous studies have shown the presence of a complex of biological components, because isolates of differing pathogenicity could be separated from a single plant through several procedures (1, 2, 9, 11, 12, 14, 15). Usually, mild isolates were separated from severe sources, and these were identified by indexing on selected indicators. The results in this study confirm the presence of a CTV complex in some trees by an independent method.

While M27A was highly transmissible by *T. citricidus*, the $M27X_n$ component apparently could not be transmitted by aphids. The titer of $M27X_n$ in the original source (M27) was not low, because extracts from M27-infected plants reacted strongly to 3DF1 and MCA13. The poor transmissibility of $M27X_n$ is apparently intrinsic.

Similar separation was recognized between BOUQ and BOUQA. But, in this case, BOUQA (ATS) was nonreactive to MCA13, and showed a strong reaction to 3DF1 (Table 1).

FS 1417 from rough lemon showed a strong reaction with 3DF1 and MCA13, while 1417A from rough lemon showed a slightly weaker reaction to MCA13. This suggests that 1417A may be slightly different from the parent source 1417. However, Etrog citron graft-inoculated with 1417A showed a strong reaction to both MAbs (data not shown).

Aphid transmission often has been used to obtain a CTV source free from other graft-transmissible agents. However, it is also apparent that separation of CTV serotypes easily occurs through aphid transmission. The separation of a serologically distinct isolate in this study was not detected by biological indexing. Further research is necessary to determine if differences in biological properties, particularly cross-protection ability, can correlated to serotype separation.

ACKNOWLEDGMENTS

The authors wish to thank Shinkichi Komazaki, Kuchinotsu Branch, FTRS, Japan, for teaching the technique of rearing aphids on artificial diet, and also Chieko Azeyanagi and Chizuko Suzuki, Okitsu Branch, FTRS, Japan, for their technical assistance. The assistance of S. M. Garnsey and T. A. Permar, USDA, Orlando, FL, for providing MCA13 monoclonal antibody and of M. Cambra, I.V.I.A., Moncada, Spain, for providing the 3DF1 monoclonal is gratefully acknowledged.

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