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Limited Cross Protection by a Protective Strain of Citrus Tristeza Virus (CTV) against Challenge Inoculation by Grafting

T. Kano and M. Koizumi

ABSTRACT. Cross protection by a protective strain of CTV was monitored by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies (MAb). Yuzu seedlings were pre-inoculated with a protective strain, M27A, which reacts to a polyclonal antibody (PAb) but not to two MAbs, MCA13 (3) and 3DF1 (4). Pre-inoculated and virus-free control trees were challenged with a severe stem pitting strain, 1595A, by grafting. 1595A reacts to all the antibodies used in this experiment. Six pre-inoculated and three virus-free control trees were challenged by grafting. Graft-inoculation finally overcame cross protection. Pre-inoculation with M27A, however, delayed severe strain replication by 21-43 days.

Several citrus virologists in the United States have reported successful application of a monclonal antibody (MAb), MCA13, to monitor cross protecting ability of mild strains of citrus tristeza virus (CTV). In our previous cross protection experiment (1), likewise, superinfection of CTV strain was detected in a preinoculated field tree of Morita navel orange by ELISA. The results also indicated that the 3DF1 MAb can be used to monitor the protective ability of the CTV isolates nonreactive to 3DF1. However, since that experiment was conducted in the field, the challenge strain of CTV could not be determined. So we carried out another cross protection experiment in vector-free greenhouses using a known strain for challenge inoculation. In this experiment, plants pre-inoculated with M27A and control trees were challenge-inoculated with 1595A, a severe strain of CTV, by aphids or by grafting. Cross protection by M27A was monitored by ELISA using MAbs (1).

Pre-inoculation and challenge by aphids. Virus-free Yuzu (*Citrus junos* Sieb ex Tanaka) seedlings (one year old) were graft-inoculated with the mild protective strain M27A in May 1989. M27A reacts to a PAb, but not to two MAbs, MCA13 and 3DF1. Infection only by M27A was verified by ELISA with the two MAbs and the PAb. Pre-inoculated and virus-free control trees were challenged with a severe, stem pitting strain using viruliferous aphids (*Toxoptera citricidus* Kirk.) in December 1989, and June 1990. Four of six non-protected control trees reacted to both MAbs and the PAb, and showed severe stem pitting and stunting. Pre-inoculated trees, however, did not react to the MAbs but reacted to the PAb. These pre-inoculated trees showed only mild stem pitting.

Challenge inoculation by grafting. Two grafts were used per tree, and survival of the graft was visually determined. In September 1991, six pre-inoculated and three virus-free trees were challenged with CTV 1595A by grafting (Table 1). The grafts remained alive during the experiment. Forty-eight days after graft challenge inoculation, tissue samples were collected from each tree and assayed by ELISA. Pre-inoculated trees (Code. Y-11 through Y-22) reacted to the PAb, but not to the MAbs, whether challenged by grafting or not. However, the three non-protected control trees (Code Y-23, 24, 25) all reacted well to the MAbs and the PAb. Sixty-nine days after challenge ionoculation, two of six pre-inoculated trees showed positive reaction to the MAbs. Ninety-one days after graft challenge, all six pre-inoculated trees reacted positively to the MAbs, although severe symptoms induced by the challenge strain were not observed on new shoots. All pre-in-

TABLE 1
SEROLOGICAL ASSAYS FOR YUZU TREES CHALLENGED BY GRAFT OR APHID INOCULATION IN A CROSS PROTECTION EXPERIMENT IN THE GREENHOUSE

Tree code	Protecting[z]	Challenge[y]	ELISA[x] Nov. 13 1991[w]	ELISA Dec. 4 1991	ELISA Dec. 26 1991	ELISA Jan. 17 1992	Symptom[v] Jun. 30 1992
Y-11	M27A	Graft	(−−+)	(−−+)	(+++)	(+++)	sSP
Y-12	M27A	Graft	(−−+)	(−−+)	(+++)	(+++)	sSP
Y-13	M27A	Graft	(−−+)	(−++)	(±++)	(+++)	sSP,LC
Y-14	M27A	Graft	(−−+)	(−−+)	(+++)	(±++)	sSP
Y-15	M27A	Graft	(−−+)	(+++)	(+++)	(+±+)	sSP
Y-16	M27A	Graft	(−−+)	(−±+)	(+++)	(+++)	sSP,LC
Y-17	M27A	–	(−−+)	(−−+)	(−−+)	(−−+)	no
Y-18	M27A	–	(−−+)	(−−+)	(−−+)	(−−+)	mSP
Y-19	M27A	–	(−−+)	(−−+)	(−−+)	(−−+)	mSP
Y-20	M27A	–	(−−+)	(−−+)	(−−+)	(−−+)	no
Y-21	M27A	–	(−−+)	(−−+)	(−−+)	(−−+)	no
Y-22	M27A	–	(−−+)	(−−+)	(−−+)	(−−+)	mSP
Y-23	–	Graft	(+++)	(+++)			sSP
Y-24	–	Graft	(+++)	(+++)			sSP
Y-25	–	Graft	(+++)	(+++)			sSP,LC

[z]M27A = graft-inoculated with a mild, protective strain M27A on May 4, 1989. Challenged with 1595A by aphids in December, 1989 and June, 1990, but not superinfected (see text).
– = not inoculated.
[y]Graft = challenged with 1595A by grafting on September 25, 1991.
– = not challenged.
[x]Reaction of each sample to MCA13, 3DF1, PAb was shown in parentheses in that order. For example, (−±+) means negative to MCA13, questionable to 3DF1 and positive to the PAb.
[w]Date for the collection of test sample from trees.
[v]mSP = mild stem pitting, sSP = severe stem pitting, LC = leaf curl, no = no symptom.

oculated trees and control trees which reacted to the MAbs and PAb, showed severe stem pitting and leaf curl in June 1992.

It is commonly considered that cross protection can be broken down by challenge using graft inoculation. It is difficult to know relative concentration of the protecting and the challenge strains in a tree by biological methods using indicator plants. In this experiment, we used MAbs and a CTV isolate non-reactive to these MAbs, and demonstrated that multiplication of a challenging severe strain after graft inoculation was slower in protected trees than in unprotected ones. Nevertheless, cross protection was finally broken down in the graft inoculated trees.

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