TRISTEZA

Biological Characterization and Evaluation of Cross Protection Potential of Citrus Tristeza Virus Isolates in Venezuela

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ABSTRACT. Ten citrus tristeza virus (CTV) isolates were biologically characterized on the basis of vein clearing, vein corking, leaf cupping and stem pitting symptoms in several citrus hosts. Serological reactivity was studied using monoclonal antibody MCA-13 to detect severe CTV strains and monoclonals 3DF1 and 3CA5 to detect all CTV strains. Five mild and three severe Venezuelan isolates were tested, and three known Florida mild CTV isolates were included for comparison. Cross protection experiments were established under field conditions with Valencia sweet orange on sour orange rootstock. Trees were graft inoculated with mild CTV test isolates and challenge inoculated with severe CTV isolates using *Toxoptera citricidus*. Trees protected with the Florida mild isolate T30a have grown satisfactorily for the past three years.

The first outbreak of the citrus tristeza virus (CTV) was observed in Venezuela in 1980, four years after the *Toxoptera citricidus* Kirk. detection (1, 3). CTV may have been introduced into the country during the 1950s since its presence was reported in 1960 by Knorr *et al.* (4). Alternatively the destructive strains of CTV may have been carried into Venezuela by *T. citricidus*. However, more than six million trees on sour orange rootstock were killed due to CTV-induced decline between 1980 and 1989 (5, 7, 13, 14). The Venezuelan citrus industry was then re-established on CTV tolerant rootstocks. Today the tristeza problem is still unsolved since severe seedling yellow (SY) and stem pitting strains affect scions regardless of rootstock. Other viruses and viroids which were latent when trees were on sour orange rootstock, as well as diseases of unknown etiology such as citrus blight or the Venezuelan sudden citrus decline, have appeared and increased. The Venezuelan citriculture program is actively in a rootstock transitional stage (11). The presence of severe CTV strains is confirmed by severe symptoms on Mexican and Tahiti limes and grapefruit. A very severe CTV strain, called "Macapo", is able to induce vein clearing and vein corking symptoms in Valencia sweet orange under field conditions (10).

This paper reports the results of biological indexing of several Venezuelan CTV isolates and the results of the first field experiment to evaluate mild strain cross protection in Venezuela.

MATERIAL AND METHODS

Biological characterization. Ten CTV isolates were biological characterized on the basis of vein clearing, vein corking, leaf cupping and stem pitting symptoms in four citrus hosts: Mexican lime, Eureka lemon, sour orange, and Marsh grapefruit. Seven CTV isolates (five mild-moderate: AM1R1, AM2Rs, AM3R1, AM3R3 and MR1R1 and two severe: GM1R1 and 'Macapo' came from the Venezuelan citrus growing areas of Carabobo and Yaracuy States. Three known mild isolates (T26, T30 and T30a) from Florida were used as reference isolates in this study (8, 9). The vein clearing symptoms

were quantified by a vein clearing index (VCI) which indicated the ratio of the number of symptomatic leaves to the total number of leaves. The leaf cupping index (LCI) was determined by counting the number of cupped leaves divided by the total number of leaves of each test plant, which were permitted to grow only three branches. The time elapsed before the first symptoms appeared was taken into account for vein corking symptoms. All CTV isolates were bud inoculated via buds, and treatments replicated using three test plants. Evaluation was carried out every five days after the appearance of the first symptoms and continued for 50 days. Stem pitting was evaluated on Mexican lime eight months after inoculation and was quantified by counting the number of pits per centimeter of stem. The Wilcoxson and Kruskal-Wallis statistical test was carried out to analyze the results.

Serological test. An *in planta* collection of CTV isolates was established in an aphid protected screenhouse. Serological reactions of the isolates were studied from the Mexican lime hosts using monoclonal antibody MCA-13 (12) to detect and confirm the presence of severe CTV strains, and the monoclonal antibodies 3DF1 and 3CA5 were mixed and used to detect all CTV strains (15, 16). The DASI-ELISA assays were run as previously described (12). CTV isolate T36 was used as a severe CTV control when using MCA-13 monoclonal antibody assays. ELISA reader values higher than 0.1 optical density at $A_{405\ nm}$ were considered positive. Young bark was sampled for serological assay. Samples from the screenhouse and the cross protection experiment in the field were collected in August 1991, the month with the highest rainfall in Venezuela.

Cross protection. A small field trial was established in 1989 to determine the behavior of mild CTV isolates under field conditions and their potential cross protection ability. Two mild CTV isolates, T30a from Florida and AM3R3 from Venezuela, were used. The mild isolates were bud inoculated into nucellar Valencia grafted onto sour orange rootstock. Three plants were inoculated with each mild isolate. The challenge was made using more than 60 *T. citricidus* on each plant following feeding on a plant infected with severe isolate GM1R1. After the artificial challenge with aphids, the trees were subject to a continuing natural challenge condition due to the presence of high aphid populations and occurrence of severe CTV strains in the field. Two healthy plants (virus-free when planted in the field) and two plants each infected with a severe (GM1R1) and a moderate (AN3R1) CTV isolate were included for comparison.

RESULTS

Biological characterization. Growth differences were observed in Mexican lime and Marsh grapefruit plants which were inoculated with the mild and severe CTV isolates. Vein clearing usually appeared around 27 days after inoculation under tropical conditions (6). The mild isolates induce minor VCI. T30a, T30, T26 and AM3R3 were the mildest, while isolates AM3R1, MR1R1, AM2R2, and AM1R1 were moderate to severe. GM1R1 and Macapo isolates were very severe and induced dwarfing, vein clearing, vein corking and leaf cupping on Mexican lime and dwarfing and leaf distortion on Marsh grapefruit. The Macapo isolate of CTV induced stem pitting and vein corking on Valencia sweet orange under field conditions, and dwarfing, vein corking and leaf cupping in Marsh grapefruit in the screenhouse. SY symptoms and wilt were induced on sour orange by the Macapo isolate (Tables 1, 2).

Serological tests. The MCA-13 monoclonal antibody reacted positively with AM3R1, GM1R1, Macapo and the T36 control. The mixture of 3DF1 and 3CA5 monoclonal antisera reacted with AM3R3, T26, T30, T30a, AM3R1, GM1R1, Macapo and the T36 control. The reactivity of AM3R1, GM1R1, Macapo, and T36 with MCA-13 agrees with the biological characterization indicating these to be severe isolates (Table 3).

TABLE 1
SYMPTOMS EXPRESSED IN FOUR CITRUS HOSTS BY DIFFERENT ISOLATES OF CITRUS TRISTEZA VIRUS

	Mexican lime						Grapefruit					Sour orange					Lemon				
Isolate	VC[z]	LC	SP	VCK	SY	D	VC	LC	VCK	SY	D	VC	LC	VCK	SY	D	VC	LC	VCK	SY	D
AM1R	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AM2R2	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AM3R1	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AM3R3	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
MR1R1	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GM1R1	+	+	+	+	+	+	+	–	–	–	+	+	–	–	–	–	–	–	–	–	–
T-26	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
T-30	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
T-30a	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Testigo	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Macapo	+	+	+	+	+	+	+	+	–	+	+	+	–	–	+	+					

[z]VC = vein clearing; LC = Leaf cupping; SP = stem pitting; VCK = vein corking; SY = seedling yellows; D = dwarfing.

TABLE 2
VEIN CORKING REACTION ON MEXICAN LIME

CTV Strain	Vein Corking	DAI[z]
AM1R1	–	
AM2R2	–	
AM3R1	–	
MR1R1	–	
GM1R1	+	153
T-26	–	
T-30	–	
T30a	–	
Testigo	–	
Macapo	+	84

[z]DAI = Days after inoculation that vein corking symptoms first appeared.

Cross protection. In the cross protection experiment, challenged plants showed interesting results. During the first 18 months, the plants grew satisfactorily, whereafter plants inoculated with AM3R3 and AM3R1 began to decline. One plant with AM3R3 died. Decline symptoms appeared later in plants inoculated with GM1R1, but plants inoculated with isolate T30a still appeared healthy in October 1992. The healthy control plant, which was planted next to one challenged with severe isolate AM3R3 showed dwarfing while the other control plant which was planted next to a plant inoculated with T30a appeared healthy (Fig. 1).

The trees in the cross protection test were evaluated serologically in 1991. Two of the plants originally inoculated with AM3R3 as well as two of the plants inoculated with T30a reacted positively against MCA-13 monoclonal antibody. One plant originally inoculated with AM3R3 was dead.

DISCUSSION

Differences amongst some Venezuelan CTV isolates have been established on the basis of biological reactions on four citrus hosts as well as their reaction with MCA-13 and the mixture of 3DF1 and 3CA5 monoclonal antibodies. In Venezuela, the serological classification of mild or severe depending on the reaction of the isolate to the MCA-13 antibody agreed well with the biological reaction obtained from the biological index. This indicates that the severe Venezuelan CTV isolates, Macapo, GM1R1 and AM3R1, have an epitope in common with the Florida isolates which cause decline on sour orange rootstock, such as T36. The AM3R1 isolate was considered moderate because it induced vein clearing on Mexican lime 31 days after inoculation, even though the VCI score is similar to isolate AM3R3.

In the cross protection tests, the Florida mild isolate T30a gave promising results, providing protection under the Venezuelan challenge conditions at least for 3 yr. Poor protection was offered by AM3R3 under similar conditions. The serological analysis in 1991 indicated that the plants were indeed challenged by severe CTV strains by their reactivity with MCA-13, however, very interesting when compared with the 1992 results. However, even though trees inoculated with isolate T30a reacted with MCA-13 in 1991, they are still performing well and looking healthy in October 1992. Apparently the MCA-13 has the ability to detect severe CTV strains in mixed infections. Further studies on the mechanism of mild strain cross protection will help explain the basis of multiple strains coexistence in a single tree where the mild strain does not totally inhibit replication of the challenge strain.

Regarding the plants originally planted in the field as virus-free healthy control, the tree planted next to the AM3R3 inoculated plants declined, but the tree next to the T30a inoculated plant still appears healthy. Its condition, and the normal wind direction suggest that T30a may have been introduced into this tree by *T. citricidus* (Fig. 1).

The preliminary data obtained from this first cross protection trial provides information which will be helpful to maintain citrus production in Venezuela. It indicates that some mild CTV isolates, such as T30a from Florida, have the ability to protect against quick decline and stem pitting strains of CTV in plants on sour orange rootstock. However, such tests should be per-

TABLE 3
SEROLOGICAL REACTIVITY OF SEVERAL CITRUS TRISTEZA VIRUS ISOLATES

CTV Strain	Monoclonal Antibodies 3DF1 + 3CA5[z]	Monoclonal Antibodies MCA-13[y]	Host[x]	Biological Characterization[w]	1992 Evaluation[v]	Source[u]
AM3R3	+	–	M.L.	Mild	good	I.P.C.
AM3R1	+	+	M.L.	Moderate	good	I.P.C.
T-30a	+	–	M.L.	Mild	good	I.P.C.
T-26	+	–	M.L.	Mild	good	I.P.C.
GM1R1	+	+	M.L.	Severe	dwarf	I.P.C.
MACAPO	+	+	M.L.	Severe	dwarf	I.P.C.
T-36 (Check)	+	+		Severe		Florida
(3) AM3R3 (Challenged)			VO/SO	Mild	died	F.C.P.P.
(2) AM3R3 (Challenged)	+	+	VO/SO	Mild	dwarf	F.C.P.P.
(1) AM3R3 (Challenged)	+	–	VO/SO	Mild	dwarf	F.C.P.P.
(1) T-30a (Challenged)	+	–	VO/SO	Mild	good	F.C.P.P.
(2) T-30a (Challenged)	+	+	VO/SO	Mild	good	F.C.P.P.
(3) T-30a (Challenged)	+	+	VO/SO	Mild	good	F.C.P.P.

[z] – = no CTV present using polyspecific monoclonal antibodies, + = CTV present.
[y] – = no reaction with MCA-13, + = positive reaction with MCA-13
[x] M.L. = Mexican lime; VO/SO = Valencia sweet orange grafted onto sour orange.
[w] Overall rating of severity of symptoms on the host indicated.
[v] Rating of the isolates in the indicated host during the 1992 evaluation.
[u] I.P.C. = *in planta* collection, maintained aphid free; F.C.P.P. = field cross protection plot, refer to Fig. 4 for guide to tree numbers.

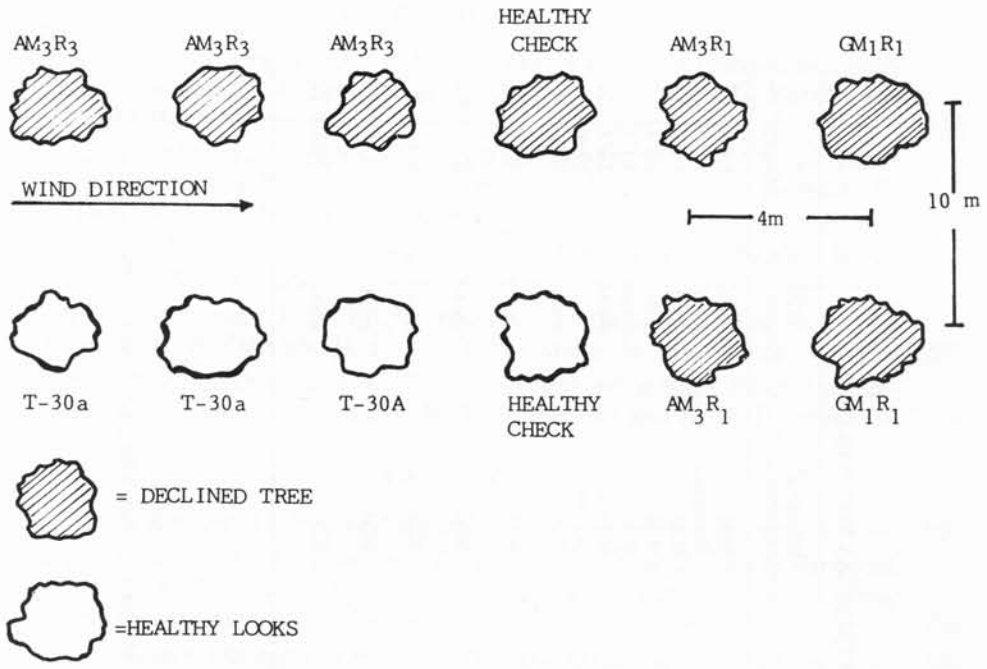

Fig. 1. Plot map of the field cross protection plot with Valencia sweet orange on sour orange rootstock. Declining trees and good performing trees are indicated.

formed with caution. Information on performance and mildness of exotic mild isolates from other countries should be obtained under quarantine conditions, and then on a small scale field plot isolated from the larger commercial plantings before considering release and use of these potentially useful mild isolates on a large scale.

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