

# Evaluation of Tristeza Tolerance of 45 Citrus Types

Zhao Xueyuan, Zhou Changyong, Jiang Yuanhui, He Xinghua,  
Cheng Jiezhong and Cheng Zhusheng

**ABSTRACT.** The tristeza (CTV) tolerance of 45 citrus types, including trifoliolate orange, mandarin, sweet orange, sour orange, shaddock, grapefruit, rough lemon and others was evaluated after graft-inoculation of CTV to their seedlings and to budded plants of sweet orange grafted onto them. Evaluation was made for stem pitting, yellowing, stunting or dwarfing of the seedlings and the tristeza reaction of budlings. CTV replication in seedlings was also examined by ELISA. No stem pitting or abnormal growth was observed in the four types of trifoliolate orange or of sweet orange budded to them. Only one of the 16 seedlings inoculated showed a positive ELISA reaction. Sweet orange grafted onto Dao-xian-ye-ju mandarin showed a tristeza decline reaction. However, no tristeza reaction was observed for the other 11 varieties of mandarin, for the 12 varieties of sweet orange, the two varieties of Chengzi, or the three varieties of rough lemon and Limeng. Of the six varieties of sour orange (including its hybrid Gou-tou-cheng), three showed a tristeza reaction, whereas no symptoms were observed in the other three varieties (Gou-tou-cheng, Xiao-hong-cheng and Zhu-luan). Grapefruit and shaddock varieties showed the maximum susceptibility to tristeza when inoculated either as seedlings or as rootstocks.

Citrus tristeza virus (CTV) is widely distributed in China (1,3). However, since the rootstocks which are commonly used are tolerant or immune, tristeza decline is not generally apparent.

Because of the concern for the appearance of severe isolates of CTV, studies were proposed to determine the pathogenicity of different CTV isolates present in China, their cross protective effects and CTV tolerance of different citrus varieties. In this experiment, a group of citrus cultivars and rootstocks which are important in China, some non-cultivated varieties found in the Chinese mountains and some cultivars imported from abroad were evaluated for their tolerance to CTV by inoculation of seedlings or budded plants of sweet orange on these seedlings as a rootstock.

## MATERIALS AND METHODS

Seeds of 45 citrus varieties were obtained from the National Citrus Genetic Resources Collection at Chongqing. These included four selections of trifoliolate, 12 of mandarin, two of Chengzi, 12 of sweet orange, two of rough lemon, one of Limeng, five of shaddock, two of grapefruit and its hybrid, and six of sour orange and its hybrids. Seeds were sown in February, 1988 and seedlings transplanted

singly in clay pots 23 x 24 cm. The soil mix was 1/3 common soil, 1/3 natural forest topsoil and 1/3 waste of earthworms. Sixteen potted seedlings of each variety were divided into two groups of eight. The first group was tested as seedlings and the second group was tested as a rootstock grafted with a sweet orange scion in April of 1990.

Four plants of each seedling group were graft-inoculated with two pieces of CTV-infected stem bark in November, 1989. The other four plants were grafted with stem bark from non-infected sweet orange seedlings and kept as controls. The budding group was inoculated in the same way in April, 1990. All plants were held in a screenhouse and given the same fertilizer, irrigation and pruning treatments. The screenhouse was located in Beibei, Chongqing. The local mean temperatures ranged from 7.4 C in January to 28.5 C in July, with an annual mean of 18.3 C.

The CTV isolate TR-L514 used in the above inoculation, was obtained by aphid transmission and kept in a Xinhuicheng (sweet orange) seedling as the inoculum source in the screenhouse. This sweet orange seedling did not show stem pitting, but when TR-L514 was graft-inoculated to grapefruit seedlings, a seedling yellows response was observed.

Seedlings of different citrus varieties were evaluated for stem pitting, yellowing, stunting, and CTV replication, as determined by ELISA. The tristeza reaction was evaluated on the budded plants of sweet orange on the various seedling varieties.

Stem pitting in seedlings was recorded in April, 1992, as -, +, ++, +++ representing negative, mild, moderate and severe, respectively.

Replication of CTV in the seedlings was determined by double-antibody sandwich ELISA with polyclonal antibodies (2), using leaf samples. Extracts (1:10 w/v) were prepared in 0.02M PBS-Tween, pH 7.4, containing 0.05% TGA and 2% PVP buffer, using a mortar and pestle. Two repetitions were examined for each sample. Plants were analyzed in April, 1992, and a part of them reexamined in July, 1992. Since ELISA results were similar in both tests, when available, only the second evaluation is presented. CTV-antiserum and alkaline phosphatase-conjugated antibody used were made in August, 1990, by the Plant Protection Assoc. Res. Inst. of Japan.

The decline (yellowing and dwarfing) of seedlings and the tristeza reduction of budlings were recorded in October, 1992, on the following visual scale: - (negative and equal to the controls); ± (slight dwarfing); + (the canopy of the inoculated plants was about 3/4 that of the controls); ++ (about 1/2 of the controls); and +++ (about 1/3 or less of the controls). The dwarfing of seedlings was accompanied with yellowish, smaller leaves, and dwarfing of budlings was accompanied with smaller leaves and leaf yellowing near the midrib.

## RESULTS

The results of this experiment are presented in Table 1. The four trifoliate orange types showed no stem pitting in the seedlings, no adverse effect on seedling growth, no tristeza reaction in the budlings and the ELISA readings were negative indicating no CTV replication. These four types of trifoliate could be classified as immune.

However, an exception was found in one of 16 inoculated trifoliate seedlings which reacted positive to ELISA in both tests.

Mandarins showed no stem pitting, no important effect on seedling growth and generally no tristeza reactions in the budlings. The ELISA readings were moderate to strong. Mandarins are classified as highly tolerant to CTV infection. An exception was found in the inoculated plants of sweet orange on the Dao-xian-ye-ju variety as a rootstock. This combination showed decline.

The performance of sweet orange, Chengzi, rough lemon and Limeng was similar to that of the mandarins although some pits were observed in the seedlings (Table 1). ELISA readings of sweet orange were usually higher than that of the mandarins.

Shaddocks were susceptible to CTV infection. Seedlings showed severe stem pitting, stunting, and yellowing and gave high ELISA readings. When used as a rootstock under a sweet orange scion, they showed a severe tristeza reaction. The performance of grapefruit was similar to shaddock and the Minneola tangelo (a grapefruit hybrid) reacted similarly to grapefruit in seedling decline, but it did not show stem pitting, and when used as rootstock, budlings did not show any tristeza reaction.

Sour orange and its hybrids performed in different ways. Gou-tou-cheng and Xiao-hong-cheng did not show a tristeza reaction when used as rootstocks although the seedlings of Xiao-hong-cheng showed stunting. The Zhu-luan variety was tentatively put in the above group. Dai-dai, Xing-shan-suan-cheng and Ban-gan used as rootstocks under sweet orange showed the tristeza reaction.

## DISCUSSION

The general concept that the trifoliate orange is immune to CTV and that mandarins are tolerant has been confirmed in this experiment. However, there are two outstanding exceptions: one out of 16 trifoliate orange

TABLE 1  
PERFORMANCE OF 45 CITRUS TYPES AFTER CTV INOCULATION

Citrus types	Seedling			Budling (Sweet orange scion)	
	Stem pitting (a)	Yellowing and dwarfing (b)	ELISA values OD410 (c)	Tristeza reaction (b)	
Trifoliolate orange	Large leaf - large flower	-	-	0.00-0.07	-
	Small leaf - small flower	-	-	0.00-1.64	-
	Small leaf - large flower	-	-	0.00-0.07	-
	Large leaf - small flower	-	-	0.00-0.06	-
Mandarin	Gou-shi-gan	-	-	0.25-0.55	-
	Dao-xian-ye-ju	-	-	0.61-1.67	++ , +++
	Yuan-jiang-jian-gan	-	-	0.32-0.83	-
	Shan-tou-suan-ju	-	-, +	0.46-0.86	-
	Hong-pi-suan-ju	-	-	0.62-0.81	-
	Ben-di-zau	-	+	0.77-1.57	-
	Ponkan	-	-	0.49-1.19	-
	Da-hong-pao	-	+	0.79-1.25	-
	Cui-pi-gan	-	-	0.10-1.23	-
	Tankan	-	-	1.57-1.79	-
	Man-ju	-	-	0.00-0.91	-
	Bian-gan	-	-	0.65-0.83	-
Chengzi ( <i>C. junos</i> )	Zhen-cheng	-, +	-	0.00-1.00	-
	Xie-cheng	-	-	0.57-0.77	-
Sweet orange	Jin-cheng	-, +	-	1.19-1.70	-
	An-liu-cheng	-, +	-	0.52-0.61	-
	Xin-hui-cheng	-, +	-	0.91-1.13	±
	Xue-gan	-, +	-	0.02-1.29	-
	Hua-zhou-cheng	-, +	-	0.55-0.89	-
	Tao-ye-cheng	-, +	-	0.39-0.61	-
	Gai-liang-cheng	-, +	-	0.77-0.99	-
	Valencia	-, +	-	0.00-0.80	-
	Hamlin	-, +	-	0.83-1.57	-
	Pineapple	-	-	0.01-1.01	-

TABLE 1 (CONTINUED)  
PERFORMANCE OF 45 CITRUS TYPES AFTER CTV INOCULATION

Citrus types	Seedling			Budling (Sweet orange scion)
	Stem pitting (a)	Yellowing and dwarfing (b)	ELISA values OD410 (c)	Tristeza reaction (b)
Jaffa	-	-	0.00-0.64	-
Ruby blood	-, +	-	0.35-0.77	-
Roughlemon and Limeng	Roughlemon Hong-li-meng	- -, +	0.90-1.59 0.04-1.15	- -
	Tu-ning-meng	-	0.54-0.92	-
Shaddock	Liang-ping-you	+++	1.67-1.95	+, ++
	Si-ji-pao	+, +, +, +, +	0.44-0.82	+, +, +, +
	Wan-bai-you	+++	1.11-1.73	+++
	Lu-you	+, +, +, +	0.00-0.86	+
Grapefruit and hybrid	Duncan grapefruit	+, ++	0.64-1.04	+, ++
	Minneola tangelo	-	0.35-0.86	-
Sour orange and its hybrid	Daidai	-	0.66-1.13	+, ++
	Xing-shan-suan-cheng	-	0.01-0.99	+, ++
	Ban-gan	-, +	0.58-0.94	+, ++
	Xiao-hong-cheng	-	0.58-0.93	-
	Gou-tou-cheng	-, +	0.69-1.42	-
	Zhu-luan	-	0.63-1.07	-, +

<sup>a</sup>Minimum-maximum stem pitting intensity observed: - = no stem pitting, + = mild pitting, ++ = moderate pitting, +++ = severe pitting.

<sup>b</sup>Minimum-maximum symptom intensity observed: - = no difference from the control, ± = slight dwarfing, + = the canopy of the inoculated plants was about 3/4 that of the controls, ++ = about 1/2 of the controls, +++ = about 1/3 or less of the controls.

<sup>c</sup>ELISA values (OD410) measured. These values are the average of two repetitions. ELISA values of negative controls ranged from 0.00 to 0.10.

plants inoculated with CTV gave a positive ELISA reaction indicating that CTV was replicating in this seedling, and one out of 12 mandarin varieties (Dao-xian-ye-ju) expressed a severe tristeza reaction when it was used as a rootstock under a sweet orange scion. Though the trifoliate orange and the Dao-xian-ye-ju appeared morphologically true-to-type, the possibility of these plants coming from sexual embryos and having a hybrid nature cannot be excluded. The mechanism of their respective susceptibility to CTV would need further study and clarification.

Shaddock seedlings inoculated with the seedling yellows isolate TR-L514 showed severe stem pitting and decline. Shaddock trees in the field show stem pitting but generally do not decline. The cause of this variation in the performance of seedlings and field trees has not been determined. Wallace and Drake (4) observed that seedlings of Eureka lemon inoculated with a tristeza-seedling yellows isolate were initially stunted and showed severe seedling yellow symptoms, but eventually they recovered and developed normal growth. A similar recovery has been also observed in shaddock seedlings (Zhao, unpublished data). This recovery phenomenon might explain the differential behavior of shaddock seedlings compared to field trees.

In the sour orange and hybrids group, Gou-tou-cheng, Xiao-hong-

cheng and Zhu-luan did not show the tristeza reaction when used as rootstocks. The CTV tolerance of Guotuo-cheng rootstock has been previously observed (1). According to leaf morphology and fruit peel colour, Goutou-cheng is not a true sour orange, but a hybrid. The three varieties of sour orange which showed tolerance to tristeza, particularly Gou-tou-cheng, are commercially used as rootstocks in Zhejiang Province, especially in the coastal area. Other than CTV and salt tolerance, tolerance to tatter-leaf virus (CTLV) might be another factor which caused Gou-tou-cheng to become an important rootstock in that area. CTLV has been found infecting all the main citrus cultivars of Zhejiang (6).

Gou-tou-cheng usually shows severe stem pitting in the field (4), but only mild or no stem pitting was found in this experiment. This variation was probably caused by differences in the CTV strains involved. In this experiment only one CTV isolate, TR-L514, which does not cause stem pitting in sweet orange seedlings, was used for evaluation. CTV-tolerant citrus types found in this experiment should be further evaluated by inoculating more severe CTV isolates.

#### ACKNOWLEDGEMENTS

The antiserum and enzyme-linked antibody used for ELISA were kindly supplied by Dr. A. Sasaki from Japan.

#### LITERATURE CITED

1. Chao, H. Y., Y. H. Chaing, C. B. Chang, C. S. Chiu and W. F. Su  
1979. Distribution of seedling yellows tristeza in citrus and the tristeza susceptibility of six sour orange rootstocks. *Acta Phytopathologica Sinica* 9: 61-73.
2. Clark, M. F., and A. M. Adams  
1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34: 475-483.
3. Ke, C., S. M. Garnsey and J. H. Tsai  
1984. A survey of citrus tristeza virus in China by enzyme-linked immunosorbent assay, p. 70-75. *In: Proc. 9th Conf. IOCV. IOCV, Riverside.*
4. Roistacher, C. N.  
1986. Recommendation on the citrus virus-free propagation in Zhejiang Province. *Zhejiang Citrus* 4: 19-23.
5. Wallace, J. M. and R. J. Drake  
1961. Seedling yellows in California, p. 141-149. *In Proc. 2nd Conf. IOCV. Univ. Florida Press, Gainesville.*
6. Zhang, T. M., X. Y. Liang and C. N. Roistacher  
1988. Occurrence and detection of citrus tatter-leaf virus (CTLV) in Huangyan, Zhejiang Province, China. *Plant Dis.* 72: 543-545.