Stem-pitting Strains of Citrus Tristeza Virus in Indonessia

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ABSTRACT. In the Indonesian Citrus Variety Improvement Programme all varieties are being cleaned up by shoot-tip grafting (STG) without preliminary heat treatment. Amongst mandarins, 80% of regrafted plants originating from STG are negative for citrus tristeza virus (CTV) by enzyme-linked immunosorbent assay (ELISA), whereas the corresponding figure for sweet oranges is only 60%. Of the sweet oranges showing positive for CTV by ELISA 31% eventually show stem-pitting. Although some plants without stem-pitting give a high optical density (OD) readings by ELISA there is a significant difference in mean OD between those plants with stem-pitting and those without. The average time of recording for stem-pitting is 18 months after STG. There appears to be varietal differences in the ability of STG to separate out stem-pitting strains.

Severe stem-pitting is commonly seen on many sweet orange cultivars but never in mandarins, with the exception of the cultivar Siem. This cultivar closely resembles Som Khe Wan in Thailand where it also shows stem-pitting and it is suggested that this cultivar is not a mandarin species. Very severe stem-pitting is occasionally also seen on pommelo, and has been seen on the main rootstock Japanese Citroen. All these strains are being characterized on a range of citrus species.

In 1937 Toxopeus reported an incompatibility of sweet orange on sour orange rootstock in Java (16) but it was not until 1951 that Terra suggested that this could be due to citrus tristeza virus (CTV) (15), with confirmation that the virus was present by inoculation of Mexican lime seedlings (16).

In more recent years the focus of attention on citrus diseases in Indonesia has been on citrus vein-phloem degeneration (CVPD), more widely known as greening disease, and other systemic diseases have been ignored. Whilst the yield effects of CTV in Indonesia, where rootstock use is now limited to rough lemon and Japanese Citroen, has yet to be determined, it is clear that CTV is endemic in Indonesia (9). ELISA was first used for CTV detection in Indonesia in 1983 and is now in routine use in the indexing activities supporting the Indonesian Citrus Variety Improvement Programme (10).

Tristeza reactions are broadly classified in three categories, i.e., phloem necrosis at bark union in sweet orange on sour orange rootstocks, stem-pitting and seedling yellows (7). Numerous strains have been described for various citrus-growing regions (18). In some countries, such as Brazil (3), Japan (6, 8), California (13), Florida (20) and India (1), attempts have been made to solve CTV problems by using cross-protection with protective mild strains.

In this paper we report on the use of the enzyme-linked immunosorbent assay (ELISA) for CTV detection in regrafted (RG) plants originating from shoot-tip grafting (STG), and observations of stem-pitting on field trees of sweet oranges used as STG sources and on RG plants originating from those trees.

MATERIALS AND METHODS

Citrus extracts (1/10 w/v) were prepared from leaf-vein samples of RG plants, with negative controls being healthy Madam Vinous sweet orange seedlings and positive controls being potted plants of sweet orange showing severe stem-pitting. The method used was the double antibody sandwich ELISA method, developed by Bar-Joseph *et. al.* (2), available as a kit (Plantest ELISA, Sanofi, France). Results were read using an ELISA plate reader (Minireader II, Dynatech, USA), with a positive result being indicated by an optical density (OD) of more than twice that of healthy controls.

The stem-pitting observations were made in a collection of sweet orange cultivars at the Tlekung Horticultural Research Station, Malang, East Java. This 17-yr-old collection was originally planted with each cultivar being included as two trees on rough lemon and two on Japanese Citroen, though various trees have since been lost for various reasons. From each tree eight budsticks (15-20 cm long, 0.6-1.0 cm diam.) were collected at random, the bark removed, and the degree of stem-pitting scored. The severity of stem-pitting was rated in five catagories i.e. 0 (no stem-pitting), 1 (mild, several small pits), 2 (moderate, some scattered small pits), 3 (severe, many small and elongated pits), and 4 (very severe, many elongated deep pits) (4) (Fig. 1). To obtained a stem-pitting score (TS) for a tree the numbers of budsticks in each category were multiplied by the category, the total summed and divided by 8, i.e.

$$\text{Tree SP score (TS)} = \frac{x_1(0) + x_2(1) + x_3(2) + x_4(3) + x_5(4)}{8}$$

Regrafted plants shown by ELISA to be CTV positive were initially maintained in a screenhouse at Punten Experimental Station (max 29 C/min 17 C) for a minimum of six months. Observations for stem-pitting was then done by peeling bark from scions, interstock and rootstocks of the CTV-infected RG plants. In this context interstock is the rootstock seedling used for STG and subsequently retained in the regraft.

RESULTS AND DISCUSSION

ELISA tests. Six hundred and fifty-two RG plants were subjected to ELISA for CTV detection (Table 1). CTV is regarded as a virus easy to eliminate by STG (12). Whilst the shoot-tip size used in Indonesia is 0.1 to 0.2 mm, consisting of the apical meristem and three leaf primordia (17), as recommended by Navarro (12), this relative high level of CTV transmission is clearly unusual, even allowing for the fact that no deliberate heat-treatment was employed prior to STG. Most shoot-tips were collected directly from field trees though some were collected from potted plants maintained in a screenhouse in Malang Horticultural Research Station (max 35 C/min 20 C). Almost all sweet orange trees from which shoots were collected for STG exhibited stem-pitting. A clear exception was in the pommelo cultivar, Bali Nambangan; shoot-tip were collected for this cultivar from a tree not showing stem-pitting although an adjacent tree of the same cultivar showed very severe stem-pitting.

Stem-pitting observations. Field trees of the sweet orange collection. All of the 25 sweet orange cultivars examined showed stem-pitting, though the severity varied; among the most severely affected were Pineapple and Carter navel (Table 2). There was also a distinct variation of severity within the eight budsticks, and between the four trees. Thus, the aggregate TS score for Valencia Late ranged from 1.3 (mild) to 2.6 (severe) for Waturejo from 0.6 (mild) to 2.9 (severe).

TABLE 1
RESULTS OF ELISA TESTS FOR CITRUS TRISTEZA VIRUS (CTV) DETECTION IN REGRAFTED PLANTS

Regrafted plants	No. of cultivars	No. of regrafted plants tested	CTV-infected regrafted plants	
			Number	%
Mandarins	37	444	88	19.8
Sweet oranges	18	184	74	40.2
Pommelo	1	24	0	0

TABLE 2
STEM-PITTING SCORE OF CITRUS TRISTEZA VIRUS-INFECTED SWEET ORANGES

Cultivar	Trees No.	Stem-pitting rating[z] 1	2	3	4	Average
Country		1.4	0.6	1.1	—[y]	1.0
Yoppa		0.6	1.1	—	—	0.9
Punten 604		0.9	0.6	0.3	—	0.6
Lord Howe		—	1.6	1.4	—	1.5
Centra orange		1.5	1.8	1.3	0.8	1.4
Orange?		2.3	3.1	3.1	2.5	2.8
Thomson navel		2.0	—	1.5	2.6	2.0
Bary Valencia		—	3.0	2.3	1.8	2.4
White Selecta		3.1	—	1.6	3.1	2.6
Punten		1.5	1.8	1.5	1.3	1.5
Pineapple		—	2.5	2.1	2.8	2.5
Noris		1.6	1.8	2.4	2.8	2.2
Maosambi		2.8	—	1.6	0.6	1.7
Laorang		2.6	2.0	2.4	2.5	2.4
Rosemiel		2.9	1.3	1.6	2.0	2.0
Miel		1.8	0.9	0.5	—	1.1
Punten 598		2.0	2.0	2.6	2.9	2.4
Waturejo		2.9	0.6	2.8	1.5	2.0
Italy		2.3	2.1	—	—	2.2
Huan		1.1	1.3	1.1	1.3	1.2
Carter Navel		3.3	1.9	2.5	3.0	2.7
Ruby		—	2.4	2.1	—	2.3
Kupang		2.6	2.4	1.9	2.6	2.4
Grovery		2.8	2.0	2.0	2.1	2.2
Valencia Late		1.3	1.6	2.6	1.9	1.9

[z]Stem-pitting rating: 0 = no stem-pitting, 1 = mild, several small pits, 2 = moderate, some scattered small pits, 3 = severe, many small and elongated pits, and 4 = very severe, many elongated deep pits.
[y]— = trees no longer exist.

TABLE 3
STEM-PITTING EXAMINATION OF CITRUS TRISTEZA VIRUS (CTV)-INFECTED REGRAFTED PLANTS OF MANDARINS AND SWEET ORANGES

Regrafted plant code	Period after STG[z] (mo)	OD_{410}[y]	Result of stem-pitting examination on:[x] Scion	Interstock		Rootstock	
Mandarins							
Batu 55							
T00 006	24	0.41	–	JC	–	JC	–
T00 007	16	0.18	–	JC	–	JC	–
T00 035	11	0.18	–	JC	+ + +	JC	+
T00 038	11	0.28	–	JC	+ + +	JC	+
Garut							
T00 001	23	0.14	–	JC	–	JC	–
T00 002	29	0.35	–	JC	–	JC	–
T00 005	29	0.58	–	JC	–	JC	–
T00 132	9	0.16	–	JC	+ + +	TC	–
Madura							
T00 001	24	0.57	–	JC	+	JC	+ + +
T00 005	16	0.10	–	JC	–	JC	–
T01 005	16	0.54	–	RL	–	JC	–
T01 006	16	0.40	–	JC	–	JC	–

TABLE 3 (CONTINUED)
STEM-PITTING EXAMINATION OF CITRUS TRISTEZA VIRUS (CTV)-INFECTED REGRAFTED PLANTS OF MANDARINS AND SWEET ORANGES

Regrafted plant code	Period after STG[z] (mo)	OD_{410}[y]	Result of stem-pitting examination on:[x]				
			Scion	Interstock		Rootstock	
Kacang Cingkalak							
T00 001	19	0.54	−	JC	+ + +	JC	+
T00 003	19	0.13	−	JC	−	JC	−
T00 006	19	0.10	−	JC	−	JC	−
T00 007	19	0.52	−	JC	+ + +	JC	+
Batu							
T13 001	22	0.55	−	JC	+	JC	+ +
T13 002	22	0.50	−	JC	+ + +	JC	+
T13 003	22	0.55	−	JC	+ + +	JC	+
Kedu							
T00 001	20	0.15	−	JC	−	JC	−
T00 002	20	0.14	−	JC	−	JC	−
T00 003	16	0.16	−	JC	−	JC	−
T00 005	16	0.19	−	JC	−	JC	−
Sweet oranges							
Punten							
T00 001	16	0.53	+ +	RL	−	JC	−
T00 012	15	0.16	−	JC	−	JC	−
T00 021	15	0.43	+ +	JC	−	JC	−
P02 015	16	0.57	+ +	JC	+ +	JC	+ + +
Punten 168							
T00 001	18	0.48	−	JC	−	JC	−
T00 002	18	0.49	−	JC	−	JC	−
T00 004	18	0.42	−	JC	−	JC	−
Pacitan							
T00 006	15	0.57	+ + +	JC	−	JC	−
T00 007	15	0.11	−	JC	−	JC	−
T00 008	15	0.10	−	JC	−	JC	−
T00 009	15	0.51	−	JC	+ +	JC	+
Valencia Late							
T00 001	19	0.53	+ + +	JC	−	JC	−
T00 005	19	0.47	−	JC	−	JC	−
T00 007	19	0.54	+ +	JC	−	JC	−
T00 023	15	0.48	+ +	JC	−	JC	−
Washington navel							
P01 003	16	0.17	−	JC	−	JC	−
P01 004	16	0.15	−	JC	−	JC	−
P02 011	16	0.15	−	JC	−	JC	−
P02 013	16	0.21	−	JC	−	JC	−
Waturejo							
T00 003	19	0.43	−	JC	−	JC	−
T00 004	19	0.44	−	JC	−	JC	−
T00 006	19	0.43	+ + +	JC	−	JC	−
Kali Bali							
T00 011	15	0.43	−	JC	−	JC	−
T00 012	15	0.44	+ +	JC	+ + +	JC	+ +
T00 013	15	0.16	−	JC	−	JC	−

[z]Time period from shoot-tip grafing to stem-pitting examination.
[y]Optical density range of positive control is 0.40 to 0.54 and negative control is 0.03 to 0.05.
[x]JC = Japanese Citroen, RL = rough lemon, TC = Troyer citrange; − = no stem-pitting, + = mild, + + = moderate, + + + = severe.

No stem-pitting has been observed in mandarins though it has been seen in a cultivar called Siem Pontianak in West Kalimantan (19) and in other clones of Siem, all of which are considered as synonymous with Som Khe Wan in Thailand and suggested to be *Citrus sukuiensis*

Hort. ex Tanaka (5). Severe stem-pitting has also been observed on Japanese Citroen, which account for 50% of Indonesian rootstocks; this cultivar is phenotypically similar to Rangpur lime.

In Brazil a very virulent strain of CTV known as Capao Bonita, is spreading in particular areas, and is extremely destructive to certain sweet oranges and Rangpur lime rootstooks (13).

CTV-infected RG plants. The result of observations on 48 CTV-infected RG plants is summarized in Table 3. No stem-pitting was observed on mandarins although some clearly contained stem-pitting CTV strains as shown by the development of symptoms on the Japanese Citroen interstocks and rootstocks. The presence of the stem-pitting strain in the scion was not strongly correlated with high OD values, as shown by some plants of Batu 55 and Garut. Although mandarins are regarded as tolerant to CTV it appears that they may contain strains capable of causing stem-pitting on the most widely used Indonesian rootstock.

On sweet orange cultivars there was a general relationship between higher OD values and scion stem-pitting but stem-pitting on the Japanese Citroen interstocks and rootstocks was less frequent and less severe than was the case with mandarin scions. In the case of Pacitan 009 the virus did not induce stem-pitting on the scion but did so on the Japanese Citroen whereas with Punten 168 no stem-pitting occurred on any part of the composite plant despite the scion having a high OD by ELISA.

CONCLUSIONS

Based on these results it can be seen that STG can eliminate certain components of the CTV complex, particularly those inducing stem-pitting. In certain cases RG plants had a high OD but no stem-pitting despite the original shoots having been obtained from trees exhibiting stem-pitting. Navarro (12) stated that STG can separate CTV strains, and that plants infected with a mild strain were obtained from plants infected with severe seedling yellows.

Almost all sweet orange cultivars contain stem-pitting strains which are not easily eliminated by STG. This is possibly due to the increased ability of severe CTV strains to invade meristematic tissues (10).

ELISA has demonstrated that CTV is present in almost all citrus trees in Indonesia but apparently this consists of a complex of strains, some of which have a potential to be damaging despite the use of rootstocks other than sour orange.

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