Inactivation of Citrus Tristeza Virus (CTV) with Heat Treatment: Heat Tolerance and Inactivation of CTV on Rootstock-Scion Combinations

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More than 80 per cent of the citrus plantings in Japan are satsuma mandarins, and most are infected with citrus tristeza virus (CTV). More than half the satsuma mandarins are infected with severe strains of CTV, and most carry seedling yellows (CTV-SY) (Yamada *et al.*, 1969, 1979). Because satsuma mandarins are very tolerant of CTV, they grow well and produce good-quality fruit. The brown citrus aphid (*Toxoptera citricida*), which transmits CTV, is widely distributed in citrus in Japan. Tristeza-free budded trees of most CTV-susceptible varieties of citrus (except Satsuma mandarin) are usually affected by severe strains of CTV in a short time. Inoculation of CTV-free citrus with a mild strain of CTV to protect it against infection by severe strains is being considered.

In this paper, basic experiments to obtain CTV-free citrus by heat treatment were carried out as the first step toward this purpose.

MATERIALS AND METHODS

Two-year-old trees of 10 rootstock varieties (Tachibana, Yuzu, Yamamikan, Rusk citrange, Mexican lime, trifoliate orange, Sunki, rough lemon, Cleopatra and Shekwasha) and four scion varieties (Morita navel orange, Miyauchi-Iyo, Seminole and Okitsu No. 14 [Miyagawa-wase X Temple]) were planted in pots. Four trees were used for each rootstock-scion combination. Trees that already had young shoots before heat treatment were placed in a naturally lighted phytotron cabinet at 45°C daytime and 35°C nighttime temperature, but were kept at 40/30°C until the third week.

After heat treatment for 5, 7 and 9 weeks, several shoots from the four scion varieties were cut and grafted onto trifoliate orange. Then, the fluorescent antibody technique was used to index for CTV in the petioles of the shoots (Tsuchizaki *et al.*, 1978). All trees, after heat treatment, were kept in the greenhouse, and the fluorescent antibody technique was used to check for CTV in the petioles of newly developed shoots 5, 9, 16 and 25 weeks after removal. Trees in which the characteristic fluorescent bodies were not observed were indexed for CTV on Mexican lime and Eureka lemon.

RESULTS

The appearance of heat-treated trees was evaluated after 5, 7 and 9 weeks (table 1). Morita navel orange appeared very tolerant of heat, Miyauchi-Iyo was tolerant, Seminole was susceptible, and Okitsu No. 14 was very susceptible. Differences in the heat tolerance among the 10 rootstock varieties were difficult to detect, but Yama-mikan and Tachibana appeared slightly tolerant. At 45°C daytime and 35°C nighttime temperature, heat tolerance was related more to scion variety than to rootstock variety. Trees on Mexican lime rootstock grew poorly even before heat treatment.

After 5 weeks, the characteristic fluorescent bodies in the petioles of Seminole and Okitsu No. 14 almost disappeared, and the number in Miyauchi-Iyo and Morita navel orange decreased (table 2). Budwood from heat-treated trees was grafted on trifoliate orange, and CTV-free budlings of Morita navel orange, Miyauchi-Iyo, and Okitsu No. 14 were obtained.

After 7 weeks, characteristic fluorescent bodies in the petioles of Miyauchi-

TABLE 1
COMPARISON OF HEAT TOLERANCE OF VARIOUS ROOTSTOCK-SCION COMBINATIONS

Rootstock	Scion and period of treatment*											
	Miyauchi-Iyo			Morita navel			Seminole			Okitsu No. 14		
	5†	7	9	5	7	9	5	7	9	5	7	9
	weeks			weeks			weeks			weeks		
Tachibana	G‡	B	B	VG	G	G	B	VB	D	B	D	D
Yuzu	B	B	D	VG	VG	VG	VB	D	D	VB	D	D
Yama-mikan	G	B	VB	VG	VG	G	B	B	VB	B	D	D
Rusk citrange	B	VB	D	VG	VG	G	B	VB	D	VB	D	D
Mexican lime	G	B	VB	G	G	G	B	B	D	B	D	D
Trifoliate orange	G	B	VB	VG	VG	G	VB	D	D	D	D	D
Sunki	G	B	VB	VG	G	G	B	D	D			
Rough lemon	B	D	D				B	D	D	B	D	D
Cleopatra	G	G	VB	VG	VG	G	B	D	D	B	D	D
Shekwasha	B	D	D	G	G	B	D	D	D	VB	D	D

* Heat treatment was begun on June 7, 1978 at 45°C daytime and 35°C nighttime, but plants were kept at 40/30°C until the third week.
† Weeks from the start of heat treatment.
‡ Condition of citrus trees: VG = very good; G = good; B = bad; VB = very bad; D = dead or nearly dead.

TABLE 2
PROGRESSIVE CHANGE OF CTV WITHIN THE PETIOLE DURING HEAT INACTIVATION, AS DETECTED BY THE FLUORESCENT ANTIBODY TECHNIQUE

Scion	Mean number* before heat treatment	5 Weeks		7 Weeks		9 Weeks	
		A	B	A	B	A	B
Morita navel orange	31.2	23.6*	5/5† (15)‡	6.8*	11/18† (42)‡	1.0*	9/14† (72)‡
Miyauchi-Iyo	21.4	6.5	5/5 (14)	0.5	4/4 (42)		
Seminole	46.2	1.0	0/0 (14)	0.6	1/1 (47)		
Okitsu No. 14	about 20	0.8	3/3 (19)				

* Mean number of characteristic fluorescent bodies.
† CTV-free budlings/number successfully grafted.
‡ Number grafted.

Iyo nearly disappeared, but a few remained in the Morita navel orange. Budlings from heat-treated Miyauchi-Iyo and Seminole were CTV-free, but some of those from Morita navel orange were not completely CTV-free.

After 9 weeks, characteristic fluorescent bodies in Morita navel orange nearly disappeared, but, again, some of the budlings were not completely CTV-free.

After heat treatment, all trees were kept in the greenhouse. Leaves of newly developed shoots from heat-treated trees were observed using the fluorescent antibody technique 5, 9, 16 and 25 weeks after heat treatment. There were no characteristic flourescent bodies of CTV in one tree of Miyauchi-Iyo (table 3). Budwood of this tree was indexed on Mexican lime and Eureka lemon, and symptoms of CTV did not appear. So, the tree is considered to be CTV-free, but characteristic fluorescent bodies were observed in 19 trees of Morita navel orange, six of Miyauchi-Iyo, and one of Seminole.

DISCUSSION

There are many methods to obtain virus-free plants (Nyland and Goheen, 1965; Rossetti *et al.*, 1965; Stubbs, 1968; Calavan *et al.*, 1972; Murashige *et al.*, 1972; Roistacher and Calavan, 1972, 1974; Roistacher *et al.*, 1972; Omori *et al.*, 1974; Navarro *et al.*, 1975), and heat treatment is generally used. There are two methods for heat treatment. One is keeping plants directly in a high temperature chamber for long periods and the other is keeping plants at high temperature for a short time, after preconditioning at a lower temperature. Roistacher and Calavan (1972) inactivated citrus viruses by the latter method, combining several temperatures and times.

The degree of heat tolerance of citrus trees was different among scion varieties in this experiment. A rootstock was sought to promote heat tolerance of citrus trees, but distinct differences were not seen among the 10 rootstock varie-

TABLE 3
MULTIPLICATION OF CTV IN TREES AFTER HEAT TREATMENT

Scion	Rootstock and tree number		No. characteristic fluorescent bodies: Just after heat treatment	No. characteristic fluorescent bodies: 16 weeks after termination of heat treatment (exceptions noted)
Morita navel orange	Trifoliate orange	-1		>29
		-2		>13
		-3		12
		-4		5
	Shekwasha	-1	1.7 (14)*	>20
		-3		>15
	Cleopatra	-1	1.5 (11)*	>35
		-4		>24
	Yama-mikan	-1		>50
		-2		>44
		-3	0.7 (13)*	>41
	Sunki	-1	0.5 (10)†	>31
		-2		>32
	Yuzu	-2		>0.2
		-3	1.2 (17)*	>26
		-4		>50
	Tachibana	-2		>19
		-4	0.3 (7)*	>25
	Mexican lime	-2		>53
	Rusk citrange	-4		>64
Miyauchi-Iyo	Trifoliate orange	-1		0‡
	Cleopatra	-2	0.2 (6)†	30
	Yama-mikan	-3		39
		-4	0.9 (10)†	14
	Sunki	-1	0.2 (8)†	26
	Tachibana	-2		>50§
		-3		4§
Seminole	Yama-mikan	-3	1.1 (14)†	60

* Heat treatment for 9 weeks (no. samples tested are in parentheses).
† Heat treatment for 7 weeks.
‡ 5, 9, 16, and 25 weeks after termination of heat treatment.
§ 25 weeks.

ties. Therefore, for efficient heat treatment of heat-intolerant varieties, preconditioning at a low temperature for several weeks is considered desirable. Calavan *et al.* (1972) inactivated CTV with heat, using 40°C for 16 hours (day) and 30°C for 8 hours (night) for 8 weeks. Omori and Matsumoto (1974) also inactivated CTV with heat treatment. For inactivation of virus and good growth of the plant, alternating temperatures appeared superior to a constant temperature (Nyland 1969; Calavan *et al.*, 1972). In this experiment, four varieties of citrus were placed at alternating temperatures (45/35°C) for 5, 7 and 9 weeks, and CTV-free trees were obtained, but some of the heat-tolerant Morita navel orange trees were not completely freed.

From this result, it appears that heat tolerance of CTV in plant tissue is closely related to the heat tolerance of the citrus tree. So, inactivation of virus in heat-tolerant plants may require a longer period than in heat-intolerant plants. Characteristic fluorescent bodies tended to decrease and disappear more rapidly in heat-intolerant varieties than in tolerant ones. Even when characteristic fluorescent bodies did not completely disappear, CTV-free plants were obtained. Antiserum may react with inactivated CTV. CTV is also inactivated as the host plant declines from the heat. Host plants must be kept at sufficiently high temperature to cause the plants to decline somewhat. Occasionally, a whole plant, as Miyauchi-Iyo, will become CTV-free after being held at high temperature for a long period.

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