Effective Methods for the Elimination of Citrus Tatter Leaf Virus by Thermotherapy and Shoot-Tip Grafting

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ABSTRACT. Citrus tatter leaf virus (CTLV) is an endemic citrus pathogen in Zhejiang Province, China, and, consequently, the use of the CTLV-sensitive trifoliate rootstock is precluded. Therefore, research was conducted on the elimination of CTLV from the main citrus varieties in Zhejiang. Thermotherapy alone and in combination with shoot-tip grafting (STG) were tested for CTLV elimination from propagation budwood. STG alone was unsuccessful, as was pre-heat treatment of 35° to 40°C for 10 to 30 days prior to STG. However, heat treatment of 40/30°C for 110 days, or 40° to 42° to 44/30°C for 6 + 2 + 2 wk, or 44/30° to 34°C for 8 or 9 wk effectively eliminated CTLV from budwood from a number of citrus cultivars.

Roistacher et al. (11) eliminated citrus tatter leaf virus (CTLV) from Meyer lemon budwood treated with hot moist air at 50°C for 3 to 22 h; whereas Calavan et al. (1) were unable to eliminate the virus in treated buds propagated on citrange in a heat chamber at 40/30°C (16-h day/8-h night) for 12 wk, but an additional 2 wk at 44/30°C was successful. Japanese scientists found that maintaining infected plants at 40/30°C or 41/31°C regime for 17 or 15 wk, respectively, rendered the plants CTLV-free (4, 6). Attempts to eliminate CTLV by shoot-tip grafting (STG) have been unsuccessful (10, 12). Okudai et al. (9) obtained CTLV-free plants by pre-heat treating at 40/30°C for 40 days prior to grafting 5-13 mm shoots. Koizumi (5) found that Satsuma mandarin could not tolerate these temperatures, but that the virus was eliminated when he took shoot tips from plants after 9 days at 40/30°C followed by 13 to 20 days at 35/30°C. He et al. (3) obtained CTLV-free plants by varying the day temperatures from 35° to 42.5°C with 30°C nights for 102 days followed by STG.

Because of the prevalence of CTLV in Zhejiang Province, China (13), the preferred trifoliate rootstock cannot be used. It is, thus, essential to develop methods to eliminate CTLV from citrus cultivars important in this province. This paper reports on the testing of various combinations of temperature treatments with and without STG on these cultivars.

MATERIALS AND METHODS

STG. All source plants obtained from trees in Zhejiang were found by indexing on citrange to be infected with CTLV (13). Troyer citrange seedlings grown in the dark were used for the rootstock in the STG procedures which followed the methods described by Navarro et al. (7). Six to 10 days after grafting, the emerging shoot and its rootstock were grafted to established Troyer citrange seedlings as described by de Lange (2). The varieties included: Bendizao mandarin (*Citrus succosa*); Ponkan mandarin; Xuekan sweet orange; and Washington navel.

Thermotherapy. Plants were maintained in a controlled environmental chamber at varying temperatures or in a wood-frame structure with two layers of glass and controlled humidity of approximately 85%. The treatments were: 1) 40/30°C (16-h day/8 h night) on Ponkan budwood for 56 days, and on Ponkan, Washington navel and blood orange plants for 60 to 110 days; 2) 40/30°C for 6 or 10 wk followed by 42/30°C for 2 wk and an additional

44/30°C for 2 wk on Hongyukan mandarin (*C. succosa*), Washington navel, Newhall navel, Kaixuanhan tangor and Ponkan plants; and 3) 44/30° to 34°C for 8 or 9 wk using Newhall navel, Skagg's bonanza navel and Omishima navel plants (to increase heat while shortening exposure time).

Thermotheropy plus STG. Okitsu Wase and Bendizao mandarins were pre-conditioned at 35/30°C or at 40/30° plus 35/30°C for 10, 20 or 30 days prior to STG.

RESULTS AND DISCUSSION

STG. Five STG plants of the four varieties grafted were indexed on Troyer citrange and all were positive for CTLV. Therefore, STG alone was unsuccessful.

Thermotheropy. Heat treatment at 40/30°C (16/8 h) of Ponkan buds for 56 days, Ponkan plants for 60, 90 or 100 days, Washington navel for 90 or 100 days, and blood orange for 100 days failed to eliminate CTLV. Only the treatment of 40/30°C for 110 days eliminated the virus from Ponkan, but not from the sweet orange varieties. However, progressively increasing the heat treatment from 40/30°C for 6 wk, plus 2 wk at 42/30°C and then 2 wk at 44/30°C successfully eliminated CTLV from Hongyukan mandarin and Washington navel. Treatment of Newhall navel at 40° to 42° to 44/30°C for 10 + 2 + 2 wk also eliminated CTLV. However, treatment of Ponkan and Kaixuankan tangor at 40° to 42° to 44/30°C for 6 + 2 + 2 wk was unsuccessful. Heat treatment of Newhall and Skagg's bonanza navels at 44/30° to 34°C for 8 and 9 wk, respectively, was successful, but shorter times used with Omishima navel were not.

Thermotherapy plus STG. Pre-conditioning for up to 30 days at the elevated temperatures followed by STG failed to eliminate the virus from the two varieties tested.

The results presented here confirmed that STG alone fails to eliminate CTLV. Although pre-heat treatment prior to STG was effective in eliminating psorosis-like pathogens (8), the treatments in these experiments failed the eliminate CTLV. Heat treatment for 40/30°C for 110 days was effective, a result similar to that of Miyakawa (6), however, shorter treatments were unsuccessful. Heat treatments for 40° to 42° to 44/30°C for 6 + 2 + 2 wk were also effective, but the time can be shortened by treating at 44/30° to 34°C for 8 or 9 wk.

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