EFFECT OF HOT-AIR TREATMENT ON LIKUBIN. TRISTEZA VIRUS AND EXOCORTIS VIROID DISEASES OF CITRUS[1]

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Abstract: Buds from likubin diseased citrus trees were grafted to rangpur lime seedlings. They were exposed to alternate temperatures of daytime ca. 40°C for 16 hr and nighttime 30° C for 8hr for a period of 4 weeks or longer, such treated plants produced no likubin symptoms in new growth after two-year period of observation. All treated plants indexed in mexican lime seedlings showed tristeza virus symptoms. These results indicated that the main component of likubin pathogen was inactivated, but the tristeza virus was not completely inactivated. Young shoots from tristeza-infected mexican lime seedlings which had been placed at 40/30°C for 15 weeks or 39-45/29-33°C for 10 weeks could be free from tristeza virus. Temperatures at 38/28°C was not sufficient for virus inactivation. None of hot-air treated cuttings were free from exocortis pathogen even the treatment was prolonged up to 52 weeks.

Introduction

Heat therapy has been extensively used for the elimination of graft transmissible pathogen in citrus (1, 10) and many other perennial plants (8) . Early in 1941, Fawcett and Cochran (3) reported that the resistance of citrus tissue and psorosis virus A to heat. Their results indicated that hot water treatment had no direct practical value for virus inactivation. Similar results were also reported by Grant in 1957 (6) . Hot-air treatment, on the other hand, was reported to be efficient for obtaining some virus-free sources of citrus by many workers (1, 5, 10). Desjardins et al (4) stated that tristeza virus symptoms were suppressed when the infected mexican lime seedlings were held for a period of 3—4 weeks at 40°C. Moreover, placing the infected plants at 98—104°F for 86—100 or more days were sufficient for getting psorosis-and tristeza-free tissues (5) .

Recently, Calavan et al (1) found that alternating temperatures were superior to constant temperature for plant survival as well as for virus inactivation. Since virus and virus-like diseases of citrus have been widespread and become important in Taiwan,

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it is of urgent need to obtain disease-free budwood sources for commerical uses. The purpose of this study was to realize a suitable hot-air treatment method for obtaining likubin-tristeza- and exocortis-free budwood sources for the establishment of the citrus variety improvement program in Taiwan.

Materials and Methods

Two types of thermotherapeutic chambers were used in this experiment : one is a 'Hotpack' model 352720 refrigerated incubator and another one is a 2.7m ×2.7m×2.0m woodmade chamber with aluminium-sheathed. The former was divided into two layers with automatic temperature control at ca. 40°C for daytime 16 hr and 30°C for nighttime 8 hr in the upper layer and about 38/28°C in the lower layer. The latter was equipped with heating system with maximum temperatures at 39—45/29—33°C in summer season.

Sources of likubin-infected budwood were collected from 10-year-old ponkan and tankan trees in Taichung and Chiayi orchards. About 2 diseased buds were bud-grafted to a healthy rangpur lime seedling and placed in the thermotherapeutic chamber one week after graft inoculation. 35 seedlings were treated with various intervals and then removed to the greenhouse. The seedlings with survived buds were then forced to develop new growth and reindexed in suitable indicator plants.

The tristeza-infected mexican lime and exocortis-infected rangpur lime seedlings were held in heat chamber. After treated with different intervals, young shoots about 1 cm in length with 2 new developing leaves were taken and grafted onto suitable size of indicator plants to determine the presence of virus in selected tissue. Observation for symptom development was continued at least for one year.

Results

I. Effect of hot-air treatment on diseased buds of likubin complex.

23 out of 35 rangpur lime seedlings were with buds survived. The results are summarized in Table 1. In general, new growth from diseased buds grew very vigorous and healthy after a period of hot-air treatment, but these plants indexed in mexican lime indicator plants showed tristeza virus symptoms. Likubin-diseased buds without any treatment produced typical symptoms 4—5 months after graft inoculation.

2. Effect of hot-air treatment on tristeza-infected mexican lime seedlings and exocortis-infected rangpur lime seedlings.

Data are summarized in Table 2. The results indicated that young shoots taken from tristeza-infected seedlings, which had been treated with 40/30°C for 15 weeks or with 39—45/29—33°C for 10 weeks, were free from tristeza. None of plant tissues taken from exocortis-infected seedlings were free from exocortis pathogen under the same temperature conditions.

Table 1. Effect of hot-air treatment on diseased buds of likubin pathogen complex

Sources	Variety	Period of treatment (weeks)	Temperature (°C)	Results		
				No. plants with bub survived	No. plants with likubin symptom	No. plants with tristeza virus
Chiayi	Ponkan	4	40/30	2	0	2
Taichung	Ponkan	4	40/30	4	0	4
Chiayi	Ponkan	8	40/30	2	0	2
Taichung	Ponkan	8	40/30	5	0	5
Taichung	Ponkan	13	40/30	3	0	1
Taichung	Tankan	8	39—45/29—33	4	0	4
Taichung	Tankan	18	39—35/29—33	3	0	0

Table 2. Results of young-shoot (YS) tests for presence of tristeza and exocortis diseases after infected seedlings with various interval hot-air treatment

Temperature (°C)	Period of treatment (weeks)	Results	
		No. YS free from tristeza / No. YS survived	No. YS free from exocortis / No. YS survived
38/28	15	1/3	—
38/28	20	4/11	—
40/30	10	0/8	0/7
40/30	15	9/10	0/9
40/30	20	6/7	0/8
40/30	30	—	0/6
40/30	52	—	0/8
39—45/29—33	10	9/10	—
39—45/29—33	16	9/9	0/7
39—45/29—33	20	8/8	0/9

Discussion

Likubin (also called Huanglungpin) is the most serious disease of citrus trees in Taiwan. The causal agent was formerly considered to be a virus closely related to tristeza (7) . Recent study revealed that the main component of likubin pathogen was probably mycoplasma-like organisms(2, 14). According to Su et al (14), likubin pathogen was readily inactivated by dipping the diseased scion in hot water at the temperature higher than 48°C for 10 min. Diseased Ponkan and Tankan seedlings treated at 30/40°C on an 8/16 hour cycle for one month showed remarkable recovery by producing healthy

normal shoots (15) . Data from the present tests also showed that diseased buds grafted to rangpur lime seedlings treated at 40/30°C for four weeks or longer showed no likubin symptoms after two-year period of observation. However, the hot-air treated plants indexed on mexican lime seedlings could produce tristeza virus symptoms. If the treatment was prolonged for a period of 13 weeks, the tristeza virus could be inactivated. It indicated that the main pathogen of likubin could be inactivated easier than tristeza virus.

The absence of tristeza virus symptoms in plants grown under warm temperature condition was a masking effect(4, 11). The disease seemed more difficult to be eliminated at warm temperature of 23—40°C maximum in daytime and 25.6°C minimum in nighttime, but a supplemental treatment in hot-moist air at 50°C for 3—7 hours could eliminate seedling yellows-tristeza virus (10) . A heat exposure period of 107 days was sufficient to inactivate the virus in young tissues, but it could not get rid of the entire source plants of tristeza virus (5). The results from the tests showed that most young shoots cut from infected plant treated with 40/30°C for a period of 15 weeks or 39 —45/29—33°C for 10 weeks were free from tristeza virus. Young shoot about 2—3 month old containing 2 new small leaves gave a higher percentage of survival rate. Temperature at 38/28°C seems to be insufficient for inactivation of tristeza virus even if the treatment is prolonged for 20 weeks. Therefore, young shoots developed under warmer temperature condition may show no tristeza symptoms, but soon after the infected plants were removed to greenhouse temperature, tristeza symptoms would reappear on new developing leaves.

The inactivation of exocortis by a combination of heat therapy and shoot-tip propagation had reported by Stubbs (16) . but the pathogen seemed very tolerant to heat (9) and was a infectious form of free nucleic acid (12, 13) . It remained active from a sweet orange plant treated for 32 weeks at 40/30°C (9). The results from this test confirmed that the heat treatment could not satisfactory inactivate the exocortis pathogen and obtain exocortis -free bud for further propagation.

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熱處理對柑桔立枯病、Tristeza及鱗砧病等病原之影響[1]

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摘要：以感染柑桔立枯病之病穗芽接於二年生健全廣東檸檬幼苗上，每天給於照光16小時40°C/ 無照光 8 小時30°C之交替溫度處理，經過四週或較長時間處理後，新生枝條均呈現健全，顯示熱處理已將立枯病病原不活化。但若採取此等植物之植株經墨西哥雷木檢定，則可發現有 Tristeza virus 之存在。由是可知。，立枯病主要病原比 Tristeza virus 較易於利用熱處理而使之不活化。

感染Tristeza virus之墨西哥雷木幼苗，同樣置於40/30°C之交替溫度處理，經過 3 至 4 週後，新生雷木葉片上 Tristeza 病徵被抑制，但病毒仍然存在。病植物繼續處理，經過十週，尚未使病原不活化，但延長至15週，則採取之嫩穗，大部份已無 Tristeza 。溫度越高，處理效果越佳，時間亦可縮短，如在 39—45/29—33°C之溫度下處理病植物10週後，即可獲得無 Tristeza病之嫩穗。在植 38/28°C下，雖然處理時間長達20週，對 Tristeza 乃然無效。Exocortis 病原對熱抵抗性很强，病物雖然處理時間長達一年，切取之嫩穗乃含有其病原。

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