

# Elimination of Citrus Pathogens by Shoot-Tip Grafting and the Establishment of Citrus Germplasm in Fujian Province, China

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**ABSTRACT.** Since 1983, a program for the elimination of graft-transmissible pathogens of citrus has been in operation in Fujian Province, China. A total of 37 excellent cultivars were collected mainly from Fujian Province and were grafted to trifoliolate orange rootstock. Young shoots from these cultivars were subjected to shoot-tip grafting (STG) and later indexed. Citrus huanglongbing liberobacter, citrus tristeza virus and exocortis viroid were eliminated by STG with an efficiency of 100, 81.3 and 40 to 60%, respectively. Tatter leaf virus was eliminated by combining thermotherapy followed by STG. Experiments for improving the success of STG were carried out. Graft survival was improved by applying 0.1 ppm zeatin or 0.1 ppm kinetin to the excised surface of 15-day-old rootstock seedlings with the cotyledon intact before STG. Grafting the newly forming shoot directly from the test tube to established seedlings was somewhat successful. Pathogen-free plants were propagated in an insect-proof screenhouse and are being maintained as a germplasm repository in Fujian.

In recent years, the area planted to citrus in Fujian Province, China has expanded from 45,000 ha in 1984 to 160,000 ha in 1994 with production increasing from 200,000 metric tons (MT) to 970,000 MT during this period. There are four major graft-transmissible agents present in Fujian citrus: huanglongbing (HLB) liberobacter, citrus tristeza virus (CTV), citrus exocortis viroid (CEVd) and citrus tatter leaf virus (CTLV). These pathogens cause important economic losses to our citrus industry. A key measure for preventing these losses is the elimination of the pathogens in propagative budwood and producing disease-free nursery trees.

In the early 1970s, research developments in the USA, Spain and other countries resulted in technology to eliminate graft-transmissible agents from propagative budwood by shoot-tip grafting (STG) (10, 13, 16, 17, 20, 21). After that, the STG technique has been popularly used in China (2, 4, 5, 18, 19). Since 1983, we have conducted extensive research on STG and its application in eliminating the four main diseases to obtain disease-free mother trees of the citrus cultivars important in Fujian.

## MATERIALS AND METHODS

**Pathogen sources.** Source plants held in the greenhouse and used as positive controls were Ponkan mandarin infected with HLB, Mexican lime or Ponkan infected with CTV, Rusk citrange infected with CTLV and Valencia late or Eureka lemon infected with CEVd.

**Plant material.** Budwood of 37 citrus cultivars was collected mainly in Fujian Province but also included a few from Sichuan and Guangdong. They were grafted to mandarin and trifoliolate orange rootstocks and held in the greenhouse for later STG.

**Experiments to increase STG survival rate.** Five experiments were conducted to try and improve survival rate. They were:

1. Pre-heating plants at 40/30°C (day/night) for 30, 60, 90 and 120 days prior to the collection of shoots for STG. The STG technique described by Navarro et al. (14) with modifications by Su et al. (20) were used.
2. Application of 0.1 ppm kinetin and 0.1 ppm zeatin to the cut surface of the rootstock.
3. Varying the time from 12, 15 and 20 days prior to STG.

4. Leaving cotyledons attached to the rootstock seedlings compared with the usual removal.
5. Grafting the young emerging shoot onto an established seedling as described by de Lange (3).

**Pathogen detection.** Indicator plants used to detect the following pathogens were as follows: Etrog citron Arizona 861-S1 for CEVd; Mexican lime for CTV; Ponkan mandarin for HLB; and Rusk citrange for CTLV. In addition, ELISA was also used for CTV and electron microscopy for the HLB liberobacter.

## RESULTS

From 1983-1990, many experiments were carried out to evaluate and improve elimination of citrus pathogens by combining STG with pre-heat treatments and are summarized in Table 1. HLB liberobacter in Ponkan mandarin could be

eliminated by STG with a 100% efficiency; CTV from Mexican lime by STG alone with 81.3% efficiency; and CEVd in Valencia late or Eureka lemon at 40 and 60% efficiency; respectively. CTLV was eliminated in Ponkan and Bendizao in only a few plants held at 40/30°C (day/night) for 90 to 120 days; whereas none were eliminated when the treatments were held for 30-60 days. However, combining STG with 30-60 days heat treatment resulted in a 50-89% elimination efficiency (Table 1). When the pre-treatment was extended to 90-120 days, CTLV was eliminated from all the STG plants. These results are similar those of Navarro et al. (12) for eliminating the more difficult psorosis-like pathogens from mandarin and Koi-zumi (6) for eliminating CTLV from satsuma.

The application of kinetin or zeatin to the cut surface of the rootstock increased the graft survival

TABLE 1  
EFFECT OF SHOOT-TIP GRAFTING (STG) AND STG WITH PRE-HEATING ON ELIMINATING CITRUS PATHOGENS

Pathogen	Host	Treatment <sup>a,b</sup>	No. of pathogen-free plant/ total no. of plant	Pathogen-free plant rate (%)
HLB	Ponkan	STG	19/19	100
CTV	Mexican lime	STG	13/16	81.3
CEVd	Valencia late	STG	4/10	40.0
CEVd	Eureka lemon	STG	9/15	60.0
CTLV	Ponkan	30	0/8	0
		60	0/12	0
		90	1/3	33.3
		120	2/9	22.2
CTLV	Bendizao	30	0/6	0
		60	0/5	0
		90	2/6	33.3
		120	1/7	14.3
CTLV	Ponkan	STG+30	8/9	88.8
		STG+60	5/6	83.3
		STG+90	7/7	100
		STG+120	9/9	100
CTLV	Bendizao	STG+30	3/6	50.0
		STG+60	7/8	87.5
		STG+90	6/6	100
		STG+120	4/4	100

<sup>a</sup>= Days of pre-heating treatment at 40/30°C (day/night).

<sup>b</sup>= Rootstock for STG was trifoliate orange.

TABLE 2  
EFFECT OF SEVERAL TREATMENTS ON SHOOT-TIP GRAFTING SURVIVAL

Treatment	Scion variety <sup>a</sup>	No. surviving plants/ no. STC plants	Survival rate (%)
0.1 ppm Zeatin	Mandarin	17/36	47.2
0.1 ppm Kinetin	Mandarin	18/40	45.0
Control	Mandarin	3/18	16.7
Rootstock age:			
12 days	Orange	4/17	23.5
15 days	Orange	9/20	45.0
20 days	Orange	5/20	25.0
Cotyledon left	Mandarin	20/25	80.0
Cotyledon left	Orange	20/24	83.3
Control	Mandarin	16/25	64.0
(cotyledon removed)	Orange	15/24	62.5
Regrafting	Mandarin or Orange	69/84	82.1
Control (Direct potting)	Mandarin or Orange	13/78	16.7

<sup>a</sup>Rootstock was trifoliolate orange.

from 16.7% (control) to 45 and 47.2%, respectively (Table 2). Table 2 also shows the effect of the age of rootstock seedlings used. Growing trifoliolate seedlings *in vitro* at 25 to 30°C for 15 days prior to grafting appeared to increase efficiency when compared to 12 or 20 days (Table 2).

There also seemed to be a slight improvement in successful grafts for both mandarin and sweet orange when cotyledons were left on the grafted seedlings. Regrafting directly onto larger plants gave a higher success rate than transplanting directly into soil (Table 2).

**Development of a pathogen-free citrus germplasm mother block.** Relying mainly on cultivars which underwent STG and were indexed negative for the four pathogens previously mentioned, we have now established a collection of 34 cultivars from Fujian and three from other provinces (Table 3). These originally were found infected by CTV (67%), HLB (12%), CTLV (8%) and CEVd (5%). After STG and indexing, two pathogen-free plants of each selection were transferred to a screenhouse as mother plants. In addition, 12 pathogen-free cultivars

were introduced from the USA. These were Ruby red orange, Cutter and Rhode red Valencia, Carter, Leng, Dream and Summerfield navels, Marsh and Star Ruby grapefruits, Ross Eureka and Frost Lisbon lemons and Satsuma mandarin.

## DISCUSSION

The propagation and planting of disease-free nursery trees are the main measures taken in the development of citrus production in many countries (8, 13, 15, 17, 20, 21). In China, disease-free citrus plants were first obtained by thermotherapy (7, 8, 9). However, exocortis cannot be eliminated by thermotherapy (1, 15). STG developed by Murashige et al. (10) and improved on by Navarro et al. (14) effectively eliminated many viruses and viroids (11, 16). Our experimental results showed that we could effectively eliminate HLB, CTV and CEVd by STG, and that CTLV was eliminated by combining STG with heat treatment.

Various techniques were tested to increasing the graft success rate, and all were found to have a beneficial effect.

TABLE 3  
RESULTS OF INDEXING FOR GRAFT-TRANSMISSIBLE CITRUS PATHOGENS ON STG  
PLANTS OF SELECTED CULTIVARS

Cultivar	Collection place <sup>a</sup>	Indexing			
		HLB	CTV	CEVd	CTLV
Sweet orange					
Chuang-Xia	Minhou	-	-	-	-
Chuang-Xia 1	Minhou	-	-	-	-
Shaohe-Xuekan	Najing	-	-	-	-
Xuekan 2	Fuzhou	-	-	-	-
Xuekan 6	Fuzhou	-	-	-	-
Taipinglong	Jianou	-	-	-	-
Xhekan 330	Fuzhou	-	+	-	-
Jingcheng	Sichuan <sup>b</sup>	-	-	-	-
Nippon	Fuzhou	-	-	-	-
Shehui Kan	Guangdong <sup>c</sup>	-	-	-	-
Hamlin	Fuzhou	-	-	-	-
Valencia	Fuzhou	-	-	-	-
Navel	Fuzhou	-	-	-	-
Mandarin					
Shehui kan	Guangdong <sup>c</sup>	-	+	+	-
Huimi	Sanming	-	-	-	-
Wenmi 3	Sanming	-	-	-	-
Wenmi 28	Sanming	-	-	-	-
Miyakawa	Fuzhou	-	-	-	+
Bendizao 3	Longyan	-	-	-	-
Tianma Ponkan	Yongchun	-	-	-	-
Shaohe Ponkan	Najing	-	-	-	-
Yanhou Ponkan	Chentai	-	-	-	-
Zhanna 1	Zhangpu	-	+	-	-
Zhanna 2	Zhangpu	-	-	-	-
Zhanna 3	Zhangpu	-	-	-	-
Baiye 2	Yongchun	-	-	-	-
Late Ponkan	Chengtai	-	-	-	-
Angeru	Fuzhou	-	+	-	-
Murcott	Fuzhou	-	+	-	-
Bailianzi	Yongchun	-	-	-	-
Wenmi	Liancheng	-	+	-	-
Early Wenmi	Yongden	-	+	+	-
Pummelo					
Tai yu	Fuzhou	-	-	-	+
Nippon yu	Fuzhou	-	-	-	-
Lemon					
Eureka	Fuzhou	-	-	-	-
<i>Fortunella</i>					
<i>F. japonica</i>	Sanmin	-	-	-	-
Other					
Gailonchen	Zhangpu	-	-	-	-

<sup>a</sup>= Counties in Fujian Province except (r)

<sup>b</sup>= Other provinces

The establishment of mother trees in an insect proof greenhouse should ensure the supply of disease-

free material to Fujian province, and will be available for safe international exchange of budwood.

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