Rehabilitation of Neck orange Production in Southern Thailand by Distribution of Diseased-Free Mother Tree and Budwood to Nurserymen

Ratana Sdoodee, Nipon Sriboonkong and Yuwadee Sutdhikaranya
Faculty of Natural Resources Prince of Songkla University, Hat Yai, Thailand 90112.

Abstract

Shoot-tip grafting (STG) *in vitro* was used to recover diseased-free citrus for producing healthy primary budwoods which would be developed into healthy mother trees in the Neck orange rehabilitation program. In our experiment, STG Neck oranges were 100% free from greening pathogen because greening DNA was not detected by PCR in midrib of 20 STG plants tested. In addition, the higher rate of successful STG was obtained with 'Troyer' Citrange than with 'Somkeawan' Mandarins and Neck Orange rootstocks. The percentage of successful graft were 38%, 19% and 5%, respectively. Since STG Neck oranges were still in their early stage to produce primary budwoods, the mother trees for budwood production experiment were then developed from citrus tristeza virus (CTV) and greening bacterium-free Neck orange cuttings. Shoot flush of the mother tree for producing budwood was induced by branch trimming. The average of 36, 29.1, 19.6 and 10.5 budwoods per tree per month were obtained by trimming the tree with 4, 3, 2 and 1 branches, respectively. According to our results under 6x9 m^2 insect proved nursery, 15 mother trees are likely to produce 3,240 diseased-free budwoods within 6 months. Consequently, these budwoods will be grafted on rootstocks to produce 2,500 healthy Neck orange nursery trees at 80% successful grafting rate.

Introduction

Neck orange (*Citrus reticulata* Blanco) was one of the popular indigenous fruit crop in southern Thailand. The origin of its planting area was in Jana district in Songkhla province. Since 1964, Neck orange decline, a fatal disease has reduced the production area in Songkhla and near by (Lim *et al*., 1987). The Neck orange decline was a disease syndrome of mixed infection of citrus tristeza virus (CTV) and greening bacterium (GB) (Sdoodee and Garnett, 1994; Sdoodee,1994). CTV was first reported in South America in 1946 and million of citrus trees were destroyed by the pathogen in Argentina, Brazil, California, Florida and Spain. The disease was then distributed to citrus growing area in Asia and Africa (Roistacher and Moreno, 1991 and Roistacher, 1991). In Thailand, Somkeawan mandarin, 'Shogun' mandarin, Neck orange, sweet orange, pummelo and acid lime were affected from CTV (Pradornuwat *et al.*, 1984). The disease symptoms caused by CTV are vein clearing, corky vein, stem pitting, decline and die back. Most of citrus cultivars also affected from greening, a fastidious bacterium in genus *Liberobacter* (Planet *et al*., 1995). The prominent greening symptoms are interveinal chlorosis, small fruit with bitter taste, fruit drop and decline. Mixed infection of CTV and GB in mandarins such as Shogun, Somkeawan and Neck orange were commonly detected in Thailand and the symptoms were severe.The life span of Neck orange was shortened by mixed infection of the pathogens as reported by Sdoodee (1994). CTV and GB did not transmit through seed but they were transmitted by grafting or through vegetative propagation materials. Moreover, insect vectors were the major contribution to widely distribution of the pathogens. The effective control measures for the disease caused by CTV and GB were eradication of infected trees and the replanting of certified disease-free nursery tree and followed by the control of insect vectors in citrus orchards (Lin and Ke, 1985; Rucks, 1994; Carvalho and de Carvalho, 1988; Anderson, 1999; Barry *et al*., 1999).

In our research program, we intend to develop disease-free Neck orange mother trees to produce clean budwood and distribute the budwood to nurserymen for production of healthy Neck orange nursery trees. This will lead to rehabilitation of Neck orange production in the South of Thailand.

Material and Method

1. Shoot-tip grafting (STG) for diseased-free primary budwood.

'Troyer' Citrange, 'Somkeawan' mandarin and Neck orange were tested as rootstock cultivars. Seed of rootstock cultivars were soaked in 0.7% sodium hypochloride for 10 min. and rinsed 3 times in sterile distilled water. The seed coats were removed aseptically and were planted *in vitro* in modified semisolid MS (Murashige and Skoog) medium (Lim *et al.,*1992) in the dark at 27°C (Fig 2A). Rootstock seedlings were removed from the medium after 2 weeks and were decapitated, leaving 1.5 cm of the epicotyl and 4-6 cm of the root. Cotyledons and axillary were also removed from the seedlings. Shoot tips (0.1-0.2 mm) of Neck orange (*Citrus reticulata* Blanco) composed of an apical meristem and 1-2 pairs of leaf primodia were isolated from young flushes of selected Neck orange trees grown at Klong Hoi Khong Research Station (KHK). The excised shoot tips (Fig 2B) were grafted into triangle incision 1-2 mm below the decapitated epicotyl of the rootstock (Fig 2C). The grafted plants were acepticcally cultured in the MS medium kept at 27°C and exposed to 16 hour daily to 1000 lux illumination. Successful STG plants having at least two expanded leaves (Fig 2D) were transplanted to the soil and kept in glasshouse or insect prove nursery.

2. Production of diseased-free budwood using Neck orange mother tree propagated by cutting.

1. Preparation of diseased-free Neck orange mother trees.

One year old Neck orange nursery trees propagated by cutting of the trees grown at KHK were assayed for citrus tristeza virus (CTV) and greening bacteria by ELISA (Enzyme-Linked Immunosorbent Assay,Fig 1A) and PCR (Polymerase Chain Reaction,Fig 2B) respectively. The nursery trees free from both pathogens were then transplanted to 45 litre cultivated containers. These trees developed into diseased-free mother trees (Fig. 3) for budwood production experiment and they were cultivated under insect prove nursery (Fig 3B). Fertilization was applied to the mother tree in soluble form (20-0-0) once a week and the slow released form of fertilizer (15-15-15) was applied 100 g/tree every 3 months and after 6 months application rate increased to 200 g/tree Overhead

sprinkler irrigation was used 2 times a day. Fungicide and bacteriocide were applied to control melanose and canker diseases , respectively.

2. Flush induction for budwood production.

Branch trimming was applied to the mother trees 3 months after being transplanted to the large container to induce flushing. The treatments were 0, 1, 2, 3 and 4 branch-trimming/plant/month. New flushes were counted prior to the next trim and budwoods were counted from trimmed branches.

Results and Discussion

1.Shoot-tip grafting (STG) for diseased-free primary budwood.

The percentage of successful STG using Neck Orange scion grafted on Troyer Citrange, Somkeawan mandarin and Neck orange rootstock were 38%, 19% and 5%, respectively. The highest grafting rate was obtained with Troyer rootstock and it was similar to that reported by Navarro *et al*. (1975). Troyer Citrange is widely used as a rootstock because it has several favourable attributes such as Phytophthora and CTV resistance, flood tolerance and moderate drought tolerance (Davie and Albrigo, 1994). However, Troyer Citrange is susceptible to exocortis viroid but there was no report of exocortis disease in Thailand. Therefore, using Troyer as a rootstock for a grafted citrus tree is suited for condition in the South of Thailand where the rainfall is about 700 inches a year and Phytophthora and CTV diseases are endemics. Total of 23 STG Neck oranges were transferred to soil and they grew well under glasshouse condition (Fig.4B). PCR tests indicated that greening bacteria were found 0/20 of STG plants. Ten of STG plants were tested when they were *in vitro* (Fig.4A) and another 10 plants were tested at glasshouse stage (Fig.1B). The significance of these results were that Neck oranges propogated through STG technique were free (100%) from greening infection.With comparison to previous report, STG eliminated greening(100%), CTV (80.6%) and exocortis viroid (54.1%) from the citrus plant (Song *et al.*, 1999). Determination of CTV in the STG plants will be performed prior to the budwood collection to ensure that they are free from major graft transmissible pathogens. A clean Neck orange budwood taken from the STG plant will be grafted onto a compatible rootstock to develop a healthy mother tree for budwood production in the future.

2. Production of diseased-free budwood using Neck orange mother tree propagated by cutting.

Since STG Neck oranges propagated in our experiment were still in their early stage and their budwoods had not fully developed yet, the mother trees used in the budwood production experiment were then developed from one-year-old diseased-free cuttings.One and two branch-trimming of the mother trees resulted

in increasing of shoot flushes in comparison with untrimmed trees which were 77% and 67%, respectively.However, budwoods harvested from the treatment of 4 branch-trimming gave the highest total number of 546 buds, although shoot flushes induced by this treatment were less than of those using 1 and 2 branch-trimming treatments (Table 1). According to our results under 6X9 m^2 insect proved nursery, 15 mother trees with 4 branch-trimming/month are likely to produce 3,240 budwoods within 6 months.These budwoods will be grafted onto compatible rootstocks to produce 2,500 healthy Neck orange nursery trees at 80% successful grafting rate. In our experiment, a climatic condition was also a factor enhancing shoot flush. Furthermore, flushing was decreased in October due to the reduction of light quantity caused by the heavy rainfall. In contrast, number of shoot flushes were dramatically increased in January-February because of the decline of rain and increase of light quantity (Fig.5 and 6).

References

Anderson, C.M.1999. Procitrus: the citrus budwood improvement program for Argentina. Proceedings 5th World Congress of the International Society of Citrus Nurserymen, Montpellier, France, 5-8 March, 1997, 149-153.

Barry, G.H., Veldman, F.J., Esselen, L.L., Esselen, F., Burdette, S.A. and Aubert,B.1999. Practical results of a citrus improvement program: the South African experience. Proceeding 5th World Congress of the International Society of Citrus Nurserymen, Montpellier, France, 5-8 March, 1997, 137-148

Carvalho, S.A.de and de Carvalho, S.A.1998. Restructuring of the citrus mother tree program and revision of the rules for production of certified citrus nursery tree of the Sao Paulo State, Brazil. Laranja 19(2): 399-409

Davie, F.S. and Albrigo, L.G. 1994. Citrus. CAB International, Wallingford, UK. 254 pp.

Lin, X.Z and Ke, C. 1985. A preliminary study on the establisment of a disease-free grafted seedings. Fujian-Agricultural Science and Technology Fujian Nongye-Keji 2:2-6

Lim, M., Pantahiran, V.,Lim, S. and Nuansri, J. 1987. Study on problems and improvement of Neck Orange (*Citrus reticulata* Blanco) cultivation. Research report, Faculty of Natural Resources, Prince of Songkla University. 20 pp.

Lim, M., Techato, S., Muangkaewkarm, A. and Kungsakoon, A. 1992. Improved shoot tip grafting of Neck Orange. Songklanakarin J. Sci. Technol. 14(3) :239245.

Nakashima, K., Prommintara, M.,Ohtsu, Y., Kano, T., Imada, J. and Koizumi, M. 1996 Detection of 16s rDNA of Thai isolates of bacterium-like organisms associated with greening disease of citrus. JIRCAS Journal No.3: 1-8.

Navarro, L., Roistacher, C.N. and Murashige, T. 1975 Improvement of shoot tip grafting *in vitro* for virus free citrus. Journal of American Society of Hortricultural Science 100: 471-479.

Planet, P., Jagoneix, S., Bove', J.M. and Garnier, M. 1995. Detection and characterization of the African citrus greening Liberobacter by amplification, cloning and sequencing of rpl KAJL- rpo BC operon. Current Microbiology 30:137-141.

Pradornuwat, A., Korpraditkoon, V., Kamjaipai, V., Attatum, S. and Taweechai, N. 1984. Citrus Diseases in Thailand. Funny Publishing, Bangkok. 126 pp.

Roistacher, C.N.1991. Graft-Transmissible Disease of Citrus: Handbook for Detection and Diagnosis. Food and Agriculture Organization of the United Nation, Rome, 286 pp.

Roistacher, C.N. and Moreno, P. 1991. The Worldwide threat from destructive isolates of citrus tristeza virus, a review. Proceeding of the 11th Conference of International Organization of Citrus Virologist, p 7-19.

Rucks, P.1994. Quality tree program for Florida citrus. Proceeding of the Florida State Horticultural Society No.107: 4-8

Sdoodee, R. 1994. Neck Orange (*Citrus reticulata* Blanco) decline: causal pathogens and factor enhancing disease severity. Songklanakarin J. Sci. Technol. 16(4) : 353-367.

Sdoodee, R. and Garnett, H. 1994. Detection of citrus greening bacterium by immunoblotting. Songklanaklin J. of Sci. and Technol. 16(3): 291-300.

Sdoodee, R. Sutdhikaranya, Y, Hongsa, W, and Luang-Aram, T. 1999. Detection and differentiation of bacterium-like organism associated with citrus greening disease in Thailand by RFLP. APPS 11th Biennial Conference, Canberra, Australia 27th - 30th September, 1999

Song, R. L., Wu, R. J., and Ke, C. 1999. Elimination of the main citrus virus and virus-like diseases by shoot-tip grafting. Acta-Phytopathologica-Sinica, 29(3): 275-279

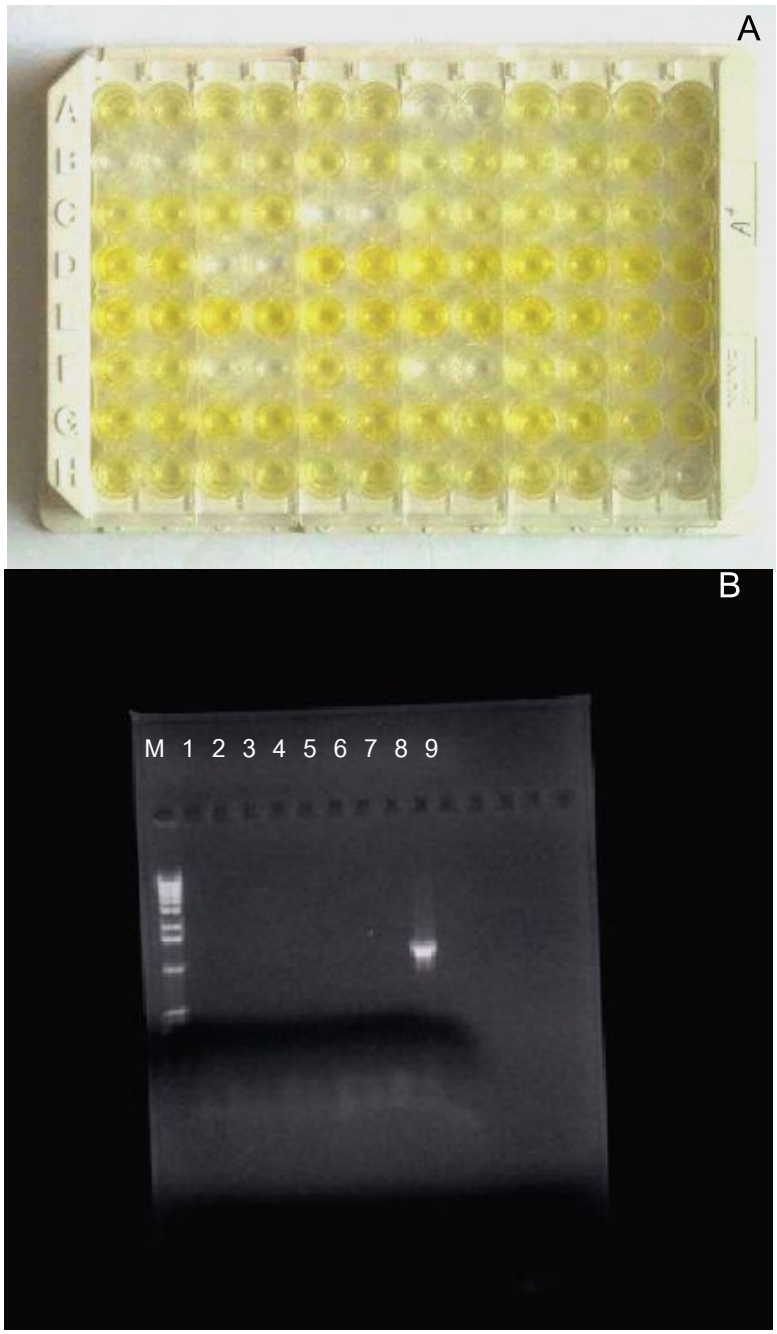

Fig 1. Detection of citrus tristeza virus (CTV) and greening bacteria.

A. ELISA test for CTV : positive reaction (yellow)negative reaction (colorless).

B. PCR test for greening.

Lane M 1 kb

Lane 1-8 DNA extract from STG plant

Lane 9 DNA from greening infected neck orange

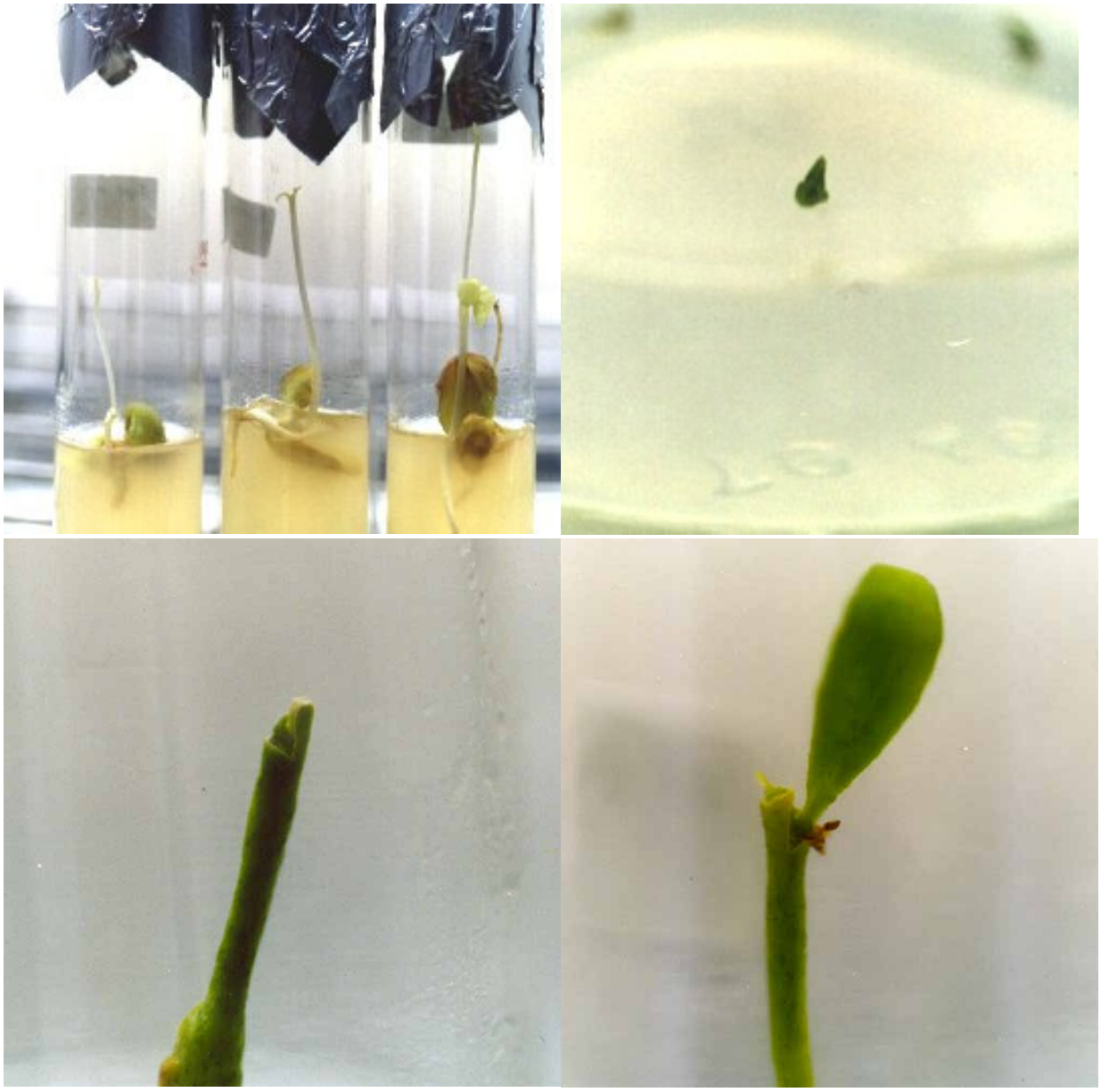

Fig 2. Propagation of Neck orange by shoot tip grafting (STG) to produce diseased-free plant.

A. Troyer citrange root stock 2 weeks after germination *in vitro.*

B. Shoot tip excised from Neck orange tree.

C. Grafted plant *in vitro.*

D. Leaf expanding from Neck orange shoot tip grafted on Troyer citrange root stock.

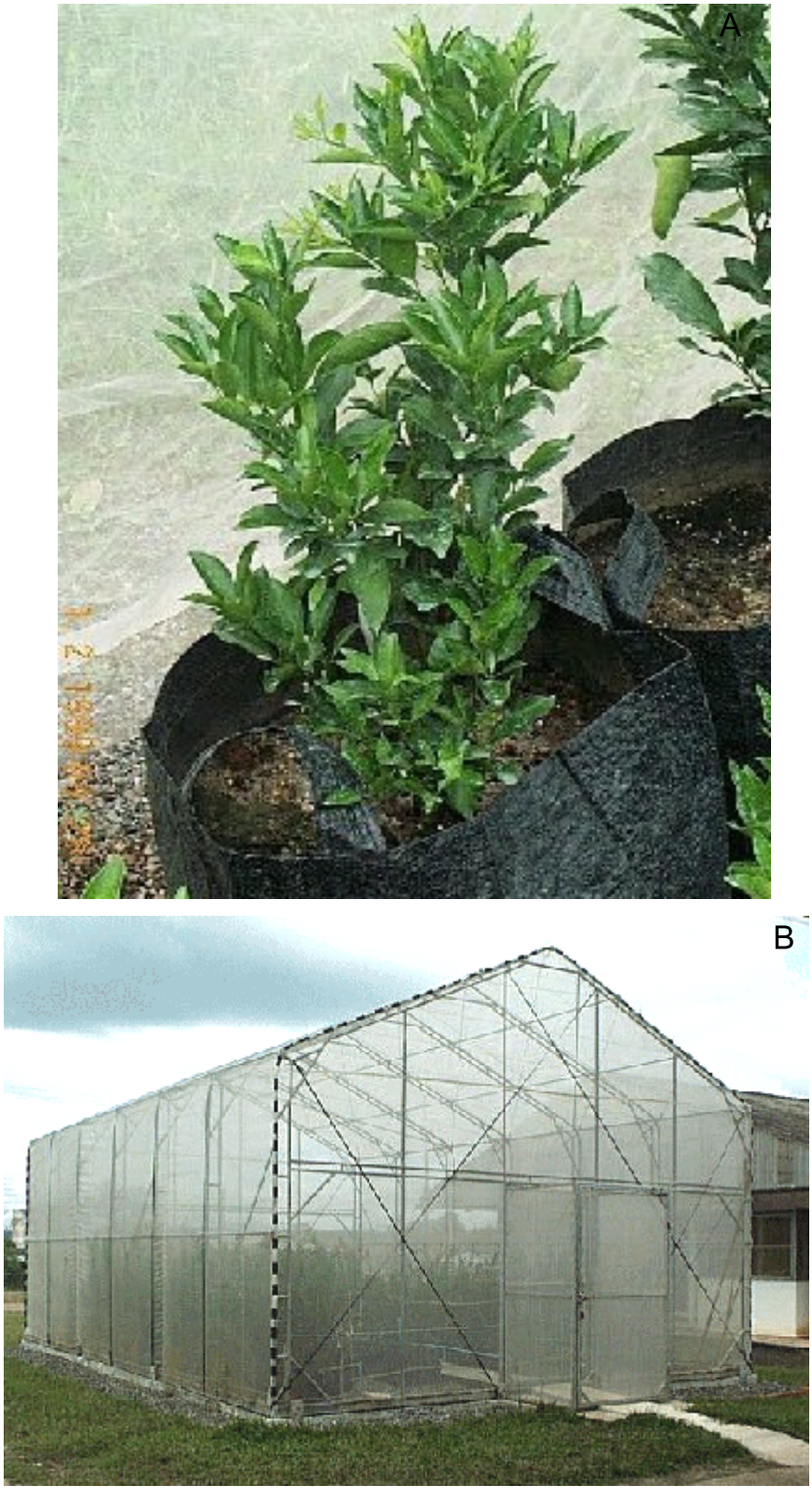

Fig.3 A. One-year-old diseased-free Neck orange mother plant grown from cutting.
B. Insect insect proved net house using for budwood production.

Fig4. A. STG Neck orange *in vitro* 3 months after grafting.

B. STG Neck orange 3 months after transferring to soil.

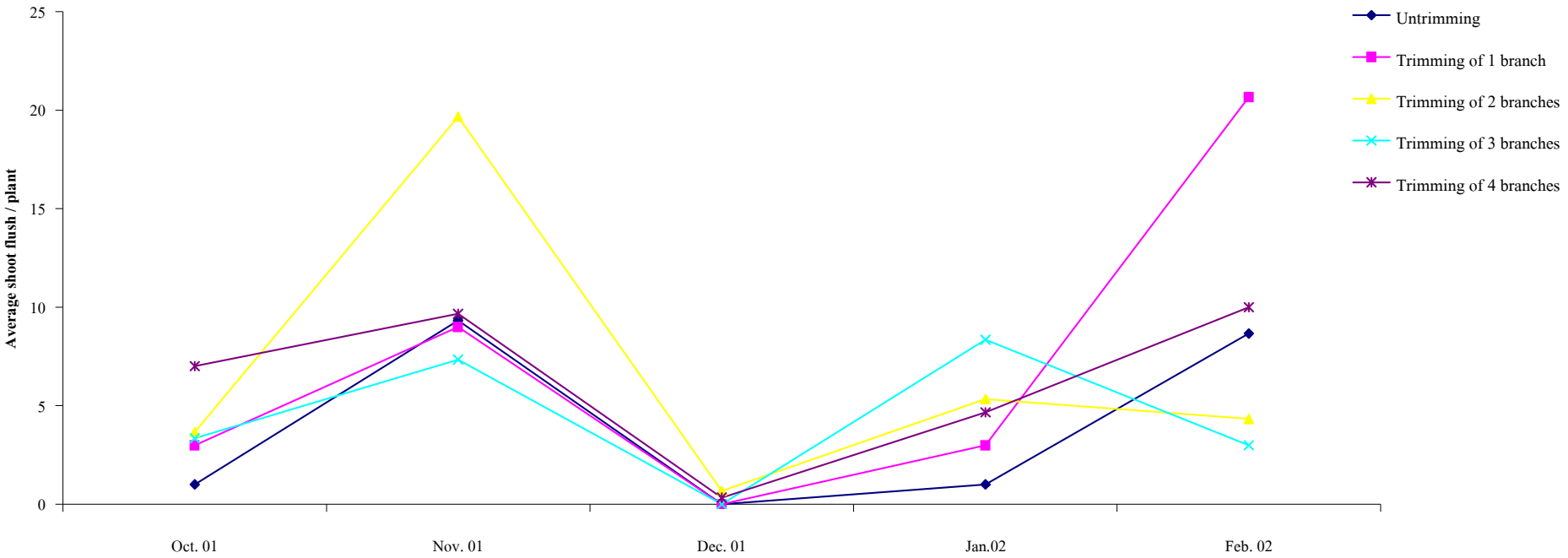

Fig.5 Shoot induction by branch trimming / month of Neck orange

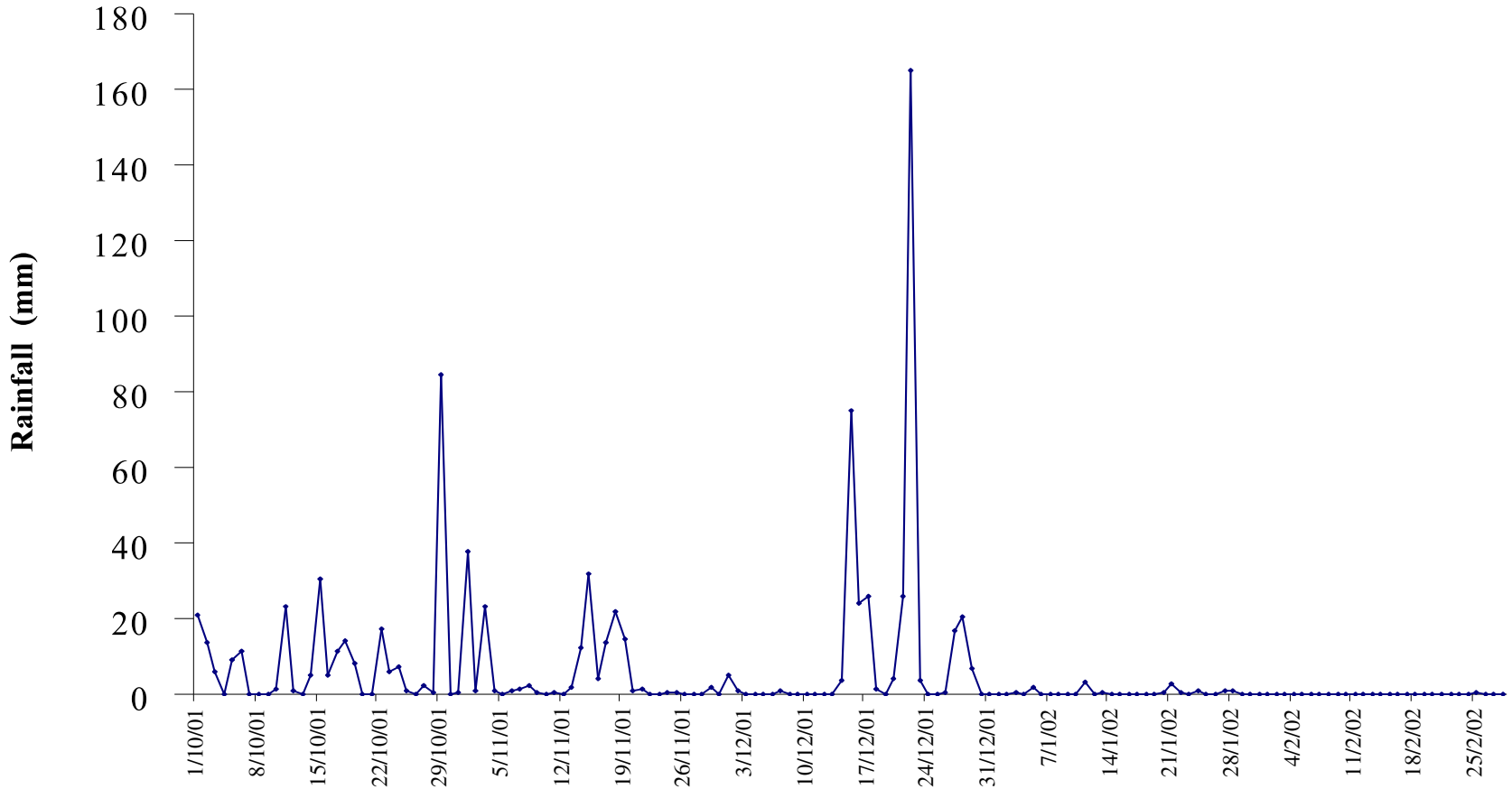
Rainfall (mm)
180
160
140
120
100
80
60
40
20
0
1/10/01
8/10/01
15/10/01
22/10/01
29/10/01
5/11/01
12/11/01
19/11/01
26/11/01
3/12/01
10/12/01
17/12/01
24/12/01
31/12/01
7/1/02
14/1/02
21/1/02
28/1/02
4/2/02
11/2/02
18/2/02
25/2/02

Table1. Induction of shoot flush for budwood production of Neck orange mother tree by branch trimming.

No. of branch trimming/month	Shoot Flush [1]		Budwood Production [2]	
	Average	%Increase	Total	Average/plant/month
0 (Control)	4	0	N	N
1	7.1	77.5	157	10.4
2	6.7	67.5	294	19.6
3	4.4	10.0	437	29.1
4	6.3	57.5	546	36.4

(1) Shoot flush were counted every month prior to the next branch trimming for 5 months and 3 mother trees were used per treatment.

(2) Fully grown budwoods were counted per trimmed branch, N= not applicable