リバビリン噴霧処理によるカンキツタターリーフウイルスのカンキツ苗条からの除去

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Elimination of Citrus Tatter Leaf Virus from Shoots of Potted Citrus Plants by Ribavirin

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Abstract

Ribavirin was applied as a foliar spray to the growing shoots of potted citrus plants of several cultivars infected with various sources of citrus tatter leaf virus (CTLV). The buds on the treated shoots were grafted on rough lemon rootstocks top-worked with rusk citrange for indexing immediately after the applications. Six or eight weekly applications of 500 ppm ribavirin eliminated all but one source (Kanp-90 in Ponkan mandarin) of CTLV from the buds with high efficiency. Kanp-90 could be eliminated from Ponkan mandarin by application of 500 ppm ribavirin in combination with heat treatment. Application of 50 ppm of ribavirin did not eliminate any source of CTLV.

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Key words: chemotherapy, citrus tatter leaf virus, ribavirin.

INTRODUCTION

Citrus tatter leaf virus (CTLV) is one of the most difficult infectious agent to eliminate from citrus plants[10]. CTLV can not be eliminated by shoot-tip grafting alone. Long-term heat treatment of infected plants for more than 92 days at 41°C/31°C (day/night) produced CTLV-free plants[5,9]. Shoot-tip grafting combined with heat treatment produced CTLV-free plants with only 30-50% success[2,7]. Furthermore, elimination by long-term heat treatment were not very efficient for heat-sensitive cultivars such as satsuma mandarin (*Citrus unshiu* Marc., cultivar Niu and Noda) and pummelo (*Citrus grandis* Osb., cultivar Suisho),[5,9] because most of the scions of these cultivars died during the long-term heat treatment.

CTLV-free shoots were obtained with high efficiency when *in vitro*-cultured shoot-tips were given application of ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide)[6]. This showed that ribavirin could inhibit replication of CTLV in citrus tissue.

This study was undertaken to investigate foliar application of ribavirin for elimination of CTLV from buds of infected potted citrus plants. Our special interest was the effect of ribavirin on heat-sensitive cultivars.

MATERIALS AND METHODS

Citrus plants and virus sources. Potted rough lemon seedlings were used as rootstocks for the experiments. CTLV-infected citrus scions were grafted on the rootstocks in order to produce potted plants. The sources of CTLV and their original host (shown in parenthesis) were : FURU-882 (satsuma mandarin, cultivar unknown), KANP-88 (Ponkan mandarin, *Citrus reticulata* Blanco, cultivar Ideyu), N-297 (satsuma mandarin, cultivar Niu), SU-1 (pummelo, cultivar Suisho).

Unless specifically noted, tests were conducted in an air-conditioned greenhouse where the tempera-

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tures were kept at 25°C/20°C (day/night).

Foliar application of ribavirin. Ribavirin was dissolved in distilled water to 50 ppm and 500 ppm. CTLV-infected citrus potted plants were cut back to force new growth. The sprouting buds were trained to three to five shoots per a potted plant a few weeks later. Repeated applications of the ribavirin solution to the expanding shoots were made at weekly intervals using a sprayer. As a control, distilled water was sprayed to the infected plants which had been subpropagated (Experiment 1).

Ponkan mandarins infected with Kanp-90 were kept in an air-conditioned greenhouse with temperatures of 35°C/30°C (day/night) and received weekly applications of 500 ppm ribavirin as in Experiment 1 (Experiment 2).

CTLV-free satsuma (cultivar Niu) and Ponkan (cultivar Ideyu) mandarin trees, which had been obtained by shoot-tip grafting in combination with heat treatment, were graft-inoculated with sources of Kanp-90 and N-297, respectively. These plants received weekly applications of 500 ppm ribavirin as in Experiment 1 (Experiment 3).

Virus indexing. The buds on the treated shoots were grafted immediately after the applications onto rough lemon rootstocks previously top-worked with rusk citrange[8]. The buds on the shoots which appeared after ribavirin treatment were also indexed in Experiment 1. One bud was always put into one rootstock. All inoculated plants were kept in the air-conditioned greenhouse and observed for symptoms for at least six months.

RESULTS

Effect of ribavirin on growth of shoot

In preliminary experiments, application of 500 ppm ribavirin induced severe distortion of leaves of the treated shoots of satsuma mandarin, when they were grown under high temperature conditions of 30°C/25°C (day/night). This chemical injury was not observed when the temperatures were kept at 25°C/20°C. Therefore, the temperatures were kept at this condition during the ribavirin applications. The shoots of Ponkan and satsuma mandarins, and pummelo matured enough for grafting within several weeks under this temperature condition.

Effect of 500 ppm ribavirin on CTLV (Experiment 1)

The buds on the treated shoots were very vigorous and easily grafted onto rough lemon rootstocks previously top-worked with rusk citrange.

The indexing showed that six or eight weekly applications of 500 ppm ribavirin eliminated all but one (source Kanp-90 in Ponkan mandarin) sources of CTLV from the buds with high efficiency. Four applications eliminated CTLV with lower efficiency. Application of 50 ppm ribavirin did not eliminate any source of the virus. Up to eight weekly applications of 500 ppm ribavirin had no effect on the elimination of source Kanp-90 from Ponkan mandarin. CTLV was found present in the shoots which appeared after the ribavirin application (Experiment 1 in Table 1).

Elimination of source Kanp-90 from Ponkan mandarin (Experiment 2)

We tried to eliminate the source Kanp-90 from Ponkan mandarin by ribavirin in combination with heat treatment after Experiment 1 showed that Kanp-90 was not eliminated by application of ribavirin alone.

Unlike satsuma mandarin, Ponkan mandarin did not show any chemical injury of ribavirin under high temperature conditions of 35°C/30°C (day/night). Buds on shoots grown under this condition were vigorous and successfully grafted on rough lemon root-stocks. The indexing showed that the source Kanp-90 was eliminated after six or eight applications of ribavirin in combination with heat treatment, while the virus from this source was not eliminated by spraying water and heat treatment (Experiment 2 in Table 1).

Effect of ribavirin on CTLV in Ponkan mandarin (Experiment 3)

Up to eight weekly applications of 500 ppm ribavirin had no effect on the elimination of N-297 from Ponkan mandarin, while Kanp-90 was easily eliminated from satsuma mandarin by the same treatment. When the ribavirin application was repeated 12 times, N-297 was eliminated from two out of three buds

Table 1. Effects of ribavirin treatment: results of indexing of the buds from CTLV-infected, ribavirin-sprayed shoots

CTLV Source/Cultivar	Treatment	Number of indexed buds[a]		
		Grafted	Survived	With symptoms
(Experiment 1)				
FURU-882/satsuma	Ribavirin			
	500 ppm 4 wk	5	5	2
	6 wk	5	5	0
	8 wk	5	5	0
	50 ppm 8 wk	3	3	3
	Water 8 wk	5	5	5
	After ribavirin[b]	3	3	3
KANP-90/Ponkan	Ribavirin			
(Ideyu)	500 ppm 4 wk	5	5	5
	6 wk	5	5	5
	8 wk	5	5	5
	Water 8 wk	5	5	5
	After ribavirin	3	3	3
N-297/satsuma	Ribavirin			
(Niu)	500 ppm 4 wk	5	5	3
	6 wk	5	5	0
	8 wk	5	5	0
	50 ppm 8 wk	3	3	3
	Water 8 wk	5	5	5
	After ribavirin	3	3	3
SU-1/pummelo	Ribavirin			
(Suisho)	500 ppm 4 wk	5	5	0
	6 wk	5	5	0
	8 wk	5	5	0
	Water 8 wk	5	5	5
	After ribavirin	3	3	3
(Experiment 2)				
KANP-90/Ponkan	Ribavirin with heat treatment[c]			
(Ideyu)	500 ppm 6 wk	3	3	0
	8 wk	3	3	0
	Water with heat treatment			
	8 wk	3	3	3
(Experiment 3)				
KANP-90/satsuma	Ribavirin			
(Niu)	500 ppm 6 wk	3	3	1
	8 wk	3	3	0
	Water 8 wk	3	3	3
N-297/Ponkan	Ribavirin			
(Ideyu)	500 ppm 6 wk	3	3	3
	8 wk	3	3	3
	12 wk	3	3	1
	Water 8 wk	3	3	3

a) The presence of CTLV was indexed using rusk citrange as described in the text.
b) The buds on the shoots appeared after ribavirin treatment were also indexed using rusk citrange.
c) Heat treatment was conducted at 35°C/30°C (day/night) as described in the text.

of Ponkan mandarin (Experiment 3 in Table 1).

DISCUSSION

Ribavirin was first described in 1972[12] as an antiviral agent with a broad spectrum activity. This chemical inhibits replication of many plant and animal viruses *in vitro* and *in vivo*[1,3,4,11,13]. It was pointed out that foliar application of ribavirin is less expensive, faster, and simpler than thermotherapy, meristem culture, or micrografting in the treatment of stone fruit trees, which are especially sensitive to thermotherapy[3]. However, there was no report on the effect of foliar application of ribavirin for the elimination of CTLV from citrus.

In this study, application of 500 ppm ribavirin to the growing citrus shoots eliminated CTLV, except source Kanp-90 in Ponkan mandarin. Kanp-90 could be eliminated from Ponkan mandarin by ribavirin combined with heat treatment. In this case, the period of treatment could be reduced to 42 days (six weeks), which is much shorter than that of conventional heat treatment (more than 100 days). Furthermore, application of ribavirin eliminated CTLV from heat-sensitive cultivars such as Niu (satsuma mandarin) and Suisho (pummelo) very efficiently. These results show that ribavirin chemotherapy is a good alternative to the conventional shoot-tip grafting or long-term heat treatment. This chemotherapy will be especially useful for heat-sensitive citrus cultivars.

Sources N-297 and Kanp-90 were readily eliminated from satsuma mandarin but not from Ponkan mandarin under temperature condition of 25°C/20°C (day/night). These results suggest that ribavirin is not very effective on CTLV in Ponkan mandarin regardless of sources of the virus under this condition.

All of the citrus plants used as source of CTLV in this study were simultaneously infected with citrus tristeza virus (CTV). All of CTLV-free plants obtained by ribavirin treatment were still infected with CTV (data not shown). This may be a disadvantage of ribavirin treatment, when both CTLV and CTV need to be eliminated. However, CTV is readily eliminated by heat therapy which is mild and short enough for survival of heat-sensitive cultivars and by shoot-tip grafting.

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和 文 摘 要

岩波 徹・家城洋之：リバビリン噴霧処理によるカンキツタターリーフウイルスのカンキツ苗条からの除去

抗ウイルス剤リバビリンをカンキツタターリーフウイルス（CTLV）保毒カンキツへ噴霧処理した場合の効果を調べた。供試したウイルス株およびカンキツ品種は，FURU-882（ウンシュウミカン，品種不明），KANP-90（いでゆポンカン），N-297（丹生系温州），SU-1（水晶文旦）である。ポット植えの保毒カンキツの伸長中の苗条にリバビリン500 ppmを1週間に1回の割合で噴霧した。処理後ただちに苗条より1芽ずつ切り出し，ラスクシトレンジ検定を行ったところ，KANP-90保毒いでゆポンカンを除いた3品種では，6回以上の噴霧できわめて効率よく無毒化されていた。KANP-90保毒いでゆポンカンからは35°C/30°C（昼/夜）の高温度条件下で，500 ppm，6回以上の噴霧を行うと効率よく無毒個体が得られた。50 ppmの噴霧では，いずれの品種からも全く無毒化個体は得られなかった。このリバビリン噴霧による無毒化の方法は，熱処理単独や茎頂接ぎ木と熱処理の組み合わせなどの方法に比べ，非常に簡単で効率がよく，耐熱性が弱く熱処理が困難な品種において特に有効であると考えられた。