Inactivation of Citrus Tristeza Virus by Gamma Ray Irradiation*

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家城洋之**・山口 昭*** : γ -線照射によるカンキツトリステザウイルスの不活性化

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Citrus tristeza virus (CTV) is distributed in almost all the citrus growing areas of Japan. Satsuma mandarin which occupies more than 70 per cent of citrus plantings in Japan was found to be infected with CTV as well as all other citrus varieties. Fortunately, Satsuma mandarin on Trifoliate orange tolerant to CTV stem pitting grows well and produces good quality fruits. Recently, because of the oversupply of Satsuma mandarin, farmers are replacing Satsuma mandarin with other late-ripening varieties such as Hassaku, Navel orange, Iyo, Kawano natsudaïdai etc.. However, most of these varieties are susceptible to CTV and the tree vigor gradually decreases, and hence fruit production declines¹⁻⁵⁾.

Since the brown citrus aphid (*Toxoptera citricidus*), the principal insect vector of CTV, is widely distributed in the citrus growing areas of Japan, it was presumed that cross-protection with mild strains of CTV against severe strains may be an effective control for the stem pitting disease by CTV.

The authors are presently conducting experiments on inactivation of CTV by heat treatment and on disease control by cross-protection with mild strain. This paper describes about total exposure of gamma ray and intensity of gamma ray per hour for inactivation of CTV, and effect to citrus tissues, because of more utilization of gamma ray on inactivation and production of mild strain of virus.

The budwoods (length: ca. 10 cm) of Morita navel orange (*Citrus sinensis*) infected with a severe seedling-yellow strain of CTV were subjected to irradiate with gamma ray (⁶⁰Co source) for 20~52 hours. All irradiations were performed in the gamma room of the Institute of Radiation Breeding, the Ministry of Agriculture, Forestry and Fis-

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Table 1. Inactivation of CTV by gamma ray irradiation (First experiment)

Total exposure (kR/20hrs)	B / A ^{a)}	Number of CTV- free plants ^{b)}	Number of CTV- infected plants ^{b)}
5	9/20	0(0) ^{c)}	12 ^{d)}
10	6/20	0(0)	6
20	2/20	2(100)	0
30	1/20	1(100)	0
50	0/20	0(0)	0

a) A : Total number of Mexican lime grafted.

B : Number of Mexican lime with living grafts.

b) CTV was detected with ELISA.

c) percentage of CTV-free plants in parenthesis.

d) includes three without living grafts, but infected with CTV.

Date of gamma ray irradiation : June, 4~5, 1980

Table 2. Inactivation of CTV by gamma ray irradiation (Second experiment)

Total exposure (kR/20hrs)	B / A ^{a)}	Number of CTV- free plants ^{b)}	Number of CTV- infected plants ^{b)}
10	23/30	11(48) ^{c)}	13 ^{d)}
13	26/30	16(62)	10
15	28/30	27(96)	1
18	27/30	26(96)	1
20	22/30	22(100)	0

a), b), c) see Table 1.

d) includes one without living grafts.

Date of gamma ray irradiation : April, 14~15, 1981.

Table 3. Inactivation of CTV by gamma ray irradiation (Third experiment)

Total exposure (kR)	B / A ^{a)}	Number of CTV- free plants ^{b)}	Number of CTV- infected plants ^{b)}
10 kR/20 hrs	13 ^{d)} /50	7(58) ^{c)}	5
13 kR/20 hrs	25 /50	8(32)	17
13 kR/29 hrs	29 /50	14(48)	15
13 kR/52 hrs	35 /50	20(57)	15
15 kR/20 hrs	21 /50	11(52)	10

a), b), c) see Table 1.

d) includes one died before indexing of CTV.

Date of gamma ray irradiation : May, 12~14, 1982.

heries. Buds or small tissue pieces of the irradiated budwoods were subsequently grafted onto Mexican lime (*Citrus aurantifolia*). The Mexican limes were maintained for symptom expression and tested for CTV by the enzyme-linked immunosorbent assay (ELISA).

In the first experiment (Table 1), the budwoods were irradiated at intensity of 5, 10, 20, 30 and 50 kR/20 hrs of total exposure of gamma ray. The buds or tissues irradiated for 5, 10, 20 and 30 kR/20 hrs were successfully grafted onto Mexican lime,

but the rate of success of grafting was low. The budwoods irradiated with 50 kR/20 hrs were apparently damaged and could not survive after grafting. Therefore, the higher the total exposure to gamma ray, the lower the success rate of grafting. Citrus tristeza virus was inactivated with 20 and 30 kR/20 hrs of irradiation, but not with 5 and 10 kR/20 hrs. As a result, subsequent experiments were performed by irradiating with 10~20 kR of total exposure of gamma ray.

Citrus tristeza virus was not inactivated by 10 kR/20 hrs irradiation in the first experiment. Whereas, it was inactivated in the second and the third experiments (Table 2 and 3). The rate of inactivation in these experiments was about 50 %. This was supposedly due to the high success rate of grafting on Mexican lime by careful treatment of the grafted plants. The rate of inactivation of CTV by gamma ray was variable in these experiments. CTV was easily inactivated with irradiations of 10~18 kR/20~52 hrs. The higher the total exposure, the higher the rate of inactivation. CTV in the budwoods was almost inactivated after irradiation with 20 kR.

On the other hand, when the total exposure to gamma ray on budwoods was the same, CTV was more efficiently inactivated by irradiation for long period with low intensity of gamma ray per hour than that for short period with high intensity per hour (Table 3).

There are several reports⁶⁻¹¹⁾ on heat therapy of virus diseases of citrus. CTV in shoots was easily inactivated by heat treatment, resulting in CTV-free plants^{12,13)}. Other methods such as micrografting¹⁴⁾, tissue culture and apical meristem culture are also available for preparation of virus free tissues. We have shown that gamma ray irradiation was also an effective method to eliminate CTV from citrus tissues. However, it will be necessary to check the irradiated materials to determine that it is still satisfactory for propagation, because gamma ray have been used as a tool to obtain mutations in plant breeding.

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