

Mutation of Citrus Variety *Tachima-wase* Induced By X-rays

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A bud mutation was induced from *Tachima-wase* by X-ray irradiation. This mutant is characterized by vigorous growth.

There are many papers reporting bud mutation induction in fruit trees by X-rays.^{1-5,7)} Stadler first pointed out the possible uses of X-rays in the development of bud mutation in apples.¹²⁾ Experiments in irradiation of dormant scions of apples were conducted by Bishop,¹⁻³⁾ Granhal,^{5,6)} and Zwintzsher.¹³⁾ However, there is no reference concerning bud mutation induction of citrus.

In this experiments, *Tachima-wase* (bud mutation from *C. Unshiu var. Owari*) was irradiated by X-rays, and the characteristics of induced mutant were examined.

MATERIALS AND METHODS

The upper parts of two-year-old *Tachima-wase* trees were irradiated by various dosages of X-rays, 500, 1000, 1500, 2000, 2500, 3000, 4000 and 5000R, on April 24 in 1964. The trees irradiated at each exposure level were transplanted in pots and arranged in field.

In the treated year, shoot growth was measured at ten or two weeks intervals from June to September and the number of deformed leaves was counted in October. In the following years, tree vigour and fruit bearing age were observed. At 3000R exposure, a tree which is very vigorous in growth first came to bear flowers five years after irradiation while most tree for each exposure bore flowers at four years after treatment. Pollen fertility of the vigorous mutant was examined, and the flowers were isolated with paper bags to determine whether they are parthenocarpic or not. The fruit quality and leaf oils of the mutant were also examined. Percentage of free acids was determined by titration with NaOH, and amount of sugars was decided by Somogyi method. Essential oils were extracted by distillation from fresh rinds. A gas chromatographic analysis was done by using Hitachi K53 Gas Chromatography, the parameters for this analysis were as follows:

| | |
|----------------------------------|--|
| Column | 6 mm × 200 pyrex with 10% LAC 446 on Chromosorb P |
| Carrier gas | N ₂ 40 ml/min. |
| Column temperature | 120°C |
| Injection & detector temperature | 230°C |
| Chart speed | 1 cm/min. |

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RESULTS AND DISCUSSION

(Shoot growth) The average shoot growth at each exposure is given in Table 1. Dormant buds of trees irradiated at more exposures than 1000R were considerably later than the control, and the reduction in shoot growth is more severe with increased exposure. Average shoot growth at 5000R exposure is the half of the control, while no difference in shoot growth is observed between 500R exposure and control. A vigorous mutant was induced at 3000R exposure, and this tree stood out clearly from other plants in growth (Fig. 2-a).

Table 1. The average shoot growth of *Citrus Unshiu var. Tachima-wase* irradiated by X-rays*.

| Total exposure (R) | Seedling length (cm) | | | | | | | |
|--------------------|----------------------|------|------|------|------|------|-------|-------|
| | Date | 5/14 | 5/26 | 6/6 | 6/26 | 7/15 | 7/31 | 8/21 |
| Control | | 0.09 | 4.12 | 5.53 | 5.53 | 5.55 | 10.90 | 16.80 |
| 500 | | 0.99 | 5.43 | 6.72 | 7.79 | 7.84 | 10.00 | 17.71 |
| 1000 | | 0.13 | 2.95 | 5.63 | 6.18 | 6.36 | 10.28 | 17.19 |
| 1500 | | 0.13 | 2.43 | 4.67 | 4.88 | 5.18 | 14.40 | 17.72 |
| 2000 | | 0.03 | 1.68 | 4.01 | 5.09 | 5.20 | 10.34 | 16.38 |
| 3000 | | 0.05 | 1.55 | 3.77 | 4.64 | 4.67 | 9.99 | 14.49 |
| 4000 | | 0.00 | 0.41 | 0.82 | 1.19 | 2.23 | 9.75 | 12.67 |
| 5000 | | 0.01 | 0.44 | 0.54 | 1.03 | 2.76 | 4.07 | 9.69 |

* Mean value of ten trees.

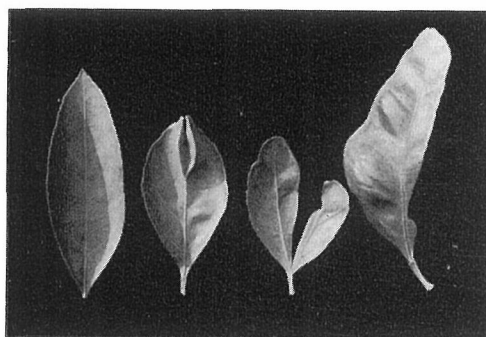


Fig. 1. Deformed leaves induced by X-rays.

(Leaf formation) A kind of the morphological effects noted in early stages of growth after X-irradiation were frequent leaf abnormalities (Fig. 1). Most of the leaf changes were confined to the first and second leaves, later sprouted leaves apparently were normal. Number of deformed leaves per plant in each exposure are shown in Fig. 3. Thus the percentage of deformed leaves increases with increasing exposure.

(Mutant' characteristics) Five years after irradiation, the characteristics of a vigorous mutant were examined. The mutant is very vigorous in growth, and its size reaches about twice as normal tree. Such vigorous in growth is a desirable characteristic for *Unshiu-mandarin*, because this variety tends to be weak in tree growth. The earliest tree in all exposures bore flowers three years after irradiation, and most came

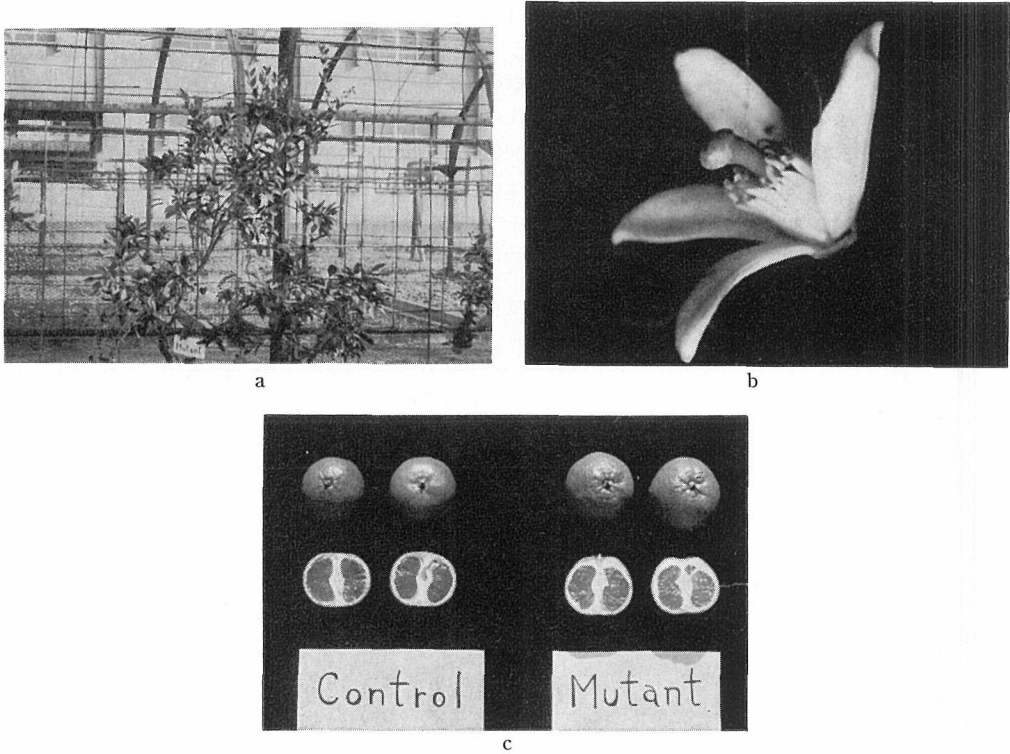


Fig. 2. Characteristics of mutant a) mutant tree (left-large), b) a flower with degenerated anthers, c) fruits with thick rind.

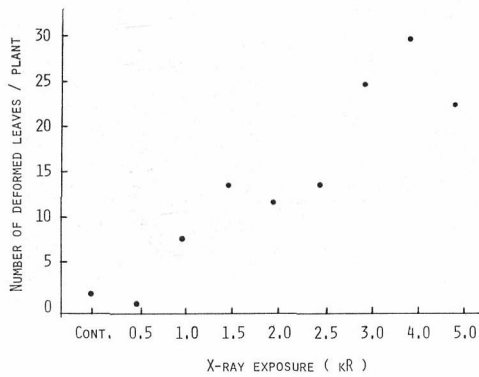


Fig. 3. Correlation diagram between number of deformed leaves and X-ray exposures.

to bear flowers four years after treatment. The mutant bore first 10 flowers five years after exposure. Its lag in bearing flowers is considered to be due to its vigorous vegetative growth, anthers of this mutant had degenerated and there was no pollen (Fig. 2-b). Percentage of fruit set amounted to 80, but there was no seed in fruits. This fact shows that the mutant is parthenocarpic like other *Unshiu mandarin*. Fruits of the mutant started to ripen in early November. Fruits of the mutant are a little larger and have a thicker rind than normal ones (Fig. 2-c). Free acids, soluble solids and

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Table 2. The percentage of free acids, soluble solids and sugars in juice of the mutant induced by X-rays*.

| | Free acid | Soluble solid | Reducing sugar | Non-reducing sugar | Total sugar |
|--------|------------|---------------|----------------|--------------------|-------------|
| Mutant | 1.12±0.004 | 9.83±0.000 | 2.94±0.000 | 3.54±0.017 | 6.49±0.018 |
| Normal | 1.02±0.062 | 11.26±0.010 | 3.80±0.160 | 5.03±0.567 | 8.83±1.321 |

* Mean value of three fruits.

Table 3. The essential oils in rind of the mutant induced by X-rays*.

| | Peak number | | | | | | | | | | | | | | | |
|--------|-------------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Mutant | 0.46 | 14.50 | 1.00 | 0.30 | 1.07 | 5.13 | 0.34 | 0.26 | 0.14 | 0.16 | 1.03 | 1.46 | 0.36 | 0.18 | 0.29 | — |
| Normal | 0.46 | 11.35 | 1.00 | 0.23 | 0.53 | 4.26 | 0.16 | 0.27 | 0.27 | 0.13 | 0.08 | 0.10 | 0.10 | 0.10 | 0.46 | 0.18 |

* Mean value of three fruits, figure shows the ratio of peak area to peak number 3.

sugars of mutant are given in Table 2. The mutant has a tendency to be rich in free acids and less in soluble solids and sugars than normal. These tendency are usual in young trees, but the quality can be expected to become when vegetative and reproductive growth are on balance.

It is known that essential oils are determined by genetic factors.⁸⁻¹⁰ The rind oils of the mutant were analysed by gas-liquid chromatography. Table 3 shows the relative values of each peak on the ground of peak No. 3 (γ -terpinene). It is evident that there is considerable differences in patterns of essential oils between the mutant and normal trees.

These results indicate that this vigorous tree is a desirable mutant, and this suggests a certain possibility of mutation by X-rays.

SUMMARY

Young plants of citrus variety *Tachima-wase* were irradiated by X-rays at 500, 1000, 1500, 2000, 2500, 3000, 4000 and 5000R. Dormant buds of trees irradiated at more exposures than 1000R come out considerably later than control, and the reduction in shoot growth was more severe with increased exposure. Typical abnormalities in leaves of irradiated trees were observed in early sprouts. Percentage of deformed leaves increased with increasing exposure.

X-rays induced a vigorous mutant. Five years after irradiation, the mutant bore 10 flowers and showed parthenocarpy. The fruit of the mutant tends to be rich in free acids. The experimental results suggest that the mutation induction by X-rays has a considerably high possibility in citrus.

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