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STUDIES ON THE VIRUSES OF RADISH MOSAIC

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ABSTRACT

KIM, Woon Soo\* & CHOI, Yae Chin\*\*(University of Ill., U.S.A.\* & Chunchon Agricultural Coll.\*\*\*) Studies on the viruses of radish mosaic. Kor. Jour. Bot. VI (2):9—21, 1963.

A mosaic diseased radish collected from the suburb of Seoul, in November, 1961 was used for studying the host range, physical properties, purification, insect transmission, and electron microscopy.

A Japanese strain of radish mosaic (RPV) was also used with Korean strain of radish mosaic (KRMV) for a comparative study.

The two viruses, KRMA and RPV, were identified by the difference in host range, insect transmission and electron microscopy. The KRMA was severely infective on tobacco and *Nicotiana glutinosa*, while on *Gomphrena globosa* was immune to the virus. RPV produces necrotic local lesions on *Gomphrena globosa* but did not infect tobacco and *N. glutinosa*. Among varieties of radish, *Seoul*, *Akamaroo*, *Akanagea*, *Koong-Joong* showed more severe symptoms than *Simoo*, *Minong*, *Paek-soo*, which appeared to be faintly resistant. In a number of tests, it was found that the virus KRMA retained its infectivity until to a dilution of 1:2,000, heating at 58° C for 10 minutes, and aging *in vitro* for 7 days at room temperature. The RPV was not inactivated until it was diluted to 1:2,000, heated to 56° C, and aged for 6 days.

The KRMV was readily transmitted by the aphid (*Myzus persicae* Sulz). The virus RPV was not transmitted by the aphid in a number of tests. Partially purified viruses using ammonium acetate buffer, salting-out by ammonium sulfate and centrifugation of high and low speed were highly infective. Electron micrographs showed that the KRMV particles are of spherical particles whereas the RPV particles are rod-shaped.

INTRODUCTION

Since Tompkins<sup>(30)</sup> first noted the mosaic disease of radish in California, U.S.A. in 1939 similar diseases have been found in several other parts of the world. and a number of viruses or virus strains that cause mosaic symptoms in radish have been reported. Some of such viruses are Turnip mosaic

virus<sup>(29)</sup>, Cabbage black ring spot virus<sup>(13)</sup>, and Cauliflower mosaic virus<sup>(26)</sup>, Works have already been made in the U.S. and other countries on Radish mosaic virus<sup>(7, 8, 24, 30)</sup>; there have also been several reports from Japan on this virus<sup>(21, 25, 32)</sup>. From a review of the literature, there are several viruses which attack radish crops (Table 1). Ishiyama et al.<sup>(8)</sup> reported the stunt disease in radish from Japan: Severin<sup>(19)</sup>, Kasai<sup>(9)</sup> and others (7, 8, 21, 30) reported that the transmission of this virus was done by aphids.

Takahashi<sup>(24)</sup> observed the particles of radish mosaic virus under the electron microscope and found that it is of rod-shape and about 25 x 120 mu in size. Tochiara<sup>(25)</sup> in Japan classified the isolated viruses from radish, by host range into three strains P, Q, and R. He made further studies on the p virus-host range, purification, electron microscopy. The physical properties of this virus vary among reporters. The radish mosaic virus (RMV), along with the tobacco mosaic virus (TMV) and the potato virus X (PVX), is one of the most important pests to the main crops of Korea. To prevent the loss of the crops research works on the virus pathogens and on their control have been carried on in the U.S. and other countries of the world. Recently vegetable crops in Korea have decreased in yield by the virus pathogens; the damage in yield of radish and chinese cabbage amounts to over 40% yearly. Isolation of the virus and the discovery of the measure of its control would be an urgent problem in this country. In this study, some preliminary researches have been done on the host range, physical properties, transmission, purification, and the electron microscopy.

## MATERIALS AND METHODS

A collection of six radish plants showing mosaic symptoms was made from important radish growing areas in the outskirts of Seoul. The Korean radish mosaic virus (KRMV) used in this study was isolated from these mosaicdiseased radish. The Japanese radish mosaic virus (RPV) was used as control; it was originally isolated from radish by Tochiara, at the National Institute of Agricultural Science in Japan. Saps from these diseased radishes were inoculated mechanically on the chinese cabbage (*B. pekinensis* Makino), turnip (*B. campestris* L.), *Nicotiana glutinosa* L. tobacco (*N. tabacum* L.), radish (*Raphanus sativus* L.), cabbage (*B. oleracea* var. capitata), cucumber (*Cucumis sativus* L.), *Chenopodium Koreanse* Nakai and other hosts. Local lesion symptoms were produced on the inoculated leaves of chenopodium. From these lesions the KRMV was isolated. The RPV also produced a local lesion symptoms on the inoculated leaves of chenopodium. Both of the viruses KRMV and RPV have been maintained by successive inoculation on radish and turnip, which were kept in the wire net insect cages.

For the most part, plants to be tested were grown on compost soil in 3''-5'' earthen pot. Plants were inoculated mechanically with a glass spatula after carborundum had been sprinkled on the leaves. The turnip variety Kanamachi and chenopodium were used for most of the green house studies.

Physical properties were determined in a manner similar to that described by Tochiara<sup>(25)</sup>.

The aphids (*Myzus persicae* Sulz) used in the insect transmission studies were collected from the radish nursery in the vegetable farm and were manipulated by methods similar to those described by Tochiara<sup>(25)</sup>.

In purification, the International Centrifuge Model PR-2 was used. Chinese cabbage harvested 20 days after inoculation was used for purification.

For the electron microscopy, Hitachi Model HS-6 electron microscope and Hitachi HUS-3 Vacuum evaporator were used.

**Table 1.** Viruses Infecting on Radish

| Virus                         | Author and reference            | Place found     | Plant isolated        | Symptoms on radish | Host range & differential hosts   | Methods of transmission                  | Physical properties                     |
|-------------------------------|---------------------------------|-----------------|-----------------------|--------------------|---|--|---|
| Aster yellow virus            | Severin & Frazier 1945*(2)      | U.S. Calif.     |                       | Systemic infection | LIC *(1)<br>Onion, Potato<br>Celery   | MCT<br>IT                                |   |
| Brassica nigra virus          | Sylvester 1953                  | U.S. Calif.     | Brassica nigra        | SM*(3)             | (-) Tobacco<br>(-) Cabbage<br>(+) Turnip  | MCT<br>IT: Aphid                         | TI: 60°C.<br>DE: 1:1,000<br>AI: 96 hrs. |
| Broccoli mosaic virus         | Caldwell & Prentice 1942        | Devonshire      | Broccoli              | SM                 | (+) Cabbage<br>(+) Rape<br>(+) Turnip   | MCT<br>IT: Aphid                         | TI: 75°C.<br>DE: 1:2,000<br>AI: 7 days  |
| Cabbage black ring virus      | Tompkins, Gardner & Thomas 1937 | U.S. Calif.     | Cabbage               | SM                 | (+) Chenopodium<br>(+) Turnip<br>(+) Tobacco<br>(+) N. glutinosa  | MCT<br>IT: Aphid                         | TI: 59°C.<br>DE: 1:1,000<br>AI: 3 days  |
| Cabbage black ring spot virus | McClellan & Cowin 1952-3        | S. Africa       | Cabbage               | SM                 | (+) Poppy<br>(+) Zinnia<br>(+) Tobacco  | MCT<br>IT: Aphid                         | TI: 60°C.<br>DE: 1:1,000<br>AI: 48 hrs. |
| Cauliflower mosaic virus      | Tompkins 1934                   | U.S.            | Cauliflower           | SM                 | (-) Tobacco<br>(+) Turnip<br>(+) Cabbage  | MCT<br>IT: Aphid                         | TI: 75°C.<br>DE: 1:2,000<br>AI: 15 days |
| Chinese cabbage mosaic virus  | Tompkins & Thomas 1938          | U.S. Calif.     | Chinese cabbage       | SM                 | (+) All cruciferae<br>(+) N. glutinosa (LL)<br>(+) Tobacco (LL)* (4)  | MCT<br>IT: Aphid                         | TI: 73°C.<br>DE: 1:5,000<br>AI: 3 days  |
| Chinese rape mosaic virus     | Wei et al. 1958                 | China           | Rape                  | VC*(5)             | (+) Ch. cabbage<br>(-) cabbage,   | MCT<br>IT: Aphid                         |   |
| Crucifer virus                | Hoggan & Johnson 1935           | U.S.            | Horse radish & Turnip | VC                 | (+) Tomato<br>(+) Tobacco<br>(+) N. glutinosa   | MCT<br>IT: Aphid                         | TI: 54°C.<br>DE: 1:1,000<br>AI: 2 days  |
| Crucifer virus                | Oliveira & Borges 1944          | Portugal Lisbon | Papist spp.           | SM                 | G9: (-) N. glu. (+) Cab.<br>G8: (-) Cab. (+) Tob.<br>G3: (-) Tob.<br>G1: (+) N. glu. (+) C. semp.<br>G5: (-) C. semp.<br>G4: (+) N. glu. (+) Radish<br>G7, 2, 6: (-) Radish |  |   |
| Crucifer virus                | Dale 1948                       | Trinidad        | Radish                | SM                 | (-) Cabbage<br>(+) Tobacco  | MCT<br>IT: Aphid                         | TI: 58°C.<br>DE: 1:1,000<br>AI: 4 days  |
| Crucifer virus                | Hean 1949                       | S. Africa       | Cauliflower           | VC                 | (+) Cabbage<br>(+) Turnip   | MCT<br>IT: Aphid                         | TI: —<br>DE: 1:50<br>AI: 2 days         |
| Horse radish mosaic virus     | Pound 1948                      | U.S. Wiscons.   | Horse radish          | VC                 | (+) Ann stock<br>(+) Brassicae  |  |   |
| Malva yellows virus           | Costa et al. 1959               | U.S. Calif.     | Radish                |                    | (+) Cabbage<br>(+) Tobacco<br>(+) Petunia<br>(+) Zinnia<br>(+) Hibiscus   | MCT: (-)<br>IT: Aphid<br>Grafting<br>(+) |   |

|                            |                               |                  |                |                        |   |                             |  |
|----------------------------|-------------------------------|------------------|----------------|------------------------|---|-----------------------------|--|
| Radish mosaic virus        | Tompkins 1939                 | U.S. Calif.      | Radish         | Chlorotic lesion       | (+) Tobacco (LL)<br>(+) Cauliflower<br>(+) Turnip   | MCT<br>IT: Aphid<br>B. Bra. | TI: 68°C.<br>DE: 1:14,000<br>AI: 14 days               |
| Radish stunt virus         | Ishiyama & Mizawa 1943        | Japan            | Radish         | VC<br>VB*(6)<br>EN*(7) | (+) Cabbage   | MCT<br>IT: Aphid            | TI: 70°C.<br>DE: 1:15,000<br>AI: 23 days               |
| Radish mosaic              | Takahashi 1952                | U.S. Calif.      | Radish         | SM                     | (+) B. spp.   |                             | Rod-shape<br>25×120 mu                                 |
| Radish mosaic virus        | Horton 1956                   | U.S. Wiscons.    | Radish         | VC<br>VB               | G1, 3: LIC<br>G2, 4, 5, 6:<br>CR&NC*(8)             | MCT<br>IT: Aphid            | TI: 70-80°C.<br>DE: —<br>AI: —                         |
| Radish mosaic virus        | Raychaudhuri & Pathanian 1956 | India            | Radish         | Necrosis & stunting    | LIC   | MCT<br>IT: ?                | TI: 85°C.<br>DE: 1:10,000<br>AI: 17 days               |
| Radish mosaic virus        | Shimahama 1957                | Japan            | Radish         | SM                     | Clsfd 5 groups by inf. to tobacco & cabbage         | MCT<br>IT: Aphid            |  |
| Radish mosaic virus        | Tochihara 1959                | Japan            | Radish         | VC                     | (+) Brassica spp.<br>(-) Tobacco<br>(-) Cabbage (+) | MCT<br>IT: (±)              | TI: 55-60°C.<br>DE: 1:2,000<br>AI: 7 days<br>Rod-shape |
| Rape mosaic virus          | Ling & Yang 1940              | China            | Rape           | VC                     | (+) Turnip<br>(+) Ch. cabbage                       | MCT<br>IT: Aphid            | TI: 60-65°C.<br>DE: 1:6,000<br>AI: 6 days              |
| Stock mosaic virus         | Tompkins 1939                 | U.S.             | Stock          | SM                     | (+) M. incana                                       | MCT<br>IT: B. bra.          | TI: 58-60°C.<br>DE: 1:5,000<br>AI: 144 hrs.            |
| Swede yellow mosaic virus  | Kristensen 1957               | Denmark          | Swede          |                        | (+) Turnip<br>(+) Radish<br>(+) Ch. cabbage         |                             | TI: 75°C.<br>DE: 1:10 <sup>5</sup><br>AI: 14 days      |
| Turnip mosaic virus        | Tompkins 1938                 | U.S. Calif.      | Turnip         | SM                     | (+) Cabbage<br>(+) Ch. cabbage<br>(+) Ann stock     | MCT<br>IT: Aphid            | TI: 60-63°C.<br>DE: 1:3,000<br>AI: —                   |
| Turnip yellow mosaic virus | Borges 1947                   | Portugal Lisbon  | Turnip         | Chlorosis of veins     | (+) Mustard<br>(+) Lepidium<br>(+) Ch. cabbage      | MCT<br>IT: (-)              | TI: 60°C.<br>DE: 1:200,000<br>AI: 9 days               |
| Turnip yellow mosaic virus | Markham & Smith 1949          | England Edinbur. | Turnip         | SM                     | (+) Ch. cabbage<br>(+) Cabbage                      | MCT<br>IT: Beetle           | TI: 70-75°C.<br>DE: 100,000<br>AI: 14 days             |
| Wasabia mosaic virus       | Ohzima 1954                   | Japan Hokkaido   | Wasabia radish | SM                     | (+) Turnip<br>(+) Ch. cabbage<br>(+) Napus          | MCT<br>IT: Aphid            | T: 75°C.<br>DE: 1:1,000<br>AI: 3 days                  |

\*1.....Limited in cruciferae

\*2.....Referenle number

\*3.....Systemic mosaic

\*4.....Local lesion symptom

\*5..... Vein-clearing

\*6..... Vein-banding

\*7.....Enation

\*8.....Cruciferae & non-cruciferae

## EXPERIMENTAL RESULTS

Symptoms on radish; Seven to 14 days after inoculation the KRMV caused mosaic symptoms on the apical young leaf at temperatures ranging from 13 to 19° C. Slight vein-clearing and chlorotic lesions sometimes occur on the inoculated leaf 5 to 7 days after inoculation. Necrotic lesions and stunting of infected plants have not been known to occur either in the field or in the green house.

When the RPV was inoculated mosaic symptoms were produced 5 to 10 days after inoculation. Vein-clearing and chlorosis were sometimes observed on the leaves of infected radish. Stunting of the plant has not been observed during the experiment.

Symptoms on chinese cabbage; The KRMV infected on to the plant systemically and developed yellowish pin-points or chlorotic lesions around edges. Seven to 10 days after inoculation mosaic symptom appeared on a young leaf. Leaf distortion was observed sometimes on infected chinese cabbage plants a month after inoculation.

When the RPV was inoculated mosaic symptom appeared on a young leaf 5 to 7 days after inoculation. Leaf distortion was also observed 20 days after inoculation. No pin-points were developed.

Symptoms on turnip; The mosaic symptoms tend to be more pronounced on turnip with KRMV. After five to 10 days of inoculation the mosaic symptoms began to appear, the dark areas being often raised. Vein-clearing and vein-banding were sometimes observed clearly on the leaves developing after infection.

Upon inoculation of RPV on turnip vein-clearing and vein-banding were produced on the newly appearing leaves of the infected plant 5 to 10 days after inoculation. Dark green areas were sometimes observed 20 days after inoculation. Under temperature of above 28° C. the symptoms were milder than under the temperatures ranging from 15 to 20° C.

Symptoms on *Nicotiana glutinosa*; After 3 to 5 days of inoculation pin-point like lesions appeared on the leaves inoculated. After 5 to 10 days the infection became systemic with some stunting especially on the upper part of the plant. RPV did not infect on the plant.

Symptoms on chenopodium; Both KRMV and RPV caused local lesion on inoculated leaves 3 to 4 days after inoculation. After 7 to 10 days, the lesions have a tinge of red and look quite distinct under the sun light. When inoculated on field grown chenopodium, or kept at high temperature above 28° C., the symptom started very slow taking more than two weeks. (Fig. 1)

**HOST RANGE** Fifty two members of cruciferae, Solanaceae, Cucurbitaceae, and other families of plants were tested for susceptibility to the two viruses. The plants tested and the results obtained are shown in table 2. Twenty-three members of plants were not susceptible to both the KRMV or RPV. None of the 7 *Nicotiana* species including *N. glutinosa* were susceptible to the RPV. KRMV was infective on *Nicotiana* species except to *N. rustica* L. *N. sandrae* L. *B. juncea* was susceptible to both KRMV and RPV.

The Pathogenicity to cabbages were very low with both of the viruses. KRMV was susceptible to *Cucurbita pepo* L., but it was not susceptible to RPV. *B. oleracea* var. capitata, succession, *B. oleracea* var. botrytis L., *Cucurbita moschata*, *Cucurbita pepo* L., *Cucumis sativus* L., *Impatiens balsamina* L., *Lycopersicum esculentum* Mill, *Zinnia elegans* Jacq were shown very mild infectivity to KRMV, but they

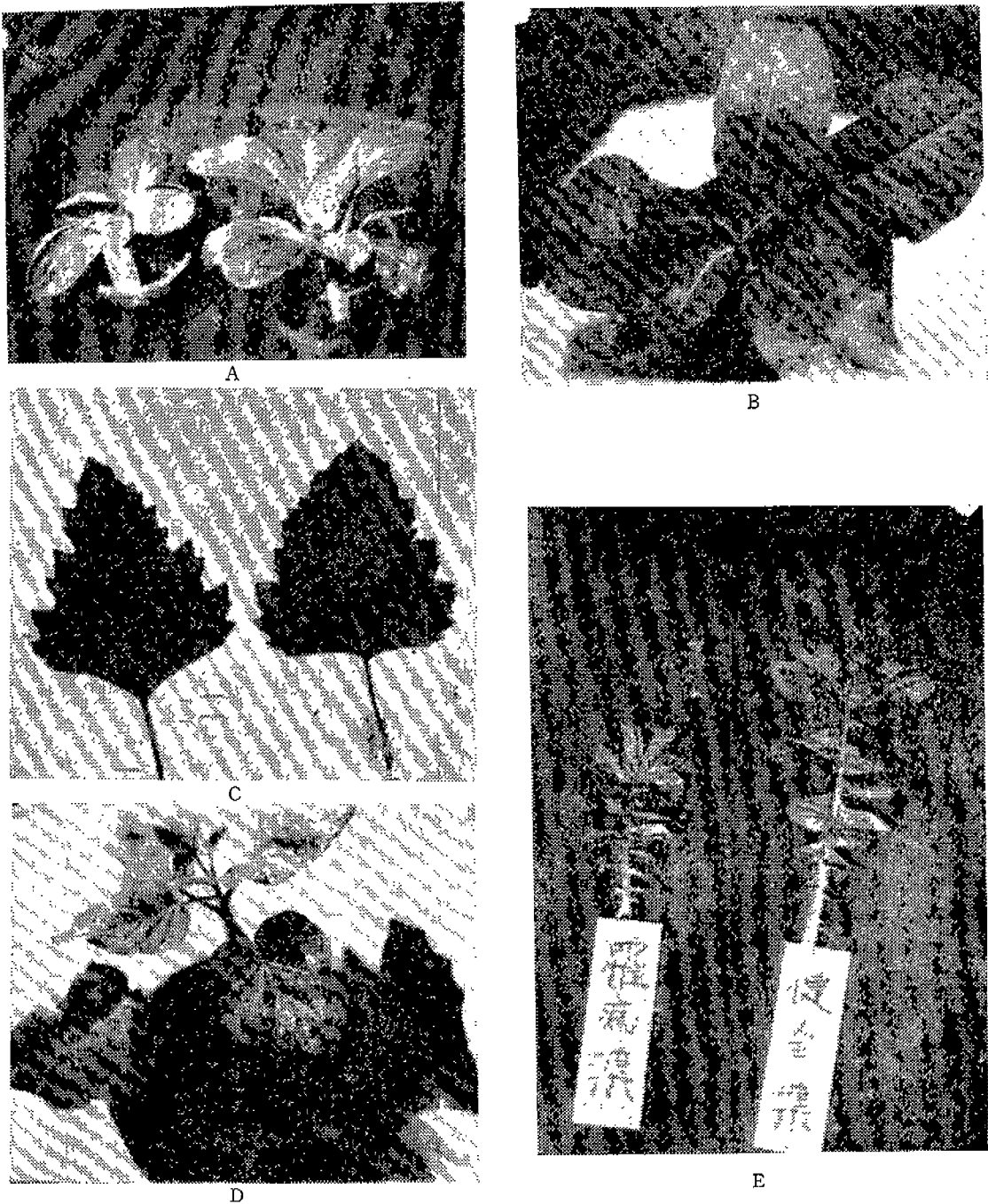


Fig. 1. Symptoms of KRMV on various hosts:

- A. Chinese cabbage: infected (left) and healthy (right).
- B. White Burley tobacco: systemic infection.
- C. *Chenopodium Koreanse* Nakai, local lesions on inoculated leaf (right) and healthy (left.)
- D. *N. glutinosa* L., systemic infection.
- E. Radish; infected (left) and healthy (right).

were not susceptible to RPV. *Gomphrena globosa* L., was shown necrotic local lesion to RPV, but it was not susceptible to KRMV.

Table 2. Host range of two mosaic viruses of radish, KRMV (Korean strain) and RPV (Japanese strain).

| Host   | KRMV  | RPV   |
|--|-------|-------|
| <i>althaea rosea cavanilles</i> .....                            | 0/5*  | 0/3*  |
| <i>Beta vulgaris</i> L.....                                      | 0/3   | 0/3   |
| <i>B. compensis</i> .....  | 10/10 | 10/10 |
| <i>B. juncea</i> L., Florida Broad Leaved.....                   | 5/5   | 5/5   |
| <i>B. juncea</i> L., Black mustard.....                          | 5/5   | 5/5   |
| <i>B. juncea</i> L., Yellow mustard.....                         | 5/5   | 5/5   |
| <i>B. juncea</i> Coss.....                                       | 5/5   | 5/5   |
| <i>B. napus</i> L.....   | 10/10 | 2/5   |
| <i>B. oleracea</i> L.....  | 1/10  | 0/3   |
| <i>B. oleracea</i> var. <i>capitata</i> , Copenhagen.....        | 0/10  | 0/3   |
| <i>B. oleracea</i> var. <i>capitata</i> , Succession.....        | 1/10  | 0/3   |
| <i>B. oleracea</i> var. <i>capitata</i> , Yop-sim.....           | 0/5   | 0/3   |
| <i>B. oleracea</i> var. <i>botrytis</i> L.....                   | 1/10  | 0/3   |
| <i>B. oleracea</i> var. <i>capitata</i> , Early round dutch..... | 0/10  | 0/5   |
| <i>B. pekinensis</i> .....                                       | 20/20 | 20/20 |
| <i>B. rapa</i> L.....  | 10/10 | 10/10 |
| <i>Callistephus chinensis</i> Nees.....                          | 0/5   | 0/3   |
| <i>Capicum annum</i> L.....                                      | 0/5   | 0/3   |
| <i>Chenopodium Koreanse</i> Nakai.....                           | 10/10 | 5/5   |
| <i>Chrysanthemum coronarium</i> L.....                           | 2/5   | 1/3   |
| <i>Citrullus vulgaris</i> schrader.....                          | 0/3   | 0/3   |
| <i>Cucumis melo</i> L.....                                       | 0/3   | 0/3   |
| <i>Cucumis sativus</i> L.....                                    | 1/10  | 0/8   |
| <i>Cucurbita moschata</i> .....                                  | 1/3   | 0/3   |
| <i>Cucurbita pepo</i> L.....                                     | 5/5   | 0/3   |
| <i>Datura tatula</i> L.....                                      | 0/5   | 0/5   |
| <i>Gomphrena globosa</i> L.....                                  | 0/6   | 4/5   |
| <i>Impatiens balsamina</i> L.....                                | 2/5   | 0/3   |
| <i>Lactuca sativa</i> L.....                                     | 0/10  | 0/5   |
| <i>Lagenaria vulgaris</i> Seringe.....                           | 0/2   | 0/2   |
| <i>Lycopersicum esculentum</i> Mill.....                         | 2/5   | 0/3   |
| <i>Malva olitoria</i> Nakai.....                                 | 0/3   | 0/3   |
| <i>N. glutinosa</i> L.....                                       | 5/5   | 0/3   |
| <i>N. rustica</i> L.....   | 0/3   | 0/3   |
| <i>N. sandrae</i> L.....   | 0/3   | —     |
| <i>N. tabacum</i> L., Gold leaf.....                             | 5/5   | 0/3   |
| <i>N. tabacum</i> L., White Burley.....                          | 5/5   | 0/3   |
| <i>N. tabacum</i> L., Yellow special.....                        | 3/3   | 0/3   |
| <i>N. tabacum</i> L., Turkey.....                                | 3/3   | 0/3   |
| <i>Petunia hybrida</i> .....                                     | 3/4   | 3/3   |
| <i>Pharbitis</i> Nill Choisy.....                                | 0/5   | 0/3   |
| <i>Phaseolus vulgaris</i> L.....                                 | 0/6   | 0/5   |
| <i>Pisum sativum</i> L.....                                      | 0/6   | 0/5   |
| <i>Plantago major</i> L.....                                     | 0/6   | 0/3   |
| <i>Raphanus sativus</i> L., Bom-moo.....                         | 30/30 | 10/10 |
| <i>Soja max</i> Merill.....                                      | 0/5   | 0/3   |

|                                    |     |     |
|------------------------------------|-----|-----|
| <i>Solanum nigrum</i> L.....       | 2/3 | 2/3 |
| <i>Spinacia oleracea</i> L.....    | 1/3 | 1/3 |
| <i>Taraxacum platycarpum</i> ..... | 0/5 | 0/3 |
| <i>Vicia Faba</i> L.....           | 0/6 | 0/5 |
| <i>Vigna sinensis</i> L. ....      | 0/5 | 0/8 |
| <i>Zinnia elegans</i> Jacq .....   | 1/5 | 0/3 |

\* Number of plants infected/Number of plants inoculated

VARIETAL SUSCEPTIBILITY in radish and Chinese cabbage.—With 16 varieties of radish and Chinese cabbage tested, all were susceptible to KRMV and RPV, and the mosaic was most common on the plants, but 3 varieties of radish and one of Chinese cabbages showed more resistance to mosaic than the others. Radish varieties, Minong Early, Simoo (Tokinashi) were most resistant, while Paek-soo, and Extra Early (Chinese cabbage) were moderately resistant. (Table 3).

Table 3. Varietal Susceptibility Test

| Radish and Chinese cabbage variety |                        | % of infection of 10 plants |      |
|------------------------------------|------------------------|-----------------------------|------|
|                                    |                        | KRMV                        | RPV  |
| Akamaroo 20 day                    | (radish).....          | 100%                        | 100% |
| Akanaga 20 day                     | (radish).....          | 100                         | 100  |
| Chosun Moo                         | (radish).....          | 100                         | 100  |
| Koong-Joong-Dae-Kun                | (radish).....          | 100                         | 100  |
| Minong Early                       | (radish).....          | 20                          | 30   |
| Paek-Soo, Summer                   | (radish).....          | 50                          | 50   |
| Seoul Bom-Moo                      | (radish).....          | 100                         | 100  |
| Simoo (Tokinashi)                  | (radish).....          | 40                          | 30   |
| White Icicle                       | (radish).....          | 100                         | 100  |
| Chong Bang No. 9                   | (chinese cabbage)..... | 100%                        | 100% |
| Chunpa Whasim                      | (chinese cabbage)..... | 100                         | 100  |
| Extra Early                        | (chinese cabbage)..... | 50                          | 40   |
| Kaesong                            | (chinese cabbage)..... | 100                         | 100  |
| San-Dong                           | (chinese cabbage)..... | 100                         | 100  |
| Seoul                              | (chinese cabbage)..... | 100                         | 100  |
| Spring No. 2                       | (chinese cabbage)..... | 100                         | 100  |

**PHYSICAL PROPERTIES:** Tolerance to dilution, thermal inactivation, and resistance to aging *in vitro* results are shown in Table 4. Both of the viruses, KRMV and RPV, were not inactivated at a dilution of 1: 2,000. In thermal inactivation tests— heating 10 minutes— the KRMV was not inactivated at 58° C. but the RPV inactivated at 56° C. In trials of resistance to aging *in vitro*, the KRMV was inactivated in 7 days, and the RPV in 6 days.

Table 4. Physical Properties

| Tolerance to dilution: |       | times |       |       |       |       |       |
|------------------------|-------|-------|-------|-------|-------|-------|-------|
|                        |       | 0     | 10    | 100   | 1,000 | 2,000 | 5,000 |
| KRMV:                  | 10/10 | 10/10 | 10/10 | 10/10 | 5/10  | 0/10  | 1/10* |
| RPV:                   | 10/10 | 10/10 | 10/10 | 10/10 | 5/10  | 0/10  | 0/10  |

\* Number of plants infected out of 10 plants in 3 trials on the *Chenopodium Korcense* Nakai



## Thermal inactivation:

|       | Control | 55    | 56   | 57   | 58   | 59   | 60   | 62 | 65° C. |
|-------|---------|-------|------|------|------|------|------|----|--------|
| KRMV: | 10/10   | 10/10 | 5/10 | 2/10 | 1/10 | 0/10 | 0/10 | —  | 0/10*  |
| RPV:  | 10/10   | 10/10 | 2/10 | 0/10 | 0/10 | 0/10 | 0/10 | —  | 0/10   |

\* Number of plants infected out of plants on the *Chenopodium Koreanse Nakai*

## Aging in vitro:

|       | 0   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10   |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| KRMV: | 8/8 | 8/8 | 8/8 | 7/8 | 7/8 | 5/8 | 2/8 | 1/8 | 0/8 | 0/8 | 0/3* |
| RPV:  | 8/8 | 8/8 | 8/8 | 8/8 | 6/8 | 2/8 | 2/8 | 0/8 | 0/8 | 0/8 | 0/8  |

\* number of plants diseased out of 8 plants inoculated in 3 trials on the *Chenopodium Koreanse Nakai*

**INSECT TRANSMISSION:** The green aphid (*Myzus persicae* Sulz) was used as the test insect. After starvation of 2-3 hours, the aphids were allowed on the leaves of diseased plant. After 10 minutes feeding the aphids were transferred to healthy seedlings of turnip and other hosts, 5-10 aphids per plant. After 10 hours the aphids were killed by spraying insecticide.

The results of test are shown in Table 5. With the RPV, not a single infection was obtained in all tests, whereas the KRMV showed comparatively high degree of transmission.

Table 5. Aphid transmission test

| Host                        | KRMV  | RPV  |
|-----------------------------|-------|------|
| Chinese cabbage, Seoul..... | 8/10* | 0/7* |
| Radish, White Icicle .....  | 8/10  | 0/7  |
| Turnip, Kanamachi.....      | 9/10  | 0/7  |
| Tobacco, White Burley ..... | 3/3   | —    |
| Tobacco, Turkey.....        | 2/2   | —    |

\* Number of plants/infected Number of plants treated with aphids, 5-10 per plant.

**PURIFICATION:** After 3 weeks of inoculation, plants were harvested, partial washed, weighed in lots of 150 gm, wrapped in vinyl sack, and frozen, The frozen plants were passed through a mixer while still frozen with adding weight by weight of ammonium acetate buffer (pH 7.0). The juice was pressed from the macerate, and melted after 3 hours freezing at  $-15^{\circ}\text{C}$ . This was then clarified by centrifuging it for 30 minutes at 3,500 r.p.m. To the supernatant liquid, while stirring, 20% of ammonium sulfate was added and, after standing for 30 minutes, centrifuged it for 30 minutes at 3,500 r.p.m. at  $0^{\circ}\text{C}$ . To the supernatant liquid, 30% of ammonium sulfate was added and, after standing one hour, centrifuged it for 30 minutes at 3,500 r.p.m. at  $0^{\circ}\text{C}$ . The precipitate was resuspended in ammonium acetate buffer (pH 7.0). one tenth of volume to the original juice. This procedure was repeated until the bound components were removed.

To remove the dark pigmented material, involved the drop-by-drop addition of saturated ammonium sulfate solution to the virus concentrate at  $20^{\circ}\text{C}$ . until the first signs of opalescence be appeared. The preparation was chilled to  $0^{\circ}\text{C}$ . and allowed to stand for a few hours. Low speed (1,500 r.p.m./1 hr. / $0^{\circ}\text{C}$ .) centrifugation removed the brown precipitate. Saturating with ammonium sulfate (50%), the suspension was centrifuged for 30 minutes at 3,500 r.p.m./ $0^{\circ}\text{C}$ . It was then resuspended in distilled water, dialyzed against tap water for 24 hours. Centrifuged at 4,00 r.p.m./30 min./ $0^{\circ}\text{C}$ . The isoelectric point of the KRMV and RPV was around pH 4.0. (Table 6).

Table 6. Purification of KRMV and RPV

| <u>Infected plants (Chinese cabbage, 150 g)</u>           |  |
|---|--|
|   | Store over night in $-20^{\circ}\text{C}$ . Freezer<br>Grinding with 0.1 M ammonium acetate buffer (w/w) at pH 7.0<br>Press out juice<br>Melted after 3 hours' freezing at $-15^{\circ}\text{C}$ . |
|   | Juice  |
|   | Centrifuge.....3,900 r.p.m./30 min/ $0^{\circ}\text{C}$ .  |
| Repeat several times                                      | ppt      SN  |
|   | Add $(\text{NH}_4)_2\text{SO}_4$ (20%)<br>Stand 30 min.  |
|   | Centrifuge.....3,500 r.p.m./30 min/ $0^{\circ}\text{C}$ .  |
|   | ppt      SN  |
|   | Add $(\text{NH}_4)_2\text{SO}_4$ (50%)<br>Stand 1 hour   |
|   | Centrifuge.....3,500 r.p.m./30 min/ $0^{\circ}\text{C}$ .  |
|   | SN      ppt  |
|   | Add ammonium acetate buffer (pH 7.0)<br>Add $(\text{NH}_4)_2\text{SO}_4$ 15%<br>Stand several hours in $0^{\circ}\text{C}$ .   |
|   | Centrifuge.....1,500 r.p.m./1 hr/ $0^{\circ}\text{C}$ .  |
|   | ppt      SN  |
| Add $(\text{NH}_4)_2\text{SO}_4$ (50%)<br>Stand 1 hour    |  |
| Centrifuge.....3,500 r.p.m./30 min/ $0^{\circ}\text{C}$ . |  |
| SN      ppt   |  |
| Resuspend in $\text{H}_2\text{O}$                         |  |
| Centrifuge.....2,000 r.p.m./10 min.                       |  |
| Dialyze.....against tap water for 24 hours                |  |
| Centrifuge.....4,000 r.p.m./30 min/ $0^{\circ}\text{C}$ . |  |
|   | Virus suspension   |

**ELECTRON MICROSCOPY:** For electron microscopy, Hitachi model HS 6 electron microscope was used. Purified viruses were mounted on 200-mesh wire grid that had been previously covered with a collodion membrane. Dried grids were shadowed with aluminium, and observed the shape of the virus particles. The KRMV particles, spherical in shape, were measured 55 mu in size. The RPV was about  $650 \times 12$  mu in size with rod-shape.

## DISCUSSION

The radish mosaic virus isolated from Korea (KRMV) causes severe mosaic symptoms on turnip, radish, mustard, chinese cabbage, rape, and tobacco; moderate symptoms were developed on *C. pepo* L., *C. coronarium* L., tomato and *Impatiens balsamina* L. and symptoms on cabbage were even milder. In physical properties, the thermal inactivation point of the virus was  $58^{\circ}\text{C}$ ., dilution end-point, 1:2,000 and aging *in vitro*, 7 days. Like KRMV, the RPV severely infected upon radish, turnip, mustard,

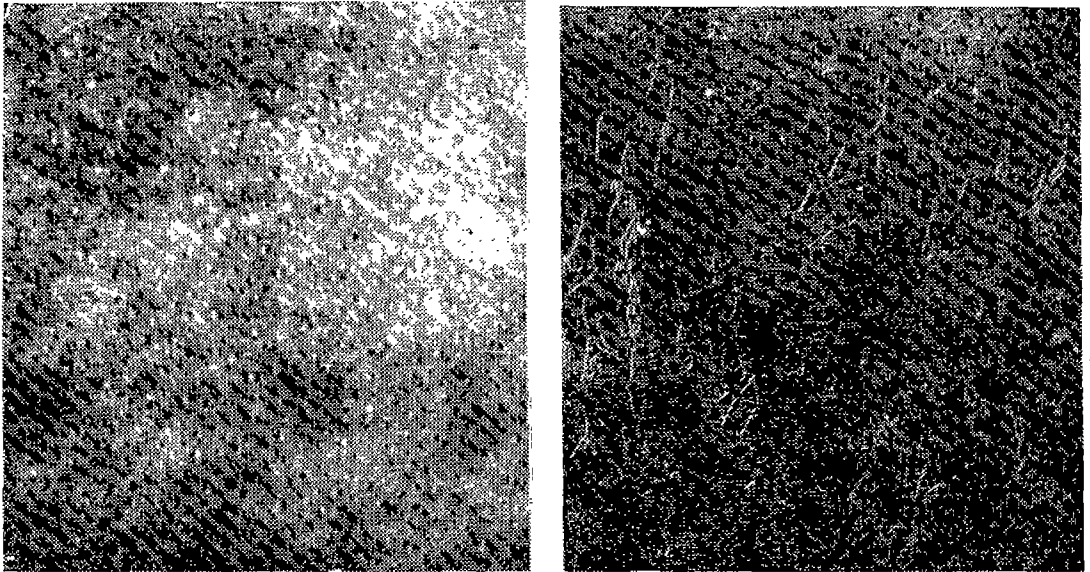


Fig. 2. Electron micrographs of KRMV & RPV; spherical ( $\times 15,000$ ) KRMV and rod-shape RPV. ( $\times 15,000$ ) chinese cabbage and rape, but on cabbage its infectivity was mild as in the case of KRMV. KRMV infected on tobacco, but RPV showed no infection. The physical properties of RPV were found  $56^{\circ}\text{C}$ . in thermal inactivation point, 1 : 2,000 in dilution end-point, and of days in aging *in vitro*, which are similar to those of KRMV.

In the tests of insect transmission it was found that the KRMV was readily transmitted by the peach aphids, whereas RPV was not.

The shape of virus particles of KRMV was spherical, while those of RPV are rod-shaped. Thus, KRMV is quite different from RPV in many respects.

According to the report by Tompkins et al. (27), the infectivity of cabbage black ring virus was severe on cabbage, turnip and tobacco, but that of KRMV was quite mild on cabbage. The thermal inactivation point of cabbage black ring virus was of  $59^{\circ}\text{C}$ ., which is similar to KRMV, but the dilution end-point of the virus was 1 : 1,000 and aging *in vitro* was only 3 days, which is different from KRMV. Furthermore the black ring virus is of rod-shape.

According to another report by the same author (28) on the chinese cabbage mosaic virus, it infected on tobacco and *Nicotiana glutinosa*, but in physical properties, dilution end-point was of 1 : 5,000 and aging *in vitro* 3 days, thermal inactivation point  $73^{\circ}\text{C}$ ., which are quite different from KRMV.

Dale's Crucifer virus (4), also infected on tobacco, but not on cabbage. In the physical properties of the virus, thermal inactivation point was of  $58^{\circ}\text{C}$ ., dilution end-point 1 : 1,000 and aging *in vitro* 4 days, which is not exactly same as KRMV.

There are several more reports on the radish mosaic virus; i.e. Tompkins, (30) radish mosaic virus, Ishiyama and Mizawa's (8) stunt disease of Japanese radish, Takahashi's (24) Rod-shaped virus of radish mosaic, Horton's (7) Radish mosaic virus, and Raychaudhuri's (18) mosaic disease of radish, but their host ranges and physical properties are different from those of KRMV.

Crucifer viruses have been classified into several groups according to the pathogenicity on the *Nicotiana* genus and cabbages (32).

Tsujiya et al. (32) classified the crucifer viruses into 5 groups on the basis of host range differences. Considering no pathogenicity to cabbage, the KRMV may belong to the 4th group.

Pound and Walker (16, 17) classified the crucifer viruses into 2 major groups, turnip mosaic group and cauliflower mosaic group on the basis of host range, physical properties and temperatures affecting infection. If we are to follow them, KRMV may belong to turnip mosaic group, considering for positive pathogenicity to tobacco.

As to the shape of virus particles infecting on cruciferous plant; Takahashi's (23, 24) *Brassica nigra* virus (12 x 700 m $\mu$ ), Radish mosaic virus (12 x 754 m $\mu$ ) etc. are all of rod-shape, but KRMV is spherical, diameter being about 55 mu.

From the above observations the writer believe that the KRMV isolated in this work, is a new strain of radish mosaic virus. For the purpose of distinction the author will name the virus as the Korean Radish Mosaic Virus (KRMV).

### 摘 要

日本農林省 農業技術研究所에서 보내온 “무 모자이크 바이러스” (RPV)와 韓國의 서울 近郊의 菜蔬園에서 病에 걸린 “무”를 蒐集하여 그로 부터 分離한 “무 모자이크 바이러스” (KRMV)를 對照로 하여 宿主範圍 및 症狀, 物理的 性質, 昆蟲傳染 및 電子 현미경 觀察 등으로 檢討하였다. 이 두 바이러스, KRMV와 RPV는 서로 다른 系統이라는 것이 밝혀졌다. 卽

1. 宿主範圍에서 KRMV는 담배 植物에 感染性이 있으나 RPV는 그에 感染性이 없다.
3. KRMV는 昆蟲傳染이 되나 RPV는 되지 않았다.
3. KRMV 粒子는 球形이며 RPV는 棒狀이었다.
4. 物理的 性質은 大體로 비슷하였다.

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