Necrotic Ring Disease of Japanese Iris (Iris kaempferi Sieb.), A New Disease Caused by Japanese Iris Necrotic Ring Virus.

Bull. Univ. Osaka Pref., Ser. B, Vol. 43,
大阪府立大学紀要，農学・生物学，1991

Necrotic Ring Disease of Japanese Iris (*Iris kaempferi* Sieb.), A New Disease Caused by Japanese Iris Necrotic Ring Virus.

Ko YASUKAWA*, Takeshi OSAKI and Tadao INOUYE

Laboratory of Plant Pathology, College of Agriculture

*Yamanouchi Pharmaceutical Co. Ltd.

(Received, 1990)

Abstract

A previously undescribed virus, named Japanese iris necrotic ring virus (JINRV), was detected in 27 out of 52 cultivars of Japanese iris (*Iris kaempferi* Sieb.). The virus was transmitted mechanically but not by the aphids *Myzus persicae* and *Rhopalosiphum padi* or through soil. It caused necrotic spindle-shaped streaks or rings in the leaves of Japanese irises and latent local infection in *I. sanquinea* and *I. laevigata*, but did not infect any other species out of all 40 species of thirteen plant families tested in total.

JINRV particles were isometric about 35 nm in diameter and showed rounded outlines with rough surfaces when negatively stained in uranyl acetate. In ultrathin sections, virus particles could be consistently observed in the cells of most tissues of infected Japanese iris leaves. They occurred in the ground cytoplasm of which they were scattered or in clusters in the central vacuoles in some cells.

Introduction

Japanese iris (*Iris kaempferi* Sieb.) originates in Japan where it is widely cultivated as a traditional ornamental plants. A spherical virus causing spindle-shaped necrotic rings of the leaves of them was first recorded in 1982 and named Japanese iris necrotic ring virus (JINRV) on the basis of characteristic symptoms in leaves[1].

JINRV is sap-transmissible with a narrow host range in the Iridaceae[1]. Some of the other biological and physicochemical properties of the virus have been described by Yasukawa *et al*[1]., and Ozaki *et al*.[2] but not in any detail.

This paper describes an investigation of the symptomatology and host range of the virus.

Materials and Methods

Field survey : To assess the field distribution of the disease, we examined Japanese iris cultivars grown in the University farm, College of Agriculture, University of Osaka Prefecture. An estimate of the percentage of infected plants was made on visual symptoms and transmission studies with representative samples.

Virus isolate and transmission test : The original culture of JINRV was obtained from infected plants of Japanese iris growing in the field that were verified to contain no viruslike particles other than 35 nm spheres. Leaves with necrotic rings were ground in

0.1 M phosphate buffer (pH 7.0). The homogenate was rubbed on carborundum-dusted leaves of test plants including the seedlings of Japanese iris. The virus obtained was thereafter maintained in Japanese iris cv. Kamiyo-no-shirabe in which it induced systemic necrotic rings and were used as source of inoculum for all experiments.

Myzus persicae and *Rhopalosiphum padi* were tested by standard techniques for ability to transmit in the non-persistent, semipersistent and persistent modes. The aphids were fed on infected Japanese iris and tested on healthy seedlings of Japanese iris cv. Kamiyo-no-shirabe with 5-15 aphids per plants.

Soil transmission tests were done by planting healthy seedlings of Japanese iris in field soil collected where infected Japanese iris was growing.

Ears from three infected cultivars (Yume-no-sato, Suiten-issyoku, Kamiyo-no-shirabe) in the field were dried and the harvested seeds were sown in steamed soil in the greenhouse to check for seed transmission.

Virus identification : The identity of viruses in naturally field-infected plants or in plants inoculated artificially was determined by electron microscopy, immunosorbent electron microscopy (ISEM)[3] or combinations of these methods.

Electron microscopy : Samples of leaves from field-infected and manually inoculated cultivars of Japanese iris were ground with 2 % potassium phosphotungstate (pH 6.5) and were examined in a Hitachi H-300 electoron microscope for the presence of virus particles. The size of the particles was determined by comparison with purified CMV. Preparations of JINRV and CMV were mixed, negatively stained with 0.5 % uranyl acetate and photographed. Particle sizes were compared, taking the diameter of CMV particles of 29 nm. Preparations were also examined using the clumping technique of serologically specific electron microscopy as described by Milne[3].

Leaf tissue of Japanese iris was prepared for ultrathin sectioning by fixation in 2.5 % gultaraldehyde in phosphate buffer (PH 7.0), postfixation in 2 % osmium tetroxide in the same buffer, dehydration in an alcohol series, and then embedding in Spur's medium. Sections were stained with uranyl acetate and lead citrate before electron microscopic examination.

Results

Field occurrence and symptomatology : In surveys conducted in the University farm between 1979 and 1980, JINRV but no other virus, was detected in 27 of 52 cultivars. In Japanese iris plants naturally infected with JINRV, three types of symptoms which varied among cultivars were distinguished in leaves : 1) necrotic rings, 2) mosaic, 3) a mixture of the two (Table 1).

Spindle-shaped necrotic rings were prominent in cultivars such as in Ama-no-ukikusa and so on (Table 1). The young leaves of these cvs. developed spindle-shaped chlorotic areas and were later turned brown and necrotic (Plate I-1, 2). Conspicuous necrotic ring symptoms were developed on the flower stalk and spathe early in the flowering season (Plate I-3). At this stage, the remainder of the leaf was either symptomless or developed mild mosaic depended on the cultivar (Table 1). In such cultivars as Akatuki-no-sono and Hato-no-tsukai developed distinct fine yellowish green mosaic or a faint chlorotic mosaic (Table 1). The floweres were normal in size and shape. The economical value and the vigour of the plants did not seem to be markedly reduced.

Numerous isometric particles, about 35 nm in diameter, were found in negatively

Table 1. The symptoms in Japanese iris cultivars naturally infected with Japanese iris necrotic ring virus

Cultivar	Symptoms		Cultivar	Symptoms	
	Necrotic ring	mosaic		Necrotic ring	Mosaic
Ama-no-ukikusa	+	−	Syōzyōden	+	+
Matsunami	+	−	Akatsuki-no-sono	−	+
Rurityō	+	−	Hato-no-tsukai	−	+
Ryūgū	+	−	Hititoku-no-mai	−	+
Sennyo-no-hora	+	−	Keiga	−	+
Shikōuden	+	−	Komadome	−	+
Shyokō-nisiki	+	−	Mikasayama	−	+
Fuzi-no-wa	+	+	Murakumo	−	+
Hakuhōkan	+	+	Nishiki-ba	−	+
Hide-murasaki	+	+	Suikintei	−	+
Kamiyo-no-shirabe	+	+	Tōkai-no-hi	−	+
Sakura-no-haru	+	+	Yamato-hime	−	+
Shihōden	+	+	Yume-no-ukihashi	−	+
Suiten-isshoku	+	+			

+ : Symptoms produced, − : not produced

stained leaf dip preparations of all of 27 cultivars collected at the University farm, and the virus was identified serologically by ISEM test with JINRV antiserum[7].

Seedlings from seven Japanese iris cultivars (Shirayuki, Hakusetu, Yume-no-sato, Tōkai-no-hi, Hakuhōkan, Kamiyo-no-shirabe and Suiten-isshoku) were mechanically inoculated from each of infected cultivars (Sakura-no-haru and Ama-no-ukikusa), all of which developed necrotic rings in the field. All test plants of each cultivar showed necrotic rings on inoculated leaves and overall symptom pattern was similar to that observed in the field. By contrast, when the same test plants were inoculated with extracts from naturally infected leaves of two other cultivars (Yamatohime and Yume-no-ukikusa) which developed mosaic symptoms, plants of each cultivar developed necrotic rings instead of mosaic. Thus, the variations in symptom expression seemed due to differences in the tolerancy of cultivar.

Host range : In addition to the 7 Japanese iris cultivars, the virus was transmitted by inoculation with sap to two other iridaceous species, *Iris sanguinea* Horn and *I. laevigata* Figh. The virus induced symptomless infection in only the inoculated leaves of these plants. However, in one of the two tests, one out of ten plants of *I. laevigata* developed systemic spindle-shaped necrotic rings. The virus failed to infect any of the following 40 species from 12 other families; *Achyranthes bidentata*, *Allium fistulosum* var. *giganteum*, *A. tuberosum*, *A. porrum*, *Avena sativa* cv. Victory, *Beta vurgaris* var. *Saccharifera*, *Brassica rapa* cv. Tokinashi Kokabu, *Capsicum annuum* cv. California wonder, *Chenopodium amaranticolor*, *C. murale*, *C. quinoa*, *Cucumis sativus* cv. Sagami -Hanziro, *Cucurbita pepo*, *Datura stramonium*, *Daucus carota* cv. Tokinasi-Gosun, *Glycine max* cv. Sapporo-Midori, *Gomphrena globosa*, *Gossypium arboreum* var. *rugosa*, *Hordium distichum* cv. Haerhpin-Nizyou, *Lactuca sativa*, *Lillium longiflorum*, *Lycopersicon esculentum* cv. Marglobe, *Nicotiana debneyi*, *N. tabacum* cv. Xanthi, *N. glauca*, *N. glutinosa*, *N. rustica*, *N. sylvestris*, *Petunia hybrida*, *Phaseolus vulgaris* cv. Top Crop, *Physalis floridana*, *Pisum sativum* cv. New Season, *Solanum melongena* cv. Benkei,

Spinacia oleracea cv. Sapporo-Ooba, *Tetragonia tetragonoides*, *Triticum durum*, *Vicia faba* cv. Kawachi-Issun, *Vigna sesquipedaris cv.* Kurodane-Sanzyaku, *Zea mays* var. *rugosa*, *Zinnia elegans.*

Transmission tests : *M. persicae* and *R. padi* failed to transmit the virus from infected Japanese iris to healthy one after any of the three acquisition access periods used. In seed transmission tests no virus like symptoms were observed in plants produced from seeds collected from infected Japanese iris cultivars Yume-no-sato, Kamiyo-no-shirabe and Suiten-issyoku after such plants were kept in the greenhouse for two months after transplanting. No symptoms appeared on healthy Japanese iris seedlings grown in field soil containing infected plants.

Electron microscopy : Crude sap from Japanese iris and other hosts contained small isometric particles with rounded outlines and rough surfaces (Plate I-4). When particles were exposed to 2 % PTA at pH 6.0, most of the particles were either partially or totally penetrated by stain or partially disrupted although undameged particles were well contrasted (Plate I-5～7). The penetrative and disruptive effects of PTA were overcome by fixing the virus in 0.2 % formaldehyde for 2-4 h (Plate I-4, 8). Using CMV particles as an internal size standard, eighty-three particles were measured; the diameter of individual particle ranged from 28-38 nm, but fourty-nine (59 %) were 32-35 nm wids.

In thin sections of JINRV-infected Japanese iris, leaf cells had particles throughout in cytoplasm (Plate II-1) but not in nuclei. They occurred in clusters in the central vacuoles in some cells (Plate II-2, 3). Crystalline arrays of virus particles were rare, but those occured in necrotic cells (Plate II-4). Most of the cell organelles were apparently unaffected but sometimes mitochondria adopted an unusual shape.

Discussion

The question of homogeneity of our virus preparations is particularly crucial because the necessity to use naturally infected plants as sources of virus. However, the uniform appearance of the particles in the electron microscope indicate that our preparations contained only one virus.

For diagnosis, the distinct necrotic ring can be useful but it developes late and does not occur in all cultivars. Therefore, serological tests are necessary for reliable detection in all cultivars.

JINRV showes similarties to the carmoviruses which recently approved the establishment of the group by the International Committee on Taxonomy of Viruses (ICTV)[4] in particle size and morphology, mechanical thransmissibility, ultrastructural feature, and failure of transmission by aphids[5,6]. Furthermore, as shown in our next paper[7], the properties of nucleic acid and coat protein of JINRV indicate that the virus is a member of the carmovirus group. Despite of these similarities, JINRV is serologically unrelated to carnation mottle virus, the type member of the carmovirus group[7], and differs from any of recognized carmoviruses in host range. Therefore, our results suggest that JINRV is hitherto underscribed virus belong to the carmovirus group.

At least 10 other viruses have been recorded in other irises. However, these have either flexuous filamentous virus particles (*e. g.* iris severe mosaic[8], iris mild mosaic[9], iris fulva mosaic[10,11], bean yellow mosaic[12], turnip mosaic[13] potyviruses, and narcissue latent[14,15] carlavirus), isometric virus particles (*e. g.* broad bean wilt[16] fabavirus and tobacco ringspot[14] nepovirus), or rigid rod-shaped virus particles (*e. g.* tobacco mosaic[14]

tobamovirus and tobacco rattle[14] tobravirus). Furthermore JINRV is readily distinguishable from these viruses in host range, serology and other properties.

References

1) YASUKAWA, K., OHKI, S. T., OSAKI, T. and INOUYE, T. (1982). Japanese iris necrotic ring disease, a newly recognized disease caused by Japanese iris necrotic ring virus. *Ann. Phytopath. Soc. Japan* **48**, 113-114 (*Abstr. in Japanese*).

2) OZAKI, K., YASUKAWA, K., OHKI, S. T., OSAKI, T. and INOUYE, T. (1987). Physicochemical properties of Japanese iris necrotic ring virus. *ibid.* **53**, 422 (*Abstr. in Japanese*).

3) MILNE, R. G. (1984). Electron microscopy for the identification of plant viruses *in vitro* preparations. In "*Methods in Virology Vol. 7*". (Ed. by MARAMOROSCH, K. and KOPROWSKI, H.). Academic Press, New York. 87-120.

4) BROWN, F. (1989). The classification and nomenclature of viruses : Summary of reseelts of meetings of the International Committee on Taxonomy of Viruses in Edmonton, Canada 1987. *Intervirology* **30**, 181-186.

5) FRANCKI, R. I. B., MILNE, R. G. and HATTA, T. (1985). Tombusvirus group. In "*Atlas of Plant Viruses*". Vol. 1, CRC Press, Boca Raton, Florida. 181-197.

6) MARTELLI, G. P. (1981). Tombusviruses. In "*Handbook of Plant Virus Infections and Comparative Diagnosis*" (Ed. by KURSTOK, E.). Elsevier/North-Holland Biomed. Press, Amsterdam 61-90.

7) OSAKI, T., OZAKI, K., YASUKAWA, K., OHKI, S.T. and INOUYE, T. (1991). Purification and properties of Japanese iris necrotic ring virus, a possible new carmovirus. *Ann. Phytopath. Soc. Japan* (*in subscription*).

8) BRUNT, A. A., DERKS, A. F. L. M. and BARNETT, O. W. (1988). Iris servere mosaic virus. *CMI/AAB Descriptions of Plant Viruses* No. 338.

9) BRUNT, A. A. (1986). Iris mild mosaic virus. *AAB Descriptions of Plant Viruses* No. 324.

10) BARNETT, O. W. and ALPER, M. (1977). Characterization of iris fulva mosaic virus. *Phytopathology* **67**, 448-454.

11) BARNETT, O. W. (1986). Iris fulva mosaic virus. *AAB Descriptions of Plant Viruses* No. 310.

12) ALPER, M. and LOEBENSTEIN, G. (1981). Bean yellow mosaic virus in bulbous irises in Isurael. *Plant Disease* **65**, 694-695.

13) INOUYE, N. and MITSUHATA, K. (1978). Turnip mosaic virus isolated from iris. *Nogaku Kenkyu* **57**, 1-16.

14) ASJES, C. J. (1979). Viruses and virus diseases in dutch bulbous iris (*Iris hollandica*) in the Netherlands. *Neth. J. Pl. Path.* **85**, 269-279.

15) BRUNT, A. A. (1977). Some hosts and properties of narcissus latent virus, a carlavirus commonly infecting narcissus and bulbous iris. *Ann. appl. Biol.* **87**, 355-364.

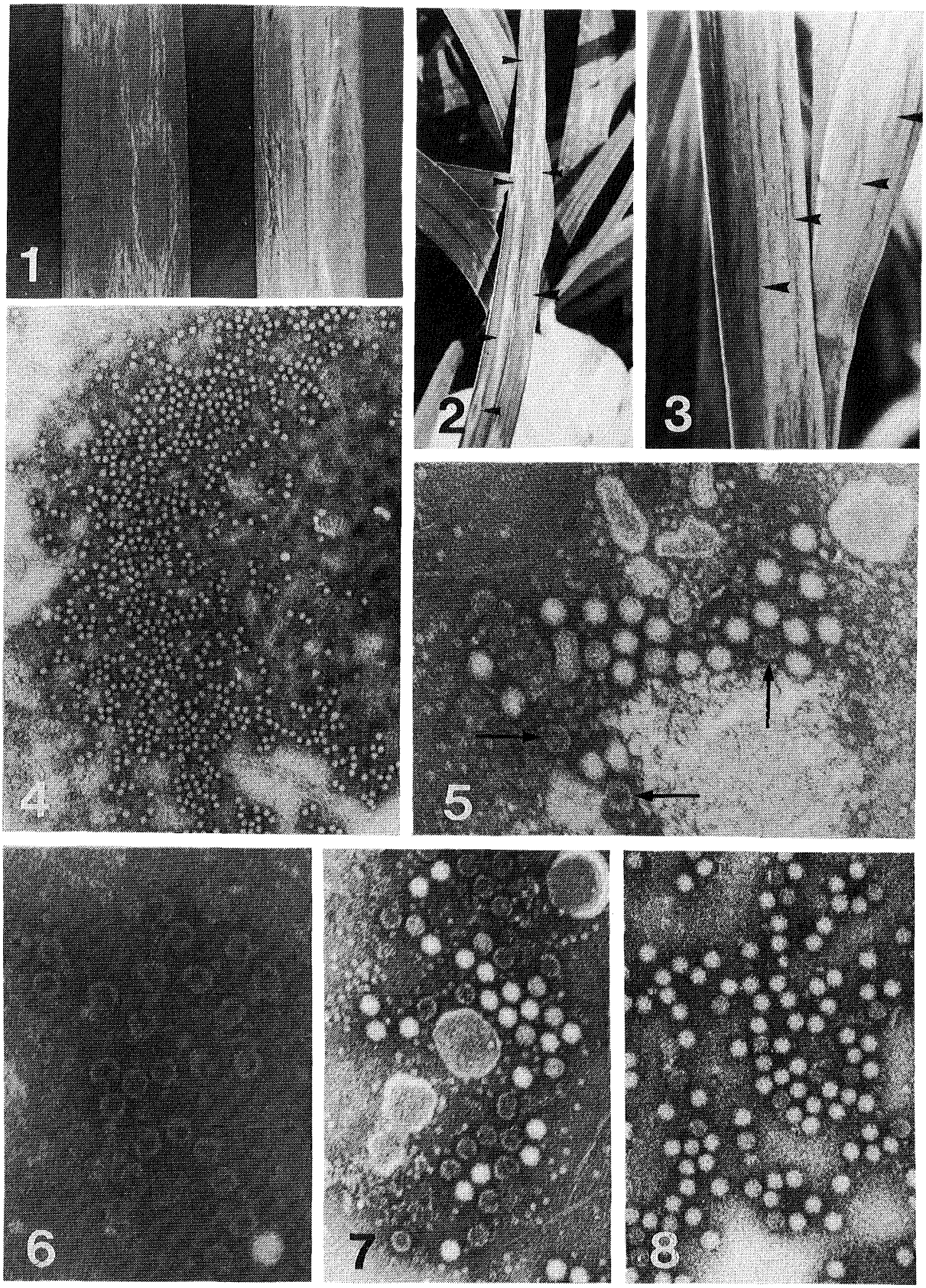
16) BAILISS, K. W., BRUNT, A. A. and DALE, W. T. (1975). The natural occurrence of broad bean wilt virus in rhizomatous iris and spinach. *Plant Pathology* **24**, 60-61.

Explanation of Plates

Plate I 1-3. Japanese iris with necrotic ring symptoms after natural infection.
1, 2. Necrotic rings developed on leaves (arrow head).
3. Necrotic rings developed on spathes (arrow head).
4-8. JINRV particles in leaf dip preparations of infected Japanese iris leaves.
4. Particles stained with potassium phosphotungstate after pretreatment with glutaraldehyde. × 30,000.
5. Particles stained with potassium phosphotungstate. Some particels appear to be disintegrating (arrows). × 128,000.
6. Particles penetrated by the stain. Stained with potassium phosphotungstate. × 130,000.
7. Particles in potassium phosphotungstate. A few particles penetrated by the stain. × 75,000.
8. Particles stained with uranyl acetate. Particles show rounded outlines with rough surfaces. × 75,000.

Plate II Electron micrographs of systemically infected Japanese iris cell.
1. Mesophyll cell showing small groups of virus particles in crystalline array (V). × 30,000.
2. Aggregate of virus particles (V) in the vacuole (Va). × 25,000.
3. Virus particles in the vacuole (Va). Virus particles in the cytoplasm (arrow head) are readily distinguished from the ribosomes. × 40,000.
4. Aggregate of virus particels (V) in the necrosed cell. × 35,000.

Plate I



PlateII