VIRUS DISEASES OF RICE AND LEGUMINOUS CROPS IN JAPAN

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ABSTRACT

Virus diseases of rice and leguminous crops are one of the major problems hampering the cultivation of these crops in Japan. These diseases are of great interest to plant pathologists, breeders and biochemists, due to the importance of the crops, and the biological problems involved.

Eight viruses attack rice plants in Japan, i.e. dwarf, stripe, black-streaked dwarf, grassy stunt, transitory yellowing, ragged stunt, Waika and necrosis mosaic viruses. Except for fungal-borne necrosis mosaic, most rice viruses are transmitted by leafhoppers. Ecological research on vector insect populations is, therefore, highly significant for virus control. Distribution of the rice virus diseases in Japan and the recent progress in the basic studies on the viruses will be discussed.

Seven leguminous crops are commonly cultivated in Japan, including soybean, azuki bean, French bean, cowpea, peanut, pea and broadbean. Soybean is most widely cultivated throughout Japan. A total of 27 different viruses that infect the leguminous crops in Japan is listed, of which, 11 affect soybean, 8 French bean, 4 azuki bean, 5 cowpea, 3 peanut, 10 pea and 9 broadbean, including 10 viruses specifically detected in Japan. Occurrence and distribution of these viruses will be discussed.

Rice viruses

1 Virus diseases of rice in Japan

So far, a total of 8 viruses that cause diseases on rice plants has been reported in Japan, i.e. dwarf (RDV), stripe (RSV), black-streaked dwarf (RBSDV), grassy stunt (RGSV), transitory yellowing (RTYV), ragged stunt (RRSV), waika (RWV) and necrosis mosaic (RNMV) viruses.

Following the isolation of a vectorless strain of RDV (Kimura, 1976), a severe strain of RDV, which caused extreme dwarfing on rice with a high transmissibility, was eventually selected (Kimura *et al.,* 1985).

A severe strain S of RWV was identified. It caused conspicuous symptoms of discoloration, brown blotches, severe stunting, reduced number of tillers of the susceptible rice cv. Reiho, with a shorter incubation period than that of the common strain C (Inoue, 1978).

Three strains of RGSV, mild, common and severe strains, have been isolated in Kyushu (Iwasaki *et al.,* 1985).

2 Vectors

Based on the vector species, three different groups of viruses have been classified: a. Persistent leafhopper-borne viruses, b. Semi-persistent leafhopper-borne viruses, c. Fungal-borne viruses.

Table 1 lists the viruses and their know vectors. The principal vectors of those viruses in Japan are: *N. cincticeps* for RDV and RWV; *L. striatellus* for RBSDV and RSV; *N. lugens* for RGSV and RRSV; *Polymyxa graminis* for RNMV.

N. cincticeps is distributed in Honshu, Kyushu and Shikoku, but not in Hokkaido. *L. striatellus* is widely distributed throughout Japan. On the other hand, *N. lugens* seems to migrate over long distances from southern China or Southeast Asia, and is distributed throughout Japan. *P. graminis* carrying necrosis mosaic is found throughout Japan.

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Table 1 Rice viruses and their insect vectors in Japan

Viruses	Vectors
	Leafhopper-borne (persistent)
Dwarf	*Nephotettix cincticeps*
	N. nigropictus
	Recilia dorsalis
Transitory yellowing	*N. cincticeps*
	N. nigropictus
	Planthopper-borne (persistent)
Black-streaked dwarf	*Laodelphax striatellus*
	Unkanodes albifascia
	U. sapporona
Stripe	*L. striatellus*
	Terthron albovittatus
	U. albifascia
	U. sapporona
Grassy stunt	*Nilaparvata lugens*
	(N. muiri)
	(N. bakeri)
Ragged stunt	*N. lugens*
	Leafhopper-borne (semi-persistent)
Waika	*N. cincticeps*
	N. virescens
	N. nigropictus
	Fungal-borne
Necrosis mosaic	*Polymyxa graminis*

3 Distribution and epidemiology

Distribution of the rice viruse diseases is as follows:

Dwarf,	Honshu, Shikoku and Kyushu
Stripe,	Hokkaido, Honshu, Shikoku and Kyushu
Black-streaked dwarf,	Honshu, Shikoku and Kyushu
Waika,	Kyushu
Transitory yellowing,	Okinawa
Grassy stunt,	Kyushu, Shikoku and Honshu (Chugoku)
Ragged stunt,	Kyushu
Necrosis mosaic,	Honshu (Chugoku), Shikoku and Kyushu

RDV has been endemic in Kyushu for the past 10 years. It occurred over 66,000 ha of rice fields in Kyushu in 1983, hence accounting for half of the total acreage of rice fields affected with RDV in Japan. *N. cincticeps* in Kyushu has a high affinity to RDV (Shinkai, 1984).

RSV causes the most severe damage to rice throughout Japan. It occurred in Hokkaido, the northernmost island having long snowy winter, and in Kyushu, south-western Japan which is characterized by a short winter season. RSV also occurred severely in the Kanto-Tozan and Kinki-Chugoku districts in central Japan. The outbreak of the disease highly depended upon the infective population of *L. striatellus.* In Hokkaido, virus source in the spring originates from the infective adults of the overwintering generation and those which acquired the virus through eggs. On the other hand, in Kanto or Chugoku, where RSV is prevalent, the virus is transmitted to rice plants by the adults of the first generation which increase their population on winter wheat.

RBSDV was recently recorded in Aomori and Yamagata in Tohoku on maize but not on rice. The disease on rice is mostly prevalent in Kanto and occasionally in Shikoku. The virus source in the spring in those areas originates from the infected insects that acquired the virus from the diseased weeds in early spring (Ishii and Yoshimura, 1973).

RWV was first recognized in Kyushu in 1967, and was prevalent in 1972 to 1973 in the western and southern parts of Kyushu, where the most susceptible rice cultivar, Reiho had been distributed. After cultivation of resistant cultivars and pesticide control for the green rice leafhoppers, the disease was totally eradicated. It is not clear as yet how the causal virus was introduced to Japan (Shinkai, 1979).

RTYV occurred only in Okinawa in 1977. The disase did not spread up to western Japan (Inoue, 1979).

RGSV was found in Fukuoka and Kagoshima in Kyushu in 1978. The vector, *N. lugens* a long distance flying insect from southern China or Southeast Asia, could not overwinter in these areas (Iwasaki and Shinkai, 1979). The disease has appeared all over Kyushu since 1979, Kagawa in Shikoku and Yamaguchi in Chugoku in 1980, but was not endemic.

RRSV-infected rice was first detected and Kagoshima and Nagasaki in Kyushu in 1979. Later the disease was found to be scattered over the paddy fields in Kyushu. The virus was carried by the long distance flying insect, *N. lugens* (Shinkai, 1984).

RNMV, which was first recorded in Okayama in Chugoku in 1976, is caused by a soil-borne virus transmitted by *P. graminis* (Fujii, 1978). The most endemic record was in Okayama, where about 269 ha paddy fields were infected. The damage caused by RNMV involved almost half of the average yield. The disease is usually prevalent in paddy fields after transplantation of rice seedlings grown in upland nursery beds. But it does not occur commonly when seedlings grown in flooded paddy are transplanted.

4 Virus particles

Remarkable progress in the basic research concerning the structure and properties of rice viruses has been made recently in Japan.

A revised model of RDV shows that the virion consists of 180 morphological units which are arranged in such a way that two of them are shared by each one of the neighboring holes.

A model of RRSV revealed that the virus particle is covered with 20 large spikes and has no double-shelled capsid. Therefore, it was concluded that the morphology of RRSV is distinct from that of RDV and RBSDV.

Electrophoretic analysis indicated that the genomes of RDV and RGDV consist of 12 double-stranded RNA segments, while those of RBSDV and RRSV comprise 10 segments, and that the total molecular weights of the genomes of those viruses are different.

It was thus confirmed that plant reoviruses can be divided into 3 subgroups, such as PHYTOREOVIRUS, FIJIVIRUS, AND RICE RAGGED STUNT VIRUS.

Research on in vitro transcription and hybridization has progressed using RDV and RDGV.

Protein structure of RDV, RGDV and RRSV was analysed. Each of the viruses consists of 7 polypeptides with different molecular weights.

Electrophoretic analysis of RTYV revealed that the virion consists of 4 structural proteins, G, N, NS, and M. The virus, thus, belongs to the subgroup A of plant rhabdoviruses, which corresponds to the genus Vesiculovirus including viruses with bullet-shaped particles maturing in the cytoplasm in the infected cells. In the case of TRYV, maturation site is observed in the perinuclear space. Further detailed investigations will be required to confirm these aspects.

5 Detection and control

It is known that RDV, RSV and RBSDV are highly immunogenic. In addition, antisera against RGDV, RRSV, RTYV, RGSV and RWV are now available.

Practically, latex test (for RDV, RGDV, RRSV, RGSV, RSV and RWV) has been employed for detecting the viruses.

Recently, enzyme-linked immunosorbent assay (ELISA), which has been shown to be more sensitive for detecting RSV, can also easily detect RSV and RGSV in an individual insect.

Results of immunoelectron microscopy (IEM) indicated that RSV is closely related to maize stripe virus. It was confirmed by agar gel diffusion test that RSV and RGSV are serologically related. These results made it possible to include these three viruses in a new group characterized by long thread-like particles (rice stripe virus group).

Monoclonal antibodies against RDV, RGDV and RDV-RNA have been obtained.

Control measures of rice virus diseases consist of:

1) Eradication of the vector populations to decrease the number of active transmitters.
2) Use of resistant rice cultivars.
3) Cultural practices adapted to local conditions, cultivars and vector dynamics.
4) Elimination of virus source.
5) Exclusion of weeds and crops that increase vector density.

6 Other topics

Kawano *et al.* (1984) described a virus carried by rice tarsonemid mites which inhabited the inside surface of leaf sheaths. The virus appeared in the sheath cells, adult mites and eggs.

Another topic is the isolation of two types of virus-like particles, 30nm and 32nm in diameter, from rice plants infected with downy mildew (*Sclerophthora macrospora*).

Several cell lines of *N. cincticeps* were established from embryonic tissue culture. The cultured celles have been inoculated with RDV and RGDV. These cell cultures will be very useful for further rice virus research (Kimura, 1984).

Legume viruses

There are 7 leguminous crops commonly cultivated in Japan, i.e. soybean, azuki bean, French bean, cowpea, peanut, pea and broadbean, of which, soybean is most widely cultivated throughout Japan.

1 Virus diseases of legumes in Japan

As shown in Table 2, at least 11 viruses that infect soybean have been reported, i.e. 8 viruses for French bean, 4 viruses for azuki bean, 5 viruses for cowpea, 3 viruses for peanut, 10 viruses for pea, and 9 viruses for broadbean (Yora *et al.,* 1983; Kitajima *et al.,* 1984).

Of these, soybean mosaic (SMV), soybean stunt (SSV) and soybean dwarf (SDV) viruses are the major agents of soybean diseases.

Seed-borne viruses (indicated by s in Table 2) cause serious problems for legume production.

2 Viruses and vectors

Vectors, particles and strains of the legume viruses are indicated in Table 3. Most of the legume viruses are transmitted by aphids in a non persistent manner.

3 Distribution

SMV is distributed from Honshu to Kyushu. It has also been observed in Hokkaido, but occasionally and in a limited area.

SSV is reported to occur in Honshu (Koshimizu and Iizuka, 1963; Takahashi *et al.,* 1980).

SDV was first observed in southern Hokkaido in 1952, and named in 1969. It has appeared all over Hokkaido since 1971, and has spread to northern Tohoku. A yellow strain of SDV causes yellow disease on French bean. Following the spread of SDV, the number of infected clover plants around the soybean fields has increased, which may play a role as a virus source (Tamada, 1975).

Table 2 List of legume viruses occurring in Japan

	Soybean	Azuki bean	French bean	Peanut	Cowpea	Pea	broad-bean
Alfalfa mosaic	+						
Azuki-bean mosaic		s					
Bean common mosaic		×	×s				
Bean yellow mosaic	×	×	×		×		×s
* Broadbean necrosis							×
Broadbean wilt			×		×	×	×
* Broadbean yellow ringspot							×
* Boradbean yellow vein							×
* Clover yellows						×	×
Cowpea aphid-borne mosaic		×	×s				
Cucumber mosaic			×		×	×	×
Lettuce mosaic						×	
* Milk-vetch dwarf	×		×		×	×	×
Pea seed-borne mosaic						×s	×
* Pea stem necrosis						×	
Peanut mottle					×s	×s	
Peanut stunt	×s		×	×			
Southern bean mosaic	×s						
* Soybean chlorotic mottle	×						
* Soybean dwarf	×		×				
* Soybean mild mosaic	×s						
Soybean mosaic	×s						
* Soybean stunt	×s						
Tobacco rattle	×						
Turnip mosaic				×			
Watermelon mosaic			×			×	
White clover mosaic						×	

* specific to Japan
s seed-borne

In Japan, several strains of bean yellow mosaic virus (BYMV) were identified, including necrotic strains (Inouye,T., 1968). Damage caused by a necrotic strain of BYMV to French beans was reported in Kyushu in 1978, and later the occurrence of top necrosis disease caused by necrotic strains of BYMV was frequently observed in Hokkaido.

The legume viruses specific to Japan are marked by * (asterisk) in Table 2. Ten viruses among the 27 listed include broadbean necrosis, broadbean yellow ringspot, broadbean yellow vein, clover yellows, milk-vetch dwarf, pea stem necrosis, soybean chlorotic mottle, soybean mottle, soybean dwarf and soybean stunt viruses.

So far, no precise and conclusive survey on the distribution and damage caused to legumes by these viruses throughout Japan is available.

It is considered that SMV is the most widely distributed virus throughout Japan, and perhaps azuki bean mosaic virus and BYMV are also common in Japan.

But, occasionally, in certain localities where specific leguminous crops are cultivated, severe damage by the corresponding legume viruses has been recorded.

4 Detection and control

Since the first report on the application of immunoelectron microscopy (IEM) for detecting legume viruses in 1978, this method has been employed for routine detection and identification

Table 3 Vectors, particles, groups, and strains of legume viruses

Virus	Vector	Particle	Group	Strain
Alfalfa mosaic	aphids	56, 43, 35, 30×16	alfalfa mosaic virus	common, systemic to bean and cowpea, or to bean
Azuki-bean mosaic	aphids	750	poty	
Bean common mosaic	aphids	705×13	poty	common, S(soybean)
Bean yellow mosaic	aphids	705×13	poty	N(necrotic), B(broad bean), P(pea)
Broadbean necrosis	fungus	150,250×25	tobamo	
Broadbean wilt	aphids	25		unknown in Japan
Broadbean yellow ringspot	unknown	28		
Broadbean yellow vein	unknown	240×120	rhabdo	
Clover yellows	*Aphis craccivora* (semi-persistent)	1700×12	clostero	
Cowpea aphid-borne mosaic	aphids	750	poty	several
Cucumber mosaic	aphids	28—30	cucumo	many, legume isolate
Lettuce mosaic	aphids	750×13	poty	
Milk-vetch dwarf	aphids (persistent)	26	luteo	
Pea seed-borne mosaic	aphids	750×13	poty	unknown in Japan
Pea stem necrosis	*Olpidium*	34		
Peanut mottle	aphids	750	poty	unknown in Japan
Peanut stunt	aphids	30	cucumo	J strain
Southern bean mosaic	*Ceratoma trifurcata*	28—30	sobemo	cowpea, Mexican
Soybean chlorotic mottle	sap (not by aphids)	50	caulimo?	
Soybean dwarf	*Acyrthosiphon solani* (persistent)	25	luteo	dwarf, yellow
Soybean mild mosaic	aphids	26—27		
Soybean mosaic	aphids	750	poty	several
Soybean stunt	aphids	25—28	cucumo	4 strains
Tobacco rattle	*Paratrichodorus minor*	50—80, 180×22	tobra	M. NM
Turnip mosaic	aphids	750×13	poty	several
Watermelon mosaic	aphids	750×13	poty	strains 1,2
White clover mosaic	aphids	480×13	potex	unknown in Japan

procedures in Japan. IEM is extremely useful for differentiating mixed virus infection in crops as well as viruses with the same size and shape such as BYMV and bean common mosaic virus (BCMV). It is applicable to a small quantity of leaf materials. Detection of BCMV in bean seeds by IEM was found to be 100 to 1,000 times more sensitive than biological tests (Hagita and Tamada, 1984). The virus was detected in a mixture of 0.1% (w/w) of the infected seeds.

ELISA (direct, double sandwich) was first applied for detecting BYMV in 1980 and was found to be useful for virus detection. Since then, this assay technique has been widely and is now routinely applied to legume virus detection as a reliable and sensitive method.

A characteristic reaction in ELISA, which is useful for differentiating strains of the viruses, created problems for practical use of this assay. The application of indirect ELISA enables to overcome this shortcoming.

Control measures for legume viruses are as follows:

1) Chemical control of insect vectors, including application of insecticides to soil.
2) Use of resistant varieties.
3) Production of virus-free seeds for seed-borne viruses.
4) Early or late cultivation of legumes to avoid infection.
5) Eradication of weeds as virus reservoir (which is practically difficult to achieve).

Conclusion

Even though progress has been made in basic studies and ecological investigations, viruses of rice and legumes still pose a major threat for the production of these crops.

During the past 10 years, more than 10 new legume viruses have been identified in Japan. Some of them caused serious problems and yield losses on leguminous crops in certain localities.

It is highly regrettable that stripe disease of rice has been occurring in Hokkaido for the past 15 years, where no rice virus had been recorded during the past 100 years since rice cultivation started in this district.

The fact that several so-called "tropical rice viruses" have landed in the south-western part of Japan during the past 10 years, apparently indicates the close relationship of virus spread among the countries of Asia.

In this regard, emphasis should be placed on studies of virus diseases and their distribution as well as ecological aspects of vector insects in each country, including mainland China, which encompasses tropical, subtropical and temperate zones.

We may also anticipate a new approach for viral control as a result of recent advances in molecular biology of viral genomes in the near future. Accordingly, international collaborative studies and investigations are being increasingly required to overcome virus problems.

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Rice viruses

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Discussion

Disthaporn, S. (Thailand): In your report you mentioned that tarsonemid mites inhabiting rice leaf sheaths carried a virus. We observed that when ragged stunt occurred severely, rice often showed secondary infection with sheath rot which is a fungal disease transmitted by the tarsonemid mite *Steneotarsonemus spinki* (conidial transmission). I would like to know whether it is possible to demonstrate that the mite carries virus particles in the case of rice ragged stunt.

Answer: The mite virus was isolated from rice plants infected with RRSV, RDV and RBSDV in the greenhouse. It was not possible to detect the virus in the sheath rot fungus. No positive results were obtained when the virus was inoculated through the mite. Fungus spores are carried by the mite but mite cultivation was not possible. The virus identified in the mite is different from tungro virus.

Mochida, O. (IRRI): With regard to rice necrosis mosaic virus, does it occur in the tropics and how can you prevent the disease?

Answer: I do not know whether this virus occurs in the tropics. The incidence of the disease was lower when the soil moisture decreased. The disease was not commonly observed when the seedlings grown in flooded paddy where transplanted.

Dollet, M. (CIRAD)*: Did you characterize the necrosis mosaic virus transmitted through *Polymyxa graminis?* Are there any serological relationships with other viruses transmitted by the same fungus?

Answer: Saito, Y. (FFTC): There are serological relationships among wheat yellow mosaic virus, barley yellow mosaic virus, wheat spindle streak virus and rice necrosis mosaic virus.

Abu Kassim, A.B. (Malaysia): Is it possible to differentiate serologically bean yellow mosaic virus from bean common mosaic virus?

Answer: Yes it is possible to differentiate the two viruses by immunoelectron microscopy.

Anjaneyulu, A. (India): I believe that rice necrosis mosaic virus has been reported in India.

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