TRANSMISSION AND SOME PROPERTIES OF RICE GALL DWARF VIRUS

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ABSTRACT

Rice gall dwarf disease was observed for the first time in 1979 in Thailand. The symptoms consisted of stunting of rice plants, galls along veins of leaves and sheaths, reduced number of tillers, and retardation of plant growth. The disease which was transmitted by *Recilia dorsalis, Nephotettix nigropictus, Nephotettix cincticeps, Nephotettix malayanus* and *Nephotettix virescens* in a persistent manner, was also transmitted through the eggs of *N. nigropictus*. Incubation period of the virus in *R. dorsalis* and *N. nigropictus* was about one week in Thailand and that in *N. nigropictus* was about 2 weeks at 25°C in Japan. Transmission efficiency showed marked differences among insect vector species and colonies. Of the plants inoculated, the following were infected and back-inoculated to rice: *Oryza rufipogon, Hordeum vulgare, Triticum aestivum, Secale cereale, Avena sativa, Lolium multiflorum,* and *Alopecurus aequalis* var. *amurensis*. No seed transmission through rice plants was detected. Double-shelled polyhedral particles about 65 nm in diameter were purified from infected rice plants and the purified particles were highly infectious to rice seedlings inoculated via insect nymphs. No specific reaction was detected between the new virus and antisera against the rive dwarf and wound tumor viruses that belong to the phytoreovirus group.

Introduction

Rice gall dwarf disease was observed for the first time in Thailand by Omura and others in 1979 under the collaborative research project between the Department of Agriculture, Thailand and the Tropical Agriculture Research Center, Japan. Research on the disease was conducted both in Thailand and Japan and the major properties of the virus were determined several years after the disease was observed. This paper describes the symptoms on plants as well as host range, transmission mode, purification and serological detection of the virus.

Distribution and incidence

Incidence of rice gall dwarf disease has been reported in Thailand (Omura *et al.*, 1980; Putta *et al.*, 1980), in Peninsular Malaysia (Ong and Omura, 1982) and in China (Faan *et al.*, 1983). The affected area in Thailand and in Malaysia was not surveyed. According to Faan *et al.*, (1983), rice gall dwarf disease occurred in the western part of Guangdong Province in South China and the affected area covered 110,000 mu (about 7,000 ha) in 1981 and 500,000 mu (about 33,000 ha) in 1982. The yield loss amounted to 2,400–3,700 kg/ha in the moderately affected rice fields and 4,500 kg/ha in the most seriously affected fields.

Symptoms and host range

1 Symptoms

Infected plants are stunted and tillers are decreased. Pale green to translucent white galls are observed on the abaxial surface of the leaf blades and sheaths. The leaves are dark green as compared with healthy ones and are sometimes slightly twisted at the tip. Stunting is caused by the shortening of both leaf blades and sheaths as well as by the incomplete emergence of the

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young leaves. Stunting is conspicuous and galls abundantly appear when the seedlings are infected at an early stage of growth. The size of the galls or vein swellings ranges from 0.4 to 8 mm in length, being mostly less than 2 mm, and from 0.4 to 0.5 mm in width. Only a small number of galls appear on the leaves when rice plants are infected at the late growth stage. It is often difficult to observe galls but they can be felt as a rough zone when the leaf blades are touched with fingers from the base to the tip. Heading of the infected plants is delayed due to the retardation of growth and the plants bear small panicles.

2 Host range

Seven plant species out of 11 tested were found to be affected by this disease and back-inoculated by insect vectors to rice plants. Wild rice (*Oryza rufipogon*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), rye (*Secale cereale*), oat (*Avena sativa*), Italian ryegrass (*Lolium multiflorum*), and water foxtail (*Alopecurus aequalis* var. *amurensis*) showed typical symptoms and polyhedral particles about 65 nm in diameter were observed in negatively stained preparations.

Transmission

1 Transmission mode

Transmission mode was tested in *Recilia dorsalis, Nephotettix nigropictus* and *N. virescens*. Nymphs of *R. dorsalis* and *N. nigropictus* were allowed to feed on diseased rice plants for 1 day and they were tested for their transmission ability by daily transfers on healthy plants. Nymphs of *N. virescens* fed on the diseased plants for 1, 3 and 6 days. After being reared for 10 to 13 days on healthy rice seedlings for incubation, each insect was tested for its transmission ability by 5 to 8 transfers at 2-day intervals on test plants. The insects tested transmitted the virus in a persistent manner, and most individuals transmitted it intermittently. The incubation period was 7.9 days for *R. dorsalis* and 7.6 days for *N. nigropictus* on the average. Three out of 100 individuals of *N. virescens* transmitted the virus with a 6-day acquisition access.

2 Acquisition feeding period

The second instar nymphs of *R. dorsalis* were allowed feeding access on the diseased rice plants for 1, 2, 4, 8, 16, 24, and 48 hr. After the acquisition access, the insects were reared on healthy rice seedlings for 10 days and each insect was tested for its transmission ability by serial inoculation. The insects did not transmit the virus within 4 hr of shorter acquisition feeding period, but 3 individuals out of 50 transmitted the virus within 8 hr feeding. Transmission was more efficient with a longer acquisition period.

3 Inoculation feeding period

After a 5-day acquisition access on the diseased rice plants by the 2nd instar nymphs of *R. dorsalis*, the insects were reared on healthy rice seedlings for 10 days. Each insect was then allowed an inoculation feeding period of 1, 2, 4, 8, 16, 24, and 48 hr on individual test plants. Three individuals out of 38 transmitted the virus within 1 hr inoculation access period.

4 Effect of instar stage of nymphs on transmission

Nymphs of *R. dorsalis* at the 1st-2nd, the 3rd-4th, the 5th instar stages and adults were allowed acquisition feeding access on the diseased plants for 1 day and their transmission efficiency was tested. Young nymphs acquired the virus more efficiently than the older ones. Previously mentioned results are summarized in Table 1.

Table 1 Interaction of rice gall dwarf virus and insect vectors in Thailand

	R. dorsalis	*N. nigropictus*	*N. virescens*
Transmission mode	Persistent	Persistent	Persistent
Transmission efficiency	11.2% (11/94)	9.2% (13/120)	3.0% (3/100)
Incubation period in insect	5-11 (7.9) days	4-12 (7.6) days	13 days
Shortest acquisition feeding period	8 hr		
Shortest inoculation feeding period	1 hr		
Acquisition efficiency in nymphs	2nd instar (highest)		
Transstadial passage	yes	yes	
Seed transmission in rice		no (0/1461)	

(Data from Morinaka *et al.*, 1982)

5 Transovarial transmission

Inoue and Omura (1982) demonstrated that the virus was transmitted to the next generation through eggs in *N. nigropictus*. Eggs oviposited by the infective female were removed from rice plant tissues before being hatched and the transmission ability of the second generation of individuals was tested when they were in the 5th instar or young adult stage. Transmission of the virus through the eggs was tested on 3 successive generations on the colony derived from Amami. As shown in Table 2, in some broods the virus was transmitted to the 3rd generation through eggs, but the number of infective broods and percentage of infective individuals decreased from generation to generation.

Table 2 Transovarial transmission of RGDV over 3 successive generations in *Nephotettix nigropictus* (Amami colony)

	1st generation		2nd generation		3rd generation	
No. brood	Number of test progenies	Percentage of infective individuals	Number of test progenies	Percentage of infective individuals	Number of test progenies	Percentage of infective individuals
1	31	87	39	13	42	2
2	9	67	36	11	40	0
3	16	44	40	3	40	0
4	29	38	37	8	40	0
5	14	29	37	3	—	—
6	26	27	38	3	40	0
7	12	25	40	0	40	0
8	32	6	40	0	40	0
Average	—	39.6	—	4.9	—	0.4

(Data from Inoue and Omura, 1982)

6 Vector species range and transmission efficiency

Difference of transmission ability in 7 leafhopper and planthopper species was tested by Inoue and Omura (1982). The insects were fed on the diseased source plants for an acquisition access of 2 to 15 days and were allowed to feed individually for an inoculation access on the rice seedlings in test tubes. The insects were transferred serially to healthy test seedlings at 2-day intervals during their life span. *N. nigropictus, N. cincticeps, N. virescens, N. malayanus* and *R. dorsalis* were able to transmit the virus. No transmission was obtained in either male and female of *Nilaparvata lugens* and *Laodelphax striatellus*. The average incubation periods in the insects tested were about 2 weeks in *N. nigropictus, N. cincticeps* and *N. malayanus* and in *N. virescens*

and *R. dorsalis* the incubation period was 2-5 days longer.

Difference of transmission efficiency in *N. nigropictus, N. cincticeps* and *N. virescens* was also tested by Inoue and Omura (1982). In this test, a total of 10 colonies in 3 *Nephotettix* species from different origins were compared. As shown in Table 3, there was a considerable variation in the rate of infective individuals among the colonies within the species of *N. nigropictus* and *N. cincticeps*. There was no essential difference in the infective efficiency between 2 *N. virescens* colonies.

Table 3 Variation in transmission efficiency of rice gall dwarf virus in different colonies of 3 *Nephotettix* species

Species	Site of collection	Number of insects tested	Virus acquisition access (days)	Percentage of infective individuals
N. nigropictus	Kagoshima	40	9	47.5
	Amami	40	9	95.0
	Thailand (1)	38	3	7.9
	Thailand (2)	57	4	1.8
	Thailand (3)	20	12	10.0
N. cincticeps	Joetsu	51	4	33.3
	Chikugo	42	4	42.7
	Amami	69	4	1.4
N. virescens	Kagoshima	70	4	0.1
	Thailand	231	4	0.1

(Data from Inoue and Omura, 1982)

Purification and preparation of antiserum

1 Particles in the tissues

Polyhedral particles about 65 nm in diameter were always observed in dip preparations of leaves and roots of diseased plants. In the ultrathin sections of virus-infected plants, polyhedral particles about 65 nm in diameter were frequently observed, and no such particles were detected in healthy rice plants.

2 Detection of causal agent

In order to detect the causal agent of the disease, negatively stained specimens of viruliferous insects and diseased plants were compared with those of the virus-free insects and healthy rice plants. Many large polyhedral particles about 65 nm in diameter were detected only in the diseased plants and in the viruliferous insects. Small polyhedral particles about 35 nm in diameter were detected in both viruliferous and non viruliferous insects. These particles were not observed in healthy or diseased plants (Table 4).

3 Purification

The polyhedral particles about 65 nm in diameter which were observed only in the diseased plants and viruliferous insects were purified and considered as the causal agent of the disease. The method of purification for rice dwarf virus developed by Kimura (1976) was mainly employed for rice gall dwarf virus with a few modifications to avoid significant particle loss.

To ascertain that the purified particles were the causal agent of the disease, the purified virus preparation was injected into 3rd to 5th instar nymphs of *N. nigropictus*, and the insects were reared on healthy rice seedlings. After 12 days, individual nymphs were transferred to test seedlings in test tubes and they fed for 3 days. As shown in Table 5, 57-85% of the insects were

infective and infected rice seedlings showed typical symptoms of rice gall dwarf disease. The 65 nm particles were observed in negatively stained specimens from these diseased plants. These results demonstrate that the purified particles are the causal agent of rice gall dwarf disease.

Table 4 Transmission of particles by a colony of *Nephotettix nigropictus* viruliferous for rice gall dwarf virus

Insect colonies	Sex	Number of insects	Small[a] particle carriers	Large[b] particle carriers	Active transmitters
Viruliferous	female	19	29	12	9
	male	21	21	12	11
Virus-free	female	10	10	0	0
	male	10	10	0	0

a) Polyhedral particles about 35 nm in diameter
b) Polyhedral particles about 65 nm in diameter
(Data from Omura *et al.*, 1982)

Table 5 Infectivity test of purified rice gall dwarf virus

Experiment	Number of insects			Percentage of transmission
	Injected	Survived for 12 days	Transmitted	
I	50	7	4	57
II	50	32	26	81
III	50	13	11	85
Check[a]	120	21	0	0

a) Buffer-infected control
(Data from Omura *et al.*, 1982)

4 Preparation of antiserum

The antiserum was prepared by injection of purified rice gall dwarf virus to a rabbit. The serological relation between the particles in the insect vectors and in the diseased plants was analysed by the clumping technique. Aggregations of polyhedral particles about 65 nm in diameter were frequently observed in a mixture of viruliferous insects and the antiserum. However, no such aggregation was observed in mixtures of virus-free insects and the antiserum. Thus, the 65 nm particles in the plants and insects were shown to be serologically related.

No specific reaction was observed between the purified rice gall dwarf virus and antisera against the rice dwarf and wound tumor viruses that belong to the phytoreovirus group.

Omura *et al.*, (1984) showed that the latex flocculation test was useful for detecting virus antigens in rice plants and in insect vectors. This technique can be used as an excellent and convenient method of diagnosis for rice gall dwarf virus.

Control

Varietal differences in the resistance to rive gall dwarf disease have not been tested. Faan *et al.*, (1983) reported that hybrid rice varieties, Shan You No.1, Shan You No.2, Shan You No.6. Shan You No.30, and Shan You No.36 were seriously damaged and ordinary rice varieties, Bao Xuan Er and Gui Chao were also highly susceptible in natural infection, but no information on

resistant varieties is available. At present, the use of resistant varieties can not be applied as a method of control.

It appears that insecticides can be used for insect vectors as well as for rice virus diseases transmitted by leafhoppers or planthoppers.

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Kishimoto, R. (Japan): 1. Is the virus still spreading? Indeed it may become established in Japan as the vector population is present in Japan. 2. The rate of transovarial passage was found to decrease drastically from generation to generation. Did you collect infective females in each generation? In the case of stripe, the percentage of transovarial transmission decreased slowly from generation to generation.

Answer: 1. The incidence of the disease has decreased remarkably in Thailand. The disease has also been reported in China. 2. We obtained the progenies from viruliferous females in each generation.

Makkouk, K.M. (ICARDA): Is the virus consisting of small polyhedral particles which was detected in non viruliferous insects an insect virus?

Answer: Yes it is. This virus is transmitted transovarially in non infectious insects.

Mochida, O. (IRRI): You mentioned that rice gall dwarf disease occurs in Thailand, China and Peninsular Malaysia. In your transmission studies you used *Nephottettix cincticeps* which gives a high rate of transmission in Japan. It would be advisable to use vectors occurring locally such as *N. virescens, N. nigropictus* or *N. malayanus* for such studies.