Some Physical Properties of Rice Ragged Stunt Virus

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仙北俊弘*・周 廷光*・四方英四郎* : イネ ragged stunt ウイルスの物理的諸性質

Rice ragged stunt disease was first reported in Indonesia and the Philippines. Hibino *et al.*¹⁾, Ling²⁾, and Ling *et al.*³⁾ described the characteristic symptoms on rice and the transmission mode by brown planthopper (*Nilaparvata lugens* Stål). Electron microscopy of purified and dip preparations revealed isometric particles of 50-70 nm in diameter⁴⁾. Recently, success of artificial injection of the virus into the insects has been reported⁵⁾. This paper reports some physical properties of rice ragged stunt virus (RRSV) using insect injection method.

Virus-free brown planthoppers used in this experiment, that were collected in Japan, were offered by Dr. Kishimoto, Central Agricultural Experiment Station (Konosu, Saitama, Japan) and maintained over 5 years in the greenhouse of the Department of Botany, Faculty of Agriculture, Hokkaido University, Sapporo, Japan. Originally, the diseased rice plants (IR 3839-1) were offered by Dr. K. C. Ling, International Rice Research Institute (Philippines), and the virus maintained on diseased *japonica* rice plants (Mihonishiki) inoculated by viruliferous brown planthoppers which had fed on a disease rice plant (IR 3839-1). Extracts from the diseased rice plants grown for 1-2 months after inoculation, and from the viruliferous brown planthoppers confined for 2 weeks after 2 days acquisition feeding were used for injection. These materials were ground in 0.1 M ammonium acetate buffer (pH 7.0) in most case, and centrifuged at 3,000 rpm for 10 min. The resultant supernatants were injected into insects for infectivity assay. The injected insects were fed on healthy rice plants for 11 days, and then transferred individually to healthy young rice seedlings in test tubes for inoculation. After 3 days, they were transferred to another test plants for 3 days.

Table 1. Effect of buffers on RRSV in crude sap

Buffers(pH 7.0)	No. of insects injected	No. of insects survived for 11 days	No. of insects infected	(%)
0.1M phosphate	40	12	10	(83)
0.2M phosphate	40	12	9	(75)
0.1M ammonium acetate	40	15	12	(80)
0.05M Tris	40	15	13	(87)
Distilled water	40	13	7	(54)

The inoculated plants were transplanted to soil in the temperature controlled greenhouse at 25 C.

Effect of Buffers. Four kinds of buffer solutions at pH 7.0, such as 0.1M and 0.2M phosphate buffers, 0.1M ammonium acetate buffer, and 0.05M Tris buffer were used. Distilled water was used as

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Table 2. Longevity *in vitro* of RRSV at 4C

Days	No. of insects injected	No. of insects survived for 11 days	No. of insects infected	(%)
(control)	60	23	15	(65)
1	60	13	5	(39)
2	60	19	13	(68)
3	60	36	25	(69)
4	60	16	4	(25)
5	60	18	3	(17)
6	60	28	3	(11)
7	60	17	1	(6)

control. Diseased leaves were ground in 2 volumes (v/w) of each buffer and the extracts were injected into insects. As shown in Table 1, the infectivity of RRSV in the crude sap expressed in these buffers at pH 7.0 did not differ markedly.

Longevity *in vitro*. In order to know the longevity of the virus in crude sap, the diseased leaves were macerated in 2 volumes (v/w) of 0.1M ammonium acetate buffer. The extract was stored at 4 C and injected into healthy insects every

Table 3. Dilution end point of RRSV

Virus source	Dilution	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸
Planthoppers	No. of insects injected	—	—	—	50	50	50	50	50
	No. of insects survived for 11 days	—	—	—	21	25	25	28	26
	No. of insects infected	—	—	—	6	2	3	0	0
	(%)				(29)	(8)	(12)		
plants	No. of insects injected	60	60	50	50	50	50	50	—
	No. of insects survived for 11 days	19	13	20	25	20	20	14	—
	No. of insects infected	15	11	8	7	2	0	0	—
	(%)	(79)	(85)	(40)	(28)	(10)			

Table 4. Effect of heat on RRSV

Temperature (C)	(Control)	40	50	60	70	80	90
No. of insects injected	50	50	50	50	50	50	50
No. of insects survived for 11 days	15	19	28	19	28	21	23
No. of insects infected	9	14	16	0	0	0	0
(%)	(60)	(73)	(57)				

24 hrs.. As shown in Table 2, RRSV in the crude sap remains infective even after 7 days at 4 C.

Effect of Dilution. Diseased rice leaves and brown planthoppers fed on diseased rice plants for 2 weeks were used for the experiments. The diseased leaves were ground in 10 volumes (v/w) of 0.1M ammonium acetate buffer, and then diluted. The exposed insects (about 30 % of the exposed brown planthoppers were the active transmitters), approximately 0.2g of 110 insects, were macerated in 20ml of 0.1M ammonium acetate buffer, and then diluted. Each viral preparation was injected into healthy insects. The results shown in Table 3 indicated that dilution end point of RRSV in extracts from diseased rice leaves was 10⁻⁵, while that from viruliferous insects was 10⁻⁶.

Effect of Heat. The extracts from the diseased plants were diluted in 2 volumes (v/w) of 0.1M ammonium acetate buffer, and were filled in glass tubes (about 10 ×

Table 5. Effect of freezing and thawing on RRSV

Freezing and thawing	No. of insects injected	No. of insects survived for 11 days	No. of insects infected	(%)
1st	50	26	23	(89)
2nd	50	30	21	(70)
3rd	50	30	22	(73)

Table 6. Effect of hydrogen-ion concentration on RRSV

pH	No. of insects injected	No. of insects survived for 11 days	No. of insects infected	(%)
5	50	15	7	(47)
6	50	11	9	(82)
7	50	18	12	(67)
8	50	10	8	(80)
9	50	15	11	(73)

the preparations were injected into virus-free insects. As shown in Table 5, the infectivity was not markedly changed after three times of freezing and thawing.

Effect of Hydrogen-ion Concentration. The diseased leaves were ground in 0.1M ammonium acetate buffer at different hydrogen-ion concentration from pH 5 to 9. Each extract was then injected into virus-free insects. The result shown in Table 6 indicated that the virus was stable at pH 6 to 9, but slightly decreased its infectivity at pH 5.

Previous studies on RRSV indicated that the insect vector is capable of acquiring the virus by artificial injection⁴⁾. Therefore, it became possible to work further studies on the properties of RRSV by this method. The results obtained in this experiment represent that RRSV is seemingly stable in the buffer solutions at different pH values ranging between 5-9, at relatively low temperature (4 C), and repeated freezing and thawing. Infectivity of the extracts from the viruliferous insects is 10 times higher than that of the extracts from the diseased plants. Thermal inactivation of the virus at 60 C indicates that the virus is not highly resistant to heat.

Shikata *et al*⁶⁾, according to its transmission mode and morphology of the virions, pointed out that RRSV most likely belonged to planthopper borne sub group of plant reovirus group. Properties of RRSV obtained in this experiments almost agree with those of rice black-streaked dwarf virus, which is one of the members of the subgroup.

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0.3cm) plugged at both end with rubber stoppers. The tubes were heated at 40, 50, 60, 70, 80, and 90 C for 10 minutes in hot water, and then rapidly cooled in running tap water. Each fluid was injected into healthy nymphs. In Table 4, the infectivity of RRSV in crude extracts was lost at 60 C, but remains infective at 50 C.

Effect of Freezing and Thawing. The extract from the diseased leaves in 2 volumes (v/w) of 0.1M ammonium acetate buffer, was frozen at -30 C, and then thawed at room temperature. Freezing and thawing of the crude extract was repeated 3 times, and

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