Inheritance of Resistance of Rice to Tungro and Biotype Selection of Green Leafhopper in Malaysia

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Introduction

Tungro which is called Penyakit Merah Virus (PMV) in Malaysia, is a serious virus disease of rice in the Philippines, Thailand, India, Indonesia and Malaysia. Symptoms of this disease have been recognized since 1934 in Malaysia by the name 'Penyakit Merah,' but the viral nature was only demonstrated in 1965. The infected plants show very low grain yields and poor grain quality. The disease is transmitted mainly by green leafhopper (GLH), *Nephotettix virescens*.

In the past, serious outbreaks of PMV in Malaysia were confined to Krian area. However in 1980 off season a serious PMV outbreak was found in Province Wellesley and since then it spread to Central Kedah.

Studies on rice breeding for PMV resistance were carried out from August 1980 to July 1982 in MARDI, Bumbong Lima, Malaysia, under a cooperative research program between MARDI and TARC and details of the results are to be published in the near future. A part of the results will be presented briefly in this paper.

Inheritance of GLH and PMV resistance

1) Materials and method

F_3 and B_1F_2 lines of cross combination of IR42 (resistant to GLH and PMV) and Setanjung (susceptible to both) were tested for GLH and PMV resistance with check varieties. GLH resistance of the lines was evaluated by the difference in the number of insects survived on seedlings. Ten germinated seeds of each line were sown in a row with an inter-row spacing of 2.5 cm in a tray (30×23×2 cm). When the seedlings reached the 1st leaf stage (4–5 days after sowing), 2nd–3rd instar nymphs (about 5 GLH per seedling) were spread on the seedlings in a small cage (31×25×28 cm). The number of GLH on each line was observed one day after the caging. Artificial inoculation test method was used to evaluate PMV resistance of the lines. Twenty germinated seeds of each line were sown in a tray. When the seedlings reached the 1st leaf stage (4–5 days after sowing) they were inoculated by viruliferous GLH in a small cage. The viruliferous insects were prepared by caging adults of GLH with PMV-infected plants in a small cage (31×25×28 cm). This acquisition feeding lasted for one day just before the inoculation. The number of PMV-infected plants was counted about one month after the inoculation.

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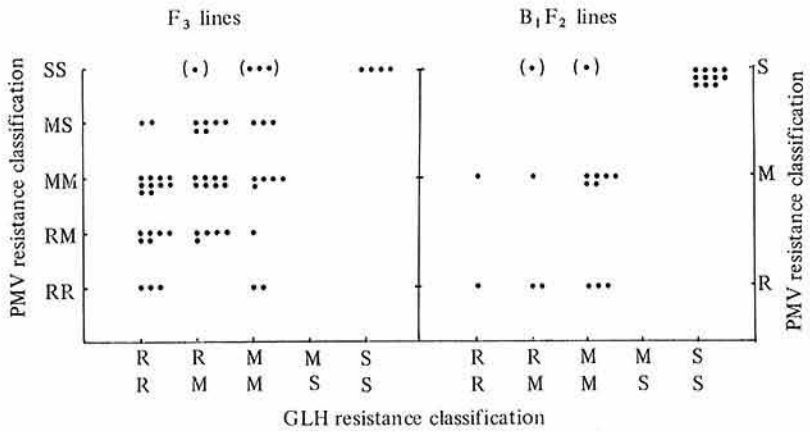

Fig. 1. Relationship between GLH resistance and PMV resistance

Table 1. R~M : S segregation ratio of GLH and PMV resistance in F_3 and B_1F_2 lines

Generation	GLH resistance			PMV resistance		
	No. of lines R~M	S	Expected ratio, χ^2, probability	No. of lines R~M	S	Expected ratio, χ^2, probability
F_3	56	4	15 : 1, $\chi^2=0.016$, $0.9<P$	52	8	15 : 1, $\chi^2=5.14$, $0.01<P<0.05$ 3 : 1, $\chi^2=4.36$, $0.01<P<0.05$
B_1F_2	16	10	3 : 1, $\chi^2=2.51$, $0.1<P<0.2$ 1 : 1, $\chi^2=1.38$, $0.2<P<0.3$	14	12	1 : 1, $\chi^2=0.16$, $0.8<P<0.9$

2) Results

The result of both tests on GLH and PMV resistance is shown in Fig. 1. The data of PMV resistance of B_1F_2 lines were taken from Rep. 1 only. Grading of R, M and S in GLH resistance was done by visual observation of insect number without counting.

The grading of PMV resistance was made as follows: data (no. of PMV-infected plants) of the susceptible check variety (SMII) minus that of the resistant check variety (IR42) was calculated, and then divided by 6 to give a "one-sixth". Lines showing the data less than (that of IR42+"one-sixth") were referred as resistant (R), those more than (data of SMII−"one sixth") as susceptible (S), and the others as medium (M). There was a clear distinction between R-M and S in the GLH resistance but not so in PMV resistance (Fig. 1).

Table 1 shows the number of R-M and S lines, expected ratio, χ^2 value and probability in both GLH and PMV test. It is concluded that the GLH resistance in IR42 is controlled by two dominant genes, because R-M:S ratio in F_3 and B_2 F_1 fit the ratio 15:1 and 3:1 respectively.

With regard to the PMV resistance, however, R-M:S ratio in F_3 does not fit either to 15:1 or 3:1, and in B_1F_2 the ratio is close to 1:1 ratio. In Fig. 1 it can be seen that all susceptible (S) lines to GLH are also susceptible (S) to PMV, but few lines among resistant lines (R-M) to GLH are susceptible (S) to PMV. This is probably caused by a high inoculation intensity. If inoculation intensity is less, only the GLH (S) lines will be shown to be susceptible to PMV, so that PMV resistance in IR42 is considered to be controlled by the same two genes as the ones

for GLH resistance.

3) Discussion

The inheritance of GLH resistance was first investigated by Athwal et al.[1] in varieties, Pankhari 203, ASD7 and IR8, and their genes were designated Glh 1, Glh 2 and Glh 3 respectively, segregating independently each other. Other varieties were investigated by Siwi and Khush[14]. One single recessive gene in Ptb8 was designated Glh 4, which is independent of Glh 1, Glh 2 and Glh 3. A dominant resistant gene of ASD8 was designated Glh 5.

On the other hand, no thorough analysis of inheritance of PMV resistance has been carried out mainly because of not small variation in varietal reaction. One study at IRRI[3] indicated that resistance in Pankhari 203 is governed by two complementary dominant genes. According to Shastry et al.[13] the resistance in Latisail is under a duplicate gene control. Seetharaman et al.[12] reported that the resistance of Pankhari 203 is controlled by two complementary dominant genes and that in Kataribhog and Kamod 253 by one dominant gene with interference of one inhibitor. However, their genetic studies were conducted by analyzing reaction of individual plants. It should be analyzed by using data of lines because some infected plants are usually seen in a resistant variety.

In the present study, GLH resistance and PMV resistance were tested on F_3 and B_1F_2 lines. The inheritance of GLH resistance in IR42 was confirmed to be controlled by two dominant genes. Comparing GLH and PMV resistance in the same lines, PMV resistance in IR42 was considered to be controlled by the same two genes as GLH resistance.

Such an inheritance of resistance to vector and virus is similar to the relationship of inheritance found by Kobayashi[5] between resistance to green rice leafhopper (GRLH) *N. cineticeps*, and to rice dwarf virus disease.

Selection of GLH biotypes in Malaysia and comparison of varietal reactions to PMV and GLH observed between MARDI and IRRI

1) Materials and method

For selecting biotypes, GLH caught in Penang and Kelantan State were bred on a resistant variety IR42. Selection process of GLH biotypes is shown in Table 2. More than thousand young nymphs of Penang and Kelantan GLH were reared on IR42. About one hundred nymphs could grow up to nearly 5 instar nymphs and then they were transferred to susceptible variety Anak Dara or Seribu Gantang for their multiplication. Rearing on IR42 and susceptible variety was repeated several times.

The 4 selections of GLH obtained: selection I (5 times reared on IR42), selection II (4 times), selection III (5 times) and selection IV (4 times) were compared in surviving rate tests with ordinary Penang GLH used as control on two varieties, IR42 and Seribu Gantang. In these tests one leaf, second from the newest leaf, was cut and 10 cm of the basal part was used. Ten GLH nymphs were caged with the cut leaf in a test tube covered by net. The number of surviving GLH was counted daily after caging.

To compare the varietal reactions to PMV and GLH between MARDI and IRRI, 10 varieties included in the IRRI's collaborative study with some tropical countries were used. They were tested for resistance to PMV and GLH in MARDI, Bumbong Lima, Malaysia. Test methods of PMV and GLH resistance were the same as mentioned above.

2) Results

After 4–5 times rearing on the resistant variety IR42, it was found that GLH could survive and multiply on IR42. The result of surviving rate tests of the 4 selections of GLH and ordinary Penang GLH on the two varieties is shown in Table 3. Seribu Gantang,

Table 2. Selection process of GLH biotype by caging on a resistant variety IR42

Caging period	Growing & oviposition of GLH	4 GLH Selections			
		I Bum.	II Bum.	III Kel.	IV Kel.
1981					
Oct. 10 — Nov. 2	2nd i.n. — 5th i.n.			①	①
Oct. 24 — Nov. 20	2nd i.n. — 5th i.n.	①	①		
Nov. 2 — Dec. 9	5th i.n. — adult. ovipo. — adult			1—2	1—2
Nov. 20 — Dec. 9	5th i.n. — adult	1	1		
Dec. 9 — Jan. 4	ovipo. — hatching — 4th i.n.	②	②	③	③
1982					
Jan. 4 — Jan. 23	4th i.n. — adult	2	2	3	3
Jan. 23 — Feb. 25	ovipo. — hatching — 5th i.n.	③	3	④	4
Feb. 25 — Mar. 20	5th i.n. — adult. ovipo. — 4th i.n.	3—4	3—4	4—5	4—5
Mar. 20 — Apr. 3	4th i.n. — adult	④	④	⑤	⑤
Apr. 3 —	ovipo. — hatching — 3rd i.n.	⑤	⑤	⑥	⑥

Note: Number 1, 2, 3, ... indicate the ordinal number of generation during the selection process for each of the selections. Circle indicates the rearing on IR42. Number without circle shows the rearing on a susceptible variety.
i.n.: instar nymph, ovipo.: oviposition, Bum.: from GLH collected in Bumbong Lima, Kel.: in Kelantan

Table 3. Surviving rate (%) of four selected types of GLH and ordinary GLH on two rice varieties

Variety	Selected or ordinary GLH		Exp. I			Exp. II		
			Days after caging			Days after caging		
			1	2	3	1	2	3
IR42	Ord.	Bum.	*66	*26	*18	87	*33	*27
	I	Bum.	97	80	67	95	90	74
	II	Bum.	—	—	—	89	86	79
	III	Kel	100	93	93	97	86	78
	IV	Kel.	—	—	—	90	86	83
Seribu Gantang	Ord.	Bum.	100	100	97	100	100	90
	I	Bum.	93	93	83	—	—	—
	II	Bum.	—	—	—	97	97	97
	III	Kel.	97	90	79	—	—	—
	IV	Kel.	—	—	—	100	100	93
LSD			28.3	50.6	44.6	9.2	25.7	23.9

* Shows a significance by 5%

susceptible to ordinary GLH, showed susceptibility to all 4 selections as well as ordinary GLH. However, IR42 resistant to ordinary GLH showed resistance to only ordinary Penang GLH, but susceptibility to the 4 selections. From these results it can be said that the 4 selections of GLH are different from ordinary GLH; the former and the latter belong to different biotypes. GLH used in this test were identified as *N. virescens* using description by Nasu[7].

Multiplication of these selections, however, was very low as compared with that of ordinary GLH on Seribu Gantang and Anak Dara as well as on IR42.

Table 4 shows the comparison of resistances to PMV and GLH between MARDI and IRRI. In the PMV test the data of 2 varieties were

Table 4. Comparison of varietal reactions to PMV and GLH between MARDI and IRRI

Variety	PMV resistance				GLH resistance			
	MARDI			IRRI	MARDI			IRRI
	Diseased %				Surviving %			
	Rep 1	Rep 2	Resist class	Resist class	2 days*	3 days*	Resist class	Resist class
T(N)1	90	85	S	S	100	78	S	S
IR26	67	74	MS	S	100	62	MS	S
Ambemohar 159	—	—	—	M	95	45	M	S
Habiganj DW 8	29	38	R	R	88	75	S	S
Kataribhog	—	—	—	R	100	76	S	S
Latisail	65	74	MS	S	88	75	S	S
Pankhari 203	33	56	RM	R	50	23	R	M
IR34	89	74	S	S	95	53	M	R
Gam Pai 30	45	70	M	M	76	47	M	R
Ptb 18	32	35	R	M	95	38	MR	R

* 2 or 3 days after caging (using 4–5 instar nymphs)
IRRI Resist. class according to the data in 1978 (Ling et al., 1981)

discarded because many seedlings were killed soon after inoculation. In Table 4 not much difference can be seen between MARDI and IRRI scores[4] of resistances to PMV and GLH of these varieties.

3) Discussion

Regarding tungro (PMV) strains, two strains, S and M, were reported to produce different chlorotic symptoms on two rice varieties[10]. Shastry et al.[13] reported that strains of virus collected from different parts of India produced different symptoms on several rice varieties and appeared to differ in severity. After the break down of resistance in most varieties originated from crosses with Ptb 18 as a source of resistance in the Philippines in 1979[4], IRRI started the collaborative study with some other tropical countries to determine the existence of vector biotypes and tungro strains. Ling et al.[6] reported that 10 varieties so far used in their study were unable to separate biotypes and strains. However there was slight difference in vector biotypes between AICRIP and CRRI, and the tungro strain at AICRIP might be more virulent than those at CRRI, IRRI, and LPP.

On GLH biotypes, Resaul Karim[9] reported the presence in Bangladesh of a different biotype and only 10 of the 473 varieties that showed resistance at IRRI were also resistant at BRRI while several varieties that were resistant at BRRI were susceptible at IRRI. Inheritance of GLH resistance of Ptb 18 showed a further evidence of biotype variation; Ptb 18 which showed two genes for GLH resistance at IRRI showed a single dominant gene at BRRI. They named Bangladesh biotype as Bb, and the Philippines biotype as Pb, based on their location.

For an analysis of virus strains and insect biotypes in any place, it is better to check at first biotypes of the vector. In the present study a different biotype was selected from Malaysian GLH by rearing on a resistant variety IR42. Varietal reaction to selected GLH was different from that to ordinary GLH, showing IR42 was susceptible to selected GLH. These biotypes should be named Malaysian ordinary biotype (Mb-1) and Malaysian selected biotype (Mb-2).

No clear difference in reaction of 10 varieties to both PMV and GLH was found between the above result obtained in MARDI and the result in IRRI[4]. Recently B and I particles[11] were isolated from tungro (PMV) diseased plants and it was indicated that tungro symptoms were caused by the B particles but not by the I particles. However, the presence of the I particles intensified the

symptoms, and the I particles were transmitted singly, whereas B was transmitted only when I was acquired previously or at the same time[2]. These findings suggest that virus strains as well as inheritance of PMV resistance should be studied by using each of these particles of the virus.

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