Genetic Studies on Brown Planthopper Resistance of Rice in Japan

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The brown planthopper, *Nilaparvata lugens* Stål. (abbr. BPH), is one of the most serious pest throughout tropical and temperate Asia. The development of high yielding cultivars of rice has coincided with the serious occurrence of BPH, a previously minor pest of rice in tropical Asia.[11,13]

In 1967 dozens of rice cultivars resistant to BPH were identified at IRRI. The first BPH resistant cultivar, IR 26, was released in 1973, but the breakdown of the resistance was observed in 1975 in the Philippines, Indonesia and elsewhere.

On the other hand, inheritance studies of resistance to BPH were initiated by Athwal et al. (1971).[1] They identified two genes for resistance, *Bph 1* and *bph 2*, and reported that *Bph 1* was closely linked or allelic to *bph 2*. Then, Lakshminarayana and Khush (1977)[10] identified *Bph 3* and *bph 4*, which are independent of *Bph 1* and *bph 2*, respectively.

With a view to widening the genetic base so as to enable the reliable use of BPH resistance, the identification of a large number of cultivars with BPH resistance along with that of their genotypes is desirable. Moreover, the search for new resistance genes and efforts for accumulating genes is also important for the use of resistant cultivars.

This paper reports on the screening of rice cultivars from different areas of the world and the genetic analysis of resistance to BPH in Japan.

1) *Screening of rice cultivars*

In Japan, the screening of rice cultivars was initiated by Kaneda in 1973. He modified the bulk seedling method[12] to meet the conditions of Japan and to be performed more easily and precisely.[8]

As shown in Table 1, a total of 3,287 rice cultivars excluding the breeding lines in Japan were screened during the eight year-period, 1973–1980.[9]

Almost all of the BPH resistant indigenous cultivars originated from India especially South India, and Sri Lanka. All resistant cultivars in the Far East and most of the resistant cultivars in Southeast Asia were breeding lines selected for BPH resistance. Two indigenous cultivars from Thailand and one from Burma were resistant. One each from Cambodia, Nepal and U. S. S. R. showed a comparatively low level of resistance and reacted inconsistently.

There are reports in India that several cultivars from Korea, Laos, Vietnam or U. S. A. are highly resistant to the BPH.[2,7] However, none of them were resistant in Japan, confirming that cultivars which are resistant to the BPH in Japan originate mostly from the southern part of the Indian subcontinent including Sri Lanka, and not from East Asia or other continents.

2) *Classification of the resistant cultivars by using the BPH biotypes*

Table 2 lists the selected indigenous cultivars resistant to BPH biotype I (wild type), and their reaction pattern to biotypes II and III. Most of the modern breeding lines and recommended cultivars were omitted, because their reaction to BPH biotypes could only be estimated from their parentage.

The infestation ability of our biotypes can be summarized as follows: biotype II cannot infest cultivars with resistance genes other than *Bph 1*. Biotype III infests cultivars with the gene *bph 2*

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except for a few of them. PTB 18 and its derivative CR 94–13, as well as its progeny IR 36 are resistant to biotype III. Biotype reaction of ASD 7 and PTB 18 was quite different also in Taiwan.[3]

3) *Allelism test of the four resistance genes*

Crosses, backcrosses or test crosses were made in 1977 and 1978 among the four resistant cultivars, Mudgo, ASD 7, Rathu Heenati and Babawee, representing *Bph 1*, *bph 2*, *Bph 3* and *bph 4*, respectively.[4] Reactions of F_1, F_2 and B_1F_1 or T_1 (F_1 of test cross) progenies from crosses among four resistant cultivars are presented in Table 3. The results indicate that *bph 2*, as well as *Bph 1*, segregates independently of both *Bph 3* and *bph 4*, while *Bph 3* and *bph 4* as well as *Bph 1* and *bph 2* were found to be closely linked or allelic to each other, as shown in Fig. 1. Accordingly, it appears possible to combine *Bph 1* with *Bph 3*, *Bph 1* with *bph 4*, *bph 2* with *Bph 3*, or *bph 2* with *bph 4* in a cultivar. On the other hand, it may be difficult or impossible to combine *Bph 1* with *bph 2* or

Table 1. Number of rice cultivars rated for brown planthopper (biotype I or wild type) resistance, 1973–1980

Region	Country, or group of cultivars	Reaction to BPH[a]			Total
		R	M	S	
Japan	Indigenous, scented or red rice	0	10	153	163
	Indigenous, others	0	1	195	196
	Modan	0	0	24	24
Far East	Korea and China including Taiwan	27[b]	21	495	543
Southeast Asia	Philippines through Indonesia and Burma	117[c]	27	559	703
South Asia	India and Sri Lanka	235[d]	26	618	879
	Nepal, Bhutan, Bangladesh and Pakistan	1[e]	4	156	161
Near East and East Europe	Iran through U.S.S.R.	1[e]	1	161	163
West Europe	Italy through Spain	0	0	133	133
Africa	Egypt, Malagasy and West Africa	0	0	165	165
America & Oceania	North and South America and Australia	0	0	157	157

[a] R: resistant, M: intermediate, S: susceptible
[b] All cultivars are hybrid lines bred for BPH resistance in Korea and Taiwan
[c] Most of these are hybrid lines bred for BPH resistance
[d] Out of these, all of the 172 cultivars from Sri Lanka are indigenous
[e] Not consistently resistant

Table 2. Classification of selected BPH resistant rice cultivars according to the reaction pattern to biotypes II and III (an abridged table)

Cultivar	Seed source[1]	Heading[2]	Pattern[3]	Gene
ARC 6650	IIRN 35	×	1	
ARC 6650	BPHN79-4	—	3	
ASD 7	TARC	○	2	*bph 2*
CR 94-13	BPHN 76-38	○	3	*bph 2*
Mudgo	TARC	○	1	*Bph 1*
PTB 18	67-119	×	3	*bph 2*
PTB 21	67-121	×	3	*bph 2*+*Bph 3*
PTB 33	67-133	×	3	unknown 2 genes
PTB 34	67-134	○	2	*bph 2*

1) IIRN, BPHN are from IRRI. TARC was given in 1973 by the Tropical Agriculture Research Center. 67-119 etc. are from the 7 th Lab., Div. Genetics, NIAS.
2) Heading in Konosu under the field conditions
○ : positive, × : no heading
3) Reaction pattern to biotypes II and III
1 : S(susceptible) and R(resistant), 2: R and S, 3: R and R.

Table 3. Segregation for resistance to the brown planthopper in crosses among four resistant cutivars representing *Bph 1*, *bph 2*, *Bph 3*, and *bph 4*, respectively

Genes of resistance involved	Cross	Generation	Number of seedlings			Hypothesis	X^2 value	P. value
			Res.	Sus.	Total			
Bph 1–bph 2	Mudgo/ASD 7	F_1	10	0	10			
		F_2	276	0	276	All R.(z)		
	Mudgo/ASD 7²	B_1F_1	69	0	69	All R.		
Bph 1–Bph 3	Mudgo/R.H.(x)	F_1	11	0	11			
		F_2	426	39	465	15 : 1	3.624	.05–.10
	Mudgo/R.H.// Nipponbare	T_1(y)	75	26	101	3 : 1	0.030	.80–.90
Bph 1–bph 4	Mudgo/Babawee	F_1	13	0	13			
		F_2	297	66	363	13 : 3	0.077	.70–.80
	Mudgo/Babawee²	B_1F_1	44	11	55	3 : 1	1.100	.70–.80
bph 2–Bph 3	R.H./ASD 7	F_1	10	0	10			
		F_2	557	144	701	13 : 3	1.478	.70
bph 2–bph 4	Babawee/ASD 7	F_1	0	13	13			
		F_2	237	303	540	7 : 9	0.004	.90–.95
Bph 3–bph 4	R.H./Babawee	F_1	10	0	10			
		F_2	1025	1	1026	All R.		
	Babawee/R.H.	F_1	11	0	11			
		F_2	629	0	629	All R.		

(x); Rathu Heenati, (y); F_1 of test cross, (z); No susceptible segregants.

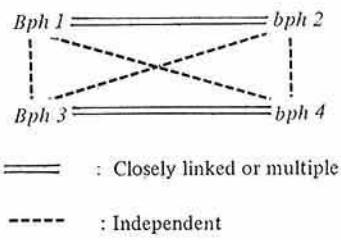

Fig. 1. Relationship among four resistance genes

Bph 3 with *bph 4*.

4) *Gene analysis of unclassified resistant cultivars*

One cultivar from Sri Lanka, Andaragahawewa and two from India, PTB 34 and PTB 21, were crossed in 1975 with resistant and susceptible testers. The F_2 data of each cross are shown in Table 4. Based on the results in Table 4, it can be concluded that Andaragahawewa and PTB 34 have *Bph 1* and *bph 2*, respectively. This conclusion was also corroborated by the reactions of the cultivars to BPH biotypes.[9]

With regard to PTB 21, Lakshminarayana and Khush (1977)[10] concluded that the resistance in this cultivar was controlled by one dominant and one recessive gene, segregating independently of each other, and one of then was either *Bph 1* or *bph 2*. Our data on the PTB 21 crosses with *Bph 3* and *bph 4* indicate that the second gene in PTB 21 may be either *Bph 3* or *bph 4*, and not a new gene, for no susceptible seedlings were observed in the F_2 populations of the three crosses except one seedling in the cross of PTB 21/Rathu Heenati. In order to determine whether the gene pair in PTB 21 is *Bph 1* and *bph 4* or *bph 2* and *Bph 3*, 12 F_3 lines of Kochihibiki/PTB 21//Asominori were tested for resistance by using the biotypes I, II and III of BPH. Six lines of them were homozygous for susceptibility to the biotype III and segregating to both of the biotypes I and II. From this result, it may be concluded that one of the two genes in PTB 21 is *bph 2*. Therefore, the resistance in PTB 21 is controlled by the gene pair *bph 2* and *Bph 3*.

Table 4. F_2 segregations for resistance to the brown planthopper in crosses of testers and unclassified resistant cultivars

Cross	Gene of tester	Number of F_2 seedlings			Hypothesis	X^2 value	P. value
		Res.	Sus.	Total			
Andaragahawewa/T (N) 1	(Sus.)	544	179	723	3 : 1	0.023	.80–.90
Andaragahawewa/IR 1414-67-3-2	*Bph 1*	872	1	873	All R.(x)		
PTB 34/T (N) 1	(Sus.)	79	233	312	1 : 3	0.017	.80–.90
PTB 34/IR 1414-67-3-2	*Bph 1*	873	8	881	All R.		
PTB 34/IR 1154-243	*bph 2*	235	3	238	All R.		
PTB 21/Rathu Heenati	*Bph 3*	517	1	518	All R.		
Rathu Heenati/PTB 21	*Bph 3*	272	0	272	All R.		
PTB 21/Babawee	*bph 4*	1039	0	1039	All R.		

(x); No susceptible segregants.

Table 5. F_2 segregation for resistance to the brown planthopper in the crosses between trisomic lines and Rathu Heenati (*Bph 3*) or Babawee (*bph 4*)

Resistance gene	Cross	Number of F_2 seedlings			X^2 for	
		Resistant	Susceptible	Total	3 : 1	1 : 3
Bph 3	Trisomic C/Rathu Heenati	78	42	120	7.600**	
	Trisomic H/Rathu Heenati	168	52	220	0.218	
	Control (disomic)	161	64	225	1.424	
bph 4	Trisomic A/Babawee	29	96	125		0.216
	Trisomic C/Babawee	8	306	314		84.420***
	Trisomic E/Babawee	70	186	256		0.750
	Trisomic F/Babawee	82	263	345		0.279
	Trisomic L/Babawee	117	337	454		0.144
	Control (disomic)	203	634	837		0.249

For the reliable use of BPH resistant cultivars, it is very important to have two or more resistance genes in one cultivar and PTB 21 is an example of this fact. This cultivar shows more stable resistance than other IRBPHN (International Rice Brown Planthopper Nursery) entries with monogenic resistance throughout the countries of Southeast and South Asia.

5) *Trisomic analysis of Bph 3 and bph 4*

To identify the chromosome on which *Bph 3* or *bph 4* is located, Rathu Heenati and Babawee were crossed in 1977 with the nine trisomic lines,[5,6] which were introduced from Kyushu University. F_1 plants were tested under the microscope at the metaphase of the first meiotic division of pollen mother cell to determine whether or not they were trisomics. Then the F_2 populations derived from trisomic F_1 plants were tested by the bulk seedling test. Due to hybrid sterility, only 7 F_2 populations of crosses involving 6 types of trisomic were examined for BPH resistance, as shown in Table 5. Disomic and trisomic portions of the F_2 populations were investigated in the lump for segregation of BPH resistance.

According to the results, the segregation of *Bph 3* or *bph 4* deviated significantly from the disomic ratio of 3:1 or 1:3 in the F_2 of crosses with C type of trisomics. On the other hand, in the crosses with A, E, F, H or L type of trisomics, the segregation of *Bph 3* or *bph 4* fitted very well to the disomic segregations. Accordingly, it is suggested that *Bph 3* and *bph 4* are carried on the extra-chromosome of C type. On the other hand, extra-chromosome of C type also

carries *fl* and *pgl* marker genes.[5] Moreover, these two genes are carried by chromosome 7.[14]

Therefore, it is concluded that *Bph 3* and *bph 4* are located on chromosome 7.

6) *Discussion*

Comparison of two different seed sources in Japan might be interesting and also important from the standpoint of utilization of germ plasm. The Seed Storage Lab. supplied us with 1,103 cultivars and the 7th Lab. of Genetics, both of the Division of Genetics, NIAS, supplied us with 1,778 cultivars. Among these, we could identify 3(0.3%) and 81(4.6%) BPH resistant cultivars, respectively. Such a marked discrepancy in recovery percentage can be ascribed to the following reason. Cultivars which do not flower and mature in the field in Japan were not included in the collection of the Seed Storage Lab., while the 7th Lab. which is responsible for germ plasm collection worldwide keeps cultivars from lower latitudes. Such cultivars cannot be sent to the Seed-Storage Lab. due to insufficient seed amount. Therefore, we consider it important to make effort to multiply the seed of these cultivars and send them to the Seed Storage Lab. for better utilization of germ plasm by a larger number of scientists. An institution, specially assigned for securing a sufficient amount of seed from each of introduced germ plasm, is needed

The biotype test is very powerful for gene analysis of the unclassified resistant cultivars. To identify *Bph 3*, *bph 4* and the other unknown resistance genes, however, the biotypes corresponding to their genes are needed. The selections of biotype IV and V are now in progress in our laboratory.

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