Japan. J. Breed., 31(3): 279~285 (1981)

Genetic Analysis of Resistance to Brown Planthopper, *Nilaparvata lugens* Stål., in Rice

Ryoichi IKEDA and Chukichi KANEDA

Central Agricultural Experiment Station, Konosu, Saitama 365, *Japan*

In order to determine whether it is possible to combine two or more resistance genes to the brown planthopper (BPH), allelic relationships among four resistance genes, *Bph* 1, *bph* 2, *Bph* 3 and *bph* 4, were studied. 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. Therefore the combination of *Bph* 1 with *Bph* 3, *Bph* 1 with *bph* 4, *bph* 2 with *Bph* 3 or *bph* 2 with *bph* 4, respectively, in a cultivar could be achieved without any difficulty. Then, in order to search for new resistace genes, unclassified resistant cultivars were analyzed. The resistance of the cultivars, 'Andaragahawewa' and 'PTB 34' was found to be monogenically controlled by *Bph* 1 and *bph* 2, respectively while the resistance in 'PTB 21' appeared to be controlled by the gene pair *bph* 2 and *Bph* 3. Thirdly, according to the results of trisomic analysis, it was assumed that *Bph* 3 and *bph* 4 are located on chromosome 7.

Introduction

For the past decade, the brown planthopper, *Nilaparvata lugens* Stål., (abbr. BPH), has become the most serious rice pest in Southeast and South Asia. The first BPH resistant cultivar, 'IR 26', was released in 1973, and the breakdown of the resistance was observed in 1975 in the Philippines, Indonesia and elsewhere. 'IR 36' and other cultivars with a new genotype were substituted for the older ones, and are still successfully being grown in these countries. The reliance on the use of the BPH resistant cultivars is of the greatest concern to plant breeders as well as rice growers in the tropics.

Inheritance studies of resistance to BPH were initiated by ATHWAL *et al.* (1971). They identified two genes for resistance, *Bph* 1 and *bph* 2, and reported that *Bph* 1 was closely linked or allelic to *bph* 2. On the other hand, MARTINEZ and KHUSH (1974) showed that the rice cultivar 'TKM 6' is homozygous for *Bph* 1 as well as for the inhibitory gene *I-Bph* 1. LAKSHMINARAYANA and KHUSH (1977) identified the third and fourth genes, *Bph* 3 and *bph* 4, which are independent of *Bph* 1 and *bph* 2, respectively.

As the search for new resistance genes and efforts for accumulating genes is important for the use of resistant cultivars, we report in this paper the results of allelism tests of the four resistance genes, gene analysis of several resistant cultivars, and trisomic analysis of *Bph* 3 and *bph* 4.

Materials and Methods

1. Allelism tests 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. F_1, F_2, and B_1F_1 or F_1 of test crosses (T_1) were tested for the BPH resistance by the bulk seedling test as stated later.

Received February 14, 1981

2. 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. 'Mudgo' or 'IR 1414-67-3-2' were used for *Bph* 1, 'ASD 7' or 'IR 1154-243' for *bph* 2, 'Rathu Heenati' for *Bph* 3, and 'Babawee' for *bph* 4, as resistant testers, and 'Kochihibiki' or 'Taichung Native 1, (T(N)1) as susceptible tester. The bulk seedling test was applied to the F_2 populations of each cross to identify the genotype.

3. Trisomic analysis of *Bph* 3 and *bph* 4

To identify the linkage group of *Bph* 3 and *bph* 4, 'Rathu Heenati' and 'Babawee' were crossed in 1977 with the nine trisomic lines (IWATA and OMURA, 1975, 1976), which were supplied by Prof. OMURA and co-workers of 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 plant were tested by the bulk seedling test.

The bulk seedling test, modified from the one described by ATHWAL *et al.* (1971), was used to test plants for BPH resistance. Two shallow trays, each containing 18 rows of hybrids and two rows of check cultivars, 15 or 17 plants per row, were accommodated in a cage. Cages were placed under fluorescent lamps in the insectary under controlled conditions at 27°C-light and 23°C-dark, with 16 Hr daylength. Seedlings were infested at the second leaf stage by the second to third instar nymphs of the wild type (biotype I) of BPH. Reactions of plants to BPH were recorded when seedlings of the susceptible check were killed, generally five to six days after the infestation. The method is described in detail elsewhere (KANEDA, 1975 a, b).

Results

1. Allelic relationships among four resistance genes

Reactions of F_1, F_2 and B_1F_1 or T_1 progenies from crosses among four resistant cultivars are presented in Table 1.

No susceptible plants were found in the F_2 and B_1F_1 populations of the cross between 'Mudgo' and 'ASD 7'. This finding is in agreement with the results of ATHWAL *et al.* (1971) who showed that *Bph* 1 was closely linked or allelic to *bph* 2.

Secondly, the proportion of resistant and susceptible seedlings fitted to the ratio of 15 : 1 in the F_2 of Mudgo/Rathu Heenati and 3 : 1 in the T_1 population of Mudgo/Rathu Heenati// Nipponbare, suggesting independent segregation of the two dominant genes. This result was in agreement with the report of LAKSHMINARAYANA and KHUSH (1977) who demonstrated that *Bph* 3 was independent of *Bph* 1.

Thirdly, according to the results of the segregation in the F_2 and B_1F_1 populations of the crosses between 'Mudgo' and 'Babawee', it appears that *bph* 4 is independent of *Bph* 1.

As the segregation in the F_2 population of Rathu Heenati/ASD 7 was found to fit to the 13 : 3 ratio expected for independent segregation of one dominant and one recessive gene, it was concluded that *bph* 2 is independent of *Bph* 3.

All of the F_1 progenies of Babawee/ASD 7 were found to be susceptible. Furthermore the segregation in the F_2 population of this cross fitted closely to the 7 : 9 ratio expected

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

Genes of resistance involved	Cross	Generation	Number of seedlings			Hypothesis	χ^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.(3)		
	Mudgo/ASD 7^2	B_1F_1	69	0	69	All R.		
Bph 1-*Bph* 3	Mudgo/R. H.(1)	F_1	11	0	11			
		F_2	426	39	465	15 : 1	3.624	.05—.10
	Mudgo/R. H.// Nipponbare	T_1(2)	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/Babawee^2	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.		

(1) R. H.;Rathu Heenati, (2) T_1 ; F_1 of test cross, (3) All R.; No susceptible segregants.

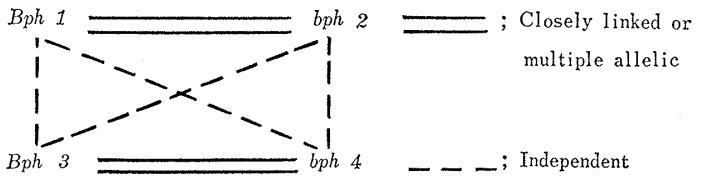

Fig. 1 Relationship among four resistance genes.

for two independent segregating recessive genes. Therefore, the relationship between *bph* 2 and *bph* 4 was also in agreement with the results of LAKSHMINARAYANA and KHUSH (1977).

Lastly, with respect to the relation between *Bph* 3 and *bph* 4, the reciprocal crosses between 'Rathu Heenati' and 'Babawee' produced only one susceptible seedlings. From this result, it may be concluded that *Bph* 3 is closely linked or allelic to *bph* 4 as reported by SIDHU and KHUSH (1979).

Therefore, from the above mentioned results, the relationships among the four BPH resistance genes can be illustrated as shown in Fig. 1.

Table 2. 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	χ^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.(1)		
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.		

(1) All R.; No susceptible segregants.

According to Fig. 1, 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 *Bph* 3 with *bph* 4.

2. Gene analysis of unclassified resistant cultivars

Table 2 shows the F_2 data of crosses between genetic testers and the three cultivars. In the cross, Andaragahawewa/IR 1414-67, only one of the 873 seedlings was classified as susceptible. On the other hand, the F_2 data of Andaragahawewa/T (N) 1 fitted to the 3 : 1 ratio expected for one dominant genic control of resistance in 'Andaragahawewa'. It can thus be concluded that the cultivar has *Bph* 1 gene.

Similarly, it may be concluded that the resistance in 'PTB 34' is controlled by *bph* 2 because the F_2 of PTB 34/T (N) 1 appeared clearly to fit to the 1 : 3 ratio expected for one recessive gene, and the presence of a few susceptible seedlings in the F_2 population of both crosses, PTB 34/IR 1414-67 and PTB 34/IR 1154-243 did not indicate genetic segregation for susceptibility.

With regard to 'PTB 21', LAKSHMINARAYANA and KHUSH (1977) concluded that the resistance in this cultivar was controlled by one dominant and one recessive gene, segregating independently of each other, and that one of them 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.

3. Trisomic analysis of *Bph* 3 and *bph* 4

To identify the chromosome on which *Bph* 3 or *bph* 4 is located, nine types of trisomics were crossed as female parent with 'Rathu Heenati' or 'Babawee'. However, 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 3. Disomic and trisomic portions of the F_2 populations

Table 3. 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	Parent or Cross	Number of F_2 seedlings			χ^2 for	
		Resistant	Susceptible	Total	3 : 1	1 : 3
Bph 3	Trisomic C, Parent	0	17	17		
	Trisomic H, Parent	0	12	12		
	Rathu Heenati, Parent	24	0	24		
	Trisomic C/Rathu Heenatl, F_2	78	42	120	7.600**	
	Trisomic H/Rathu Heenati, F_2	168	52	220	0.218	
	Control (disomic), F_2	161	64	225	1.424	
bph 4	Trisomic A, Parent	0	11	11		
	Trisomic E, Parent	0	18	18		
	Trisomic F, Parent	0	9	9		
	Trisomic L, Parent	0	7	7		
	Babawee, Parent	15	0	15		
	Trisomic A/Babawee, F_2	29	96	125		0.216
	Trisomic C/Babawee, F_2	8	306	314		84.420***
	Trisomic E/Babawee, F_2	70	186	256		0.750
	Trisomic F/Babawee, F_2	82	263	345		0.279
	Trisomic L/Babawee, F_2	117	337	454		0.144
	Control (disomic), F_2	203	634	837		0.249

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. Accodingly, it is suggested that *Bph* 3 and *bph* 4 are carried on the extra-chromosome of C type.

It has been reported by IWATA and OMURA (1975) that the extra-chromosome of C type carries *fl* and *pgl* marker genes. On the other hand, YOSHIMURA, IWATA and OMURA (1979) reported that *fl* and *pgl* genes are carried by chromosome 7.

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

Discussion

BPH resistance of the two cultivars 'Andaragahawewa' and 'PTB 34' was found to be monogenically controlled by *Bph* 1 and *bph* 2, respectively. Such results were also corroborated by the reactions of the cultivars to BPH biotypes (KANEDA, ITO and IKEDA, 1981).

For the reliable use of BPH resistant cultivars, it is very important to have two or more resistance genes in one cultivars and 'PTB 21' is an example of this fact. This cutivar shows more stable resistance than other IRBPHN (International Rice Brown Planthopper Nursery) entries with monogenic resistance throughout the countries of Southeast and South Asia (IRBPHN Report, 1976～1978). Results of our gene analysis revealed that BPH resistance of this cultivar is controlled by two sets of genes, either *Bph* 1 and *bph* 4 or *bph* 2 and *Bph* 3.

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 (KANEDA, ITO and IKEDA, 1981) of BPH. Six lines of them were homozygous for susceptibility to the biotype III and segregating to the both biotype I and II. From this results, 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.

In F_2 of some crosses between resistant cultivars, a small number of susceptible seedlings were sometimes found. As this is also found even in the resistant cultivars such as 'Mudgo', 'ASD 7', and others, those susceptible seedlings were considered not segregants originating from the recombination of resistance genes. ATHWAL *et al.* (1971) discussed the factors of the occurrence of dead seedlings in such crosses in detail. According to them, it is difficult to determine whether the dead seedlings occasionally observed in crosses between resistant cultivars are the result of genetic recombination between nonallelic genes or whether they were killed by another pathogen or by an unusually heavy infestation on some of the seedlings. In addition to the discussion of ATHWAL *et al.* (1971), it is possible that delayed growth of some seedlings in early stage or the occurrence of the damping-off to some seedlings may obscure the judgement of resistance or susceptibility in F_2 populations.

The locus (or loci) of *Bph* 3 and *bph* 4 was considered to be on chromosome 7 on the basis of trisomic analysis. Since a complete set of trisomic lines is not yet available, our assumption must be corroborated by linkage analysis using the marker genes located on

chromosome 7. However, this is realized after breeding of *japonica* lines with either *Bph* 3 or *bph* 4 achieved. The breeding is now in progress, and the question is to be solved in the near future.

Acknowledgement

The authors wish to express their sincere thanks to Prof. Takeshi OMURA and Dr. Nobuo IWATA of Kyushu University for their helpful advice and for supplying the seeds of trisomics, and to Dr. Tokuhiko MAKINO of Tohoku Agric. Expt. St. for his demonstrating the technique for checking trisomic under the microscope.

Literature cited

ATHWAL, D. S., M. D. PATHAK, E. H. BACALANGCO and C. D. PURA 1971. Genetics of resistance to brown planthoppers and green leafhoppers in *Oryza sativa* L. Crop Sci. **11** : 747～750.

IRRI 1976, 1977, 1978. Final report of the International Rice Brown Planthopper Nursery pp. 7, 10, 15.

IWATA, N. and T. OMURA 1975. Studies on the trisomics in rice plants (*Oryza sativa* L.) III. Relation between trisomics and genetic linkage groups. Japan. J. Breed. **25** : 363～368.

——— and ——— 1976. ——— VI. On the possibility of association of three linkage groups with one chromosome. Japan. J. Genet. **51** : 135～137.

KANEDA, C. 1975a. Simple testing methods of brown planthopper resistance in rice, and their application to breeding. Nogyo oyobi Engei **50** : 614～618. (in Japanese)

——— 1975b. Mass rearing of, and testing for resistance in rice to, brown planthopper in Japan. Rice Entomol. Newsl. **3** : 11～12.

———, K. ITO and R. IKEDA 1981. Screening of rice cultivars for brown planthopper, *Nilaparvata lugens* Stål., by three biotypes. Japan. J. Breed. **31** (2) ; 141～151.

LAKSHMINARAYANA, A. and G. S. KHUSH 1977. New genes for resistance to the brown planthopper in rice. Crop Sci. **17** : 96～100.

MARTINEZ, G. R. and G. S. KHUSH 1974. Sources and inheritance of resistance to brown planthopper in some breeding lines of rice. Crop Sci. **14** : 264～267.

SIDHU, G. S. and G. S. KHUSH 1979. Linkage relationships of some genes for disease and insect resistance and semidwarf stature in rice. Euphytica **28** : 233～237.

YOSHIMURA, A., N. IWATA and T. OMURA 1979. Linkage analysis by reciprocal translocation method in rice plants (*Oryza sativa* L.). On some genes located on chromosome 2, 3, 4 and 7, respectively. Japan. J. Breed. **29** (Suppl. 1): 232～233. (in Japanese)

イネのトビイロウンカ抵抗性の遺伝分析

池田　良一・金田　忠吉
（農事試験場　作物部）

トビイロウンカ抵抗性遺伝子 *Bph* 1, *bph* 2, *Bph* 3 および *bph* 4 の相互関係を明らかにするため，各遺伝子を代表する抵抗性品種 Mudgo, ASD 7, Rathu Heenati および Babawee 間で相互交配を行なった。バイオタイプⅠのウンカ（上記4品種のいずれも抵抗性を示す）を用いて，F_1, F_2 および B_1F_1 世代における抵抗性の分離を調べたところ，(1) *Bph* 1 と *bph* 2 ならびに *Bph* 3 と *bph* 4 は密接に連鎖しているか複対立遺伝子のいずれかであり，(2) *Bph* 1 と *Bph* 3, *Bph* 1 と *bph* 4, *bph* 2 と *Bph* 3 ならびに *bph* 2 と *bph* 4 は互いに独立であると推定された。このことから，互いに独立な2遺伝子ずつの集積は比較的容易であるが，密接に連鎖しているか複対立の関係にある2遺伝子の集積は困難であると結論された。

また，未同定の抵抗性3品種の遺伝子分析を行なった。まず Andaragahawewa が *Bph* 1, PTB 34 が *bph* 2 をそれぞれ持つと推定された。次に PTB 21 は *Bph* 1 と *bph* 4 か *bph* 2 と *Bph* 3 のいずれか2遺伝子を持つと考えられたので，PTB 21 由来の F_3 系統をバイオタイプ別に検定したところ，バイオタイプⅠとⅡ（*Bph* 1 をもつ品種を加害）には抵抗性（分離）を示す反面，Ⅲ（*bph* 2 をもつ品種を加害）には抵抗性を示す系統がなかった。したがって PTB 21 は *bph* 2 と *Bph* 3 をもつと結論された。

一方，*Bph* 3 および *bph* 4 の座乗染色体を明らかにするため，トリソミック分析を行なった。九州大学から導入された9種類のトリソミック系統に Rathu Heenati または Babawee を交配し，F_2 世代における抵抗性の分離をみた。*Bph* 3 および *bph* 4 はいずれもトリソミックC型系統との F_2 において，3：1 または 1：3 の比とは有意に異なる分離を示し，C型系統の過剰染色体である第7染色体に座乗すると推定された。