Status of varietal resistance to brown planthopper in Japan

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Methods of mass-rearing brown planthoppers (BPH) and of screening for varietal improvement and genetic studies were described.

Three BPH colonies, originating from catches in 1975 in Fukuoka and Konosu, and in 1976 at Chikugo, were tested for racial difference. A set of differential varieties reacted Identically to the colonies, which were considered similar to the biotype 1 at IRRI.

The growth in population of a wild colony of BPH on BPH-resistant rice was studied in the field and in the greenhouse using lines nearly isogenic for *Bph 1* gene. The colony kept on resistant lines for three generations in the field and for four generations in the greenhouse infested caged plants of the resistant lines in the field as severely as did the colony caged on susceptible varieties. In other experiments, as much as 74% and 92% of the 2 colonies reared for 4 generations on Mudgo and ASD 7, respectively, survived on these varieties. When these colonies were kept on Mudgo and ASD 7 for 10 generations and then on the susceptible variety Nihonbare for 4 generations, they did not recover their nonpreference for the resistant varieties.

Backcrossing is being adopted to incorporate *Bph 1* and *bph 2* into Japanese rice. Studies with F_2 plants with *Bph 1* suggested that the resistant gene *Bph 1* is significantly associated with longer culm, while studies with F_2 plants with *bph 2* gene, originating from IR1154-243, showed no relationship between BPH resistance and culm height, grain fertility, or threshability

Since there is more reason for suspecting degradation of the antibiosis in hybrid lInes than in donors of BPH resistance, breeding lines of a single cross and its first and second backcrosses were tested. Survival percentage was not necessarily higher, but the mean body weight of surviving insects increased nonsignificantly with repeated backcrossing.

The linkage relationship of *Bph 1* is being studied with other agronomic traits in cross-combinations involving many marker genes on 10 different chromosomes.

THE BROWN PLANTHOPPER (BPH) *Nilaparvata lugens* Stål., the most serious insect pest of rice in Japan, cannot overwinter in the country. It is believed to immigrate every year from late June to July (Kisimoto 1976). BPH multiply on rice for three or four generations and finally cause “tsubo-gare,” or hopperburn, in maturing rice. As we bring hopper-resistant rice varieties into practical use,

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information on the area from which the insect migrates, the predominant biotypes of that area, changes of biotypes, etc. becomes essential. The role of the International Rice Brown Planthopper Nursery (IRBPHN) in providing such information is important to Japan.

SCREENING FOR VARIETAL RESISTANCE

At the Central Agricultural Experiment Station (CAES), Konosu, Japan, BPH are reared and screened in an insectary at 26 to 27°C and with 15 hours of light. In insect mass-rearing, young seedlings are grown in plastic trays (26 15 × 3.3 cm) without soil. Two trays are placed in a wooden-frame cage, 30 x 34 × 43 cm, with plastic plates for the top, the bottom, and the front cover, and with nylon mesh for the other three sides. About 2,000 to 3,000 nymphs can be reared in each tray.

In mass screening, 15 germinated seeds of each entry, after 2 days of incubation at 30°C, are planted in a half row (about 6.5 cm) in the tray. Usually 20 half-rows, including two replications of check rows, are grown in the tray's shallow soil. After 2 days in a lighted incubator, seedlings at the early second-leaf stage are each infested by about five second- and third-instar BPH nymphs. Susceptible plants are killed within 5 to 7 days after infestation.

In a short-term caging method (Kaneda 1975) designed to secure susceptible plants for further experiments, infestation is discontinued after 3 days. All nymphs are cleared off, and the plants are brought into a lighted incubator again. One or two days of nurturing would make clear the difference of varietal resistance. Susceptible plants recover from the suppressed growth after several days.

During the past 4 years of screening, we have found, in addition to those already recorded at IRRI and elsewhere, varieties that are highly resistant to the BPH. They are: Ptb selections (4, 7, 10, 12, 20, 28, 29, 30, 31, 34), HS 19, H 2871, and ADT 3 of India and Chhuthana of Khmer. Kaeu N. 632 and 651 of USSR are also resistant. Dozens of traditional varieties of Japan, China, and Southeast Asian countries, which were found moderately resistant, proved to have an antibiosis as weak as that of susceptible varieties (Kaneda et al 1977).

Some varieties in the 1974 IRBPHN that had been called resistant, such as CO 13, RP9-3, and RP9-4, proved to be susceptible in repeated tests. Similar observations were made in Korea (Choi 1975). The results of the 1976 IRBPHN suggested that the BPH colony at CAES is quite similar to biotype 1 at IRRI. The varieties and selections with resistance gene showed resistance, except for segregation in several selections.

RACIAL DIFFERENCES OF SEVERAL BPH COLONIES IN JAPAN

Until August 1976, CAES used a colony collected in Kagoshima in 1972 for screening germplasm and breeding materials. The insects used for the mass

Table 1. Suppression of seedling growth of selected rice cultivars by three colonies[a] of the brown planthopper (BPH) in Japan (Konosu 1976).

Cultivar	BPH resistance gene	Growth[b] (% of growth of uninfested check)					
		Seedling ht			Leaf age		
		A	B	C	A	B	C
Mudgo	*Bph 1*	68	50	53	97	99	100
IR26	"	71	71	74	97	95	97
$F_8$262	"	60	63	63	99	99	97
$F_6$324	"	66	64	70	97	97	99
ASD 7	*bph 2*	74	79	70	95	98	98
IR1154-243	"	57	72	63	86	95	97
IR32	"	67	81	67	92	95	92
IR36	"	72	83	64	100	100	100
CR 94-13	"	76	88	68	99	99	97
Rathu Heenati	*Bph 3*	66	75	75	100	100	100
Babawee	*bph 4*	50	60	53	84	94	92
Ptb 21	unknown	82	91	85	98	100	98
Ptb 33	"	77	82	17	100	100	100
Nihonbare	none	14[c]	14[c]	14[c]			
TN1	"	31[c]	28[c]	28[c]			

[a]Insect colonies: A = ’75 Fukuoka, B = ’75 Konosu. C = '76 Chikugo.
[b]Seedling ht = $\frac{\text{Ht of infested seedlings}}{\text{Ht of uninfested seedlings}} \times 100$. [c]Killed by BPH.

screening and other tests were not re-collected for the reproduction of the next generations. Screening for 4 years gave no indication of breakdown of varieties with *Bph 1* or *bph 2* genes for resistance. Since September 1976, a new colony, ’75 Fukuoka, has been used. CAES tested three BPH colonies, including ’75 Fukuoka, for their effects on 15 rice cultivars of different genetic backgrounds (*Bph 1, bph 2, Bph 3, bph 4,* and others). Entries were randomly arranged in a tray containing shallow soil; 10 seeds of each entry were planted in a row. In mass screening, the reactions of the varieties to the three colonies were generally identical; only the susceptible check varieties were killed. The ’75 Konosu colony seemed weaker than the other colonies (Table 1) but since seedlings were not infested with exactly the same numbers of nymphs, the difference may not be essential. The experiment made clearer the similarity to biotype 1 of IRRI of the BPH that migrated to the Kyushu areas in 1975 and to the Kanto areas in 1976.

We consider it necessary to mention the yearly monitoring of the types of BPH migrating into Japan. Sampling immigrants early in the rice-growing season in western Japan will provide needed data.

BIOTYPES OF BPH

In the field, populations of a wild colony of BPH artificially released on a susceptible check variety, Nihonbare, and on susceptible lines in the two sets of nearly isogenic lines (Kaneda 1975) multiplied to such a degree that hopper-

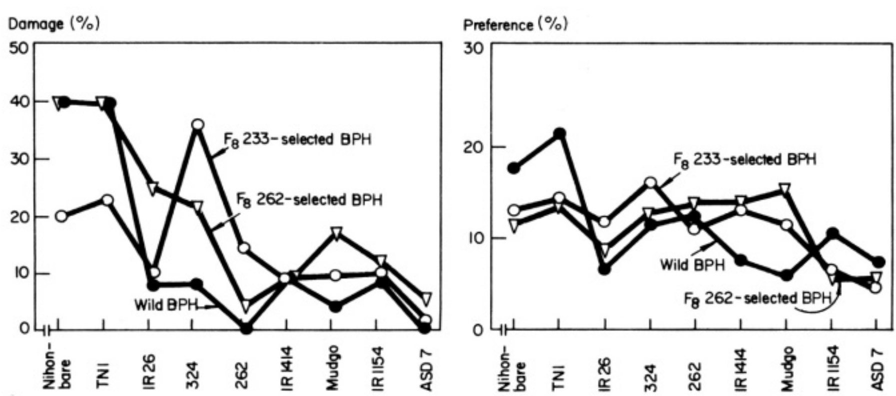
Table 2. Population growth of wild brown planthoppers (BPH) artificially released on two sets of lines nearly isogenic for *Bph 1,* and on a susceptible check.

Cultivar	Replicate	BPH population[a] (no.) 28 July	1st generation[b] (no.) 20 Aug	2nd generation[b] (no.) 26 Sept.	Damaged hills[c] (no.)			
					A	B	C	Total
Nihonbare	1	8	129	∞	38	22	4	64
S check	2	12	189	∞	34	35	21	90
F_8 262 (R)	1	0	6	25	0	0	0	0
	2	0	10	38	0	0	0	0
F_8 264 (S)	1	8	167	∞	74	23	0	97
	2	9	226	∞	63	23	7	93
F_8 223 (R)	1	0	11	23	0	0	0	0
	2	0	18	50	0	0	0	0
F_8 236 (S)	1	5	225	∞	59	19	3	81
	2	5	152	∞	54	13	2	69

[a] Number of short-winged females remaining from 27 insects introduced on 3 × 3 hills at center of plot on 24 July. [b] Number of short-winged females found in each experimental plot of 15 × 15 hills, ∞ means more than several hundreds. [c] A = completely withered hills; 13 = partly withered hills; C = no tillers withered but with sooty mold.

burn occurred in the third generation. On the other hand, on resistant lines carrying *Bph 1* from Mudgo, the multiplication was clearly suppressed but the pests were not exterminated; they stayed at a low but rather stable level for three generations until harvest (Table 2). The surviving adults were collected and kept for four generations in a glass house with ratoons of the test lines. In the next crop season they infested caged rice plants in a field of resistant lines, apparently as easily as wild BPH infested susceptible varieties.

After 10 generations of breeding on the resistant lines F_8 262 and F_8 233, each biotype colony was tested for its host preference, at 25°C in the laboratory, on two susceptible check varieties, five resistant cultivars carrying *Bph 1,* and two carrying *bph 2* (Fig. 1). Five plants of 9 cultivars, with 2 replicates (90 plants in a cage), were infested at the second leaf stage with 250 to 300 insects. Most of the insects were fifth-stage nymphs; some were adults. Preference was indicated by the average percentage of insects observed infesting each variety



1. Preference for and damage to rice lines F_8 262 and F_8 233 by three BPH biotypes.

during the first 3 days after the insects were introduced. The wild BPH population showed equal nonpreference for all resistant varieties but the selected biotypes showed impartiality for susceptible and resistant varieties carrying *Bph 1*. Nonpreference for lines carrying *bph 2* persisted. After 10 days of infestation, plant injury was determined on the basis of a scale of 0 (no visible damage) to 4 (completely withered). All resistant varieties were resistant to the wild BPH but in some cases, some of the resistant varieties carrying *Bph 1* showed as much susceptibility to feeding damage by F_8 262-selected and F_8 233-selected biotypes as did the susceptible checks. The differences in the inherent tolerance for damage among varieties caused rather variable results.

Selective breeding for biotypes infesting Mudgo and ASD 7 was carried out in the laboratory. Nymphs fed on Mudgo and ASD 7 had survival ratios of 46 and 31%, respectively, in the first generation, and 74 and 92% in the fourth generation. The preference of each colony for the resistant variety it fed on increased until it was as great as its preference for a susceptible check (Nihonbare); its preference for the other variety did not increase. At the 10th generation, a part of each colony was again released on Nihonbare to feed for two and four generations to determine if nonpreference for a resistant variety can be recovered. Preference, however, remained as high as that of a colony not so treated.

STATUS OF RICE BREEDING FOR BPH RESISTANCE

Efforts in breeding for BPH resistance had been concentrated on introducing *Bph 1* and *bph 2* genes into Japanese rice until 1976, when Rathu Heenati, Babawee, Ptb 33, and other cultivars were crossed with Japanese varieties to widen the genetic base of BPH resistance.

Breeding for *Bph 1*

Agronomic characteristics and factors related to BPH resistance of the primary parental lines derived from crosses between japonica varieties and Mudgo, have been described elsewhere (Kaneda 1976). The major defect of those parental lines was their rather low cross-compatibility with Japanese varieties. Their plant height, threshability, grain and table quality, and other traits were not as satisfactory as those of commercial varieties in Japan. Backcrossing was repeated and plant selection from the crosses was initiated in 1976.

Earlier studies with nearly isogenic lines suggested that the *Bph 1* gene is linked with some genetic factor or factors controlling greater culm height, which came from Mudgo. One of the primary parental lines, F_8 262, is about 4 cm taller than its susceptible counterpart, F_8 264.

The association between BPH resistance and culm height was also noticed in the B_1F_2 populations of Reiho/F_8 262//Reiho grown in 1976. A total of 167 F_2 plants, selected randomly from two populations of the cross, were analyzed for association between BPH resistance and maturity, culm height,

Table 3. Association between brown planthopper resistance (*Bph 1*) and culm height in random samples of F_2 plants of Reiho/F_8 262//Reiho.[a] Konosu 1976.

Culm ht (cm)	F_2 plants (no.) observed and expected (in parentheses)			
	Bph 1/Bph 1	*Bph 1/bph 1*	*bph 1/bph 1*	Total
70 or less	0 (2.5)[b]	7 (9.0)	9 (4.5)	16
71 to 80	5 (11.2)	39 (40.5)	28 (20.2)[c]	72
81 to 90	17 (10.0)	37 (36.0)	10 (18.0)	64
91 to 100	4 (2.3)	11 (8.5)	0 (4.2)	15

[a] Chi square for free recombination of resistance and culm height: x^2 = 49.18 (P > 0.005). [b]Category for F_8 262. [c]Category for Reiho.

grain fertility, threshability, grain quality, and leaf character (japonica type or indica type). Only culm height was significantly associated with BPH resistance. As shown in Table 3, none of 16 plants with culms less than 70 cm were homogeneous for the resistance, while none of 15 plants with culms over 90 cm were homogeneous for susceptibility.

In the other cross, F_6 324/Akitsuho^3, resistance was also associated, though not significantly, with taller culm or lower grain fertility.

In spite of such undesirable associations, CAES expects to have many promising lines in 1977, selected from different backcrosses. Table 4 lists the primary CAES breeding material for BPH resistance grown in 1977.

Table 4. Principal breeding material for BPH resistance at Central Agricultural Experiment Station, Konosu, Japan, in 1977.

Cross no.	Cross combination[a]
B_1f_1	
	Tsukushibare/Rathu Heenati//Tsukushibare, etc
	Tsukushibare/Babawee// Tsukushibare, etc.
	Tsukushibare/Ptb 21 or Ptb 33//Tsukushibare, etc.
BC F_2	
7605	A/Akitsuho^3, etc.
7607	B/Reiho^2, etc.
7611	C/Asominori^2, etc.
7613	Tsukushibare/C, etc.
F_3 lines	
7501	D/Tsukushibare
7503	A/Reiho//Asominori
7507	A/Akitsuho^2
7513	B/Tsukushibare, etc.
7520	A/Tsukushibare
7517	C/Asominori
7518	C/Tsukushibare
7519	C/Mizuho
F_4 lines	
7316	A
7407	A/Reiho
7408	A/Akitsuho

[a]A = F_6 324/Akitsuho, B = Reiho/F_8 262, C = Asominori/IR1154//Asominori, D = IR1414-67/Nihonrnasari^2//Tsukushibare.

Table 5. Characteristics of backcrossed F_1 plants, with or without *bph 2* gene, grown in greenhouse boxes, Konosu, Japan. winter 1975–76.

Cross no.	Population	Genotype	F_1 plants tested (no.)	Culm length[a] (cm)	Fertile grains [a] (no./ear)	Gram fertility (%)	Thresh-ability [b] (grade)
7517	4	*bph 2*/--	87	48.3 ± 6.13	18.1 ± 7.43	78	2.2
		--/--	97	47.3 ± 6.46	16.7 ± 6.22	76	2.3
7518	4	*bph 2*/--	74	48.4 ± 5.51	18.5 ± 6.51	71	2.7
			69	46.3 ± 5 42	16.8 ± 5 89	72	2.6
7519	3	*bph 2*/--	56	50.2 ± 5.24	18.0 ± 7.71	72	2.3
		--/--	90	49.5 ± 5.36	17.0 ± 6.19	68	2.8
Asominoro (check)			16	45.5	14.8	86	1-3
Mizuho (check)			16	42.1	11.1	66	2-4
IR1154-243 (check)			9	37.0	9.6	32	6-7

[a] x ± s. [b] International Rice Standard.

Breeding for *bph 2*

IR1154-243 was selected as the gene source of *bph 2* because of its short stature and earliness under temperate conditions. Besides, it seems more compatible with japonica rice than with indica rice.

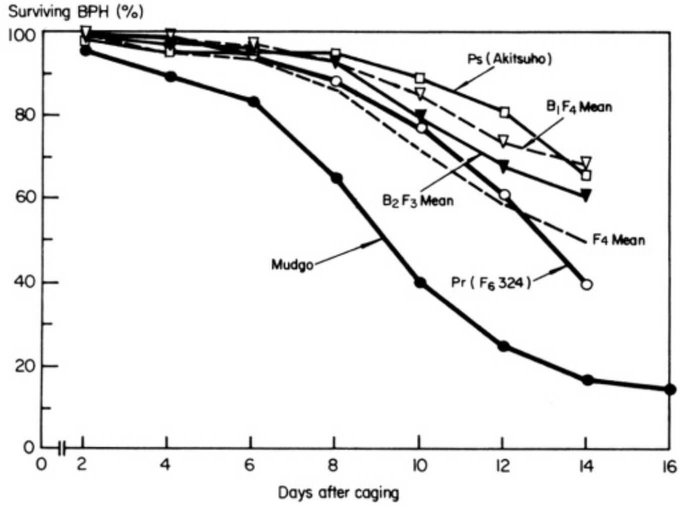
In contrast with what was done with *Bph 1* material, the backcrossed F_1 plants were grown without screening for BPH. Each F_1 plant grown in the greenhouse during winter was tested, using newly harvested F_2 seeds, for the retention of the *bph 2* gene. The presence of *bph 2* in B_2F_1 individuals did not affect culm height, grain fertility, number of grains per panicle, or threshability (Table 5; Kaneda et al 1977).

Also, the materials with *bph 2* seemed much more tolerant than *Bph 1* materials of the low temperatures and shading that cause low fertility of the grain in the winter crop produced in the greenhouse. Although resistant F_1 plants of *Bph 1* material suffered 70 to 80% sterility, *bph 2* material suffered only 22 to 29% sterility in the greenhouse during the 1975–76 winter.

We, therefore, consider breeding with *bph 2* to be much less difficult than breeding with *Bph 1,* although we have no data yet concerning the association of BPH resistance and grain quality, especially the high amylose content of IR1154-243.

EFFECT OF BACKCROSSING UPON ANTIBIOSIS

The primary CAES parental lines for BPH resistance do not retain the high levels of antibiosis retained by the donor parent Mudgo, though their reactions in the mass screening are as strong as Mudgo's. If the level of antibiosis is inevitably lowered by crossing with susceptible varieties, continued back-crossing, which is essential for breeding BPH-resistant japonica rice, would result in the selection of rices with little antibiotic effect on the BPH. In other words, rice varieties with more improved agronomic traits would not effectively hold down BPH populations.



2. Brown planthopper (BPH) survival on rice selections of different times of backcrossing compared with that on donor and recurrent parents (cf. Table 6).

Ten rice selections with different degrees of backcrossing, which were homogeneous for BPH resistance, were tested along with their donor and recurrent parents. Three second-leaf seedlings were caged with 10 second-instar BPH nymphs in glass test tubes. Insect survival, percentage of adults emerging, and mean body weight of insects surviving on the 16th day after caging, were used to determine the level of antibiosis.

As seen in Figure 2 and Table 6, insect survival was not necessarily higher for cultivars with more backcrosses in their backgrounds, and it varied even among lines of the same cross. The mean body weight of surviving BPH seemed to be increased by repeated backcrossing to japonica rice, although for some unknown reasons, it was much smaller on some single-cross lines than on their resistant parent F_6 324 (Kaneda and Jin 1977). However, variance analysis of body weight clearly differentiated the strong antibiosis of resistant breeding lines from that of susceptible parent. More tests are needed to dispel fears that repeated backcrosses to susceptible varieties may cause the degradation of antibiosis in rice selections.

Studies of the inheritance of *Bph 1*

Association of *Bph 1* with other agronomic traits is studied in the breeding process already described. In addition the research into the linkages between *Bph 1* and many marker genes has been conducted.

Five cross-combinations involving 10 marker genes on 6 chromosomes were analyzed by applying a “short-term caging method” to F_2 populations. After resistant and susceptible F_2 plants had been separated, they were transplanted

Table 6. Survival and development of BPH nymphs on rice selections derived from different doses of backcrossing to a japonica variety.

Test no.[a]	Cultivar	BPH survival (%) after caging for				Adult emergence (%)	Mean body wt (mg)
		8 days	10 days	12 days	14 days		
1	Mudgo	65	40	25	17[b]	3.7	0.96[b]
2	F_6 324	88	77	61	40[b]	36.0	1.15[b]
13	Akitsuho	95	89	81	66[c]	75.3	2.22[c]
3		90	81	70	61[c]	38.2	1.12[b]
4		86	77	65	59[c]	18.5	0.80[b]
5		81	57	42	30[b]	2.5	0.75[b]
	3–5 mean	86	71	59	50[c]	19.9	0 89
6		94	86	84	80[c]	49.0	0.91[b]
7		91	80	62	54[c]	27.4	1.16[b]
8		93	88	76	72[c]	46.6	1.05[b]
	6–8 mean	93	a5	74	69	41.0	1.04[b]
9		89	66	48	43[b]	31.0	1.24[b]
10		95	92	86	78[c]	51.8	1.12[b]
11		92	78	69	61[c]	46.0	1.18[b]
12		97	80	69	60[c]	40.0	1.22[b]
	9–12 mean	93	79	68	61	42.2	1.19

[a]3–5 = F_4 lines of F_6 324/Akitsuho; 6–8 = F_4 lines of F_6 324/Akitsuho^2. 9–12 = F_3 lines of F_6 324/ Akitsuho^3. All of these lines are homogeneous for BPH resistance. [b] Significantly different from the maximum. [c] Significantly different from Mudgo, the donor of antibiosis.

in groups to the field, and observed for segregation in the marker characters. The linkage group and the marker genes, which seemed independent of *Bph 1,* are: *lg* (liguleless) and d_{11} (dwarf) of II ; *g* (long glume) of IV, *bl* (brown-spotted leaf), *tri* (triangular spikelet). and gh_2 (gold hull) of X; and *gl* (glabrous leaf) of XII, *la* (lazy habit) and *sp* (short panicle) of VIII; and *ch* (chlorina) of XI.

Six other crosses involving 13 marker genes on 8 chromosomes were tested in F_3. *Dn* (dense panicle) and dp_2 (depressed palea) of VII, *dt* (tillering dwarf), *Sp* and *la* of XIII, gh_2 and d_w (dwarf) of X seemed independent of *Bph 1. Cl* (clustering of spikelets) and *dp* of I, *lg* of II, lux (lax panicle) of III, gh_2 of VI, and *dl* (droopy leaf) of XI are still to be studied.

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