Present Status and Prospect of Rice Breeding for Brown Planthopper Resistance in Japan

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Brown planthopper, *Nilaparvata lugens* Stål, (abbrev. as BPH) has been one of the most serious pests of rice in Japan. The famous famine in 1732, which caused death of nearly one million, was attributed to the crop damage by BPH.

The finding of plant resistance to BPH in 1967 at IRRI seemed to terminate the struggle against BPH. However, the occurrence of breakdown of resistance in recent years in tropical rice growing countries suggests that the breeding and usage of resistant varieties should be accompanied by various aspects of basic studies on the insect in relation to environments including cultivation technology.

This article briefly introduces some features on the breeding and its related research areas on BPH resistance in Japan. Fig. 1 outlines the flow or the interrelation of research areas which will contribute to the stable use of varietal resistance for controlling BPH.

Methods of mass rearing and screening for BPH resistance

In order to conduct the experiments throughout the year in a cold country like Japan, methods of mass rearing and screening should be designed as to be performed inside the insectary using young seedlings. Our insectary is conditioned at 15 hrs light, 26–27°C in the light and 22–23°C in the dark. Young seedlings are grown in plastic trays (26×15×3.3 cm) without soil for the mass rearing, and with soil for screening. Two trays are kept in

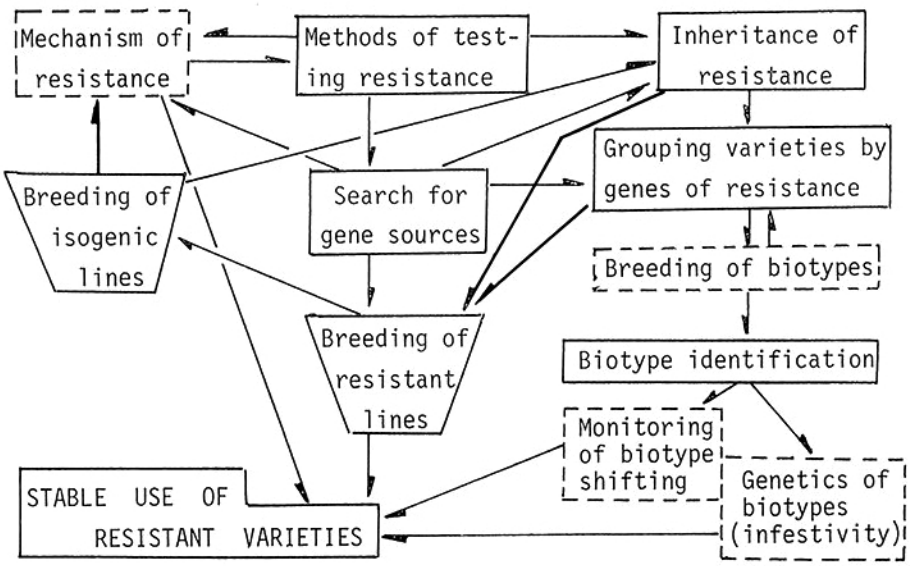

Fig. 1. Flowchart of studies on breeding for BPH resistance

a cage, 30×34×43 cm, made of wooden frames, plastic plates on top, bottom and front side, and nylon mesh on the other three sides.

For the mass screening, 15 germinating seeds per entry are planted to half a row (ca 6.5 cm) in the tray. Twenty half-rows, including two replications of check rows, are accommodated in a tray with shallow wet soil. Plants at the early second-leaf stage are infested by around five nymphs per seedling. Rating of resistance can be done 5 to 7 days after infestation.

A specific method of screening was invented in 1975 for the use in genetic studies. To secure susceptible plants for further experiments, infestation is discontinued in three days. After all nymphs are cleared off, the trays are brought into the lighted incubator again. Two days of incubation makes clear the difference of varietal resistance, and susceptible plants can recover from the suppressed growth after few days. Resistant and susceptible plants are transplanted in groups[2]. Using this "short term caging method", linkage analysis and other genetic studies are being conducted.

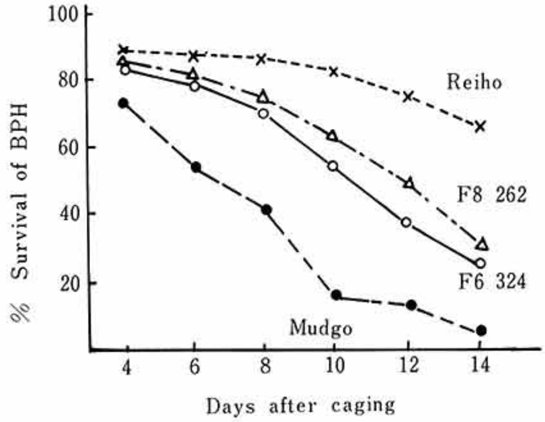

Fig. 2. Changes of survival % of BPH nymphs caged with different varieties and lines (10 second instars per 3 seedlings, means of 10 reps).

Present status of breeding

When breeding for BPH resistance was initiated in 1973 at Konosu, several primary parental lines, bred by TARC in cooperation with IRRI and the Taichung Agricultural Improvement Station in 1968 through 1972, were used as the direct source of BPH resistance with *Bph 1* gene. Performance of these lines against BPH in the field was certified at the Kyushu Agricultural Experiment Station in 1973[7]. The origin and agronomic characteristics of the two parental lines, that proved to be useful by their offsprings, are briefly described here.

F_8 262: from the cross Hoyoku/Mudgo// Kochikazé; maturity and plant height are similar to those of leading varieties in Kyushu; with longer panicles and heavier grains; very easy shattering and poor eating quality.

F_6 324: from Hoyoku/Mudgo//Kochikazé/3/IR781-1-94/4/Hoyoku; very early maturing (similar to Reimei), medium height, medium threshability, smaller grains with fair palatability; lower grain fertility.

As for the gene source of *bph 2*, IR1154-243 was selected for its earliness at Konosu. Its origin, IR 8^2/Zenith, was also considered preferable from the standpoint of possibly better affinity with japonica rice.

Since even the parental lines of *Bph 1* were still far from commercial varieties in agronomic characters and in the cross compatibility with Japanese varieties, the method of breeding was directed at the backcrossing. Usually BC F_1 plants were grown in winter in the greenhouse after screening out susceptible segregates regarding *Bph 1* gene, and without screening in case of *bph 2*. Backcrosses were made in summer season using ratoon plants of BC F_1's transferred from the greenhouse to the fields. Before transplanting of ratoons, genotypes are checked by testing F_2 panicle-lines in *bph 2* material. Backcrosses in the greenhouse were first made in April, 1977, but not quite efficiently.

The following is the list of principal breeding materials grown in 1977 in the fields at

Konosu.

Bph 1	F_4 lines :	F_6 324/Akitsuho^2, etc.
	F_3 lines :	F_6 324/Akitsuho^3, Reiho/ F_8 262//Tsukushi-baré, etc.
	F_2 pops :	F_6 324/Akitsuho^4, Reiho/ F_8 262//Reiho^2, etc.
	F_1 plants:	F_6 324/Akitsuho^5
bph 2	F_3 lines :	Asominori/IR1154-243// Asominori^2, etc.
	F_2 pops :	Asominori/IR1154-243// Asominori^2/3/Tsukushi-baré, etc.
	F_1 plants:	Asominori/IR1154-243// Asominori^4
Bph 3	F_1 plants:	Tsukushi-baré^2/Rathu Heenati
bph 4	F_1 plants:	Mizuho/Babawee, etc.
others	F_1 plants:	Kochi-hibiki/Ptb 33, Tsukushi-baré/IET 5122, etc.

At the beginning of breeding work, there was the fear that the level of antibiosis of parental lines might be degraded through the process of repeated backcrosses. As seen in Fig. 2, antibiosis level of F_8 262 and F_6 324, measured by the percent survival of BPH nymphs caged on test seedlings, was intermediate between Mudgo and the susceptible check variety Reiho, in spite of their highly resistant reaction in the mass screening tests. If the intermediate level of antibiosis was an inevitable result from hybridization of the resistant and susceptible varieties, then repeated backcrosses to susceptible varieties, which is essential for the purpose of breeding japonica rice resistant to BPH, will produce lines of resistant reaction in the mass screening, but with only low level of antibiosis.

A test was conducted to clarify this question, using single cross F_4, B_1F_4, and B_2F_3 lines, all homozygous for resistance, with the donor parent F_6 324 and the recurrent parent Akitsuho, and the resistant check Mudgo. Three second-leaf seedlings were tested in each test tube with ten second instar nymphs, with nine replications. The result is shown in Table 1 and Plate 1. Degradation of antibiosis seems not to occur linearly with the increasing number of backcrossing. There is a considerable variation in survival percent among lines of the same order of backcrosses for some unknown reason. Also percent survival and mean body weight of the surviving insects

Table 1. Survival and development of BPH nymphs on rice selections derived from different doses of backcrossing to Akitsuho

Test No.	Cross, variety	% survival of BPH			% adult emergence	Mean body wt. of survivals	
		12 days	14 days				
1	Mudgo	25	17	a	3.7	0.96	a
2	F_6 324	61	40	a	36.0	1.15	a
3	F_6 324/Akitsuho, F_4 lines	70	61	b	38.2	1.12	a
4		65	59	b	18.5	0.80	a
5		42	30	a	2.5	0.75	a
6	F_6 324/Akitsuho^2, B_1F_4 lines	84	80	b	49.0	0.91	a
7		62	54	b	27.4	1.16	a
8		76	72	b	46.6	1.05	a
9	F_6 324/Akitsuho^3, B_2F_3 lines	48	43	a	31.0	1.24	a
10		86	78	b	51.8	1.12	a
11		69	61	b	46.0	1.18	a
12		69	60	b	40.0	1.22	a
13	Akitsuho	81	66	b	75.3	2.22	b

a: significantly different from the maximum (5% level)
b: significantly different from Mudgo, the donor of antibiosis (5% level)

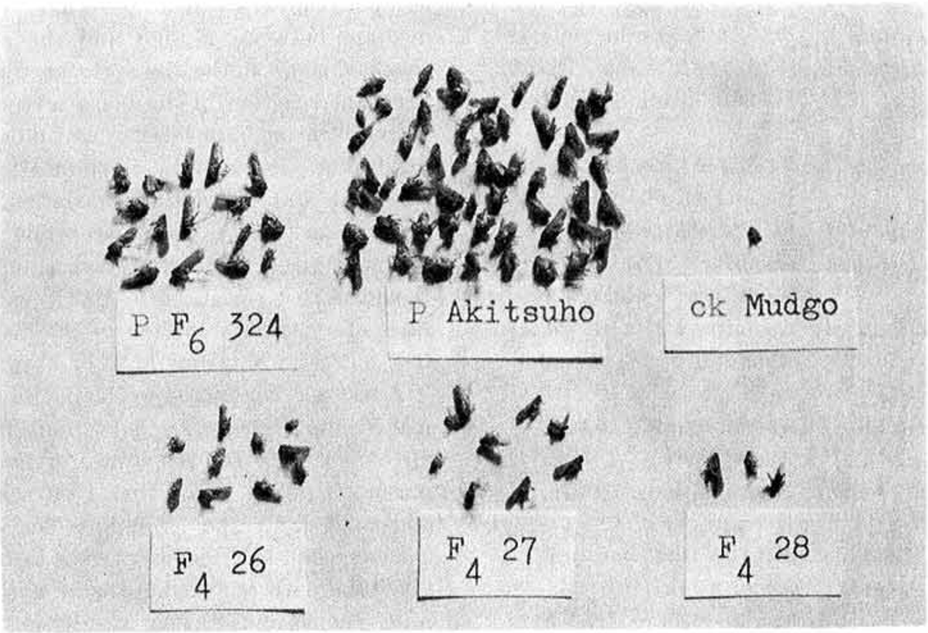

Plate 1. Number and size of surviving BPH after two week caging with seedlings of different varieties and lines in test tubes. A total of 90 second instar nymphs were initially released. F_4 26-28, from the cross F_6 324/Akitsuho, are sister lines of the same family.

were not necessarily parallel[5].

Association of resistance and other agronomic characters

In the initial step of plant selection of the primary parental lines, the association between BPH resistance and other agronomic character was noticed in F_2 populations. By the ordinary method of plant selection, based on evaluating several plant characteristics as the criteria of selection, percent of recovery of resistant F_2 plants was much smaller than the theoretically expected. Out of six traits examined, degree of grain sterility was found significantly associated with BPH resistance[1].

The association between BPH resistance and the culm height was also noticed in the B_1F_2 populations of Reiho/F_8 262//Reiho grown in 1976 at Konosu. As seen in Table 2, none of 16 plants of less than 70 cm were homogeneous for the resistance, while none of 15 plants of over 90 cm were homogeneous for the suscep-

Table 2. Association between BPH resistance (*Bph* 1) and culm height in random samples of F_2 plants of Reiho/F_8 262/Reiho

Culm height (cm)	Number of F_2 plants observed and expected (in parenthesis)			
	Bph 1/Bph 1	Bph 1/bph 1	bph 1/bph 1	Total
70 or less	§ 0 (2.5)	7 (9.0)	9 (4.5)	16
71— 80	5 (11.2)	39 (40.5)	§§ 28 (20.2)	72
81— 90	17 (10.0)	37 (36.0)	10 (18.0)	64
91—100	4 (2.3)	11 (8.5)	0 (4.2)	15

chi square for free recombination of resistance and culm height: $x^2=49.18$ ($P<0.005$)

§: category for F_8 262 §§: category for Reiho

Table 3. Characteristics of backcrossed F_1 plants with or without *bph* 2 gene grown in boxes in the greenhouse

Cross No.	No. of pops.	Geno type	No. of F_1 plants tested	Culm length (cm)		Fertile grains per ear		Grein fertility %	Grade of thresh-ability
				$\bar{x}$	s	$\bar{x}$	s		
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
Asominori (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

Rating of threshability: International rice standard

tibilty. Maturity, grain fertility, threshability, grain quality and leaf character (reaction to low temperature in autumn) was not significantly correlated with BPH resistance.

Grain fertility of BC F_1 plants with *Bph 1*, grown in the greenhouse during winter, was noticed to be much lower than japonica parents. For example, resistant segregates of B_2F_1 of F_6 324/Akitsuho[3] yielded only 20% filled grains when Akitsuho produced around 55%. The difference may not be due to the linkage of *Bph 1*, because both of the resistant and susceptible segregates in other crosses had almost same spikelet fertility[3].

Characteristics of resistant and susceptible segregates of BC F_1 populations were also compared in *bph 2* breeding materials in the greenhouse in the same year. As seen in Table 3, plants with or without *bph 2* gene did not show any significant difference in culm length, grain fertility, and threshability[3].

Breeding of isogenic lines on BPH resistance

Pairs of resistant and susceptible sister lines equal with other characteristics are highly valuable for analyzing mechanisms of varietal resistance to insect pests. Two different methods are being undertaken for breeding of such isogenic lines. One is backcrossing, and the other is the successive selection of heterogeneous lines (Fig. 3). For the latter, three generations per year are grown in the greenhouse. At present, eight populations originat ing from F_{10} segregating lines of Hoyoku/Mudgo//Kochikazé are being grown, and several sets of F_{20} isogenic lines will be prepared by 1979 summer season.

So far, two pairs of "nearly isogenic lines" selected at F_8 have been supplied to entomologists and biochemists for various studies on bionomics of BPH, or for analysis of the anti-feeding substance[6] which was found in the barnyard grass, *Echinochloa crus-galli* var. *oryzicola*. The antifeedant aconitic acid, of the barnyard grass was found not to be the chemical factor of BPH resistance in rice.

Problems on biotypes of BPH

Since 1975, breakdown of resistance in varieties became apparent in round-the-year rice growing areas of tropical countries. The process of population build-up of BPH, infestive to the so far resistant varieties, is not yet clarified; but the case may be similar to that of blast.

In Japan, BPH population build-up starts in the fields from adult BPH which migrate in every summer season, and overwintering is

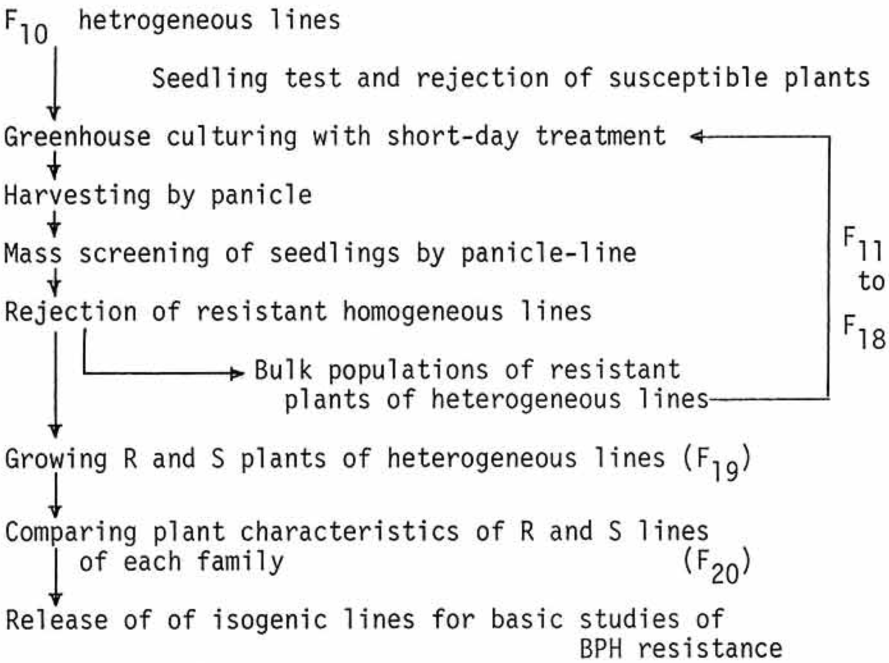

Fig. 3. Method of breeding isogenic lines of BPH resistance by successive selection of heterogeneous lines

Table 4. Growth of a rice seedling caged with a BPH nymph from the coleoptile stage for 9 days; percentage in parenthesis against the uninfested check

Line/ Variety	Second leaf			Seedling height			Leaf age		
	$\bar{x}$	(%)	cv	$\bar{x}$	(%)	cv	$\bar{x}$	(%)	cv
Mudgo	7.9	(92.2)	10.0	12.2	(93.1)	12.5	2.7	(98.5)	8.5
F_8 262	3.4	(92.1)	12.4	6.3	(86.8)	19.8	2.9	(95.3)	8.3
ASD 7	9.8	(94.8)	15.7	12.2	(93.8)	17.5	2.6	(100.4)	5.9
IR 1154	5.6	(86.7)	8.5	7.7	(83.6)	19.7	2.5	(95.8)	8.1
Nihonbaré	3.9	(81.9)	14.8	5.6***	(52.8)	32.9	2.5***	(82.8)	14.0

***: Significantly different from the uninfested control at 0.5% level. All of others are of non-significant difference.

considered negligible as the source of the pest in the following year. Therefore, the development of new biotypes due to the cultivation of resistant varieties may not be probable. However, the monitoring of the immigrating BPH biotypes will be important in the future. For the purpose of monitoring changes in biotype components of BPH populations, it would be essential to develop some method of identifying infestivity of individual insect. The method, when completed, will also contribute much to genetic studies of infestivity of different biotypes.

Preliminary experiments suggest that even a single nymph can exhibit its infestivity to a rice seedling of susceptible varieties, if infestation starts as early as the coleoptile stage. Table 4 shows that only the susceptible check variety suffered retarded growth by the "single nymph caging" treatment in which an unselected colony was used as the tester insect. The greater coefficient of variation means diversified reactions of seedlings due to the sex and wing forms of the caged insect[4)]

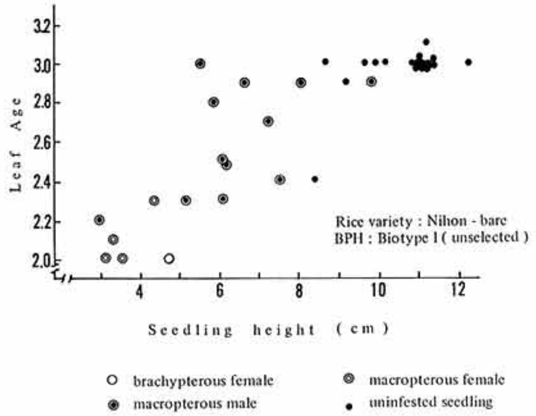

Fig. 4. Differential effect of the sex and wing form of BPH upon the degree of retarted seedling growth of the susceptible variety caged with a 4th instar nymph for 8 days. Sex and wing form at the adult stage of BPH:

(Fig. 4).

The single nymph caging method needs further studies before it can be successfully applied for analysis of biotype components of BPH colonies. Among varieties with the same resistance gene (eg. *Bph 1*, or *bph 2*), those with more tolerance to the feeding damage would not be suitable to be used as discriminative varieties.

The two different colonies are being maintained for more than 20 generations on Mudgo and ASD 7, respectively, by entomologists at Konosu. Their infestivity to varieties with *Bph 1* or *bph 2* will be assessed in the near future.

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