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RFLP Mapping of *Bph-1* (Brown Planthopper Resistance Gene) in Rice

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Summary

Restriction fragment length polymorphism (RFLP) analysis was carried out to determine the location on the chromosome and the locus of the resistance gene to brown planthopper, *Bph-1*, on the linkage map of rice, *Oryza sativa* L.. Our results showed that *Bph-1* was not located on chromosome 4 but on chromosome 12, that is, *Bph-1* was linked at the recombination value of 10.7 % with a RFLP marker *XNpb 248* on chromosome 12. Based on the recombination value between *Bph-1* and RFLP markers, the arrangement was as follows ; *Bph-1* −*XNpb 248* −*XNpb 336*. This is the first report on the tagging of a resistance gene to brown planthopper in rice.

Key Words : ***Oryza sativa*, brown planthopper, RFLP marker, resistance gene, linkage analysis.**

Introduction

Brown planthopper, *Nilaparvata lugens* Stål. (BPH) is one of the most serious pests of rice in the temperate and tropical Asian countries. The use of varieties resistant to BPH is an economical and effective way of controlling the BPH. Already nine BPH resistance genes have been identfied (Athwal *et al.* 1971, Lakshminarayana and Khush 1977, Khush *et al.* 1985, Kabir, M. A. and G. S. Khush 1988, Nemoto *et al.* 1989).In the nine known BPH resistance genes, *Bph-1* gene had been first identified by Athwal *et al.* (1971) and used in the Breeding Program at IRRI and in Asian countries. *Bph-1* was located on chromosome 4 based on trisomic analysis (Ikeda and Kaneda 1983) and by using a marker gene, *d-2* (Ikeda 1985). However, the *Bph-1* locus had not been located on the linkage map in detail. Mapping of BPH resistance genes using RFLP and RAPD markers is very important for marker-based selection in rice breeding. In the present study, we identified several RFLP markers linked to the *Bph-1* locus by the RFLP analysis method. As a result, we were able to determine precisely the location on the chromosome.

Materials and Methods

For this study, the *indica* variety, IR 28, resistant to BPH was crossed with a susceptible *japonica* variety, Koshihikari. IR 28 harbours the *Bph-1* gene derived from TKM 6. IR 28, Koshihikari and the F_3 population were screened for resistance to the BPH biotype 1. The F_2 genotype for the BPH reaction was found to be resistant homozygous or heterozygous and susceptible based on F_3 segregation. DNA was extracted from the young leaves of the parents and each F_3 line by the CTAB method (Murray and Thompson 1980). The total DNA was digested with eight restriction enzymes (*Eco* R I , *Eco* R V , *Bgl* II , *Hind* III , *Bam* H I , *Apa* I , *Dra* I , *Kpn* I). Digested DNA was subjected to electrophoresis in 0.8 % agarose gels and blotted onto a nylon membrane. For Southern hybridization, ECL^{TM} direct nucleic acid labelling and detection systems were used according to the manufacture's instructions (Amersham Corp. UK). Recombination values between *Bph-1* and RFLP markers were calculated by the maximum likelihood method (Allard 1956) and were converted into genetic map distances (cM) using the Kosambi function (Kosambi 1944).

Results and Discussion

In the screening of the BPH resistance, the difference between resistant and susceptible plant is clear. But individuals with a susceptible or intermediate reaction are rarely found in resistant lines. Therefore, we were not able to distinguish between resistant homozygous and heterozygous plant. The F_2 population from the cross Koshihikari/IR 28 segregated into 68 : 24 for resistant homozygous or heterozygous (R + H) and homozygous susceptible(S). This segregation showed a good fit to the expected 3(R+H) : 1 (S) ratio (χ^2=0.058). We first used several markers on chromosome 4, because Ikeda and Kaneda (1983) had reported that the *Bph-1* gene was located on chromosome 4 based on trisomic analysis. Our RFLP analysis showed that the *Bph-1* gene was not linked to 11 markers on chromosome 4. Ikeda (1985) reported that *bph-2* which was closely linked to or was an allele of *Bph-1* was linked at the recombination value of 39.4 % with *d-2* gene. Ideta *et al.* (1994) reported that the *d-2* gene was located on chromosome 1 using RFLP analysis. Therefore, we used several RFLP markers located on chromosome 1. However, the *Bph-1* gene was not linked to the 21 RFLP markers on chromosome 1. Then, we used several RFLP markers located on the

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other 10 chromosomes to obtain the marker linked with *Bph-1*. The results showed that the *Bph-1* gene was linked at the recombination values of 10.7 %, 11.9 %, 11.9 %, 12.1 %, 18.9 %, 30.3 % with *XNpb 248, XNpb 304-1, XNpb 319, XNpb 304-2, XNpb 336, XNpb 316* on chromosome 12, respectively (Table 1 Fig. 1). The recombination values among the RFLP markers are shown (Table 2). Thus, we concluded that the *Bph-1* gene was located on chromosome 12 based on the RFLP analysis. From the recombination values among *Bph-1* and 7 RFLP markers, the 8 loci were arranged as follows ; *Bph-1 −XNpb 248 −XNpb 304-1 −XNpb 319 −XNpb 304-2 −XNpb 336 −XNpb 316 −G 124-1* (Fig.2).

Earlier reports (Ikeda and Kaneda 1983, Ikeda 1985) differ from our results, we think that in trisomic analysis for disease and insects resistance it is possible to make a mistake. Recombination values between *bph-2* and *d-2* of 39.4 % is too distant to be sure the correct chromosome is identified. Marker genes on chromosome 12 were not used in previous reports. We used many RFLP markers on all chromosomes, and were able to find the markers closely linked with *Bph-1* and determine the location of *Bph-1* on chromosome 12.

Ishii *et al.* (1994) first described the tagging of the

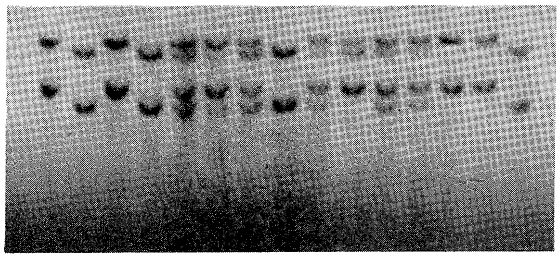

Fig. 1. RFLP pattern in F_3 population of a cross of IR 28 with Koshihikari. The total DNA was digested with *Hind* Ⅲ and probed with *XNpb 248* on chromosome 12. 1 : IR 28, 2 : Koshihikari.

Table 1. Linkage analysis between *Bph-1* and RFLP markers on chromosome 12

Gene Pair A B	Segregation mode in F_3						χ^2 [1]	Recombination value (%)	Genetic map distance (cM)
	A-BB	A-Bb	A-bb	aaBB	aaBb	aabb			
Bph-1 -XNpb 248	20	42	6	0	4	20	54.5(<0.001)	10.7±3.4	10.9±3.4
Bph-1 -XNpb 304-1	19	42	6	0	5	19	48.6(<0.001)	11.9±3.6	12.1±3.6
Bph-1 -XNpb 319	18	42	6	0	5	19	48.6(<0.001)	11.9±3.6	12.2±3.6
Bph-1 -XNpb 304-2	21	40	6	0	5	19	49.1(<0.001)	12.1±3.6	12.3±3.6
Bph-1 -XNpb 336	20	38	9	1	6	17	33.0(<0.001)	18.9±4.5	19.9±4.5
Bph-1 -XNpb 316	21	33	14	2	9	13	12.5(<0.01)	30.3±5.6	35.2±5.6
Bph-1 -G 124-1	18	36	7	2	14	8	6.6(<0.01)	32.7±5.9	38.1±6.0

[1] Calculated based on the ratio of 3 : 6 : 3 : 1 : 2 : 1 (df. 2).

Table 2. Linkage analysis among RFLP markers on chromosome 12

Gene Pair A B	Segregation mode in F_3									χ^2 [1]	Recombination value (%)	Genetic map distance (cM)
	AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb			
XNpb 248 -XNpb 304-1	19	0	0	0	46	0	0	1	25	173.5(<0.001)	0.6±0.6	0.6±0.6
XNpb 248 -XNpb 304-2	19	0	0	2	44	0	0	1	25	166.5(<0.001)	1.7±1.0	1.7±1.0
XNpb 248 -XNpb 316	16	3	1	6	32	8	1	7	18	65.7(<0.001)	16.5±3.0	17.2±3.0
XNpb 248 -XNpb 319	13	6	0	5	36	4	0	5	21	82.8(<0.001)	11.7±2.6	11.9±2.6
XNpb 248 -G 124-1	11	8	0	7	30	5	2	12	10	22.4(<0.001)	24.6±3.9	26.9±3.9
XNpb 248 -XNpb 336	18	1	0	3	40	3	0	3	23	130.0(<0.001)	5.7±1.8	5.7±1.8
XNpb 304-1 -XNpb 316	15	3	1	6	32	9	1	7	17	58.1(<0.001)	17.4±3.1	18.2±3.2
XNpb 304-1 -XNpb 319	12	6	0	5	36	5	0	5	20	74.1(<0.001)	12.6±2.7	12.9±2.7
XNpb 304-1 -G 124-1	10	8	0	7	30	6	2	12	9	17.9(<0.01)	25.9±4.1	28.7±4.1
XNpb 304-1 -XNpb 336	18	1	0	3	40	4	0	3	22	124.5(<0.001)	6.2±1.9	6.3±1.9
XNpb 304-2 -XNpb 316	15	4	2	6	31	8	1	7	17	55.5(<0.001)	18.8±3.3	19.7±3.3
XNpb 304-2 -XNpb 319	14	6	0	3	36	5	0	5	20	82.6(<0.001)	11.3±2.5	11.5±2.5
XNpb 304-2 -G 124-1	10	10	0	7	28	6	2	12	9	16.2(<0.01)	27.4±4.2	30.7±4.2
XNpb 304-2 -XNpb 336	18	3	0	3	38	4	0	3	22	117.3(<0.001)	7.4±2.0	7.4±2.0
XNpb 316 -XNpb 319	11	10	1	6	26	9	1	11	15	29.4(<0.001)	25.2±3.9	27.8±3.9
XNpb 316 -G 124-1	16	5	0	3	35	0	1	10	15	71.2(<0.001)	12.6±2.7	12.9±2.7
XNpb 316 -XNpb 336	17	4	1	4	34	4	0	6	21	94.0(<0.001)	11.6±2.5	11.8±2.5
XNpb 319 -G 124-1	9	9	0	9	28	7	2	13	8	11.8(<0.01)	29.7±4.4	34.2±4.4
XNpb 319 -XNpb 336	12	5	0	8	32	7	0	6	19	60.8(<0.001)	15.8±3.0	16.4±3.0
G 124-1 -XNpb 336	12	5	2	8	32	10	0	3	12	35.2(<0.001)	20.1±3.5	21.3±3.5

[1] Calculated based on the ratio of 1 : 2 : 1 : 2 : 4 : 2 : 1 : 2 : 1 (df. 4).

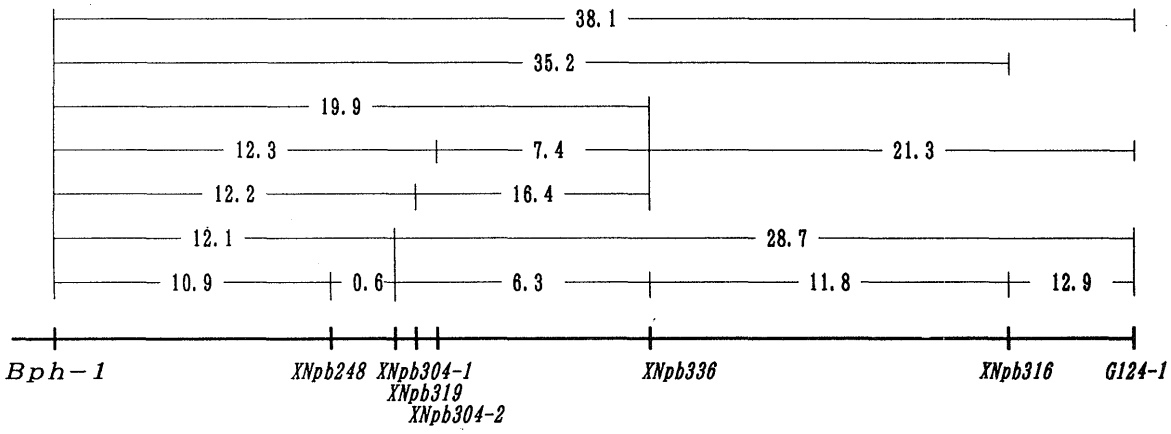

Fig. 2. Arrangement of *Bph-1* and RFLP markers on chromosome 12. The genetic distances are given in c-Morgan units.

resistance gene to the brown planthopper. They showed that the BPH resistance gene introgressed from *Oryza australiensis* was located on chromosome 12. However, our report is the first to identify the locus of a BPH resistance gene. Until now, the nine named genes for BPH resistance had not been tagged. The relationship between the *Bph-1* gene and BPH resistance gene introgressed from *Oryza australiensis* is not clear, because the RFLP markers used were different from each other. Therefore, an allele test should be carried out between the *Bph-1* gene and the resistance gene identified by Ishii *et al.* (1994). For marker-based selection in rice breeding, we will attempt to identify a closely-linked molecular marker to *Bph-1*.

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Literature Cited

Allard, R. W. (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24(10) : 235-278.

Athwal, D. S., M. D. Pathak, E. H. Bacalangco and C. D. Pura (1971) Genetics of resistance to brown planthopper and green leafhopper in *Oryza sativa* L. Crop Science 11 : 747-750.

Ideta, O., A. Yoshimura and N. Iwata (1994) Integration of convention and RFLP linkage maps in rice. V . The locus of ebisu dwarf (*d-2*). Breeding Science. 44 (Suppl.2) : 185. (in Japanese)

Ikeda, R. (1985) Studies on the inheritance of resistance to the rice brown planthopper (*Nilaparvata lugens* Stål) and the breeding of resistant rice cultivars. Bull. Natl. Agric. Res. Cent.3 : 1-54. (in Japanese)

—— and C. Kaneda (1983) Trisomic analysis of the gene *Bph-1* for resistance to the brown planthopper, *Nilaparvata lugens* Stål., in rice. Japan. J. Breed. 33 : 40-44.

—— and —— (1981) Genetic analysis of resistance to brown planthopper, *Nilaparvata lugens* Stål., in Rice. Japan. J. Breed. 31 : 279-285.

Ishii, T., D. S. Brar, D. S. Multani, and G. S. Khush (1994) Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice *O. sativa*. Genome 37 : 217-221.

Kabir, M. A. and G. S. Khush (1988) Genetic analysis of resistance to brown planthopper in rice, *Oryza sativa* L. Plant Breeding 100 : 54-58.

Khush, G. S., A. N. M. Rezaul Karim and E. R. Angeles (1985) Genetics of resistance of rice cultivar ARC10550 to Bangladesh brown planthopper biotypes. J. Genet. 64 : 121-125.

Kosambi, D. D.(1944) The estimation of map distances from recombination values. Ann. Eugen. 12 : 172-175.

Lakshminarayana, A. and G. S. Khush (1977) New genes for resistance to the brown planthopper in rice. Crop Science 17 : 96-100.

Murray, M. G. and W. F. Thompson (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8 : 4321-4325.

Nemoto, H., R. Ikeda and C. Kaneda (1989) New genes for resistance to brown planthopper, *Nilaparvata lugens* Stål, in Rice. Japan. J. Breed. 39 : 23-28.