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Trisomic analysis of the gene *Bph 1* for resistance to the brown planthopper, *Nilaparvate lugens* Stål., in rice

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Studies were undertaken to locate the gene *Bph 1* on the linkage map of rice. The trisomic lines obtained from Kyushu University were crossed with 'Kanto PL 1' or 'Kanto PL 4', which were the parental lines of japonica type rice resistant to the brown planthopper, and had *Bph 1* derived from 'Mudgo'. Segregation ratios observed in the F_2 generation of four crosses with trisomic A, C, G and H proved to be of the disomic type. But segregation ratio in the cross with trisomic E carrying the extra chomosome of 11th did not fit a disomic ratio. The 11th chromosome corresponds to the linkage group II. Among marker genes belonging to the linkage group II, *lg* and *d-11* are independent of *Bph 1*, and *lg* and *Pl-1* are also independent of *bph 2* (allelic to, or closely linked with *Bph 1*). However, it was found that *bph 2* linked with *d-2* of the linkage group II at the recombination value of 39.4%.

KEY WORDS: *Oryza sativa*, brown planthopper resistance, trisomic analysis, linkage.

Introduction

The brown planthopper, *Nilaparvata lugens* Stål., (abbr. BPH) is a most serious pest on rice throughout Asia. Since a highly resistant cultivar 'Mudgo' was found at IRRI in 1967, the breeding of rice for resistance to this pest has been going on vigorously at breeding stations in the Philippines, Japan, Taiwan, India, Korea and others.

The genetic studies of resistance to BPH were initiated by ATHWAL *et al.* (1971). They reported that *Bph 1* controlled the resistance in 'Mudgo', *bph 2* in 'ASD 7', and the two genes were closely linked or allelic. Then, LAKSHMINARAYANA and KHUSH (1977) identified *Bph 3* and *bph 4*, which were independent of *Bph 1* and *bph 2*, respectively. SIDHU and KHUSH (1979) reported that *Bph 3* was closely linked or allelic to *bph 4* as well as the relation between *Bph 1* and *bph 2*. Recently, IKEDA and KANEDA (1981) studied allelic relationships among these four resistance genes, and discussed the possibility of combining two or more resistance genes to BPH in a cultivar.

However, regarding to the loci of these resistance genes, there were only a few reports by this time. HEU and SUH (unpublished data, cited from CHOI *et al.*, 1979) reported that segregation mode of *Bph 1* did not seem to be independent of *I-Bf* and *Ps* (purple stigma) belonging to the linkage group V. IKEDA and KANEDA (1981) identified the locus or loci of *Bph 3* and *bph 4* on the chromosome 7 judging from the trisomic analysis.

The authors have been conducting the linkage analysis of *Bph 1* and *bph 2* since 1973. However, until now they could not find the clear linkage relationships between *Bph 1* or *bph 2* and any marker genes.

This paper reports the chromosome on which the *Bph 1* gene is located, estimated from

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the trisomic analysis, and the linkage relationships between *Bph 1* or *bph 2* and the marker genes on that chromosome.

Materials and Methods

'Kanto PL 1' and 'Kanto PL 4' are parental lines of japonica type rice carrying the *Bph 1* gene derived from 'Mudgo' (KANEDA *et al.*, 1979, 1980). Nine trisomic lines used in this experiment were obtained from Kyushu University (IWATA and OMURA, 1975, 1976).

Nine trisomic lines were pollinated by 'Kanto PL 1' or 'Kanto PL 4'. All F_1 plants were transplanted in 1/5000 a Wagner pots in a greenhouse.

Anthers of each F_1 plants were examined under the microscope at the metaphase I of meiosis to determine whether or not it was trisomic. Panicles of trisomic plants were covered with glassine paper bags to avoid outcrossing and were self-pollinated. Then the F_2 population from each trisomic F_1 plants was tested by the bulk seedling method of BPH (PATHAK *et al.*, 1969, KANEDA, 1975). At the time of test for resistance to BPH, nymphs of the biotype I (wild type) were used. Segregation ratios were observed for trisomic as well as disomic controls in the F_2. When the BPH resistance gene is not located on the extra chromosome of a trisomic, a disomic ratio of 3 : 1 is observed for both F_2 populations from disomic and trisomic F_1 plants. Disomic and trisomic portions of the F_2 populations were investigated in the lump for segregation of BPH resistance.

Results

The segregation ratios in F_2 of crosses between five trisomic lines and 'Kanto PL 1' or 'Kanto PL 4' together with the segregation ratio in the disomic control are shown in Table 1. Although nine trisomic lines were crossed with 'Kanto PL 1' or 'Kanto PL 4',

Table 1. F_2 segregations for resistance to the brown planthopper in the crosses between trisomic lines and *Bph 1* lines, 'Kanto PL 1' or 'Kanto PL 4'

Parent or Cross	Number of seedlings			X^2 for	Linkage	Extra
	Res.	Sus.	Total	3 : 1	group	chromosome
Trisomic A, Parent	0	17	17		?	4
Trisomic C, Parent	0	17	17		XII	7
Trisomic E, Parent	0	17	17		II	11
Trisomic G, Parent	0	17	17		VIII	9
Trisomic H, Parent	0	17	17		V, VII	1
Kanto PL 1, Parent	17	0	17			
Kanto PL 4, Parent	17	0	17			
Trisomic A/Kanto PL 1, F_2	906	292	1,198	0.251		
Trisomic C/Kanto PL 4, F_2	621	188	809	1.339		
Trisomic E/Kanto PL 4, F_2	337	182	519	28.055***		
Trisomic G/Kanto PL 4, F_2	678	222	900	0.053		
Trisomic H/Kanto PL 1, F_2	263	91	354	0.095		
Control (disomic), F_2	981	350	1,331	1.192		

*** : significant at 0.1% level.

Table 2. Linkage relations between *bph 2* and the marker genes belonging to the second linkage group in a cross between Fl 202 (*Pl-1*, *lg*, *d-2*) and F_4 203 (*bph 2*)

Segregations of the genes in F_2	Reaction to BPH in F_3				Goodness of fit				R. C. V.	Goodness of fit	
	Res.	Seg.	Sus.	Total	Item	Ratio	d. f.	X^2		d. f.	X^2
Pl-1	46	101	51	198	BPH (*bph 2*)	1 : 3	1	0. 667			
+	10	25	13	48	*Pl-1*	3 : 1	1	3. 951*			
					Independence		2	0. 133			
Total	56	126	64	246	Total	3:6:3:1:2:1	5	4. 666			
+	45	85	47	177	BPH	*ibid.*					
lg	11	41	17	69	*lg*	1 : 3	1	1. 220			
					Independence		2	3. 290			
Total	56	126	64	246	Total	3:6:3:1:2:1	5	5. 448			
+	51 (52. 0)	97 (93. 6)	45 (38. 9)	193	BPH	*ibid.*					
d-2	2 (9. 5)	29 (29. 4)	19 (22. 6)	53	*d-2*	1 : 3	1	1. 567			
					Independence		2	7. 945*			
Total	56	126	64	246	Total	3:6:3:1:2:1	5	8. 743	39. 4	5	3. 810

( ) : Expected number calculated from R. C. V. (Recombination value=39. 4).

F_2 populations of only five crosses were used for the test of resistance to BPH. In the other four crosses, the trisomic plants could not be secured in a few F_1 individuals.

The data given in Table 1 indicate that the segregation ratios fit very well to the disomic ratio of 3 : 1 in trisomic types for A, C, G and H lines, showing that the gene *Bph 1* is not associated with any of these four chromosomes.

However, in the cross with the trisomic E type, the segregation for BPH resistance did not fit to a 3 : 1 ratio, but showed the trisomic segregation. This indicates that *Bph 1* is located on the extra chromosome of the trisomic E line. According to IWATA and OMURA (1975), the trisomic E line has the extra chromosome of 11 th. The chromosome 11 corresponds to the linkage group II. Therefore, it is estimated that *Bph 1* gene belongs to the linkage group II.

The authors have been carrying out the linkage analysis of *Bph 1* and *bph 2* since 1973. However, they have never found any marker genes decisively linked with *Bph 1* or *bph 2*. Regarding to the linkage group II, it had been estimated that *Bph 1* was independent of *lg* (liguleless) and *d-11* (shinkane-aikoku dwarf).

Accordingly, it needs to analyze the linkage relationships between *Bph 1* or *bph 2* and the other marker genes belonging to the linkage group II. The F_2 segregation ratios in the cross between Fl 202 and F_4 203 are shown in Table 2. Fl 202, a marker line of Kyushu University, has *Pl-1* (purple leaf), *lg* and *d-2* (ebisu dwarf) belonging to the linkage group II. F_4 203 is a BPH resistant line derived from the cross of Asominori/IR 1154//2*Asominori, and homogeneous for *bph 2*. According to Table 2, *bph 2* is linked with *d-2*, but not with *Pl-1* and *lg*. The recombination value between *bph 2* and *d-2* was calculated to be 39. 4% by Immer's productive ratio method.

Discussion

According to the authors' results (KANEDA and IKEDA, 1976, IKEDA and KANEDA, 1977)

and unpublished data, *Bph 1* was independent of *dp-1* (depressed palea), *ws* (white stripe) and *Cl* (clustered spikelet) belonging to linkage group I, *lg* and *d-11* (II), *Rc* (brown pericarp with reddish speckels) and *g* (long empty glume) (IV), *gh-1* (gold hull & internode) (VI), *Dn-1* (dense panicle), *drp-2* (dripping-wet leaves) and *dp-2* (depressed palea) (VII), *z* (zebra stripe), *la* (lazy), *sp* (short panicle) and *dt* (tillering dwarf) (VIII), *tri* (triangular hull), *dw* (waisei-shirasasa dwarf) and *bl-1* (brown discoloration of leaves & glumes) (X), *ch* (chrolina), *dl* (drooping leaf) and *spl-3* (spotted leaf) (XI), and *gl-1* (glabrous leaf & hull) (XII), respectively. On the other hand, *bph 2* was independent of *dp-1* and *Cl* (I), *lg* and *Pl-1* (II), *Pn* (purple node) and *lax* (lax panicle) (III), *I-Bf* (inhibitor for brown furrow of glume) (V) and *gh-1* (VI), respectively.

The locus of *d-2* is the terminal of the linkage group II and the loci of *Pl-1* and *lg* are apart at a distance of 61 and 92 units from *d-2*, respectively (NAGAO and TAKAHASHI, 1963). Although *bph 2* is linked with *d-2* at the recombination value of 39.4%, it is natural that *bph 2* segregates independently with *Pl-1* or *lg*.

HEU and SUH (unpublished data, cited from CHOI *et al.*, 1979) reported that segregation mode of *Bph 1* did not seem to be independent of *I-Bf* and *Ps* (purple stigma) belonging to the linkage group V. In our unpublished data, *bph 2* was independent of *I-Bf* as mentioned above. Moreover, as shown in Table 1, *Bph 1* showed a disomic segregation ratio in the cross with the trisomic H line, which has the extra chromosome belonging to the linkage group V. SHRESTHA (1981) also reported that no linkage relationship was found between *Bph 1* and *I-Bf*.

The authors were fortunate enough to identify the chromosome carrying *Bph 1* gene, by analyzing crosses with only five trisomic lines. In the near future, we expect to analyze the linkage relationships with the other marker genes *e. g. d-3* (tillering dwarf) in the linkage group II.

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イネのトビイロウンカ抵抗性遺伝子 *Bph 1* のトリソミック分析

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イネのトビイロウンカ抵抗性遺伝子 *Bph1* の所属連鎖群を同定するために，九州大学から譲り受けたトリソミック系統に *Bph1* をもつ中間母本系統関東 PL1 または関東 PL4 を交配した。得られた F_1 株の花粉母細胞減数分裂の第一分裂中期における分裂像を検鏡してトリソミックと確認後，袋かけして異花粉の混入を防ぎ，各 F_1 株ごとに採種した。

F_2 集団の各個体については，検鏡観察によるダイソミックとトリソミックの2群に分けることなく，一括してトビイロウンカ抵抗性検定に供試した。抵抗性の検定は，バイオタイプI（野生型）のウンカを用い，集団幼苗検定法によった。

Bph1 は，岩田・大村（1975, 76）によるトリソミックA，C，GおよびH型の4系統との交配組合せの F_2 ではいずれも 3：1 の比に適合し，ダイソミックの分離を示したが，E 型系統との交配組合せでは 3：1 の比に適合せずトリソミックの分離を示した。したがって，*Bph1* はE型系統の過剰染色体である第 11 染色体に座乗するものと推定された。第 11 染色体に対応するのは第II連鎖群である。

筆者らは，1973 年以来標識遺伝子利用による *Bph1* または *bph2* の連鎖分析を実施してきたが，11 の連鎖群（第IX連鎖群は未検定）に所属する 25 の標識遺伝子とはいずれも独立の関係にあった。第II連鎖群に関しても *lg* および *d-11* と *Bph1* とは独立と推定されている．しかし，ここでトリソミック分析の結果を踏まえて再度 *bph2*（*Bph1* と密接連鎖か複対立の関係）と第II連鎖群所属の3遺伝子との連鎖について分析したところ，*bph2* は *lg* や *Pl-1* とは独立と推定されたものの *d-2* とは組換価 39.4% で連鎖していると推定された。