Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*

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Abstract **Wild rice species is an important source of useful genes for cultivated rice improvement. Some accessions of *Oryza eichingeri* (2*n* = 24, CC) from Africa confer strong resistance to brown planthopper (BPH), whitebacked planthopper (WBPH) and bacterial blight (BB). In the present study, restriction fragments length polymorphism (RFLP) and simple sequence repeats (SSR) analysis were performed on disomic backcross plants between *Oryza sativa* (2*n* = 24, AA) and *O. eichingeri* in order to identify the presence of *O. eichingeri* segments and further to localize BPH-resistant gene. In the introgression lines, 1—6 *O. eichingeri* segments were detected on rice chromosomes 1, 2, 6, or/and 10. The dominant BPH resistant gene, tentatively named *Bph13*(t), was mapped to chromosome 2, being 6.1 and 5.5 cM away from two microsatellite markers RM240 and RM250, respectively. The transfer and localization of this gene from *O. eichingeri* will contribute to the improvement of BPH resistance in cultivated rice.**

Keywords: cultivated rice, *Oryza eichingeri*, brown planthopper resistance, gene mapping.

Rice brown planthopper (BPH, *Nilaparvata lugens* Stal.) is one of the most destructive insect pests in Asian rice planting area. It has been causing serious yield reduction by directly damaging rice plants and by transferring various diseases such as rice grass stunt. The utilization of resistant gene(s) has been recognized as the most economic and effective measure for BPH management. So far, 14 BPH resistance genes have been reported[1—7], of which *Bph1*, *bph2*, *Bph3*, *bph4*, *Bph9*, *Bph10*(t), *bph11*(t), *bph12* (t), *Bph*(t) and *bph*(t) were mapped on chromosomes 3, 4, 6, 9 and 12[1,2,6,7], respectively. By using traditional and molecular methods *Bph1*, *bph2*, *Bph3* and *bph4* have been used in rice breeding program. Nevertheless, in order to cope with the variation of BPH biotypes, it is needed to find new BPH resistance genes. Wild species of *Oryza* have many useful genes for rice improvement, and it is possible to transfer these genes by wide hybridization techniques. In fact, a number of BPH resistance genes in *O. australiensis* (2*n* = 24, EE)[6] and *O. officinalis* (2*n* = 24, CC)[7,8] have been identified and transferred into cultivated rice. Some accessions of *O. eichingeri* (2*n* = 24, CC) from Uganda confer strong resistance to BPH[9]. In the present study, a set of 67 introgression lines derived from *O. sativa*-*O. eichingeri* hybrids was analyzed to evaluate the possible amount and distribution of *O. eichingeri* chromatin. Furthermore, a segregation population including 90 plants was constructed from a heterozygous resistant plant and used to localize a BPH resistant gene from *O. eichingeri*.

1 Materials and methods

(ⅰ) Plant materials. One accession of *O. eichingeri* from Uganda, acc105159, kindly provided by the International Rice Germplasm Center , IRRI, was used as the donor of resistance to BPH and white backed planthopper (WBPH); while 02428, a BPH susceptible cultivated rice variety with wide compatibility, was used as the recurrent parent. 02428 was hand emasculated and pollinated with pollens from the accession of *O. eichingeri*. By culturing 11—13-d old embryo, 15 F_1 plants were finally obtained. Both F_2 and BC_1F_1 plants, cytologically identified as allotriploids (2*n* = 36, AAC), were backcrossed to 02428. From the backcross progenies 184 diploid plants (2*n* = 24) were isolated. After being evaluated for BPH resistance, 67 introgression lines including 10 conferring BPH resistance were identified and further analyzed for identifying the amount and distribution of introgressed segment(s) in the cultivated rice background. Meanwhile, a BPH resistant introgression line, 960044-112, was selfed to produce a segregation population of 90 plants for linkage analysis of the BPH resistance gene with molecular markers.

(ⅱ) Screening for BPH resistance. The resistance of rice plants to BPH biotypes 1 and 2 was evaluated in the China National Rice Research Institute in Hangzhou for three times in February and July, 1997 and October, 1999, as described by Fu et al.[10]. The tested plants included the recurrent parent 02428, acc105159, F_1 hybrids, 67 introgression lines and the individual plants of the mapping population derived from selfed introgression line 960044-112. BPH resistance ratings were made using a 0—9 rating scale. Rice varieties Mudgo and ASD7 were used as resistant control, while TN1 as susceptible control.

(ⅲ) DNA extraction. Total DNAs from the recurrent parent 02428, donor parent *O. eichingeri*, introgression lines and individual plants of the segregation population were extracted from young leaves according to McCouch et al.[11] with minor modifications. Total DNA was digested with restriction enzymes, *Eco*RⅠ, *Eco*RⅤ, *Hin*dⅢ, *Bam*HⅠ and *Dra*Ⅰ. Southern blot and hybridization were done as described by McCouch et al.[11]. A total of 227 RFLP probes were kindly provided by Dr. McCouch of Cornell University, USA and Rice Genome

Project (RGP), Japan. 16 SSR primer pairs were synthesized by Sangon company, China, according to the sequences designed by Temnykh and Panaud et al.[12,13]. PCR reaction was done as described by Panaud et al.[13]. The PCR production was electrophoresed on a 4% agarose gel, stained with ethidium bromide (EB), and recorded on ISO 100 Kodak film.

(iv) Linkage analysis. Cosegregation of RFLP and SSR markers with BPH resistance was analyzed by using MAPMAKER/3.0 software to determine the linkage relationships between them[14]. The Kosambi function was transformed into Centimorgan (cM).

2 Results and analysis

(i) Genetic analysis of BPH resistance. The recurrent parent, donor parent and their F_1 hybrid, 67 introgression lines and individual plants of the segregation population were screened for BPH resistance. acc105159 and F_1 were highly resistant (0 and 1) while the recurrent parent was susceptible (9), indicating that BPH resistance from *O. eichingeri* was under dominant gene control. The resistance rating in the mapping population performed bimodal distribution. Except for 8 plants in which the BPH reaction results were inconsistent in three evaluations, 61 plants were resistant (1—5) while 21 plants were susceptible (7—9). The results showed a good fit ratio to the expected 3∶1 ($\chi^2 = 0.024$). Therefore, there should be a major dominant gene with modifiers governing the BPH resistance.

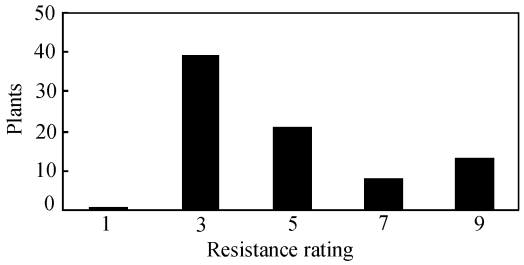

Fig. 1. Distribution of disease rating in mapping population.

(ii) RFLP analysis of introgression lines. A total of 164 RFLP probes were selected to survey polymorphisms between *O. eichingeri* and cultivated rice variety. Of them 150 (91.5%) probes were polymorphic, indicating that great genetic differentiation exists in the two species. Then the above 150 probes were used to analyze 67 introgression lines in order to monitor alien introgression. Among the probes, 6 (RG236, R210 on chromosome 1; C601, G275 on chromosome 2; G89-2B on chromosome 6 and RG752 on chromosome 10) produced *O. eichingeri* specific band pattern in 60 introgression lines, indicating that some *O. eichingeri* segments introgressed into 02428 chromosomes[12,15,16] (fig. 2). Meanwhile some probes disclosed new bands in some introgression lines which were different from those in the two parents. In 10 BPH resistant introgression lines, 1—5 probe(s) produced *O. eichingeri* specific band pattern, respectively. The *O. eichingeri* derived segments were integrated into chromosomes 1, 2, 6 and 10 of 02428. The introgressed alien segments were small, and usually detected by only one probe. On the long arm of chromosome 2, two probes, G275 and C601, were positive, namely both of them showed *O. eichingeri* specific band pattern. Four probes, including G275 and C601 on chromosome 2, G89-2B on chromosome 6 and RG752 on chromosome 10 (fig 2), were positive in the BPH resistant introgression line, 960044-112, which was used as the parent of the mapping population for BPH resistance. It is reasonable that the BPH resistance gene might reside in the detected introgressed segments though we could not determine which chromosome region contains the resistance gene. However, considering that the amount and distribution of the used probes on rice chromosomes were rather limited, some introgressions might occur in the regions where no probe was available.

(iii) Tagging the BPH resistant gene. According to the above RFLP analysis of introgression lines, we chose other 63 RFLP probes around G275, C601, G89-2B and RG752 on chromosomes 2, 6 and 10, respectively, for linkage analysis in the segregation population from the selfed 960044-112 line, to find possible cosegregation markers for the BPH resistance. The results showed that G275, C601, C424, RG256, RG654 and C560 on the long arm of chromosome 2 were related to the resistance, of which G275 linked with BPH resistant gene with a distance of 12.0 cM (fig. 3(a), (c)). Then 16 SSR primer pairs on chromosome 2 were further used to tag the BPH resistant gene. Eleven SSRs were polymorphic between *O. eichingeri* and 02428. And in the mapping population, RM250, RM240, RM6 and RM263 showed significant cosegregation with the BPH resistance. Finally, the resistance gene was located between RM250 and RM240 with a distance of 5.5 and 6.1 cM, respectively (fig. 3(b), (c)). Among the 14 known BPH resistant genes, *bph*(t) and *bph11*(t) are on chromosome 3[1,7], *Bph3* and *bph12* (t) on chromosome 4[1,7], *bph*4 on chromosomes 4[1] or 6[2], *Bph*(t) on chromosome 9[1], *Bph1*, *bph2*, *Bph9* and *Bph10* (t) on chromosome 12[1,2,6]. Meanwhile, *bph5*, *Bph6* and *bph7* only confer resistance to BPH biotype 4[6]. Therefore, the *O. eichingeri* derived BPH resistance gene on chromosome 2 should be nonallelic to these genes. It is temporarily named *Bph13*(t).

3 Discussion

Wild species of rice are an important reservoir for providing useful genes and broadening hereditary basis of cultivated rice. The difficulty of transferring useful genes from the wild species with non-AA genomes to cultivated

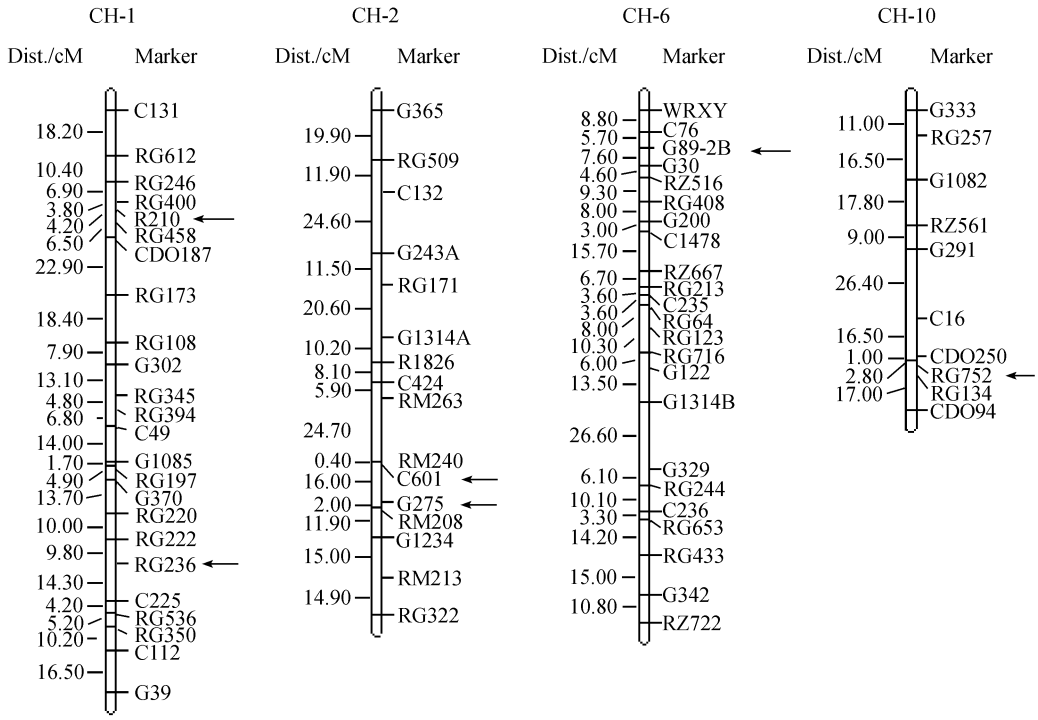

Fig. 2. RFLP map for 4 rice chromosomes on which *O. eichingeri* chromatin was detected. Numbers on the left and right of the chromosome represent distance and clone designation, respectively. The probes which detected introgression occurring were arrowed.

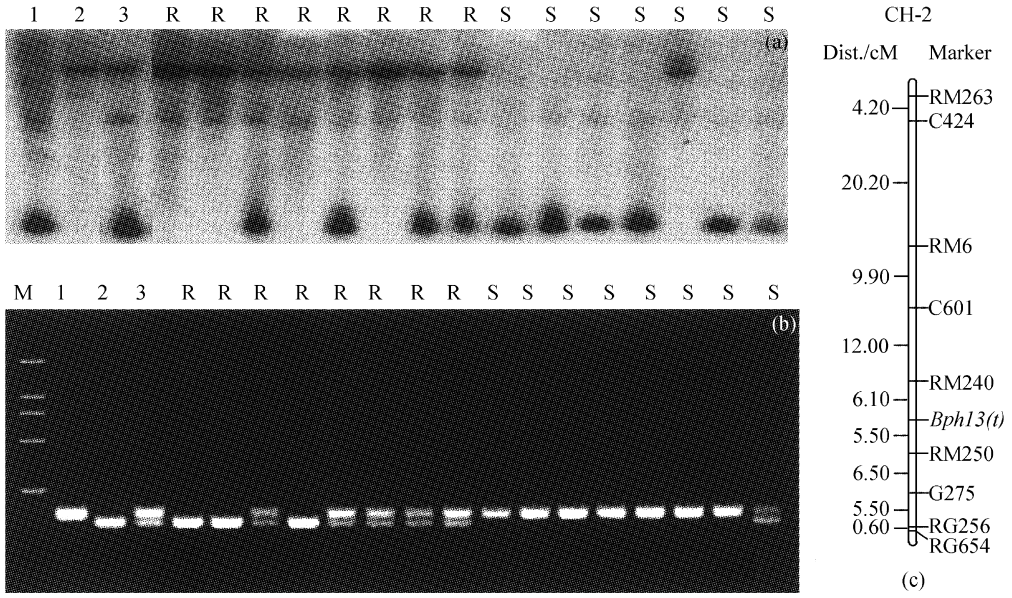

Fig. 3. (a) RFLP pattern in segregation population of BPH resistant introgression line selfed. Total DNA was digested with *Bam*HⅠ and probed with G275 on chromosome 2. 1, Susceptible parent; 2, resistant parent; 3, 960044-112; R, resistant individuals; S, susceptible individuals. (b) Gel photograph showing SSR analysis with primer RM250 on chromosome 2 in segregation population of BPH resistant introgression line selfed. 1, Susceptible parent; 2, resistant parent; 3, 960044-112; R. resistant individuals; S, susceptible individuals. (c) Molecular mapping of rice chromosome 2 showing the location of the *Bph13*(t) gene.

rice lies in cross unfruitfulness, hybrid sterility and very few recombination between chromosomes of wild and cultivated rice species. With embro rescue and other techniques, we overcome the difficulty and got introgression lines from *O. eichingeri*. RFLP probes were used for monitoring alien chromosome segments introgressed into 67 introgression lines including 960044-112, the parent of the mapping population. And the amount, size, and distri-

bution of introgressed segments from *O. eichingeri* were determined. By using more RFLP and SSR markers around the introgression occurring regions, we found the markers which were possibly linked with the BPH resistant gene. Of the 6 probes which detected introgression, G275 and C601 of chromosome 2 both produced *O. eichingeri* specific band pattern. So we deducted that it might be a relatively big introgressed segment. In the mapping population, RFLP probes including L629, R685, C370, C932, C679 and C1119[15] between C601 and G275 could not detect any introgression occurring. Therefore introgression on the long arm of chromosome 2 was not one introgressed segment detected by both C601 and G275.

In the offspring of interspecific crosses between wild and cultivated rice, new band patterns different from those of the parents were often detected. Brar and Khush[17] attributed this to the interaction between wild and cultivated genomes or/and activation of transposon elements through interspecific cross. In the present study, C424, RM240 and RM263 also disclosed new band in introgression lines, but it was noted that their segregation was as normal as other probes that could reveal *O. eichingeri* specific band pattern. There were some of these probes cosegregating with the BPH resistance.

According to Maxwell[18], the insect resistance was usually governed by a single gene, or a major gene with modifiers and mutigene. Jena et al.[8] considered that the BPH resistance from *O. officinalis* was governed by a single dominant gene while Hirabayashi[7] mapped two recessive genes from the same species. Murai[2] even found *bph2* could be dominant or recessive in different *indica* and *jopanica* genetic backgrounds. Heinrichs et al.[9] reported that the BPH resistance of IR36 was controlled by a master gene (*bph2*) and some polygenic genes. Alam et al.[19] analyzed the BPH resistance of IR64 by mapping quantitive trait locus (QTL) and identified 7 resistance related QTLs on 6 chromosomes with phenotypic contributions from 5.0% to 16.6%, respectively. In the present study, *O. eichingeri* and its F_1 showed strong resistance to BPH biotypes 1 and 2. The segregation ratio was fit to 3∶1 in the segregation population. So the *O. eichingeri* derived resistance should be governed by a major dominant gene. Because of the modifier effects obviously existing in the mapping population, we also tried mapping QTLs for the BPH resistance. It is notable that only one QTL was detected in the location of *Bph13*(t), which could account for 90% of the phenotypic variation (data not shown).

BPH is a destructive insect in rice production. Here we successfully mapped a BPH-resistant gene that was transferred from *O. eichingeri* into an elite rice variety. This resistance gene with its tagging markers could be effectively used as a new genetic resource of BPH resistance in the future rice breeding programs. We will enlarge the segregation population for its fine genetic mapping and map-based cloning.

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