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# RFLP analysis of rice (*Oryza sativa* L.) introgression lines

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**Summary.** Fifty-two introgression lines ($BC_2F_8$) from crosses between two *Oryza sativa* parents and five accessions of *O. officinalis* were analyzed for the introgression of *O. officinalis* chromosome segments. DNA from the parents and introgression lines was analyzed with 177 RFLP markers located at approximately 10-cM intervals over the rice chromosomes. Most probe/enzyme combinations detected RFLPs between the parents. Of the 174 informative markers, 28 identified putative *O. officinalis* introgressed chromosome segments in 1 or more of the introgression lines. Introgressed segments were found on 11 of the 12 rice chromosomes. In most cases of introgression, *O. sativa* RFLP alleles were replaced by *O. officinalis* alleles. Introgressed segments were very small in size and similar in plants derived from early and later generations. Some nonconventional recombination mechanism may be involved in the transfer of such small chromosomal segments from *O. officinalis* chromosomes to those of *O. sativa*. Some of the introgressed segments show association with genes for brown planthopper (BPH) resistance in some introgressed lines, but not in others. Thus, none of the RFLP markers could be unambiguously associated with BPH resistance.

**Key words:** Rice – *Oryza sativa* – *Oryza officinalis* – Introgression – RFLP – Wide cross – DNA probes

## Introduction

Rice (*Oryza sativa* L.) is the staple food of 40% of the world's population. It is grown worldwide under many different agro-climatic conditions. Rice yield has increased dramatically in recent years by the development of non-lodging, semi-dwarf varieties with genes for resistance to diseases and insects introgressed from traditional rice varieties by conventional plant breeding methods. However, changes in insect biotypes and disease races is a continued threat to increased rice production (Khush 1984). Therefore, the wild germ plasm of *Oryza* has been screened, and several accessions of the wild species have been identified as resistant to diseases and insects (Heinrichs et al. 1985). The wild species of *Oryza* with an AA genome similar to that of *O. sativa* constitute the primary gene pool. Major genes for resistance to grassy stunt virus have been transferred from *O. nivara* and for bacterial blight from *O. longistaminata* to cultivated rice by conventional plant breeding methods (Khush et al. 1977, 1990). The other wild species have BB, CC, BBCC, CCDD, EE, or FF genomes, defined on the basis of chromosome pairing in hybrids. This group of species makes up the secondary gene pool. *Oryza officinalis*, which has a CC genome, is highly resistant to brown planthopper (BPH), white backed planthopper (WBPH), and bacterial blight (BB). Genes for resistance to these diseases and insects were introgressed from *O. officinalis* into three susceptible breeding lines of cultivated rice by interspecific hybridization (Jena and Khush 1986, 1990).

Recently developed restriction fragment length polymorphism (RFLP) techniques offer a tool to monitor alien gene introgression, to carry out marker-based selection of desirable genes in plant breeding programs, and to clone genes for which the product is not currently known (Beckman and Soller 1986; Neinhuis et al. 1987; Landry et al. 1987; Tanksley et al. 1989; Paterson et al. 1988). RFLP analysis in rice for tagging disease and insect resistance genes has already begun using pairs of near-isogenic lines (McCouch et al. 1991; Yu et al. 1991), but no systematic study on a molecular basis has been made of introgression in any wide cross.

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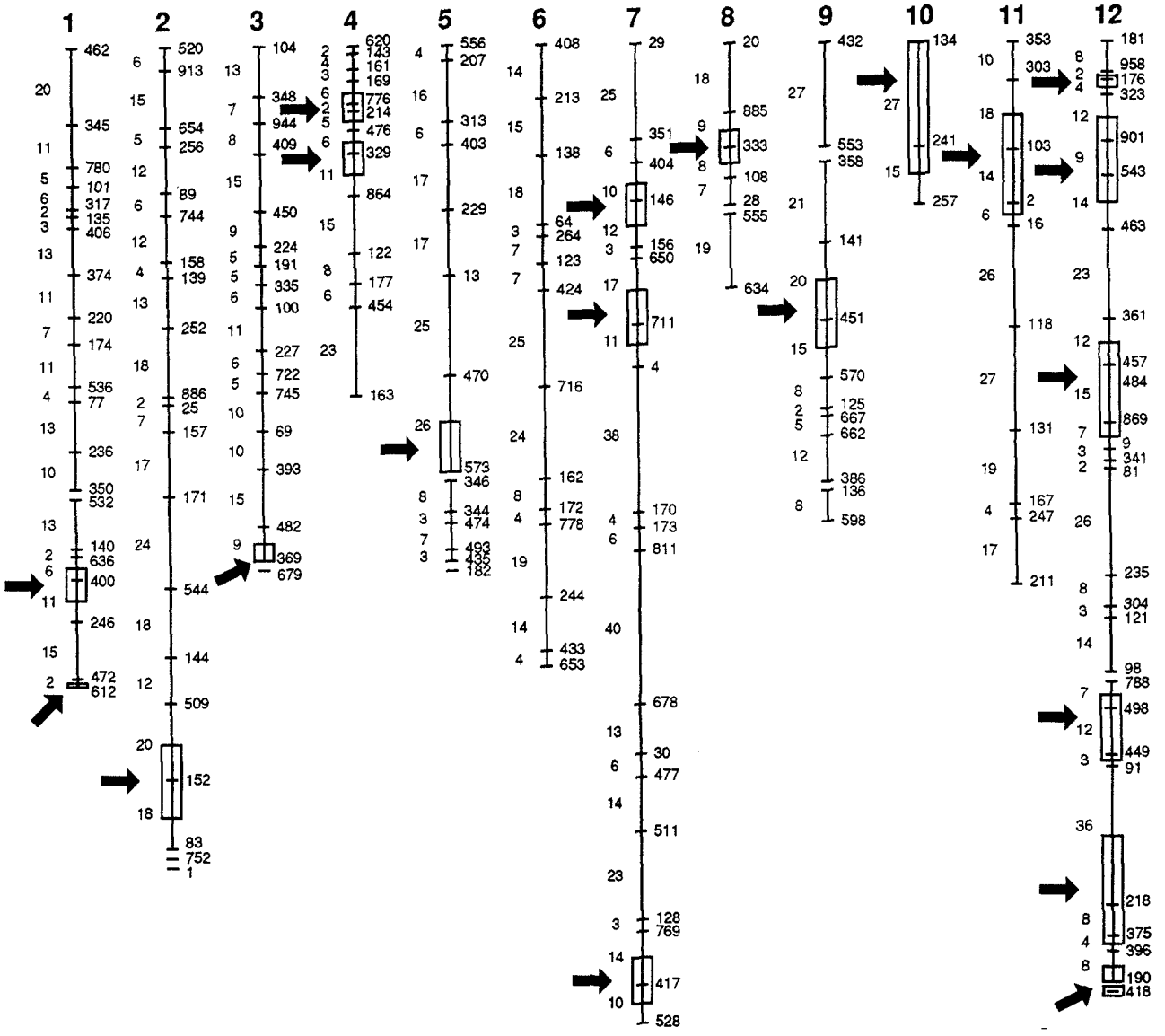


**Fig. 1.** RFLP map showing markers used in this study. Segments introgressed from *Oryza officinalis* introgression lines are identified by *boxes* and *arrows*

In this study, we report the RFLP analysis of 52 introgression lines from crosses between *O. sativa* and *O. officinalis* using a set of molecular markers (RG probes) with previously mapped chromosomal positions to detect the amount of introgression at the molecular level and to obtain insight into the mechanism of introgression.

## Materials and methods

*Plant materials*

Fifty-two $BC_2F_8$ introgression lines and their parents (Jena and Khush 1990) were used (Table 1). The original *O. sativa* parent plants were no longer available, therefore single plants grown from the same seed lot were used. The *O. officinalis* parents are perennial, so the original plants used in the crosses were sampled. Two different *O. sativa* parents and five different *O. officinalis* accessions were used in the crossing program. Each introgression line was produced from a single plant from 1 of 34 $BC_2F_3$ families, which was subsequently self-pollinated and selected for five more generations. Selection was for insect resistance and general agronomic qualities. The introgression lines have varied reactions to three BPH biotypes and appear to have inherited several monogenic traits from *O. officinalis* (Jena and Khush 1990).

*RFLP analysis*

One hundred and seventy-seven RFLP markers distributed across the 12 rice chromosomes were selected for analysis. These clones, originally selected from a *PstI* genomic library (McCouch et al. 1988), were provided by Dr. S. D. Tanksley of Cornell University, New York, USA (Fig. 1). Genomic DNA extraction, restriction endonuclease digestion, and Southern analysis were

**Table 1.** Introgression lines used for RFLP analysis

| Introgression line | Parents[a] | BC family | RFLP marker[b] | | | | | Reaction to BPH biotypes[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 776 | 214 | 329 | 241 | 449 | Bio1 | Bio2 | Bio3 |
| IR54742-1-17-12 | a | 17-1 | A | C | C | A | C | R | R | R |
| IR54742-2-1-13 | a | 19-1 | C | A | C | A | C | R | R | R |
| IR54742-5-28-5 | a | 25-1 | A | A | C | C | C | R | R | R |
| IR54742-6-20-3 | a | 25-2 | C | A | C | C | C | R | R | R |
| IR54742-9-36-1 | a | 26-4 | A | A | A | A | C | R | R | R |
| IR54742-11-8-7 | a | 29-1 | C | A | C | C | C | R | R | R |
| IR54742-15-28-38 | a | 32-1 | C | A | C | A | C | S | S | R |
| IR54742-18-3-8 | a | 39-1 | C | A | C | A | C | R | R | R |
| IR54742-1-11-17 | a | 17-1 | A | A | A | A | C | R | R | R |
| IR54742-5-36-4 | a | 25-1 | A | A | C | A | C | R | R | R |
| IR54742-11-1-9 | a | 29-1 | C | A | C | C | C | R | R | R |
| IR54742-13-29-12 | a | 30-1 | C | A | C | C | C | R | R | R |
| IR54742-18-3-8 | a | 41-1 | C | A | C | A | C | R | R | R |
| IR54742-31-9-26 | a | 46-2 | A | A | C | A | C | R | R | R |
| IR54742-48-3-1 | a | 82-1 | A | A | A | A | C | R | S | S |
| IR54742-24-38-10 | a | 43-3 | C | A | C | C | C | R | R | R |
| IR54742-31-15-20 | a | 46-2 | A | A | C | A | C | R | R | S |
| IR54742-33-18-20 | a | 47-1 | A | A | A | C | C | R | S | S |
| IR54742-11-15-3 | a | 29-1 | A | C | C | A | C | R | R | R |
| IR54742-38-37-16 | a | 51-1 | C | A | C | A | C | R | R | R |
| IR54741-3-21-22 | c | 60-3 | A | C | C | A | C | R | R | R |
| IR54745-2-28-22 | d | 54-1 | C | A | A | A | C | R | R | R |
| IR54745-1-10-20 | d | 44-2 | C | A | A | A | C | R | R | R |
| IR54748-1-6-6 | f | 74-1 | C | A | C | A | C | S | R | S |
| IR54748-1-17-12 | f | 74-1 | C | A | C | A | C | S | R | R |
| IR54748-1-17-25 | f | 74-1 | C | A | A | A | C | S | R | R |
| IR54742-4-7-9 | a | 22-1 | C | A | A | A | A | S | S | S |
| IR54742-9-4-4 | a | 26-1 | C | A | C | A | A | R | R | R |
| IR54742-19-2-3 | a | 40-1 | C | A | C | C | A | R | R | R |
| IR54742-19-6-3 | a | 40-1 | C | A | C | A | A | S | S | R |
| IR54742-19-2-3 | a | 40-1 | C | A | C | A | A | S | S | R |
| IR54742-19-6-3 | a | 40-1 | C | A | C | A | A | S | S | R |
| IR54742-20-38-1 | a | 41-1 | A | A | A | A | A | R | S | R |
| IR54745-2-34-3 | d | 54-1 | C | A | C | A | A | R | R | R |
| IR54742-44-23-9 | d | 59-2 | A | A | A | A | A | R | S | R |
| IR54742-50-19-19 | a | 82-3 | A | A | A | C | A | S | S | S |
| IR54742-23-1-29 | a | 43-2 | C | A | A | A | A | S | S | S |
| IR54743-1-10-20 | b | 66-1 | A | A | A | A | A | S | S | S |
| IR54743-2-10-26 | b | 66-2 | A | A | A | A | A | S | S | S |
| IR54743-2-9-22 | b | 66-2 | A | A | A | A | A | S | S | S |
| IR54743-5-18-26 | b | 66-5 | A | A | A | A | A | S | S | S |
| IR54743-6-45-2 | b | 66-6 | A | A | A | A | A | S | S | R |
| IR54743-11-43-12 | b | 81-2 | C | A | C | A | A | S | S | S |
| IR54743-11-43-12 | b | 81-2 | C | A | C | A | A | S | S | S |
| IR54741-2-14-5 | c | 60-2 | A | A | A | A | A | S | R | R |
| IR54741-2-2-3 | c | 60-2 | C | A | C | A | A | S | S | R |
| IR54745-4-6-11 | d | 70-2 | A | A | A | A | A | S | R | S |
| IR54745-6-2-3 | d | 70-4 | A | A | A | A | A | S | S | S |
| IR54745-8-18-6 | d | 70-7 | A | C | A | A | A | R | S | R |
| IR54745-8-1-12 | d | 70-7 | A | C | A | A | A | R | S | S |
| IR54746-4-23-3 | e | 67-1 | C | C | A | A | A | R | R | R |
| IR54746-4-23-3 | e | 67-1 | A | A | A | A | A | S | S | S |

[a] a = IR31917-45-3-2 × *O. officinalis* (100896); b = IR31917-45-3-2 × *O. officinalis* (101150); c = IR31917-45-3-2 × *O. officinalis* (100878); d = IR25587-109-3-3 × *O. officinalis* (100896); e = IR25587-109-3-3 × *O. officinalis* (102385); f = IR25587-109-3-3 × *O. officinalis* (101412)

[b] A = *O. sativa* RFLP pattern; C = *O. officinalis* RFLP pattern

[c] R = resistant; S = susceptible

similar to the methods described by McCouch et al. (1988). Plants were grown in fields at the International Rice Research Institute (IRRI), Philippines, and DNA was extracted from the leaf tissue of single plants from each of the 52 introgression lines and the parents. DNA was digested with five restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI, and *Pst*I), subjected to electrophoresis in 0.8% agarose gels, and blotted onto "GeneScreen Plus" membranes (Du Pont) by the method of Southern (1975). Two types of filters were produced: survey filters, which contained DNA of the *O. sativa* and *O. officinalis* parents, and experimental filters, which contained DNA of the 52 introgression lines. Inserts were isolated from plasmids (Tautz and Renz 1983) and radio-labelled with [$^{32}$P]-dCTP by the random hexamer method (Feinberg and Vogelstein 1984). The labelled probes were individually hybridized to the survey filters to detect polymorphism and later to the experimental filters to screen the introgression lines for chromosomal segments introgressed from *O. officinalis*. Each filter was washed at 65 °C at moderate stringency (2 × SSC, once and 1 × SSC, twice) for 20 min (all washes contained 0.1% SDS) and exposed to X-ray film with intensifying screens. In certain cases, after the film was developed (usually 24 h) the filters were washed again at higher stringencies (0.05 × or 0.02 × SSC at 65 °C) and re-exposed.

*Scoring for introgression*

On survey filters polymorphism was detected between *O. sativa* and the *O. officinalis* parents. When an appropriate polymorphism was found for a given probe/enzyme combination, filters containing DNA from all the introgression lines were analyzed. When the banding patterns of the introgression lines were similar to the *O. sativa* pattern for the RFLP markers, the result was considered "negative" and suggested no introgression at the locus concerned. If the banding patterns of the introgression lines were similar to the donor *O. officinalis* parent, the result was considered "positive" and indicated introgression of *O. officinalis* chromosome segments at that RFLP locus. The approximate size (in centimorgans) of the introgressed segments was determined by dividing the distance between two flanking markers of a putative positive marker on the RFLP map (McCouch et al. 1988). The reaction to BPH biotypes of introgressed lines with putative positive markers was known from an earlier study (Jena and Khush 1990), and the association between putative positive markers and resistance to BPH biotypes was examined.

To compare the size of introgressed segments in early and late generation lines from the wide cross program, 21 plants of one early generation ($BC_2F_3$) introgression line from a $BC_2$ family (17-1) were selected for RFLP analysis. A series of RFLP markers spanning introgressed segments identified on chromosomes 4 and 12 in the introgression lines were used to analyze the early generation lines.

## Results

*RFLPs between the parents*

One hundred and seventy-seven RFLP markers were used as hybridization probes on survey filters containing DNA digests from single plants of the two *O. sativa* parents and the five *O. officinalis* parents. Five restriction enzymes, *Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI, and *Pst*I, were used to prepare the survey filters. Of the 177 RFLP markers, 174 were polymorphic between the *O. sativa* and *O. officinalis* parents with one or more enzymes. Many of the RFLP markers also detected polymorphism between the various accessions of *O. officinalis* (Fig. 2). Most markers were polymorphic with multiple enzymes, but the highest degree of polymorphism (85.8%) was detected with *Hin*dIII digests (Table 2). Two markers, RG454 and RG511, were AA genome specific – 1

**Table 2.** Polymorphism detected by different restriction enzymes

| Chromosome | Probes tested (*n*) | Percent polymorphism with enzymes | | | | |
|---|---|---|---|---|---|---|
| | | *Eco*RI | *Eco*RV | *Hin*dIII | *Bam*HI | *Pst*I |
| 1 | 21 | 76.1 | 76.1 | 81.0 | 85.7 | 66.6 |
| 2 | 20 | 65.0 | 85.0 | 70.0 | 65.0 | 60.0 |
| 3 | 17 | 59.0 | 59.0 | 88.2 | 53.0 | 47.0 |
| 4 | 13 | 84.6 | 69.2 | 77.0 | 69.2 | 46.1 |
| 5 | 14 | 86.0 | 64.3 | 86.0 | 71.4 | 71.4 |
| 6 | 14 | 78.5 | 64.3 | 93.0 | 78.6 | 64.3 |
| 7 | 19 | 74.0 | 63.1 | 89.5 | 52.6 | 42.1 |
| 8 | 7 | 86.0 | 100.0 | 100.0 | 28.6 | 71.4 |
| 9 | 12 | 91.6 | 91.6 | 83.3 | 58.3 | 66.6 |
| 10 | 3 | 67.0 | 33.3 | 100.0 | 33.3 | 33.3 |
| 11 | 10 | 70.0 | 90.0 | 70.0 | 70.0 | 70.0 |
| 12 | 27 | 70.4 | 85.2 | 92.6 | 66.6 | 40.7 |
| Total | 177 | 75.7 | 73.4 | 85.8 | 61.0 | 56.6 |

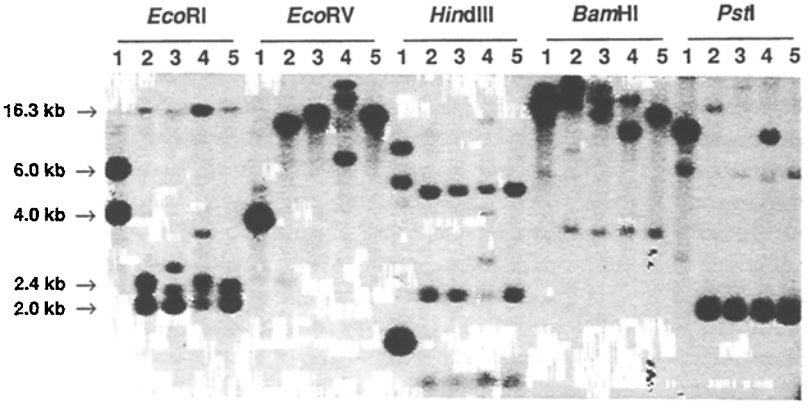


**Fig. 2.** RFLP patterns in *O. sativa* and four accessions of *O. officinalis*. DNA was digested with five restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Bam*HI, and *Pst*I) and probed with RG214. Note RFLPs between *O. sativa* and *O. officinalis* parents and among the accessions of *O. officinalis*. *Lane 1 O. sativa* '(IR31917-45-3-2)' parent, *lanes 2, 3, 4,* and *5 O. officinalis* accessions 100896, 101150, 100878, and 102385, respectively

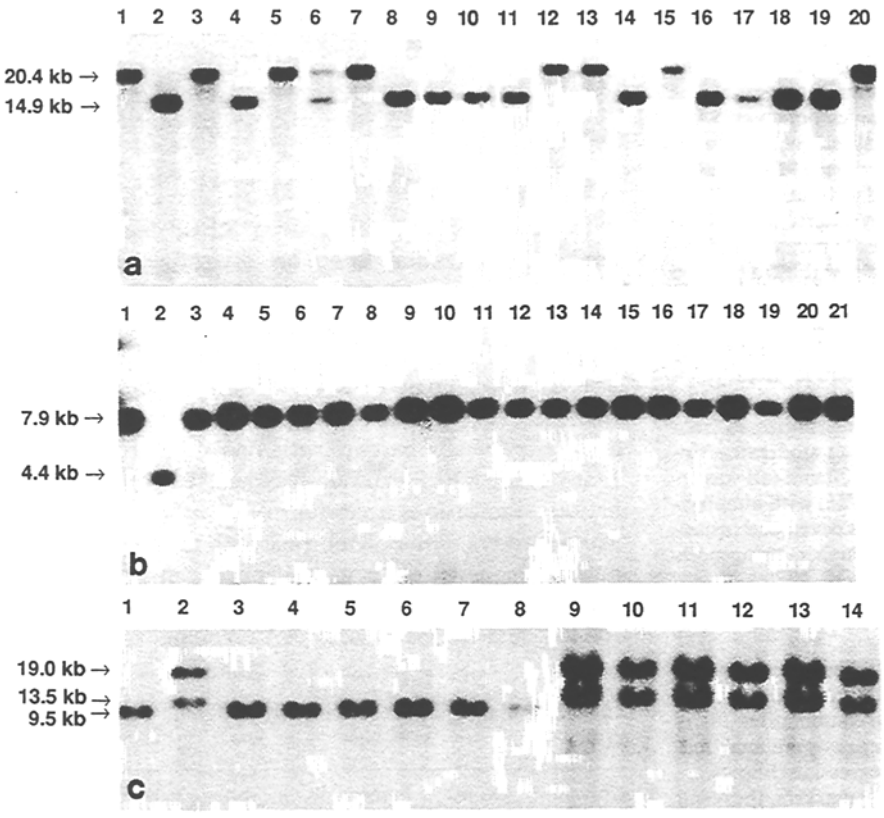


**Fig. 3a–c.** RFLP patterns of introgression lines. **a** 18 introgression lines digested with *Hin*dIII and probed with RG498. *Lane 1 O. sativa* parent, *lane 2 O. officinalis* parent, *lanes 3–20* different introgression lines. Note the replacement of 20.4-kb alleles of *O. sativa* with 14.9-kb alleles of *O. officinalis* (*lanes 4, 8, 9, 10, 11, 14, 16, 17, 18,* and *19*). *Lane 6* is a line containing RFLP alleles from both parents. **b** Introgression lines digested with *Eco*RI and probed with RG143. *Lane 1 O. sativa* parent, *lane 2 O. officinalis* parent, *lanes 3–21* are introgression lines, all of which contain the *O. sativa* allele. **c** RFLP patterns of 14 introgression lines digested with *Eco*RV and probed with RG449. *Lanes 1, 3, 4, 5, 6, 7,* and *8* show replacement of 19.0-kb and 13.5-kb alleles of *O. sativa* with 9.5-kb allele from *O. officinalis*

(RG211) detected a repeated sequence in all the plants, and individual bands were not distinguishable even after high stringency washes (Table 2). Thus, 174 markers were used for introgression line analysis.

*Amount of introgression*

Of the 174 RFLP markers, 28 (16.1%) detected *O. officinalis* alleles, and thus introgressed chromosome segments, in one or more of the introgression lines (Fig. 3a); 146 (83.9%) detected only the *O. sativa* banding pattern in all of the introgression lines (Fig. 3b). The total size of all the introgressed segments was approximately 14.0% that of the *O. sativa* genome. No individual introgression line contained all of the introgressed segments; individual lines contained 1.1–6.8% introgressed *O. officinalis* segments.

Most introgressed segments were detected by single RFLP markers, and the flanking markers were negative for introgression. For example, RG176 on chromosome 12 detected an introgressed *O. officinalis* chromosome segment in 16 introgression lines, but both flanking markers detected *O. sativa* alleles. Therefore, the size of the introgressed segment is only about 3 cM. In only 7 cases were pairs of adjacent RFLP markers jointly introgressed. For example, of the 11 positive markers identified on chromosome 12, four pairs of linked markers (RG901 and RG543, RG457 and RG869, RG498 and RG449, and RG218 and RG375) were jointly introgressed (Fig. 1). The size of the introgressed *O. officinalis* chromosomal segments on chromosome 12 ranged from approximately 11 cM to 59 cM in different introgression lines (Table 3), and 51 of the 52 introgression lines had at least 1 putative positive chromosome 12 RFLP marker.

During the survey of introgression lines with RFLP markers, RFLP alleles were detected that were not present in either of the parent plants routinely analyzed for polymorphisms. For example, in *Eco*RI digests of the introgression lines, probe RG214 detected *O. sativa* RFLP alleles (6.0 kb and 4.0 kb) in 22 lines and *O. officinalis* alleles (2.0 kb) in 6 lines, but 24 lines had one of two RFLP alleles (4.5 kb and 5.8 kb) that were not present in either parent (Fig. 4a). A 5.8-kb allele was present in 20 introgression lines, and a 4.5-kb allele was present in 4 introgression lines. These alleles appeared to replace the *O. sativa* 6.0-kb allele in all cases. Additional seeds were obtained from the original seed lot of the *O. sativa* ('IR31917-45-3-2') parent line. DNA was isolated from 28 plants, digested with *Eco*RI, and probed with the RG214 marker. Three plants contained a 4.5-kb allele of RG214 (Fig. 4b) in addition to the 6-kb allele. To determine the relationship of the 6.0- and 4.5-kb alleles, 1 of the plants containing both alleles was self-polli-

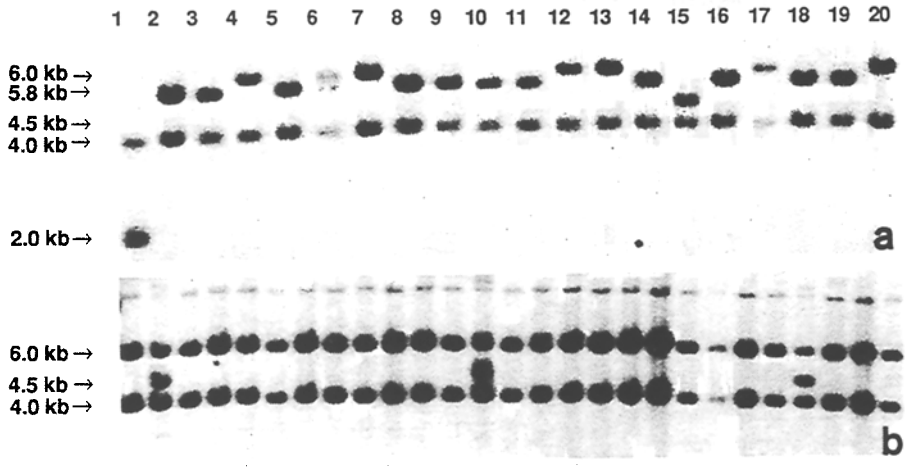


**Fig. 4.** **a** Introgression lines digested with *Eco*RI and probed with RG214, showing various alleles of RG214. *Lane 1* contains a replacement of the *O. sativa* 6.0-kb allele by the *O. officinalis* 2.0-kb allele. In the *other lanes*, 6.0-kb, 5.8-kb, and 4.5-kb alleles from the *O. sativa* parent are seen. *Lane 6* shows a plant heterozygous for the 6.0-kb and 5.8-kb alleles. **b** The detection of a 4.5-kb allele in 3 plants of 28 *O. sativa* progenitor plants digested with *Eco*RI and probed with RG214

**Table 3.** Size and chromosomal location of introgressed segments

| Chromosome | Area of introgression | Size of segment (cM) | RFLP markers |
|---|---|---|---|
| 1 | Subterminal | 5 | RG400, RG246 |
| 1 | Terminal | 1 | RG612 |
| 2 | Subterminal | 19 | RG152 |
| 3 | Terminal | 1.0 | RG369 |
| 4 | Middle | 7.5 | RG776, RG214 |
| 4 | Middle | 8.5 | RG329 |
| 5 | Terminal | 13.0 | RG573 |
| 7 | Subterminal | 11 | RG146 |
| 7 | Middle | 14 | RG711 |
| 7 | Terminal | 6.5 | RG417 |
| 8 | Middle | 8.5 | RG333 |
| 9 | Middle | 13 | RG451 |
| 10 | Middle | 34.5 | RG134, RG241 |
| 11 | Middle | 26 | RG103, RG2 |
| 12 | Middle | 3 | RG176 |
| 12 | Middle | 22 | RG901, RG543 |
| 12 | Middle | 24.5 | RG457, RG869 |
| 12 | Middle | 17.0 | RG498, RG449 |
| 12 | Subterminal | 17 | RG218, RG375 |
| 12 | Terminal | 4 | RG190 |
| 12 | ? | ? | RG418 |
| Total | | 256.0 | |

nated to produce $F_2$ seeds. Plants grown from these seeds exhibited segregation patterns for the 2 alleles that were consistent for alleles of the same locus. The original *O. sativa* line was thus found to be polymorphic for the 6.0-kb and 4.5-kb alleles. We did not find the 5.8-kb allele in any *O. sativa* plant.

The accessions of *O. officinalis* were polymorphic and displayed multiple banding patterns for several RFLP markers. However, when multiple bands were present in the *O. officinalis* parent, only one allele from *O. officinalis* could be identified in the introgression lines, where it replaced a corresponding allele of the *O. sativa* parent. For example, RG214 detected several fragments (16.3 kb, 3.5 kb, 2.4 kb, and 2.0 kb) in digests of the *O. officinalis* parent (Fig. 2), but only one of these, 2.0 kb, was incorporated into the introgression lines, where it replaced an *O. sativa* 6.0-kb allele (Fig. 4a).

*Chromosome-specific introgression*

Introgressed *O. officinalis* chromosomal segments were identified on all chromosomes except chromosome 6, which had monomorphic banding patterns characteristic of the *O. sativa* parent for each of the 14 RFLP markers of chromosome 6. Only 1 positive marker each was identified on chromosomes 2, 3, 5, 8, and 9. Chromosomes 4 and 7 had 3 positive markers, and chromosomes 1, 10, and 11 had 2 each. Introgression seemed to be most common on chromosome 12 where 11 RFLP markers detected the *O. officinalis* alleles in 1 or another of the introgression lines. The 11 positive markers are distributed all over chromosome 12 (Fig. 1).

The putative positive RFLP markers showed a significant variation in detecting *O. officinalis* introgression events in the 52 introgression lines. For example, RG333 of chromosome 8 detected *O. officinalis* alleles in 45 introgression lines, and RG869 of chromosome 12 detected 42. On the other hand, marker RG369 of chromosome 3 detected an *O. officinalis* allele in only 2 introgression lines.

*Nature of introgression*

The introgression of *O. officinalis* chromosomal segments into the genome of *O. sativa* showed two types of RFLP patterns. In the most common type, one of the *O. sativa* RFLP alleles was replaced by the corresponding *O. officinalis* allele. In the second type, RFLP alleles characteristic of both the parents were present, and the plants were apparently heterozygous at the introgressed locus. For example, RFLP marker RG498 detected a 14.9-kb *O. officinalis* allele in 19 introgression lines, and

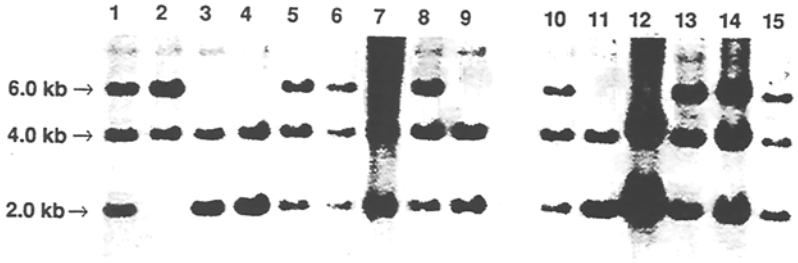


**Fig. 5.** RFLP patterns in 15 plants of a $BC_2F_3$ introgression line digested with *Eco*RI and probed with RG214. *Lanes 3, 4, 7, 9, 11,* and *12* are homozygous for the *O. officinalis* 2.0-kb allele, *lane 2* is homozygous for the *O. sativa* 4.0-kb and 6.0-kb alleles, and *lanes 1, 5, 6, 8, 10, 13, 14,* and *15* are heterozygous for the *O. sativa* and *O. officinalis* alleles

in 18 cases the 14.9-kb allele had replaced the 20.4-kb allele of the *O. sativa* parent. However, this RFLP marker detected RFLP alleles of both parents in 1 introgression line (Fig. 3a). Another marker, RG449, showed replacement of 2 *O. sativa* fragments (19.0 kb and 13.5 kb) with a single 9.5-kb fragment from *O. officinalis* (Fig. 3c). Of the 28 positive RFLP markers, 20 were heterozygous in one or more of the introgression lines (Table 4). However,with all of the positive markers the majority of the introgression lines were homozygous for the introgressed segment.

### *Association of RFLPs with BPH resistance*

The 52 introgression lines had a varied reaction to three different BPH biotypes (Table 1). There was no absolute correlation between the presence of introgressed *O. officinalis* chromosome segments and BPH resistance. However, the introgressed segments on chromosome 4 defined by RG214, RG776, and RG329 appear to be closely linked to genes for BPH resistance since all of the introgression lines that have any one of these segments are resistant to BPH biotypes 1 and 2. The chromosome 10 introgressed segment detected by RG241 also shows some correlation with resistance to BPH; every line that has this segment is resistant to biotype 3 (Table 1). All 26 introgression lines with an introgressed *O. officinalis* segment on chromosome 12 (detected by RG449) were resistant to all three BPH biotypes, suggesting correlation. None of these correlations is perfect; some of the introgression lines without positive markers are also resistant to BPH (Table 1).

### *RFLPs of early and late generation introgression lines*

To investigate changes in introgressed segment size during selfing and selection, plants were grown from remnant seed of an early generation line, $BC_2F_3$. This line reportedly segregated for resistance to BPH (Jena and Khush 1990). Of the 21 plants analyzed for RFLPs with RG214, 1 had the RFLP pattern of the *O. sativa* parent, 9 had a replacement of the *O. sativa* allele with that of *O. officinalis*, and 11 contained both the *O. sativa* and the *O. officinalis* alleles (Fig. 5).

**Table 4.** Introgression lines heterozygous for introgressed alleles

| Chromosome | RFLP markers | Heterozygous lines (*n*) | Percent |
|---|---|---|---|
| 1 | RG400 | 21 | 40.3 |
| 2 | RG152 | 1 | 1.9 |
| 4 | RG776 | 6 | 11.5 |
| 4 | RG214 | 1 | 1.9 |
| 4 | RG329 | 2 | 3.8 |
| 7 | RG146 | 1 | 1.9 |
| 7 | RG711 | 2 | 3.8 |
| 7 | RG417 | 1 | 1.9 |
| 8 | RG333 | 16 | 30.7 |
| 9 | RG451 | 1 | 1.9 |
| 11 | RG002 | 3 | 5.7 |
| 12 | RG176 | 6 | 11.5 |
| 12 | RG457 | 1 | 1.9 |
| 12 | RG869 | 1 | 1.9 |
| 12 | RG498 | 1 | 1.9 |
| 12 | RG449 | 2 | 3.8 |
| 12 | RG190 | 2 | 3.8 |
| 12 | RG418 | 8 | 15.3 |

**Table 5.** RFLP patterns of early and late generation lines

| RFLP marker | Chromosome | Early generation[a] | Late generation[a] |
|---|---|---|---|
| RG169 | 4 | A | A |
| RG776 | 4 | A | A |
| RG214 | 4 | C | C |
| RG476 | 4 | A | A |
| RG329 | 4 | C | C |
| RG864 | 4 | A | A |
| RG788 | 12 | A | A |
| RG498 | 12 | C | C |
| RG449 | 12 | C | C |
| RG91 | 12 | A | A |

[a] A = *O. sativa* RFLP pattern; C = *O. officinalis* RFLP pattern

The 21 early generation plants were also analyzed with a set of RFLP markers from chromosomes 4 and 12. The markers were selected to include those that marked introgressed segments in the later generation lines ($BC_2F_8$) and non-introgressed flanking sequences. In all 4 cases analyzed (RG214 and RG329 on chromosome 4, and RG449 and RG498 on chromosome 12), the

flanking markers that were negative in the late generation lines were also negative in the early generation lines. Thus no evidence was found that introgressed segments were larger in the earlier generations (Table 5).

## Discussion

The use of RFLP markers to detect introgression of alien chromosome segments has been demonstrated in tomato (Tanksley and Hewitt 1988; Osborn et al. 1987; Young et al. 1988; Sarfati et al. 1989). In rice, RFLP markers have recently been used to tag disease and insect resistance genes introgressed from cultivated rice germ plasm (McCouch et al. 1991; Yu et al. 1991). In our investigation, RFLP markers selected from the rice RFLP map (McCouch et al. 1988) were used to detect chromosomal segments introgressed from *O. officinalis* into 52 introgression lines derived from a wide cross program. These lines have inherited several useful genes from the *O. officinalis* parent, such as resistance to BPH, WBPH, and BB, and a number of quantitative traits (Jena and Khush 1990).

The degree of RFLP polymorphism between *O. sativa* and *O. officinalis* parents was very high irrespective of the restriction enzymes used. This made it easy to screen the introgression lines as virtually every probe could be used. Also, any segments introgressed from *O. officinalis* were easy to tag with linked RFLP markers.

In the original crosses between *O. sativa* and *O. officinalis*, the $F_1$s produced were diploid, with one chromosome set from *O. sativa* (AA genome) and one set from *O. officinalis* (CC genome). Only a limited amount of chromosome pairing (1 to 5 bivalents) was observed in the $F_1$ hybrids (Jena and Khush 1989). Thus, it would seem that opportunities for introgression by reciprocal recombination would be comparatively rare. Therefore, it was of interest to look at the RFLP patterns in the introgressed lines to see if they were consistent with introgression by reciprocal recombination. In most cases, one *O. sativa* allele was replaced with a corresponding *O. officinalis* allele, a finding consistent with introgression by reciprocal recombination. Most lines were apparently homozygous for either the *O. sativa* or the *O. officinalis* allele, but some lines had RFLP patterns of both parents and were apparently heterozygous for the introgressed segment.

The introgression lines, which we studied at the $BC_2F_8$ stage, were obtained by selfing and selection for desirable traits starting from the $BC_2F_1$ progeny. If the recombination proceeded in the expected fashion, the introgressed chromosome segments from *O. officinalis* should be relatively large in early generation progeny. During subsequent self-pollination and selection to produce the $BC_2F_8$ lines, introgressed segments might be reduced by recombination and selection against portions of the introgressed segment containing genes for undesirable traits. This would be consistent with the finding that all the introgressed chromosome segments in the $BC_2F_8$ lines were small and could be detected by only 1 or, in a few cases, 2 adjacent RFLP markers. To check this, remnant seed of some $BC_2F_3$ plants were grown, and the size of introgressed segments were compared to those found in $BC_2F_8$ plants from the same backcross family. However, the introgressed segments we checked were all the same size in the $BC_2F_3$ plants as they were in the $BC_2F_8$ plants. Thus, it appears that the introgression mechanism resulted in the transfer of only very small segments from the C to the A genome. If these transfers resulted from conventional recombination, two cross-overs must have occurred very close within 2–3 cM units from each other. This seems unlikely on the basis of observed chiasma frequency in the $F_1$ hybrids. Thus, either the observations of chiasma frequency at diakinesis do not accurately measure genetic recombination, or a nonconventional mechanism is involved in transferring small segments from C to A chromosomes.

During our screening for introgression, we occasionally found an RFLP allele in the introgression lines that was not present in either of the parent plants we used to screen for polymorphisms. For example, in *Eco*RI digests of some introgression lines, RG214 detected a novel 4.5-kb allele instead of the expected 6.0-kb *O. sativa* allele. Twenty-eight plants grown from remnant seed of the same seed lot as the *O. sativa* parent line were examined for the presence of this allele, and 3 plants were found that contained the 4.5-kb novel allele in addition to the 6.0-kb allele. When we examined self-pollinated progeny of one apparently heterozygous plant, the 2 alleles segregated in a manner consistent for alleles at the same locus. The *O. sativa* parent used to produce the interspecific cross was a breeding line that was bulk propagated at the $F_5$ stage and might still have hidden heterozygosity detectable at the molecular level. RG214 also detected a 5.8-kb allele that was not found in either parent. It could possibly have been located if more parent plants from the original seed lot were examined. Alternatively, it could represent an allele introduced into the breeding line by a chance out-cross from one of the many other rice varieties grown at IRRI.

Four of the nine known genes for BPH resistance have been located to specific chromosomes by conventional genetic analysis. In 1981 Ikeda and Kaneda (1981) located *Bph-3* and *Bph-4* on chromosome 10; in 1983 (Ikeda and Kaneda 1983) they located *Bph-1* and *Bph-2* on chromosome 4. Of the 28 putative positive RFLP markers identified in this study, 3, RG776, RG214, and RG329, belong to chromosome 4, and RG241, which is on chromosome 10, detected an *O. officinalis* allele in 9 introgression lines. However, a few introgression lines with no apparent introgression on chromosomes 4 and

10 were also resistant to BPH biotypes. No conclusions can be drawn about the association of genes for BPH resistance from *O. officinalis* with specific RFLP segments. Linkage between putative positive RFLP markers and BPH resistance gene(s) is being analyzed. For this purpose, one introgression line, 'IR54746-4-23-3-19', with *O. officinalis* chromosomal segments detected with the RFLP markers RG449, RG214, RG776, RG329, RG418, RG369, RG2, and RG869, has been crossed to the recurrent *O. sativa* parent. Analysis of $F_2$ and $F_3$ progenies of this cross will indicate the degree of linkage between RFLP markers and resistance genes.

The nature of BPH resistance genes in *O. officinalis* is not yet known. An unknown number of major genes may be responsible for BPH resistance in this wild species. In addition to positive markers that correlated with resistance to BPH in some introgression lines, 1 marker, RG449 on chromosome 12, is of particular interest. This marker detected an *O. officinalis* allele in 26 introgression lines that are resistant to all three BPH biotypes. RG449 may be linked to a resistance gene that confers resistance to all BPH biotypes. Moreover, Jena and Khush (1990) reported that of the 12 monosomic alien addition lines (MAALs), only MAAL 12 segregated for BPH resistance.

In addition to BPH resistance, *O. officinalis* is also highly resistant to WBPH. However, the chromosomal locations of the WBPH resistance genes are not yet known. It may be possible to link some of the putative positive markers identified in this study to WBPH resistance gene(s). In an earlier study of isogenic lines, McCouch et al. (1990) reported that RFLP marker RG146 is linked to WBPH resistance. In this study, RG146 detected *O. officinalis* alleles in 6 introgression lines (data not shown), and these are all resistant to WBPH. This indicates that marker RG146 on chromosome 7 might be linked to a gene for WBPH resistance introgressed from *O. officinalis*; this will have to be confirmed by linkage analysis.

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