Theor Appl Genet (1994) 87:782–788

RFLP and RAPD mapping of the rice *Gm2* gene that confers resistance to biotype 1 of gall midge (*Orseolia oryzae*)

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Received: 30 April 1993 / Accepted: 16 June 1993

Abstract. *Gm2* is dominant gene conferring resistance to biotype 1 of gall midge (*Orseolia oryzae* Wood-Mason), the major dipteran pest of rice. The gene was mapped by restriction fragment length polymorphism (RFLP) analysis of a set of 40 recombinant inbred lines derived from a cross between the resistant variety 'Phalguna' and the susceptible landrace 'ARC 6650'. The gene is located on chromosome 4 at a position 1.3 cM from marker RG329 and 3.4 cM from RG476. Since the low (28%) polymorphism of this *indica* × *indica* cross hindered full coverage of the genome with RFLP markers, the mapping was checked by random amplified polymorphic DNA (RAPD)/bulked segregant analysis. Through the use of 160 RAPD primers, the number of polymorphic markers was increased from 43 to 231. Two RAPD primers amplified loci that co-segregated with resistance/susceptibility. RFLP mapping of these loci showed that they are located 0.7 cM and 2.0 cM from RG476, confirming the location of *Gm2* in this region of chromosome 4. Use of these DNA markers will accelerate breeding for gall midge resistance by permitting selection of the *Gm2* gene independently of the availability of the insect.

Key words: *Oryza sativa* – *Orseolia oryzae* – Gall midge – Diptera – Bulked segregant analysis – Recombinant inbred lines – Insect resistance

Introduction

Gall midge (*Orseolia oryzae* Wood-Mason) is the major dipteran pest of rice (Hill 1987) and is a problem in many rice growing areas of India, Southeast Asia and Southern China (Kalode and Bentur 1989; Lai et al. 1984). A closely related species (*O. oryzivora*) is an important pest in Nigeria and other parts of tropical Africa (Ukwungwu et al. 1989). Gall midge larvae irritate and feed from the shoot meristem of the rice plant and prevent normal tiller development. The resulting "silver shoot" (a modified leaf sheath) fails to produce a panicle. Although additional tillers are induced in response to the infestation, the new tillers may themselves become damaged, with the result that yield is severely reduced (Hidaka 1974).

Gall midge has been an increasing problem in India over the last decade because of the emergence of new biotypes. A total of four distinct biotypes have been recognized for some years in various parts of India (Kalode and Bentur 1989), and a fifth biotype has recently been reported (Anonymous 1992). Four biotypes have been reported in China (Lai et al. 1984), and additional biotypes may exist in Sri Lanka, Thailand and Indonesia (Pathak and Heinrichs 1982). The screening of germ plasm collections for new sources of resistance has been a priority for several national breeding programs and has resulted in the identification of resistance genes effective against one or more biotypes. Characterization of these genes and their incorporation into high yielding cultivars are in progress.

Restriction fragment length polymorphism (RFLP) analysis may be used to map disease and pest resistance genes relative to flanking DNA markers and accelerate their use in breeding programs (Tanksley et al. 1989).

Communicated by G. S. Khush
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DNA markers have been used to identify genes for resistance to the following diseases: tobacco mosaic virus, *Fusarium oxysporum*, *Stemphylium* and *Pseudomonas syringae* in tomato (Young et al. 1988. Sarfatti et al. 1989; Behare et al. 1991; Martin et al. 1991), *Pseudomonas syringae* in *Arabidopsis* (Debener et al. 1992), maize dwarf mosaic virus in maize (McMullen and Louie 1989), potato virus X in potato (Ritter et al. 1991), powdery mildew in barley (Hinze et al. 1991), race 2 of *Plasmodiophora brassicae* in *Brassica* (Landry et al. 1992) and a nematode and rhizomania in sugar beet (Jung et al. 1990, Barzen et al. 1992). The existence of a high density genetic map of the rice genome (McCouch et al. 1988; McCouch and Tanksley 1991) makes such an approach also feasible for rice diseases and pests. Genes have been mapped for resistance to rice blast (Yu et al. 1991), bacterial leaf blight (McCouch et al. 1991a; Ronald and Tanksley 1991; Yoshimura et al. 1992) and white-backed plant hopper (McCouch et al. 1991b).

We now report the RFLP mapping of the *Gm*2 gene for resistance to biotype 1 of gall midge. The mapping population was a set of 40 F_{5-6} recombinant inbred (RI) lines derived from a cross between 'Phalguna', a high yielding resistant variety, and 'ARC 6650,' a susceptible landrace from Assam. Because of the low frequency of RFLPs in this *indica* × *indica* cross, we increased greatly the number of polymorphic markers in the study by using random amplified polymorphic DNAs (RAPDs). The RAPD technique was introduced by Williams et al. (1990) and has been used to construct genetic maps of plant species (Reiter et al. 1992; Tulsieram et al. 1992), to identify markers that are specifically linked to the Y-chromosome in *Silene latifolia* (Mulcahy et al. 1992), to tag a *Pseudomonas* resistance gene in near-isogenic lines of tomato (Martin et al. 1991) and, in combination with bulked segregant analysis, to identify markers linked to downy mildew resistance in lettuce (Michelmore et al. 1991). We used RAPD/bulked segregant analysis to check the validity of our RFLP mapping of the *Gm*2 gene.

Materials and methods

Plant and insects

The 40 RI lines were derived from a cross that was made between 'Phalguna' and 'ARC6650' in 1978 and which had been subject in the interim to periodic selfing. All lines were F_5 or F_6. A colony of biotype 1 of gall midge was maintained at the Directorate of Rice Research (DRR) at Hyderabad under glasshouse conditions. The insects were caged with Taichung-Native 1 (TN1) as the susceptible host.

Gall midge resistance in RI lines was measured under glasshouse conditions in arrays of 20 seedlings of each line regularly interspersed with 'Phalguna', 'ARC6650' and the susceptible check TN-1. Damage was scored as the percentage of plants showing silver shoots at the time 80% of the TN-1 seedings showed silver shoots. All 40 RI lines scored consistently resistant (less than 10% of plants damaged) or susceptible (more than 60% of plants damaged) in three separate trials conducted in 1990–1992. Difficulties arose with 6 additional lines that showed neither clear resistance nor clear susceptibility. Two pieces of evidence suggested that this behavior was due to mixed seeds in these lines. (1) When retested under conditions which gave 100% damage of 'ARC6650' and TN-1 (compared with 80% damage in the standard test), these lines were seen to contain both resistant and susceptible individuals. (2) The aberrant lines showed an unexpectedly high degree of apparent heterozygosity as judged by RFLPs: instead of the expected 6% heterozygosity in F_5 lines or 3% heterozygosity in F_6 lines, they showed 12–45% heterozygosity, a result most easily explained by a mixing of seed. Accordingly, these lines were not added to the mapping population.

RFLP probes

A total of 150 single-copy DNA probes distributed over the 12 chromosomes of rice were selected for analysis. These clones (the RG series) were originally selected from a *Pst*I genomic library of rice (McCouch et al. 1988) and were kindly provided by Dr. S. D. Tanksley of Cornell University, N.Y.

DNA extraction and Southern hybridization

Rice seeds were germinated in the dark for 14 days. The shoots were then harvested and kept frozen at − 80 °C until needed for DNA extraction by the method of Walbot (1988). Rice DNA (5 or 10 µg) was digested with restriction endonucleases and electrophoresed overnight at 25 V on 0.8% agarose gels containing 1 × TBE buffer (Sambrook et al. 1989). The DNA was subsequently transferred onto GeneScreen Plus nylon membranes (NEN-DuPont, USA) as described by Williams et al. (1991), and the membranes were hybridized (Bernatzky 1988) with RG plasmids that had been [^{32}P]-labelled (> 1 × 10^8 dpm/µg) using a nick-translation kit (Bethesda Research Laboratories, Life Technologies, USA). After hybridization, the membranes were washed under stringent conditions (twice in 2 × SSC, 0.1% SDS at room temperature for 5 min each; twice in 0.5 × SSC, 0.1% SDS at 65 °C for 15 min each; and twice in 2 × SSC at room temperature for 5 min each) and autoradiographed. Each blot was used for as many as ten hybridizations, between which the probe was removed according to GeneScreen protocol (20 min boiling in 10 m*M* Tris-HCl, pH 8.0, 1 m*M* EDTA, 1% SDS). In the initial survey of the two parental DNAs for RFLPs, the following restriction enzymes were used: *Alu*I, *Bam*HI, *Bgl*II, *Eco*RI, *Eco*RV, *Hin*dIII, *Not*I and *Xba*I. Probes which did not reveal an RFLP with these enzymes were then hybridized to filters containing parental DNA digested with the following enzymes: *Aat*II, *Ava*I, *Bcl*I, *Cla*I, *Hin*cII, *Kas*I, *Kpn*I, *Msc*I, *Nco*I, *Nde*I, *Nla*IV, *Sal*I and *Xho*I.

Bulked segregant analysis

DNA was bulked from 12 resistant RI lines and from 12 susceptible RI lines. The two bulks were used as target DNAs for RAPD analysis along with DNA from 'Phalguna' and 'ARC6650'. The analysis employed 160 10-base oligonucleotide primers from commercially available primer kits A, B, C, D, E, F, G and K (Operon Technologies, Alameda, Calif.) For each amplification, 20 ng DNA was used as template in 25 µl of reaction volume. The reaction conditions were as described by Williams et al. (1990) with the modification that denaturation time was kept at 5 s.

Computing

The Mapmaker Macintosh Version 1.0 program (du Pont de Nemours and Co, copyright 1990), based on the Mapmaker program of Lander et al. (1987), was used for analysis of segregation data. Although designed for use in multi-point linkage analysis with F_2 mapping populations, the program can also accommodate data from RI lines. As recommended by Burr and Burr (1991), genetic distances were calculated from recombination percentages using a formula of Haldane and Waddington (1931) to take into account the additional meioses involved in the production of RI lines compared with F_2 individuals.

Chromosome nomenclature

The rice genomic map and chromosome numbering system in McCouch et al. (1988) have been superseded. The numbering system used in this paper is that of McCouch and Tanksley (1991).

Results

Genetic analysis of gall midge resistance

Table 1 summarizes F_2 segregation data for resistance to biotype 1 of gall midge in three crosses featuring resistant variety 'Phalguna'. In crosses with susceptible varieties 'TN-1' and 'ARC6650', the population segregated 3:1 for resistance: susceptibility, indicating that resistance is determined by a single dominant gene. 'Phalguna' is derived from a cross between 'IR8' and 'Siam 29', the donor of gall midge resistance (Sastry et al. 1984), and in the F_2 population of a 'Phalguna' × 'Siam 29' cross, no susceptible segregants were observed (Table 1). The gall midge resistance gene in 'Siam 29' has been designated *Gm*2 by Chaudhary et al. (1986). Consequently, 'Phalguna' carries the *Gm*2 gene for resistance to biotype 1 of gall midge.

Table 1. Genetic analysis of gall midge resistance

Cross	Plants R	Plants S	F_2 reaction	χ^2
TN1 × Phalguna	136	44	3R:1S	0.027
Phalguna × ARC6650	73	25	3R:1S	0.013
Phalguna × Siam 29	719	0	All R	–

R, Resistant; S, susceptible

Mapping population

RFLP mapping of the *Gm*2 gene was performed with an inbred F_{5-6} population derived from a 'Phalguna' × 'ARC 6650' cross. The use of existing recombinant inbred (RI) lines advanced our study by several years, but since the population was created between 1978 and 1983 as part of a standard rice improvement program at the Directorate of Rice Research, it did not possess some of the features considered desirable in a mapping population. It had not been produced by single-seed descent, the plants had not been bagged during pollen shedding and the population was not unbiased: indeed, it had been subjected to rigorous selection for the high yield and long grain type of 'Phalguna' and the thick culm of 'ARC6650'. The fact that both parents were *indica* varieties severely reduced the frequency of useful DNA polymorphisms: out of the 150 cloned probes tested, only 43 (28%) showed RFLPs with a set of eight restriction endonucleases. As an extreme example of the paucity of RFLPs in this cross, we note that none of 23 probes mapped by the Cornell group onto chromosome 3 revealed a polymorphism with at least eight restriction endonucleases. Nevertheless, these deficiencies did not prevent the mapping of the *Gm*2 gene.

Gall midge resistance and RFLP analysis of RI lines

The 40 RI lines in the mapping population showed clear and reproducible scores for resistance to biotype 1 in three seasons of greenhouse trials at DRR, Hyderabad. The top row of Table 2 shows that 17 lines were resistant and 23 lines were susceptible. Molecular segregation data for the 'ARC6650' and 'Phalguna' alleles of 43 polymorphic RFLP markers were obtained for each RI line. Analysis of the data with the Mapmaker program (Lander et al. 1987) revealed that the *Gm*2 gene segregated closely with four RFLP markers on chromosome 4. Table 2 shows the segrega-

Table 2. Segregation data for the 'ARC6650' (A) and 'Phalguna' (P) alleles of four RFLP and two RAPD markers mapping to chromosome 4. The top row (*Gm*2) represents gall midge reaction for each of the 40 inbred lines

*Gm*2	SSSSS	RSRSR	RRRSR	SSSSS	SSRSS	SSSSR	RRRRR	RRSSR
RG 214	AAAAH	PAPAP	PPPAP	AAAAA	AAPAA	AAHAP	PPPPP	PPAAP
RG 329	AAAAH	PAPAH	HPPAP	AAAAA	AAHAA	AAAAP	PPPPP	PPAAP
RG 476	AAAAH	PAPAP	PPPAP	AAAAP	AAPAA	AAAAP	PPPPP	PPAAP
RG 776	AAAAA	PAPAP	PPPAP	PPHHA	AAHAA	AAAAP	PPPPP	PPAAP
F 08	AAAAA	PAPAP	PPPAP	AAAAP	AAPAA	AAAAP	PPPPP	PPAAP
F 10	AAAAA	PAPAP	PPPAP	AAAAP	AAAAA	AAAAP	PPPPP	PPAAP

H, Putative heterozygous state, with both RFLP alleles
R, Resistant; S, susceptible

tion data: an entry of A or P indicates that only the 'ARC6650' or 'Phalguna' allele was present at the indicated locus for the indicated line, while an entry of H indicates that both alleles were present (the putative heterozygous condition). From these data, the *Gm2* gene was calculated to reside 1.3 cM from RG329 and 3.4 cM from RG476 (Fig. 1). The susceptible phenotype was seen in 22 lines homozygous for the 'ARC6650' allele of RG329, while the resistant phenotype was seen in 14 lines homozygous for the 'Phalguna' allele and in 3 heterozygous lines (Fig. 2). Only 1 line showed evidence of recombination: line 5 was apparently heterozygous, but it was susceptible to gall midge. Thus, perhaps only one cross-over event may have occurred between the *Gm2* gene and locus RG329 during the development of the 40 recombinant inbred lines.

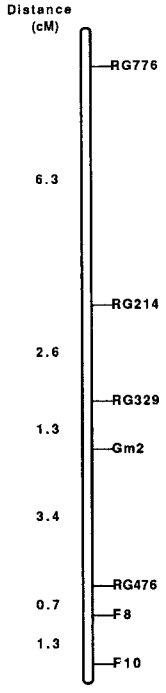

Fig. 1. Region of rice chromosome 4 in the vicinity of the *Gm2* gene for resistance to biotype 1 of gall midge (*Orseolia oryzae*). Positions are shown for flanking RFLP markers (*RG214, RG329, RG476, RG776*) and RAPD markers (*F08* and *F10*), with map distances in cM

RAPD/bulked segregant analysis

The low frequency of polymorphism seen among RFLP markers for this *indica* × *indica* cross hindered a complete survey of the genome. To place more polymorphic markers on the genome and to confirm the map location of the *Gm2* gene, we employed RAPDs and bulked segregant analysis (Michelmore et al. 1991). RAPD patterns were obtained with DNA isolated from 'Phalguna' and 'ARC6650' and with bulked DNAs isolated from 12 resistant and 12 susceptible recombinant inbred lines. Out of the 160 10-base oligonucleotides used, 159 primers were successful in amplifying loci from parental DNA. A total of 2084 bands was obtained, of which 188 were polymorphic between 'Phalguna' and 'ARC6650'. This increased very substantially the number of polymorphic markers covering the genome, from 43 to 231.

Most of the 188 polymorphic RAPD bands were also generated from both the resistant and the susceptible bulked DNA samples. This result indicates that the corresponding loci were not linked to gall midge resistance. However, two bands were clearly linked to gall midge resistance. A 0.6-kbp band from primer F10 was specifically amplified from DNA of the resistant parent and the resistant bulk (Fig. 3A), while a 1.7-kbp band from primer F08 was specific to the susceptible parent and the susceptible bulk (Fig. 3B). When DNA from each of the 12 resistant and 12 susceptible lines was amplified with these two primers, the 'Phalguna'-specific F10 band was found only in the resistant lines, and the ARC6650-specific F08 band was found only in the susceptible lines. This is evidence for very tight linkage between *Gm2* and the two RAPD loci.

To determine the map locations of the loci represented by the 0.6-kbp F10 band and the 1.7-kbp F08

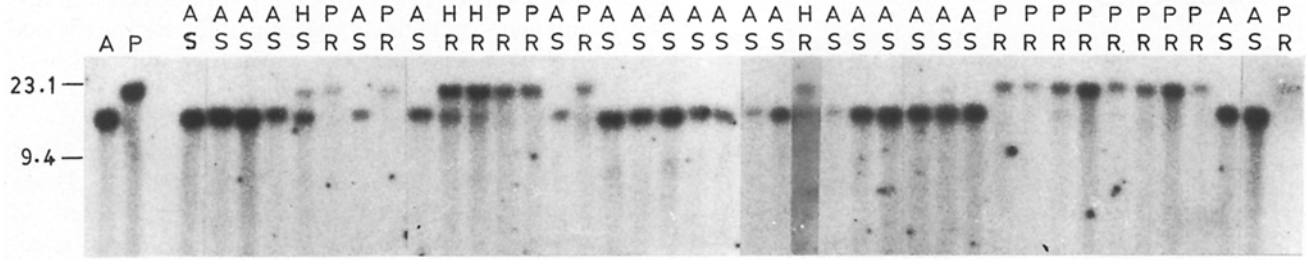

Fig. 2. Autoradiography of Southern hybridization of *Sal*I digest of rice DNAs with radiolabelled probe RG329. *Lane A* from 'ARC6650' (susceptible parent), *Lane P* DNA from 'Phalguna' (resistant parent), *remaining lanes* DNAs from 40 recombinant inbred lines. *Bottom row of letters* Entomology data (*R* resistant, *S* susceptible); *top row* interpretation of autoradiogram (*A* ARC6650 allele, *P* Phalguna allele, *H* heterozygous); *numbers at left* marker sizes in kbp

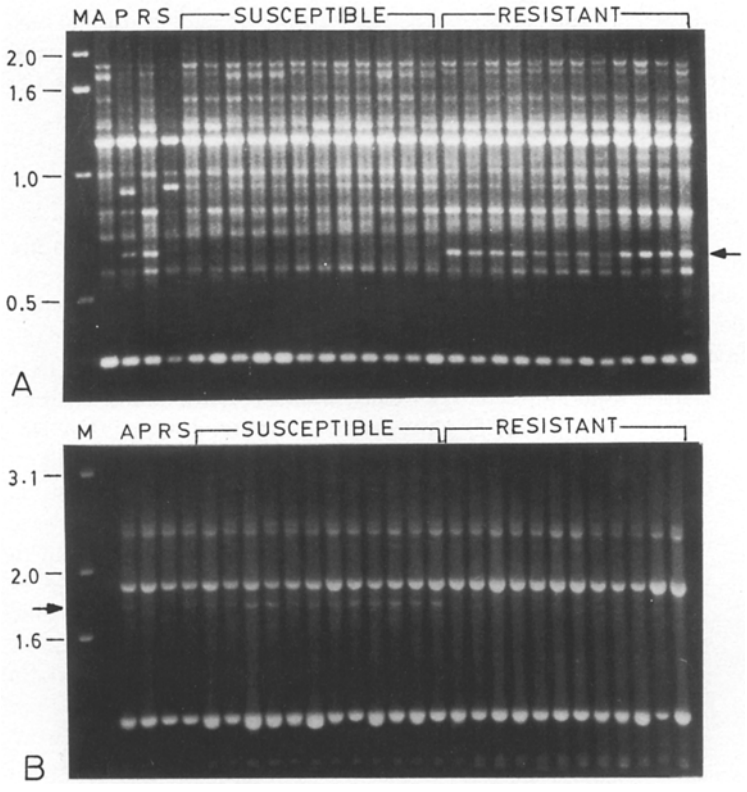

Fig. 3A, B. RAPD tagging of *Gm2* gene by bulked segregant analysis, with confirmation by co-segregation analysis. **A** RAPD data for primer F10, **B** RAPD data for primer F08. *Lanes*: DNA from 'ARC6650' (*A*), 'Phalguna' (*P*), resistant bulk (*R*), susceptible bulk (*S*), followed by 12 susceptible lines and 12 resistant lines. *Arrows* Resistant-specific RAPD (F10) and susceptible-specific RAPD (F08); *numbers at left* markers sizes in kbp

band, we eluted the bands from gels and nick-translated them for use as probes on the same RI line filters that had been used for RFLP analysis. Both RAPD loci co-segregated closely with *Gm2* and neighboring RFLP markers (Table 3). Figure 1 shows the location of the F08 and F10 markers relative to *Gm2* and flanking RFLP markers. The F08 and F10 loci lie 4.1 cM and 5.4 cM, respectively, from *Gm2*, beyond RG476. Their respective distances from RG476 are 0.7 cM and 2.0 cM. Such results support the RFLP data on the location of the *Gm2* gene in this region of chromosome 4.

Discussion

We have shown that the dominant *Gm2* gene for resistance to biotype 1 of gall midge is located on chromosome 4 of 'Phalguna' between the RFLP markers RG329 and RG476 and close to two loci amplified by RAPD primers F08 and F10. RFLP mapping took approximately 18 months and involved 43 polymorphic markers. RAPD tagging by bulked segregant analysis (Michelmore et al. 1991) took about 4 months and involved 188 polymorphic markers. These results indicate that, if suitable segregating populations are available from *indica* × *indica* crosses, sufficient DNA polymorphism may be detectable by RAPDs to tag and map major genes quickly and conveniently. In spite of their departure from the ideal for a mapping population, recombinant inbred lines derived from traditional breeding programs can constitute a useful segregating population. This would be especially true if the problem of mixed seed were removed by one cycle of seed purification before phenotyping and genotyping of the breeding lines begin.

The fact that we failed to detect polymorphisms for any of the 23 available RFLP markers from chromosome 3 indicates that large regions of the genome may be invisible in crosses between related varieties. McCouch and Tanksley (1991) commented on this phenomenon in their comparison of the sizes of the rice RFLP maps constructed from an intraspecific *indica* × *javanica* cross and an interspecific *O. sativa* × *O. longistaminata* cross.

Now that the *Gm2* gene has been tagged with DNA markers, it will be possible to accelerate breeding programs for gall midge resistance. Replacement of entomology testing by molecular marker-aided selection could enable breeders to conduct up to three cycles of breeding for this character per year. The use of RG329 as a molecular marker for *Gm2* will give a rate of false positives or false negatives of less than 2% per

generation, while the use of RG329 and RG476 together as markers will reduce the error rate to about 0.06% per generation.

Biotype 1 of gall midge is widely distributed in Madhya Pradesh and northern Andhra Pradesh. Five other biotypes are found elsewhere in India, including biotype 2 in Orissa. 'Phalguna' itself carries resistance to biotype 2. Preliminary field testing of our recombinant inbred lines at the Central Rice Research Institute in Orissa (with the late Dr. P. S. Prakasa Rao) indicated that the gene for biotype 2 resistance maps to within 2 cM of RG329. Further work will be required to determine whether the two genes are identical or very close neighbors. Probe RG329 is a useful marker for both genes. Map-based cloning will open the way to a study of the biochemical basis of gall midge resistance and biotype specificity. However, cloning will require (1) a larger mapping population to place more recombinant events between the genes and their markers, and (2) closer DNA markers. We have identified an additional 9 RAPD bands apparently linked to gall midge resistance and are in the process of determining their map locations.

The tagging of genes for resistance against other known biotypes of gall midge will follow a revised strategy. RAPDs and bulked segregant analysis will be used as the initial method of tagging each gene, and then RAPD tags will be mapped relative to RFLP markers. This should permit faster mapping than the reverse order of events followed here.

Acknowledgments. We thank Prof. K. K. Tewari, Dr. E. A. Siddiq and Dr. M. B. Kalode for their keen interest and support and the laboratory of S. D. Tanksley for probes. This research was supported in part by a grant from the Rockefeller Foundation to JB.

References

Anonymous (1992) In: Progress report, Kharif 1992: vol 2, entomology and pathology. Indian Council of Agricultural Research, New Delhi, pp 2.40–2.47

Barzen E, Mechelke W, Ritter E, Seitzer JF, Salamini F (1992) RFLP markers for sugar beet breeding: chromosomal linkage maps and location of major genes for rhizomania resistance, monogermy and hypocotyl colour. Plant J 2:601–611

Behare J, Laterrot H, Sarfatti M, Zamir D (1991) Restriction fragment length polymorphism mapping of the *Stemphylium* resistance gene in tomato. Mol Plant-Microbe Interact 4:489–492

Bernatzky R (1988) Restriction fragment length polymorphism. In: Gelvin SB, Schilperoort RA (eds) Plant molecular biology manual. Kluwer Academic Publishers, Dordrecht, pp C2: 1–18

Burr B, Burr FA (1991) Recombinant inbred lines for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Chaudhary BP, Srivastava PS, Srivastava MN, Khush GS (1986) Inheritance of resistance to gall midge in some cultivars of rice. In: Rice genetics. Proc Int Rice Genet Symp. International Rice Research Institute, Los Banos, Philippines, pp 523–527

Debener T, Lehnackers H, Arnold M, Dangl JL (1992) Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. Plant J 1:289–302

Haldane JBS, Waddington CH (1931) Inbreeding and linkage. Genetics 16:357–374

Hidaka T (1974) Recent studies on the rice gall midge, *Orseolia oryzae* (Wood-Mason), Cecidomyiidae, Diptera. Rev Plant Prot Res 7:99–143

Hill DS (1987) Agricultural insect pests of the tropics and their control, 2nd edn. Cambridge University Press, Cambridge

Hinze K, Thompson RD, Ritter E, Salamini F, Schulz-Lefert P (1991) Restriction fragment length polymorphism-mediated targeting of the *ml-o* resistance locus in barley (*Hordeum vulgare*). Proc Natl Acad Sci USA 88:3691–3695

Jung C, Kleine M, Fischer F, Herrmann RG (1990) Analysis of DNA from a *Beta procumbens* chromosome fragment in sugar beet carrying a gene for nematode resistance. Theor Appl Genet 79:663–672

Kalode MB, Bentur JS (1989) Characterization of Indian biotypes of the rice gall midge, *Orseolia oryzae* (Wood-Mason) (Diptera: Cecidomyiidae). Insect Sci Applic 10:219–224

Lai K, Tan Y, Pan Y (1984) Rice gall midge biotypes in Guangdong Province. Int Rice Res Newsl 9:17–18

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Hubert N, Crete R, Chang MS, Lincoln SE, Etoh T (1992) A genetic map for *Brassica oleracea* based on RFLP markers detected with expressed DNA sequences and mapping of resistance genes to race 2 of *Plasmodiophora brassicae* (Woronin) Genome 35:409–420

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

McCouch SR, Tanksley SD (1991) Development and use of restriction fragment length polymorphism in rice breeding and genetics. In: Khush GS, Toenniessen GH (eds) Rice biotechnology. CAB International, Wallingford, pp 109–133

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

McCouch SR, Abenes ML, Angeles R, Khush GS, Tanksley SD (1991a) Molecular tagging of a recessive gene, *xa-5*, for resistance to bacterial blight of rice. Rice Genet Newsl 8:143–145

McCouch SR, Khush GS, Tanksley SD (1991b) Tagging genes for disease and insect resistance via linkage to RFLP markers. In: Rice genetics II. International Rice Research Institute, Los Banos, Philippines, pp 443–449

McMullen MD, Louie R (1989) The linkage of molecular markers to a gene controlling the symptom response in maize to maize dwarf mosaic virus. Mol Plant-Microbe Interact 2:309–314

Michelmore RW, Paran I, Kesseli, RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Mulcahy DL, Weeden NF, Kesseli R, Carroll SB (1992) DNA probes for the Y-chromosome of *Silene latifolia*, a dioecious angiosperm. Sex Plant Reprod 5:86–88

Pathak PK, Heinrichs EA (1982) A bibliography of varietal resistance to the rice gall midge, *Orseolia oryzae* (Wood-Mason). Commonwealth Agricultural Bureaux, Slough.

Reiter RS, Williams JGK, Feldman KA, Rafalski JA, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl Acad Sci USA 89:1477–1481

Ritter E, Debener T, Barone A, Salamini F, Gebhardt C (1991) RFLP mapping on potato chromosomes of two genes controlling extreme resistance to potato virus X (PVX). Mol Gen Genet 227:81–85

Ronald PC, Tanksley SD (1991) Genetic and physical mapping of the bacterial blight resistance gene *Xa-21*. Rice Genet Newsl 8:142–143

Sambrook J, Fritsch EF, Maniatis T, (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

Sarfatti M, Katan J, Fluhr R, Zamir D (1989) An RFLP marker in tomato linked to the *Fusarium oxysporum* resistance gene *I2*. Theor Appl Genet 78:755–759

Sastry MVS, Prasada Rao U, Kalode MB, Sain M (1984) Inheritance of resistance to gall midge (*Orseolia oryzae*) in rice. Indian J Genet 44:325–328

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding: new tools for an old science. Bio Technology 7:257–264

Tulsieram LK, Glaubitz JC, Kiss G, Carlson JE (1992) Single tree genetic linkage mapping in conifers using haploid DNA from megagametophytes. Bio Technology 10:686–690

Ukwungwu MN, Winslow MD, John VT (1989) Severe outbreak of rice gall midge in the savannah zone, Nigeria. Int Rice Res Newsl 14:36–37

Walbot V (1988) Preparation of DNA from single rice seedlings. Rice Genet Newsl 5:149–151

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531–6535

Williams MNV, Pande N, Nair S, Mohan M, Bennett J (1991) Restriction fragment length polymorphism analysis of polymerase chain reaction products amplified from mapped loci of rice (*Oryza sativa* L.) genomic DNA. Theor Appl Genet 82:489–498

Yoshimura S, Yoshimura A, Saito A, Kishimoto N, Kawase M, Yano M, Nakagahra M, Ogawa T, Iwata N (1992) RFLP analysis of introgressed chromosomal segments in three near-isogenic lines of rice for bacterial blight resistance genes. Jpn J Genet 67:29–37

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. Genetics 120:579–585

Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476