Theor Appl Genet (1999) 98: 1022–1028 © Springer-Verlag 1999

A. C. Sanchez · L. L. Ilag · D. Yang · D. S. Brar
F. Ausubel · G. S. Khush · M. Yano
T. Sasaki · Z. Li · N. Huang

Genetic and physical mapping of *xa13*, a recessive bacterial blight resistance gene in rice

Received: 15 August 1998 / Accepted: 29 September 1998

Abstract The recessive gene, *xa13*, confers resistance to Philippine race 6 (PXO99) of the bacterial blight pathogen *Xanthomonas oryzae* pv *oryzae*. Fine genetic mapping and physical mapping were conducted as initial steps in an effort to isolate the gene. Using nine selected DNA markers and two F_2 populations of 132 and 230 plants, *xa13* was fine-mapped to a genomic region < 4 cM on the long arm of rice chromosome 8, flanked by two RFLP markers, RG136 and R2027. Four DNA markers, RG136, R2027, S14003, and G1149, in the target region were used to identify bacterial artificial chromosome (BAC) clones potentially harboring the *xa13* locus from a rice BAC library. A total of 11 BACs were identified, forming four separate contigs including a single-clone contig, 29I3, associated with the RG136 STS marker, the S14003 contig consisting of four clones (44F8, 41O2, 12A16, and 12F20), the G1149 contig with two clones, 23D11 and 21H18, and the R2027 contig consisting of four overlapping clones, 42C23, 30B5, 6B7 and 21H14. Genetic mapping indicated that the *xa13* locus was contained in the R2027 contig. Chromosomal walking on the R2027 contig resulted in two more clones, 33C7 and 14L3. DNA fingerprinting showed that the six clones of the R2027 contig were overlapping. Clone 44F8 hybridized with a single fragment from the clone 14L3, integrating the R2027 and S14003 contigs into a single contig consisting of ten BAC clones with a total size of approximately 330 kb. The physical presence of the *xa13* locus in the contig was determined by mapping the ends of the BAC inserts generated by TAIL-PCR. In an F_2 population of 230 plants, the BAC-end markers 42C23R and 6B7F flanked the *xa13* locus. The probes 21H14F and 21H14R derived from BAC clone 21H14 were found to flank *xa13* at a distance of 0.5 cM on either side, using a second F_2 population of 132 plants. Thus, genetic mapping indicated that the contig and the 96-kb clone, 21H14, contained the *xa13* locus.

Key words Genetic map · Physical map · Map-based gene cloning · Disease resistance · Rice · DNA markers

Introduction

Positional cloning provides a promising method for isolating a gene based only on its phenotype and genomic location. The first requirement of this strategy is to generate a high-resolution genetic map for the genomic region of a target gene such that the size of the chromosomal segment where the gene resides is suitable for the next step of physical mapping. A high-resolution map for a specific genomic region is commonly obtained by resolving the order of a number of markers tightly flanking the target gene using a relatively large mapping population. The other requirement is the availability of large insert libraries, such as yeast or bacterial artificial chromosome (YAC or BAC) libraries, for the genome of interest. When both requirements are met, a target gene can be isolated by

Communicated by G. Wenzel

A. C. Sanchez · D. Yang · D. S. Brar · G. S. Khush
Z. Li (✉) · N. Huang[1]
Plant Breeding, Genetics and Biochemistry Division,
International Rice Research Institute (IRRI), P.O. Box 933,
1099 Manila, The Philippines
Fax: 63-2 891-1292
E-mail: z.li@cgnet.com

L. L. Ilag · F. Ausubel
Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital,
Boston, Massachusetts 02114, USA

M. Yano · T. Sasaki
Rice Genome Program, National Institute of Agrobiological Resources (NIAR), Tsukuba, Ibaraki 305, Japan

Present address:
[1]Applied Phytologics Inc., Sacramento, California 95834, USA

chromosomal walking, which however is generally a tedious procedure (Hauge and Goodman 1992).

The recent development of high-density molecular-linkage maps in many species allows a much more efficient strategy, 'chromosome landing', for target gene isolation by selective enrichment of DNA markers within the sub-cM region around a target gene (Tanksley et al. 1995). Flanking DNA markers, which are physically very close to the target gene, are then used to screen large insert libraries in order to identify the small genomic fragment containing the gene. Once a candidate clone is isolated, the final step is to determine the identity of the target gene by trans-genetic complementation and DNA sequencing.

The simple monogenic inheritance and the clear and distinct phenotypic reactions of plant disease resistance genes make them well suited for gene isolation by the map-based cloning approach. Recent success in the isolation of several plant disease resistance genes has demonstrated the power of this strategy (cf. Baker et al. 1997), which represents a breakthrough in understanding the molecular basis of plant defense responses to invading pathogens.

Bacterial blight caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*), is one of the most serious rice diseases in irrigated and rainfed lowland ecologies throughout Asia. The genetic basis of host resistance to bacterial blight has been studied in depth. To-date, at least 19 host resistance genes to *Xoo* have been reported (Kinoshita 1995). The clear reactions of these resistance genes to different strains of the pathogen and their monogenic inheritance make them among the most attractive systems for studying the molecular and genetic bases of host plant-pathogen relationships. Recently, two dominant genes, *Xa21* and *Xa1*, conferring complete resistance to several races of *Xoo* have been cloned (Song et al. 1995; Yoshimura et al. 1998). Molecular studies on the two cloned genes have considerably enhanced our understanding of the defense system of rice plants to *Xoo* and the evolution of disease resistance genes in plant genomes (Song et al. 1997).

Most plant disease resistance genes are dominant, but resistance controlled by recessive genes has been reported in many plant-pathogen systems. Of more than 19 reported rice resistance genes to *Xoo*, *xa5* and *xa13* are both transmitted in a recessive manner. The *xa5* locus was mapped to rice chromosome 5 (Yoshimura et al. 1995). The *xa13* gene confers specific resistance to *Xoo* race 6 (PXO99). Originally identified in cultivar BJ1 (Ogawa et al. 1987), *xa13* was found closely linked to a RAPD marker, AC5-900, and three RFLP markers, RG136, RZ28 and CDO116, in rice chromosome 8 (Zhang et al. 1996). Resistance conferred by recessive genes such as *xa5* and/or *xa13* may represent very different and largely unknown biochemical pathways in the host defense system. If so, cloning and the molecular study of *xa13* may shed light on this important aspect of the plant defense response. Moreover, elucidation of the biochemical function of *xa13* may lead to more efficient strategies for combating *Xoo*.

We report here the results of fine mapping and physical mapping of the recessive gene, *xa13*, in an effort to clone it using the map-based cloning approach. We have identified a number of DNA markers tightly linked to *xa13* and have constructed a BAC contig containing the locus with overlapping clones, which will facilitate future marker-assisted selection of *xa13* in rice breeding programs and the final effort to clone the gene.

Materials and methods

Plant materials

Two populations were used for genetic mapping experiments. One population (hereafter referred to as the NIL population) consisted of 132 F_2 individuals derived from a cross between IR24 (*Xa13*/*Xa13*, susceptible to *Xoo* race 6) and its NIL, IRBB13 (*xa13*/*xa13*, resistant). A second F_2 population (hereafter referred to as the NPT population) consisting of 230 plants was derived from a cross between IRBB13 and a new plant type line, IR65598-112-2 (susceptible to *Xoo* race 6). The parents, the two F_1s, and the F_2 populations were maintained and evaluated for disease reaction in the greenhouse.

Bacterial inoculation and detection of *xa13* genotypes

Bacterial cells of *Xoo* race 6 (PXO99) were grown on potato semi-synthetic agar (PSA: Ou 1972) slants at 30°C for 3 days. Inoculum was prepared by suspending the bacterial mass in sterile water to a concentration of approximately 10^9 cells/ml. Leaf blades of the parents, the F_1s, and the F_2 populations at the maximum tillering stage (about 60 days after planting) were inoculated by the leaf-clipping method (Kauffman 1973). The reactions of the plants were visually evaluated 18 days after inoculation using the standard scoring system, in which plants with a lesion length < 2 cm were classified as resistant and those with a lesion length > 10 cm as susceptible.

Fine mapping of *xa13* by chromosome landing

According to the reported genomic location of *xa13* (Zhang et al. 1996) nine DNA markers, including eight RFLP markers (RG136, RZ28, CDO116, R2662, R2027, S14003, G1149 and C502) and an STS marker (RG136) around the *xa13* region from the two high-density rice molecular linkage maps constructed at Cornell (Causse et al. 1994) and in Japan (Kurata et al. 1994), were selected for chromosome landing. The STS marker of RG136 was obtained by sequencing the ends of the RFLP clone RG136 for primer design, as described by Huang et al. (1997). The polymorphism between the NIL pair IR24 and IRBB13, and between IRBB13 and IR65598-112-2 for the RG136 STS marker was determined by PCR amplification followed by *Hin*f1 digestion (Huang et al. 1997). Additional probes for chromosome walking were generated by thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR) of the positive BAC clones detected by colony hybridization (Liu and Whittier 1995). In cases when entire BAC clones were used as probes, the BAC DNA was digested with *Hin*dIII before labelling.

Rice genomic DNA was isolated from fresh leaves of plants at the maximum tillering stage using the method of Dellaporta et al. (1983). Southern-blotting analysis followed the standard procedure (Sambrook et al. 1989). Total genomic DNA was digested with the respective restriction enzymes, separated by electrophoresis on a 0.8% agarose gel, and transferred onto nylon membranes. Labelling of the probes was done using a random primer kit (Rediprime, Amersham USA) with ^{32}P-dCTP. Radioactivity was detected by X-ray films after 3–4 days exposure at $-80°$C. Linkage analysis was carried out using the program MAPMAKER/EXP 3.0 (Lander et al. 1987; Lincoln and Lander 1992). Recombination values were converted into genetic map distances (cM = centiMorgan) using the Kosambi function (Kosambi 1944).

Physical mapping

A BAC library constructed from cultivar IR64 was used for physical mapping of the *xa13* locus (Yang et al. 1997). The library consists of 18 432 clones in 48 384-well microtiter plates. High-density BAC filters (1536 BAC clones/filter and 12 filters/library) for screening were prepared as described by Yang et al. (1997). To identify BAC clones covering the target region, colony hybridization was done using the *xa13*-linked RFLP markers as probes. Each of the labelled probes was added to the pre-hybridization solution and hybridized with high-density BAC filters at 65°C overnight. The filters were washed twice with 0.2 M phosphate buffer containing 0.1% SDS at 65°C for 20 min each time with gentle shaking. The filters were then exposed to X-ray films for 4–24 h depending on the signal intensity.

All candidate BAC clones identified by colony hybridization were subjected to Southern-hybridization analysis to confirm their overlaps. BAC DNA was isolated using the alkali-lysis procedure (Sambrook et al. 1989) with some modifications. The same amount of lysis buffer (solution II) and neutralization buffer (solution III) were added sequentially after the *Escherichia coli* cells were suspended. An equal volume of chloroform : isoamyl alcohol (24 : 1) was added to the supernatants to remove proteins. A 0.7-fold volume of isopropanol was added to the supernatant and incubated for 30 min at room temperature to precipitate the DNA. Standard procedures were used to recover the DNA and remove the RNA.

Insert sizes of the BAC clones were determined by pulsed-field gel electrophoresis (PFGE). The conditions were as follows: switch time ramping from 10 to 30 s, temperature 11°C, pulse angle 110, using 0.5 × TAE buffer for 4 h. The size of each BAC clone was estimated based on its migration as compared to lambda ladder markers. For DNA fingerprinting with *Hin*dIII digestion, Southern-hybridization analysis was performed as described by Sambrook et al. (1989). The end fragments of the BAC clones to be used for chromosome walking were generated by TAIL-PCR. The set of TAIL-PCR primers was synthesized according to vector sequence pBeloBAC11 (Shizuya et al. 1992), shown as follows:

BF1 5′-GACGTTGTAAAACGACGGCCAGT
BF2 5-GTAATACGACTCACTATAGGGCGA
BF3 5′-GAGTCGACCTGCAGGCATGCA
BR1 5′-CTTCCGGCTCGTATGTTGTGTGG
BR2 5′-GAGCGGATAACAATTTCACACAGGA
BR3 5′-TAGGTGACACTATAGAATACTCA.

Three arbitrary primers (AD1, AD2, and AD3) were employed. The sequences of the AD primers were according to Liu and Whittier (1995) and Liu et al. (1995). The contig map consisting of overlapping BAC clones for the *xa13* region was constructed based on the *HindIII* fingerprint overlaps and the arrangement of the RFLP markers on the linkage map.

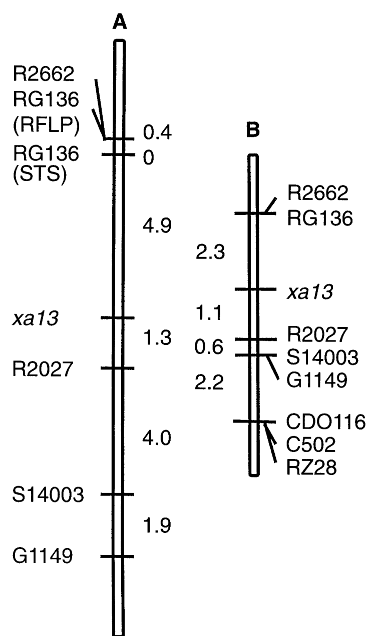

Fig. 1 The molecular map of the *xa13* region on rice chromosome 8. Map *B* was derived from 132 F_2 plants of the near-isogenic line (IR24 and IRBB13) cross. Map *A* was derived from 230 F_2 plants of the cross between IR65598-112-2 and IRBB13

Results

Fine mapping of the *xa13* region

The parental lines and the F_2 plants of the two mapping populations showed clear and distinct reactions to *Xoo* race 6 PXO99. In the NIL F_2 population, plants segregated into 106 : 26 susceptible : resistant, respectively, while in the NPT F_2 population, the segregation was 166 : 64 susceptible : resistant. Both populations fitted the expected 3 : 1 Mendelian ratio. All RFLP markers in the *xa13* region also fitted the expected segregation ratio of 1 : 2 : 1 at the 5% level.

Figure 1 shows the two genetic maps containing *xa13* and all the selected DNA markers constructed in the two mapping populations. The RFLP marker R2027 was mapped 1.1 cM from *xa13* in the NIL population (map B) and 1.3 cM away in the NPT population (map A). RG136 was located 2.3 cM on the other side of *xa13* in the NIL population and 5.3 cM away in the NPT population. The RG136 STS marker was located closer to *xa13* at a distance of 4.9 cM in the NPT population, showing 1.9% recombination with its RFLP marker. S14003 was mapped 0.6 cM distal from R2027 while R2662 completely co-segregated with RG136 in both populations. The linear order of RG136, R2662, S14003, G1149 and RZ28 in our maps was consistent with that reported by Harushima et al. (1998) using the Nipponbare/Kasalath mapping population (data not shown). Our results clearly indicated that of these markers, RG136 and R2027 were the closest ones flanking the *xa13* locus.

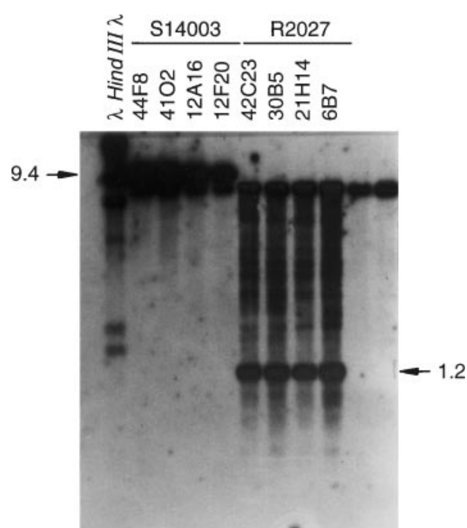

Fig. 2 Reconstruction and identification of overlapping BAC clones with RFLP markers S14003 and R2027. BAC-clone DNA samples were digested with *Hin*dIII and the resultant blot was probed with a mixture of the labelled RFLP markers. Vector DNA is the 7.4-kb band common to all clones. *Arrows* point to the fragments of BAC clone DNA hybridized by the markers

Identification and analyses of BAC clones near *xa13*

The two markers RG136 and R2027 were used to screen the IR64 BAC library in order to identify overlapping clones near *xa13*. In addition, S14003 and G1149 were used to screen the library to determine the direction of chromosome walking.

The RG136 RFLP marker did not detect any clone, but its STS marker identified a single clone, 29I3. S14003 identified four clones, 44F8, 41O2, 12A16 and 12F20 (Fig. 2), while G1149 hybridized to two clones, 23D11 and 21H8. Four overlapping clones, 42C23, 30B5, 6B7 and 21H14, were identified by R2027 (Fig. 2). These positive BAC clones identified by the four markers RG136, R2027, S14003 and G1149 resulted in four small contigs including the single-clone contig for RG136. Furthermore, the ends of the identified BAC inserts were amplified by TAIL-PCR and used as probes for hybridization with each clone in the contigs. The orientation of the clones in individual contigs was determined based on the hybridization pattern of BAC end probes. The resulting alignment of the overlapping clones in these contigs was determined based on DNA hybridization patterns using the digested clones as probes (Fig. 3).

To connect the separate contigs, the BAC end markers derived by TAIL-PCR were also used as probes for screening the library in order to extend the contigs. One marker, 42C23R, derived from the right end of clone 42C23, identified a new clone, 33C7, while 6B7F, derived from the left end of clone 6B7, identified 14L3. The extreme clones 33C7 and 14L3 were digested with *Hin*dIII, end-labeled, and used as probes for one more step of walking. Clone 33C7 did not identify any new clone. Thus, chromosome walking towards the direction of 33C7 was terminated. Clone 33C7 lies in the direction toward the telomere of the long arm of chromosome 8. Clone 44F8 hybridized with a single 10-kb band of 14L3, integrating the R2027 and S14003 contigs into one (Fig. 3B). The final contig consisted of ten clones spanning approximately 330 kb (Fig. 4).

Physical mapping of the *xa13* region

The physical relationship of the BAC clones with the RFLP markers was confirmed by Southern hybridization of the RFLP markers to the clones of the contig.

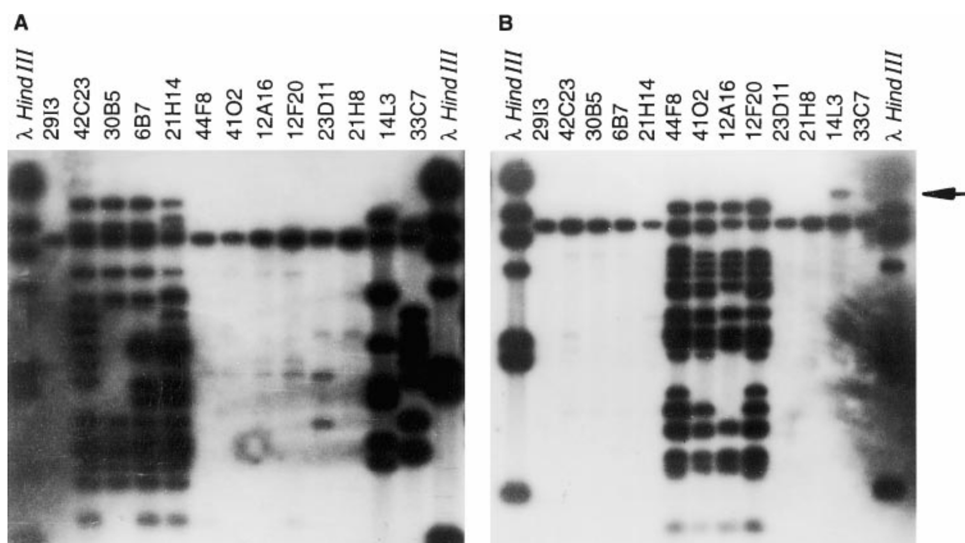

Fig. 3 Integration of the ten BAC clones into one contig using *Hin*dIII-digested 21H14 (**A**) and 44F8 (**B**) as probes. The *arrow* points to the 10-kb fragment of 14L3 that hybridizes with 44F8 integrating the two contigs into one

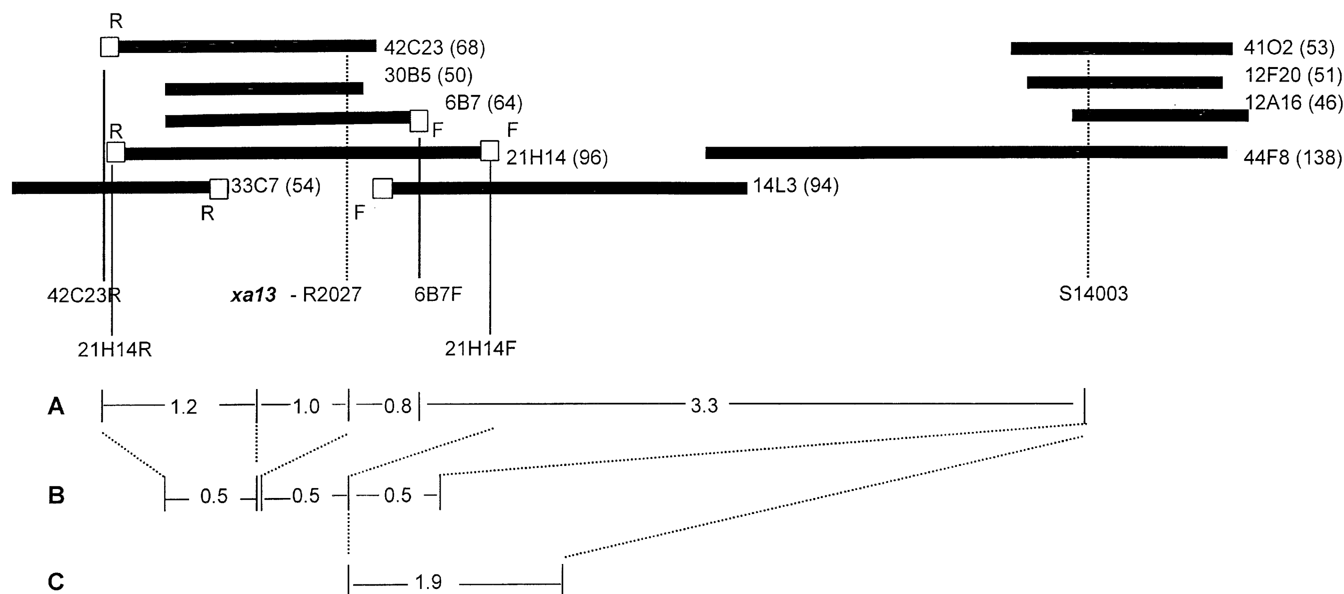

Fig. 4 Alignment of the genetic and physical maps of the *xa13* region. The physical map is shown as overlapping BAC clones represented by *solid lines* with line length equal to the insert sizes estimated through PFGE. The genetic map is shown as genetic distances in cM after inclusion of the BAC end markers: (*B*) the genetic map derived from the NIL population; (*A*) the genetic map derived from the NPT population, and (*C*) the genetic map from the Nipponbare/Kasalath mapping population

Marker R2027 was contained in a 1.2-kb band common to four clones: 21H14, 42C23, 30B5 and 6B7. These four clones shared six other common restriction fragments with a total size of about 36 kb. Marker S14003 was on a 9.4-kb fragment common to clones 44F8, 41O2, 12A16 and 12F20. These four clones also shared 11 other common fragments with a total size of about 42 kb. The physical size between R2027 and S14003 was estimated to range from 233 to 268 kb.

Figure 4 shows the genetic map aligned with the physical map of the BAC contig encompassing the *xa13* locus. The physical presence of the *xa13* locus in this contig was confirmed by mapping the BAC end markers and *xa13* in the two mapping populations. A single-copy BAC end marker, 42C23R, derived from the right end of 42C23, was 1.2 cM from *xa13* while R2027 was 1 cM on the other side in the NPT population. Another BAC-derived marker, 6B7F, is 0.8 cM beyond R2027. Two additional markers, 21H14F (left end) and 21H14R (right end), derived from clone 21H14, were found to flank *xa13* at a distance of 0.5 cM on either side using the NIL population. These results indicated that both the contig and clone 21H14 contained the *xa13* locus. The genetic distance between the insert ends of 21H14 was determined to be 1 cM while the physical size of the 21H14 insert was approximately 96 kb, giving a physical/genetic ratio of 96 kb/cM in the *xa13* region of chromosome 8.

Discussion

In an effort to clone a recessive bacterial blight resistance gene, *xa13*, employing the map-based cloning approach, we constructed a fine-genetic map and a physical map of *xa13* using two F_2 mapping populations and a BAC library constructed from rice cultivar IR64. A number of DNA markers closely linked to *xa13* identified in this study should facilitate marker-aided selection of this important gene in rice breeding programs. In addition, construction of a contig map with ten overlapping BAC clones encompassing the *xa13* locus represented a significant step toward our final goal to isolate this gene.

21H14 contains the *xa13* locus

Although the presence of *xa13* in the 330-kb contig was determined by genetic mapping of BAC-end markers derived from clones in the contig, the inclusion of this gene in a single 96-kb BAC clone, 21H14, was confirmed by the genetic mapping of its two end markers flanking *xa13*. Furthermore, three other smaller BAC clones, 6B7 (64 kb), 30B5 (50 kb) and 42C23 (68 kb), are also likely to contain the *xa13* locus according to their alignments in the contig. In particular, the three clones shared seven common *Hin*dIII restriction fragments with 21H14, which had a total size of about 36 kb. The *xa13* locus is very likely on one of these shared fragments. The succeeding steps in isolating *xa13*, including construction of the *xa13* cDNA library, the identification of the candidate cDNA clone, subcloning of clone 21H14, and genetic complementation, etc., are under way.

Lower ratio of physical and genetic distance in the *xa13* region

Our results indicated that in the *xa13* region of chromosome 8, each centimorgan of genetic distance is roughly equivalent to 96 kb, a nearly 3-fold reduction in the ratio of physical/genetic sizes as compared to the average P/G ratio of 260–280 kb/cM estimated by Wu and Tanksley (1993) and by Yoshimura et al. (1998). This reduction is apparently due to enhanced recombination in the region. Different chromosomal regions showing significant variations in recombination frequencies have been widely reported. While the heterochromatic regions near centromeres and telomeres of individual chromosomes are known to show considerable recombination suppression (Gaudet and Fitzgerald-Hayes 1990), the subtelomeric regions, by contrast, have functions that greatly enhance recombination (Rouyer et al. 1990). The *xa13* locus is in the subtelomeric region (roughly 10 cM from the telomere) of rice chromosome 8 (Harushima et al. 1998). Thus, the enhanced recombination observed is consistent with the subtelomeric location of *xa13*. The genetic basis for this phenomenon, however, remains largely unknown.

DNA markers available for MAS of *xa13*

Marker-assisted selection (MAS) is more beneficial for transferring target traits controlled by recessive genes since the desirable phenotypes of this type are not expressed in heterozygotes. In addition to the four DNA markers (AC5-900, RG136, RZ28 and CDO116) identified by Zhang et al. (1996), several other DNA markers closely linked to *xa13* were developed in this study. These included four BAC end markers, 21H14R, 21H14F, 42C23R and 6B7F. These can be of immediate use in the detection of DNA polymorphisms required for MAS of *xa13* in rice breeding programs. Among all these markers, the RG136 STS marker should be very useful in MAS involving *indica*/*japonica* crosses because it is PCR-based. The RFLP markers, including R2662, R2027, S14003, G1149 and C502 from JRGP, and RG136, CDO116 and RZ28 from Cornell, and 21H14R and 21H14F developed in this study, should be more powerful for MAS involving closely related parents since they are polymorphic even between the *xa13* NILs.

Acknowledgements We are grateful for Drs. S. D. Tanksley and S. R. McCouch of Cornell University for providing some of the RFLP markers used in this study. L. L. Ilag was supported by an NIH postdoctoral fellowship (NRSA no. 1F32GM16527). Financial support from the German Government, the Rockefeller Foundation, and Japanese Government are greatly appreciated.

References

Baker B, Zambryski P, Staskawicz B, Dinesh-Kumar SP (1997) Signalling in plant-microbe interactions. Science 276:726–733

Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, Wu K, Xiao J, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Dellaporta SL, Wood J, Hicks JB (1993) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–20

Gaudet AM, Fitzgerald-Hayes M (1990) The function of the centromere in chromosomal segregation. In: Strauss PR, Wilson SH (eds) The eukaryotic nucleus. Molecular Biochemistry and Macromolecular Assemblies. Telford Press. Caldwell, New Jersey, pp 845–881

Harushima Y, Yano M, Shomura A, Sato M, Shimano T, Kuboki Y, Yamamoto T, Lin SY, Antonio BA, Parco A, Kajiya H, Huang N, Yamamoto K, Nagamura Y, Kurata N, Khush GS, Sasaki T (1998) A high-density rice genetic linkage map with 2275 markers using a single F_2 population. Genetics 148:479–494

Hauge BM, Goodman HM (1992) Physical mapping by random clone fingerprint analysis. In: Beckman JS, Osborn TC (eds) Plant genomes: methods for genetic and physical mapping. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 101–139

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker-aided selection using RFLP and PCR. Theor Appl Genet 95:313–320

Kauffman HE, Reddy APK, Hsieh SPY, Merca SD (1973) An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae*. Plant Dis Rep 57:537–541

Kinoshita T (1995) Report of the committee on gene symbolization, nomenclature and linkage groups. Rice Genet Newslett 12:9–153

Kosambi DD (1944) The estimation of map distance from recombination values. Ann Eugen 12:172–175

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin S-Y, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang Z-X, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nature Genet 8:365–372

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

Lincoln S, Lander E (1992) Systematic detection of errors in genetic linkage data. Genomics 14:604–610

Liu YG, Whittier RF (1995) Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. Genomics 25:674–681

Liu YG, Mitsukawa N, Oosumi T, Whittier RF (1995) Efficient isolation and mapping of *Arabisopsis thaliana* insert junctions by thermal asymmetric interlaced PCR. Plant Jour 8:457–463

Ogawa T, Lin L, Tabien RE, Khush GS (1987) A new recessive gene for resistance to bacterial blight of rice. Rice Genet Newslett 4:98–100

Ou SH (1972) Rice diseases. Commonwealth Mycology Institute

Rouyer F, de la Chapelle A, Anderson M, Weissenbach J (1990). An interspersed repeated sequence specific for human subtelomeric regions EMBO J 9:505–514

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

Shizuya H, Birren B, Kim U, Mancino V, Slepak T, Tachiiri Y, Simon M (1992) Cloning and stable maintenance of 300 kilobase-pair fragments of human DNA in *Escherichia coli* using an F-factor based vector. Proc Natl Acad Sci USA 89:8794–8798

Song YL, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet Z, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 279 : 1804–1806

Song YL, Pi LY, Wang GL, Gardner J, Holsten T, Ronald P (1997) Evolution of the rice *Xa21* disease resistance gene family. Plant Cell 9 : 1279–1287

Tanksley SD, Ganal MN, Martin GB (1995) Chromosome landing: a paradigm for map-based cloning in plants with a large genome. Trends Genet 11 : 63–68

Wu KS, Tanksley SD (1993) PFGE analysis of the rice genome: estimation of the fragment sizes, organization of the repetitive sequences and relationships between genetic and physical distances. Plant Mol Biol Rep 23 : 243–254

Yang D, Parco A, Nandi S, Subudhi S, Zhu Y, Wang G, Huang N (1997) Construction of a bacterial artificial chromosome (BAC) library and identification of overlapping BAC clones with chromosome 4-specific RFLP markers in rice. Theor Appl Genet 95 : 1147–1154

Yoshimura S, Yoshimura A, Iwata N, McCouch SR, Abenes ML, Baraoidan M, Mew TW, Nelson RJ (1995) Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers. Mol Breed 1 : 375–387

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang ZX, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998) Expression of *Xa1*, a bacterial blight resistance gene in rice, is induced by bacterial inoculation. Proc Acad Natl Sci USA 95 : 1663–1668

Zhang G, Angeles ER, Abenes MLP, Khush GS, Huang N (1996) RAPD and RFLP mapping of the bacterial blight resistance gene *xa13* in rice. Theor Appl Genet 93 : 65–70