Mol Genet Genomics (2001) 265: 118–125
DOI 10.1007/s004380000382

ORIGINAL PAPER

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Chromosome landing at the bacterial blight resistance gene *Xa4* locus using a deep coverage rice BAC library

Received: 22 June 2000 / Accepted: 26 September 2000 / Published online: 27 January 2001

Abstract *Xa4* is a dominantly inherited rice gene that confers resistance to Philippine race 1 of the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* in rice. In order to isolate the gene by positional cloning, a bacterial artificial chromosome (BAC) library was constructed from genomic DNA isolated from an *Xa4*-harboring accession, IRBB56. The library contains 55,296 clones with an average insert size of 132 kb, providing 14 rice genome equivalents. Three DNA markers closely linked to *Xa4* were used to screen the library. The marker RS13, a resistance gene analogue that co-segregates with *Xa4*, identified 18 clones, of which four and six, respectively, were simultaneously detected by the other two markers, G181 and L1044. Fingerprinting and Southern analysis indicated that these clones overlapped and define an interval spanning 420 kb. In an F_2 population derived from an *indica* variety, IR24, and its *Xa4*-containing near isogenic line (NIL), IRBB4, the susceptible plants were screened in order to map the *Xa4* gene genetically and physically. Out of 24 insert ends isolated from the BACs in the contig, three revealed polymorphisms between IR24 and IRBB4. Two insert ends, 56M22F and 26D24R, flanked *Xa4* on each side. Based on the overlap of the BACs, six overlapping clones were considered to include the *Xa4* allele, one of which, 106P13, was chosen for further investigation.

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Key words Bacterial Artificial Chromosome (BAC) library · Positional cloning · Bacterial blight · Disease resistance · Rice

Introduction

Positional cloning provides a universal strategy for the isolation of a given gene based on its phenotype and genomic location. The first requirement for this strategy is the availability of a detailed genetic map for the genomic region of the target gene, so that the linked markers can be used as landmarks to access the gene. A second requirement is a large-fragment insert library containing the DNA of the target gene. When both requirements are met, the target gene can be isolated by chromosomal walking. In an alternative, and a much more efficient strategy, chromosome landing, selective enrichment for DNA markers within the sub-cM region around the target gene, can facilitate isolation of the target gene (Tanksley et al. 1995). Such DNA markers can be obtained either from high-density RFLP maps or by PCR-based approaches (Leister et al. 1996). During the past few years, the PCR-based approach using degenerate primers has been successfully utilized in the cloning of putative nucleotide binding site (NBS)-containing resistance gene analogs (RGAs) from potato, soybean, rice, maize, wheat, barley, *Arabidopsis*, lettuce and tomato (Kanazin et al. 1996; Leister et al. 1996, 1998, 1999; Yu et al. 1996; Aarts et al. 1998; Collins et al. 1998; Ohmori et al. 1998; Shen et al. 1998; Mago et al. 1999; Graham et al. 2000; Pan et al. 2000; Seah et al. 2000). Some of these RGAs have been shown to be associated with known resistance (*R*) gene loci in these plants. Thus, such RGAs can be exploited in the chromosome landing strategy for isolation of these *R* genes.

Bacterial blight (BB) is one of the most serious rice diseases in Asia. It is caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). Currently, more than 20 genes that confer host resistance against the pathogen have been identified (for reviews, see Kinoshita 1995 and Gnanamanickam

et al. 1999; Lin et al. 1996; Zhang et al. 1998). Genetic mapping of these resistance genes not only permits marker-assisted selection in rice breeding programs, but also facilitates cloning of these genes. So far, two BB resistance genes, *Xa21* and *Xa1*, have been cloned (Song et al. 1995; Yoshimura et al. 1998), and two recessive BB resistance genes, *xa5* and *xa13*, have been physically mapped (Yang et al. 1998; Sanchez et al. 1999). *Xa4* used to be the most widely exploited resistance gene in many Asian rice breeding programs and conferred durable resistance in many commercial rice cultivars (Mew et al. 1992; Li et al. 1999). It forms part of a resistance gene cluster including *Xa3, Pi-1(t), Pi-k,* and *Pi-f* on chromosome 11 (Xiao et al. 1992; Leister et al. 1998, 1999). Moreover, the use of rice accessions in which *Xa4* is combined with other BB resistance genes could result in a higher resistance level and an even wider resistance spectrum than those associated with a single resistance gene (for review, see Huang et al. 1997; Zheng et al. 1998; Gnanamanickam et al. 1999). These studies indicate a new approach to the further utilization of BB resistance genes that have been widely exploited. Therefore, we have chosen *Xa4* as a target for positional cloning.

Xa4 was first localized by Yoshimura et al. (1992) on rice chromosome 11, distal to the RFLP marker G181. Then Li et al. (1999) mapped it between RZ536 and G2132b. Recently, several RGAs amplified from rice by using degenerate primers have been mapped in this region (Leister et al. 1998, 1999; Mago et al. 1999). In order to screen for RGAs associated with *Xa4* locus, we also exploited the PCR-based approaches. One of the RGAs, RS13, which has high sequence similarity to cloned NBS-LRR genes, had been mapped to the distal flank of G181 (Zheng et al., unpublished data). By using an F_2 population of 467 individuals derived from an *indica* variety, IR24, and its *Xa4* NIL, IRBB4, RS13 had been found to co-segregate with *Xa4* and was localized between the RFLP markers G181 and L1044 (Wang et al. 2000). Thus, RS13 could be used to land at the *Xa4* locus. In order to do so, a BAC library for IRBB56, a pyramid accession with three BB resistance genes including *Xa4* (Huang et al. 1997), was constructed. The markers RS13, G181 and L1044 were used to screen the library. Then, a contig was developed that spanned approximately 420 kb and included the *Xa4* locus. Subsequently, by genetic and physical mapping, the *Xa4* allele was localized within a region of approximately 90 kb in length covered by each of six overlapping BAC clones. This result will facilitate the isolation of the BB resistance gene *Xa4*, while the linked markers can be exploited in marker-assisted breeding programs.

Materials and methods

Plant materials and phenotype scoring

The *indica* rice accession IRBB56, a NIL of IR24, pyramiding three bacterial resistance genes (Huang et al. 1997), was used to construct a BAC library. One F_2 population consisted of 1401 individuals derived from a cross between IR24 (*xa4xa4*, susceptible to race 1 of *Xoo*) and its NIL, IRBB4 (*Xa4Xa4,* resistant). The parents and the F_2 population were inoculated with Philippine race 1 of *Xoo* in the net-house using the leaf clipping method of Kauffman et al. (1973). Reaction to the pathogen was determined 18 days after inoculation. Plants were scored as being resistant or susceptible based on comparison of the lesion lengths with those found in their parents. Leaves of individuals that were fullly susceptible to *Xoo* were harvested for DNA isolation.

BAC library construction

The BAC vector pCUGIBAC1 was developed by Dr. Meizhong Luo (Luo et al., unpublished data). A single colony of DH10B containing BAC vector pCUGIBAC1 from a freshly streaked plate was inoculated into 100 ml of LB medium containing chloramphenicol and ampicillin. After incubation at 37 °C overnight, plasmids were isolated using the Qiagen Plasmid midi kit. Then the clone vector was subjected to *Hin*dIII digestion and dephosphorylated with calf intestinal phosphatase (CIP, Biolab). BAC library construction was performed essentially as described by Zhang et al. (1996) with the following modifications. The first size selection used PFGE switch times of a 1–50 s linear ramp. Fractions between 100 kb and 300 kb were cut from the gel in two pieces, one from 100 kb to 200 kb and another from 200 kb to 300 kb. Then the second size selection was run at a constant 10 s switch time to remove small, trapped DNA fragments. After removing appropriate fractions from the second PFGE run, DNA was recovered from the agarose gel by electroelution into dialysis tubing. Transformed cells were plated on 200 ml of selective medium (LB, Luria-Bertani medium) in 24×24 cm plates (Genetix) with 12.5 μg/ml chloramphenicol, 0.55 mM IPTG, and 80 μg/ml X-Gal. After about 20 h of incubation at 37 °C, white, recombinant colonies were picked robotically using the Genetix Q-BOT and arrayed as individual clones in 384-well microtiter plates (Genetix) containing 60 μl of freezing broth. After incubation at 37 °C overnight, microtiter plates were stored at −80 °C. BAC clone characterization was conducted following Woo et al. (1994).

BAC library screening

High-density colony filters for hybridization screening of the library were prepared using the Genetix Q-BOT. Clones were gridded in double spots using a 4×4 array with six fields per 22.5×22.5 cm of nitrocellulose (Hybond NT) filter. This gridding pattern allows 18,432 clones to be represented per filter. Library screening was performed using three filters, such that one filter set represented 55,296 clones. Colony filters were processed and hybridized using standard techniques (Sambrook et al. 1989). Three barley chloroplast genes, *ndhA, rbcL,* and *psbA* (provided by Dr. Jay DuBell, Texas A and M University) and four rice mitochondrial genes, *atpA, cox1, cob,* and *atp9* (provided by Sasaki T, Rice Genome Program of Japan), were used to screen the library for identification of organelle DNA contents. Screening with three markers linked to *Xa4* was performed to pick out clones for physical mapping of the *Xa4* gene locus. After determination of the address for each hit, clones were picked and analyzed as described by Tomkins et al. (1999).

Determination of the insert size in BAC clones

The insert size of each BAC clone was determined by CHEF electrophoresis after *Not*I digestion. Switch times were ramped from 5 to 10 s, at 14 °C and 6 V/cm, using $0.5 \times$ TBE buffer for 15 h. The size of each BAC clone was estimated based on its mobility in comparison to molecular weight markers.

Isolation of insert ends from BAC clones

The insert ends of BAC clones were obtained by TAIL-PCR as described by Liu and Whittier (1995). The primers for TAIL-PCR were synthesized as described by Yang et al. (1998).

DNA isolation and Southern analysis

Total DNA was extracted from individuals that were absolutely susceptible to *Xoo* using the method of McCouch et al. (1988). Southern analysis followed the standard procedure (Sambrook et al. 1989). RFLP markers G181 and L1044 were kindly provided by the Rice Genome Project of Japan (Harushima et al. 1998).

Results

BAC library construction and characterization

A rice BAC library, consisting of 55,296 clones, was constructed from the rice accession IRBB56. Analysis of a random sample of 522 BACs picked from the library during the course of library construction showed an average insert size of 132 kb, with a range of 30–260 kb. The 522 BACs were grouped by insert size and the insert size of each clone was plotted against the frequency of each group of clones represented in the library (Fig. 1). Based on this analysis, over 80% of the insert-containing clones were estimated to have an average insert size greater than 100 kb. Of the clones larger than 100 kb, 60% were between 140–170 kb and more than 85% had inserts greater than 130 kb. Considering the average insert size and a haploid genome size of 430 Mb (Arumuganthan and Earle 1991), the coverage of the library was about 14 genome equivalents, resulting in a 99.99% probability of recovering any specific sequence of interest, though some 17% of clones did not contain inserts. To determine the representation of organelle DNAs in the library, the library were screened with three different chloroplast genes spaced equidistantly around the 133 kb barley chloroplast genome and four rice mitochondrial genes, respectively. Results from this screen showed that approximately 0.85% of library sequences were chloroplast DNA and 0.11% were mitochondrial DNA homologs (data not shown).

BAC library screening with markers linked to *Xa4*

The three DNA markers G181, RS13, and L1044, which are linked to *Xa4*, were used to screen the library, resulting in 13, 18, and 106 hits, respectively (data not shown). The high hit number detected by L1044 implied that L1044 might contain multicopy sequences. The marker RS13, a resistance gene analogue that co-segregates with *Xa4*, picked out 18 candidate BAC clones, of which 1F21, 26D24, 56M22, and 111E1 were simultaneously detected by the RFLP marker G181; and 1F21, 33M8, 56M22, 61A13, 104B15, and 111E1 were simultaneously detected by L1044 (Table 1). These results implied that these clones may overlap and cover the region between L1044 and G181 on chromosome 11.

Identification of candidate BACs at the *Xa4* locus

It is difficult to handle all of the BACs picked out by the three probes linked to *Xa4*. So, we focused on the 18 clones recognized by RS13, some of which were simultaneously detected by G181 and L1044. The insert sizes

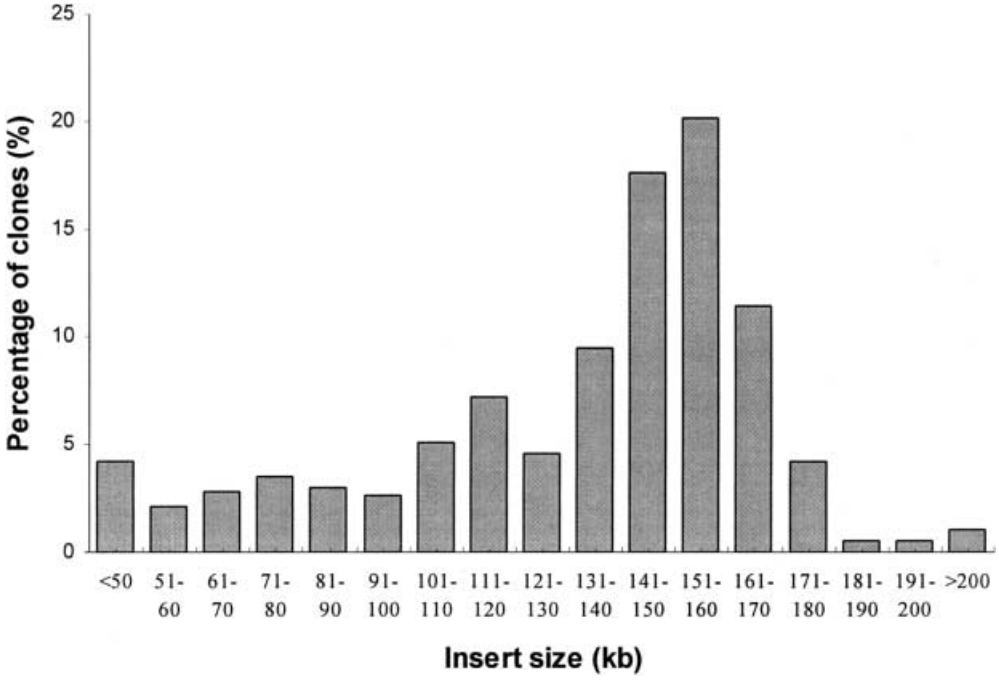

Fig. 1 Insert size distribution of BAC clones in the IRBB56 BAC library. BAC DNAs of 522 randomly selected clones were digested with *Not*I. The insert sizes were determined by CHEF electrophoresis and plotted against frequency of occurrence

Table 1 Candidate BAC clones covering the *Xa4* locus

Clone no.[a]	Clone address	Insert size (kb)[b]	Positive probe
1	56M22	140	RS13, L1044, G181
2	111E1	140	RS13, L1044, G181
3	117I22	175	RS13
4	106P13	180	RS13
5	50B1	150	RS13
6	43B6	160	RS13
7	29H13	170	RS13
8	26D24	160	RS13, G181
9	45B20	145	RS13
10	104B15	180	RS13, L1044
11	61A13	140	RS13, L1044
12	33M8	165	RS13, L1044
13	1F21	130	RS13, L1044, G181
14	37E12	170	RS13
15	81P3	150	RS13
16	95F11	160	RS13
17	99P13	160	RS13
19	110C24	155	RS13

[a] The first 12 BACs was used to construct a contig covering the *Xa4* locus

[b] The insert size was determined by CHEF electrophoresis after *Not*I digestion

in these clones were in a range of 130–180 kb, as determined by PFGE analysis of *Not*I-digested BACs (Table 1). The *Hin*dIII restriction patterns revealed that six BAC clones (1F21, 37E12, 81P3, 95F11, 99P13, and 110C24, as shown in Fig. 2A) were completely included by others, and therefore these were not used in our further investigation. The other 12 BACs were subjected to Southern analysis to confirm their identity. In *Hin*dIII digests of individual BACs, G181, L1044 and RS13 probes detected 1–5 hybridizing bands, and thus, all were indeed positive for all three probes. The hybridizing bands also revealed the locations of the three probes in the positive clones. G181 detected one band common to 56M22, 111E1 and 26D24 (Fig. 2B). Thus, G181 was located in the overlap between these clones. L1044 detected bands at two different positions: one band was common to 56M22 and 111E1, both harboring G181, the other was common to 104B15, 33M8, and 61A13 (Fig. 2C), implying that homologs of L1044 were situated in different regions. Nevertheless, according to the genetic map (Wang et al. 2000), L1044 should be located in the overlap between 104B15, 33M8, and 61A13 (see below). When RS13 was used as a probe, two to five hybridizing bands were seen in the *Hin*dIII digests of the BACs, and each band was common to at least two BACs (Fig. 2D), confirming that these BACs overlap and implying that a cluster of RS13 homologs exists in the region. In a rice accession harboring *Xa4*, RS13 could detect up to five *Hin*dIII bands (data not shown). It is noted that among the BACs recognized by RS13, the clones 106P13 and 43B6 included all the hybridizing bands detectable (Fig. 2D).

Construction of a BAC contig in the *Xa4* region

The 12 overlapping BACs were arranged to form a contig based on their *Hin*dIII and *Not*I restriction patterns. To confirm this arrangement and estimate the length of the contig, Southern analyses were conducted by using forward and reverse insert ends from the BACs as probes (Fig. 3). As the reverse insert end of 56M22 (56M22R) detected three unique extra bands in 56M22 (Fig. 3A), 56M22R should represent one of the outermost ends of the contig. The forward insert end of

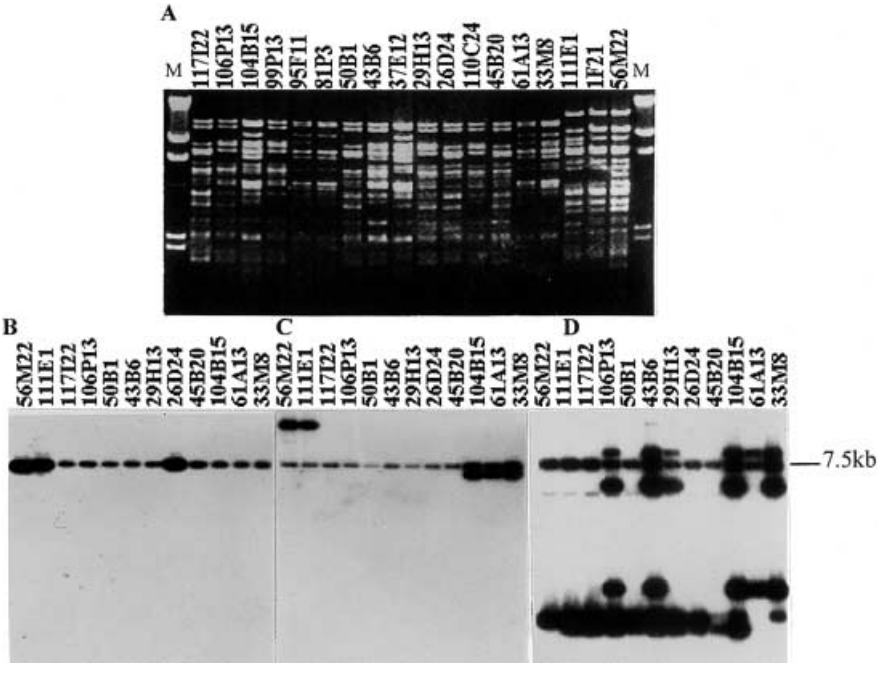

Fig. 2A–D Analysis of candidate clones covering the *Xa4* locus. **A** *Hin*dIII restriction patterns of 18 candidate BACs. M, marker (*Hin*dIII digest of λ DNA). **B–D** Hybridization patterns revealed by the probes G181 (**B**), L1044 (**C**) and RS13 (**D**)

56M22 (56M22F) detected two bands in 56M22 that were shared by 111E1, 117I22, 106P13, 50B1, 29H13, 26D24 and 45B20 (Fig. 3B); therefore, the insert end 56M22F overlapped with these BACs. Similarly, it was deduced that the forward insert end of 26D24 (26D24F) overlapped with 56M22 and 111E1 (Fig. 3C). The bands situated at both the reverse insert end of 26D24 (26D24R) and the reverse insert end of 43B6 (43B6R) were shared by 117I22, 106P13, 50B1, 43B6, 29H13, 26D24 and 45B20 (Fig. 3D and E); thus, 26D24R and 43B6R overlapped with these BACs. The forward insert end of 43B6 (43B6F) overlapped with 104B15, 61A13 and 33M8 (Fig. 3F). The forward insert end of 104B15 (104B15F) overlapped with 117I22, 106P13, 50B1, 43B6, 29H13, 45B20 and 33M8 (Fig. 3G). The reverse end of 104B15 (104B15R) detected two bands shared by 104B15 and 61A13 (Fig. 3H), indicating that their ends were probably situated at approximately the same site forming the other end of the contig. This deduction was then confirmed by probing *Hin*dIII-digested BACs in the contig with the insert ends of 61A13 (data not shown). The insert of 61A13 was shorter than that of 104B15, as shown by their *Hin*dIII-restriction patterns (Fig. 2A) and insert size determination based on PFGE analysis after *Not*I digestion (Table 1). Thus, 56M22 and 104B15 represent the two outermost clones on each end of the contig, and are 140 kb and 180 kb in length, respectively. Because both 56M22F and 104B15F overlap with 117I22 (Fig. 3B and G), which is 175 kb in length and shares 55 kb with 56M22 and 20 kb with 104B15, as shown by their *Hin*dIII and *Not*I restriction patterns (Fig. 2A, data not shown), the physical distance between 56M22 and 104B15 is 100 kb. Thus, the ordered contig spans about 420 kb. This contig is covered by three BACs, namely, 56M22, any one of the five central BACs (117I22, 50B1, 29H13, 45B20, and 106P13), and 104B15.

Identification of BACs contain the *Xa4* allele

In order to locate the *Xa4* allele in the BAC contig, an F_2 population containing 1401 plants was obtained from a cross between IR24 and its *Xa4* NIL IRBB4, in which the resistant gene *Xa4* was completely dominant to its susceptible allele. It was therefore fairly easy to score the susceptible individuals. The ratio between the resistant and susceptible individuals was consistent with the expected single-gene segregation ratio of 3:1 (339 susceptible out of 1401 plants, $\chi^2 = 0.482$, $P = 0.4$–0.5). However, the resistant phenotype might be attributable to either the resistant genotype or to the failure of *Xoo* infection. In addition, both homozygous and heterozygous individuals with *Xa4* could contribute to resistant phenotypes, so that the exchange events between the linked markers and *Xa4* might not be revealed. Therefore, only the 339 susceptible individuals were selected for linkage analyses. The NIL parents were probed with 24 isolated BAC insert-ends by Southern analysis. Three of them revealed polymorphisms between the NIL parents; for these, 56M22F detected three recombinants, 26D24R five recombinants, and 104B15R six recombinants among the susceptible individuals. Since the recombinants detected by 56M22F were different from those detected by 26D24R and 104B15R, *Xa4* should map in the region between 56M22F and 26D24R, an interval corresponding to a genetic distance of 1.1 cM and a physical length of approximately 90 kb. Based on the physical relationship between the BACs, the *Xa4* allele was located in the middle six clones of the contig as shown in Fig. 4B.

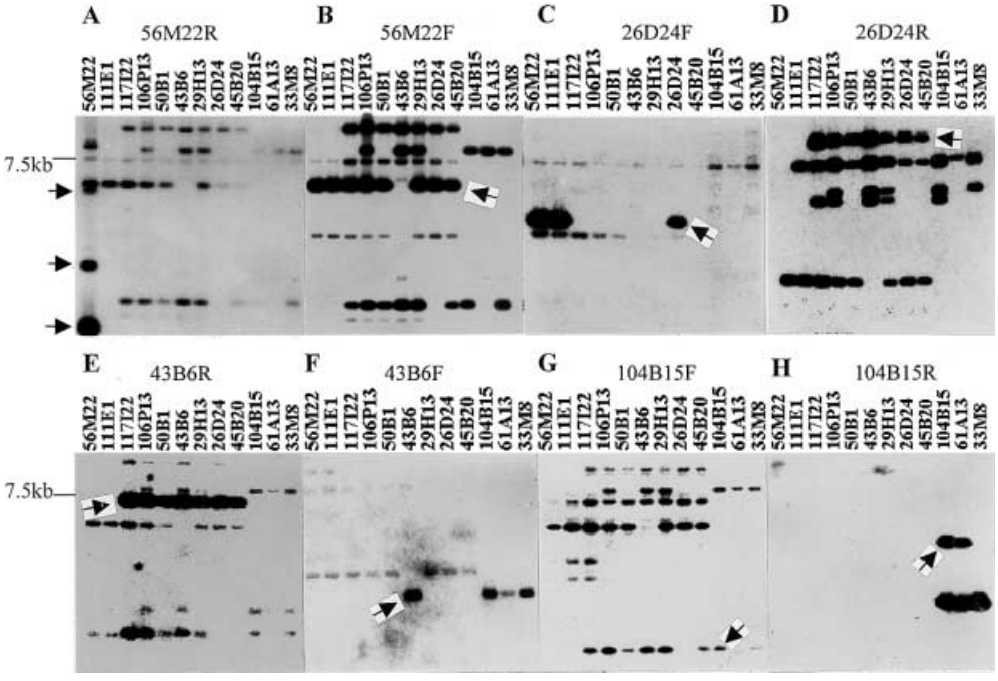

Fig. 3A–H Southern hybridization of 12 overlapping BACs digested with *Hin*dIII and probed with the insert-ends from some overlapping BACs. The insert-ends used as probes are indicated *above* the photographs. The *arrows* indicate the end fragments and their counterparts in the overlapping clones

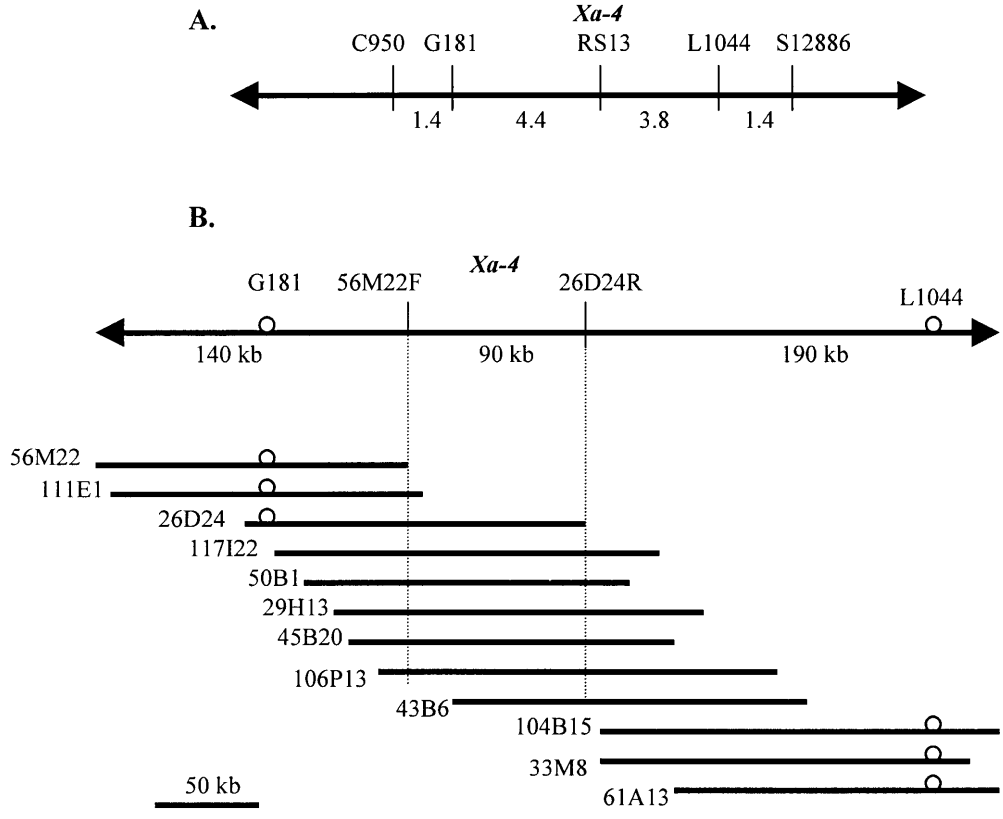

Fig. 4A, B Genetic and physical maps of the *Xa4* locus. **A** The *double arrowed line* represents chromosome 11. The *numbers under the line* are map distances in Centimorgans. **B** The *doubly arrowed line* represents the chromosome region surrounding the *Xa4* locus, covered by the three cloned DNA fragments, whose sizes were estimated by CHEF electrophoresis. The *horizontal lines below the map* represent the BACs named on the *left*. The region between the dotted vertical lines probably contains the *Xa4* locus

Discussion

Currently, quite a few BAC libraries for various rice varieties have been constructed by different laboratories (Tao et al. 1994; Wang et al. 1995; Zhang et al. 1996; Nakamura et al. 1997; Yang et al. 1997). However, the usefulness of these libraries is limited because their size is relatively small (3–8 genome equivalents), or they do not contain some specific target genes. In the present study, in order to isolate the *Xa4* gene, a BAC library, characterized by high clone number, large insert fragments, deep coverage of the rice genome, and a low level of organellar DNA, was constructed from the rice accession IRBB56. IRBB56 was chosen for BAC library construction because it carries not only the *Xa4* gene, but also *xa5* and *xa13*, two recessive genes conferring resistance to *Xoo* (Huang et al. 1997). In addition, IRBB56 had been developed from IR24, a restorer line that has been widely exploited in hybrid rice production. Thus, the library could be used, too, for screening for the restorer gene for cytoplasmic male sterility and other important genes. The library size of fourteen genome equivalents makes it one of the most comprehensive BAC libraries available in rice and provides a 99.99% probability that any desired rice sequence is represented in the library.

The deep coverage of the BAC library was confirmed by the high hit numbers for the three markers (G181, RS13, and L1044) linked to the *Xa4* gene. The hit numbers for G181 and RS13 were in the range expected based on the library coverage. However, the hit number detected by L1044 was unexpectedly high. Southern analysis of the *Hin*dIII-digested BACs indicated that G181 was a single-copy probe and RS13 was a clustered multicopy probe (Fig. 2B and D). Hybridization patterns shown in Fig. 2C clearly illustrated that there were two copies of L1044 located in each of the five positive BACs, and analysis of the other BACs that were positive with L1044 revealed that there were other copies further away (data not shown). Thus, L1044 must be a dispersed multicopy probe, which would account for its high hit number. It was also comprehensible that three BACs, 56M22, 111E1, and 1F21, did not cover the whole contig though they were simultaneously detected by all the three markers.

By making use of conserved domains of cloned *R* genes, investigators have obtained RGAs associated with known *R* gene loci (Leister et al. 1996; Mago et al. 1999; Seah et al. 2000). These RGAs can be used as a starting point for the isolation of *R* genes following the chromosome landing strategy. Previously, we had mapped the RGA fragment RS13 between L1044 and G181, cosegregating with the *Xa4* (Wang et al. 2000). In the present study, based on the construction of a deep-coverage BAC library and the development of an F_2 population containing 1401 plants from a cross between IR24 and its *Xa4* NIL IRBB4, we constructed a contig spanning approximately 420 kb with three BACs as the minimum tiling. Then the *Xa4* allele was localized

between two insert ends, 56M22F and 26D24R, on the basis of three and five recombinants in 339 susceptible individuals, respectively. The physical and genetic distances between the two insert ends were approximately 90 kb and 1.1 cM, respectively. The ratio of physical to genetic distance (< 100 kb/cM) was much smaller than the average for rice (300 kb/cM) (Arumuganathan and Earle 1991; Harushima et al. 1998). The higher recombination rate in this region, which greatly facilitated our fine-scale mapping of the *Xa4* locus, may be attributable to its location close to the telomere on the longer arm of chromosome 11.

Other studies on the cloning and mapping of dominant resistance genes have revealed that in most cases the situations were more complicated than initially anticipated. Disease resistance genes frequently occur in tightly linked clusters. Analysis of the *Xa21* gene family in rice revealed that at least seven family members are located within a 230-kb genomic region. Apart from *Xa21* itself, *Xa21D* displayed a resistance spectrum identical to that observed for *Xa21* but conferred only partial resistance to *Xoo* (Song et al. 1997; Wang et al. 1998). It has been shown that there are several resistance genes, including two BB resistance genes (*Xa4* and *Xa3*) and three rice blast disease resistance genes (*Pi-1(t), Pi-f,* and *Pi-k*), located in the region distal to G181 on chromosome 11 (Xiao et al. 1992; Leister et al. 1998, 1999). It is also noteworthy that quite a few RGAs have been located in this region (Leister et al. 1998, 1999; Mago et al. 1999; Zheng et al., unpublished data). RS13 has a sequence that is highly similar to genes of the NBS-LRR resistance gene class. In a previous study, it was used to probe varieties harboring the *Xa4* gene, and five *Hin*dIII bands were detected. In the present study, a BAC contig was derived from a BAC library for IRBB56, an *Xa4*-bearing line. When RS13 was used as a hybridization probe for *Hin*dIII digests of the BAC clones, two BAC clones (43B6 and 106P13) located in the middle of the contig, were found to include all of the five bands detectable with the probe. The multiple bands detected by RS13 imply that a family of resistance gene homologs exists at the *Xa4* locus, and complex rearrangements may have shaped this region. Recently, Li et al. (1999) identified an allele, referred to as the "defeated" resistance gene $Xa4^T$, at the *Xa4* locus in the *indica* cultivar Teqing, that also provides proof that a cluster of genes of the *Xa4* family is located in this region. Although the *Xa4* locus has thus far been considered to consist of a single dominant resistance gene, it is quite possible that the locus contains a cluster of closely linked resistance gene homologs. The BAC clone 106P13, which includes all five *Hin*dIII bands detectable by RS13 and covers the region between 56M22F and 26D24R, thus presumably carries all members of the *Xa4* gene family and is suitable for the isolation of the *Xa4* gene.

Acknowledgement The IRBB56 BAC library was constructed by W. Wang at Clemson University Genomics Institute (CUGI) financially supported by Rockefeller Foundation. Thanks to Drs. David A. Frisch, Jeff P. Tomkins, and Long Mao for their technical help and useful suggestions. Thanks also to Michael Atkins and Kerry Wilson for their help in picking up the library and preparing high-density filters. Thanks to Dilara Begum for her help with lab facilities and reagents. We are grateful to Prof. Q. Zhang and Dr. Y. Zhou for their help with phenotype scoring of the *Xa4* mapping population. We are also grateful to Drs. Daichang Yang and Guoliang Wang for their technical help in contig construction. This project was supported by grants from the Natural Science Foundation of China (Grant No.39630190), the National Key Basic Research Science Foundation of China (Grant No.G1998010207), and the Rockefeller Foundation Rice Biotechnology Program (Grant No. RF97001#590).

References

Aarts MG, te Lintel Hekkert B, Holub EB, Beynon JL, Stiekema WJ, Pereira A (1998) Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. Mol Plant Microbe Interact 11: 251–258

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9: 208–218

Collins NC, Webb CA, Seah S, Ellis JG, Hulbert SH, Pryor A (1998) The isolation and mapping of disease resistance gene analogs in maize. Mol Plant Microbe Interact 11: 968–978

Gnanamanickam SS, Priyadarisini VB, Narayanan NN, Vasudevan P, Kavitha S (1999) An overview of bacterial blight disease of rice and strategies for its management. Curr Sci 77: 1435–1444

Graham MA, Marek LF, Lohnes D, Cregan P, Shoemaker RC (2000) Expression and genome organization of resistance gene analogs in soybean. Genome 43: 86–93

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

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

Kanazin V, Marek LF, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93: 11746–11750

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

Leister D, Ballvora A, Salamini F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat Genet 14: 421–429

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, Schulze-Lefert P (1998) Rapid reorganization of resistance gene homologs in cereal genomes. Proc Natl Acad Sci USA 95: 370–375

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Graner A, Schulze-Lefert P (1999) RFLP- and physical mapping of resistance gene homologues in rice (*O. sativa*) and Barley (*H. vulgare*). Theor Appl Genet 98: 509–520

Li ZK, Luo LJ, Mei HW, Paterson AH, Zhao XH, Zhong DB, Wang YP, Yu XQ, Zhu L, Tabien R, Stansel JW, Ying CS (1999) A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonas oryzae* pv. *oryzae*. Mol Gen Genet 261: 58–63

Lin XH, Zhang DP, Xie YF, Gao HP, Zhang Q (1996) Identification and mapping of a new gene for bacterial blight resistance in rice based on RFLP markers. Phytopathology 86: 1156–1159

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

Mago R, Nair S, Mohan M (1999) Resistance gene analogues from rice: cloning, sequencing and mapping. Theor Appl Genet 99: 50–57

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

Mew T, Vera Cruz CM, Medalla ES (1992) Changes in race frequency of *Xanthonomas oryzae* pv. *oryzae* in response to rice cultivars planted in Philippines. Plant Dis 76: 1029–1032

Nakamura S, Asakawa S, Ohmido N, Fukui K, Shimizu N, Kawasaki S (1997) Construction of an 800-kb contig in the near-centromeric region of the rice blast resistance gene *Pi-ta*^{2} using a highly representative rice BAC library. Mol Gen Genet 254: 611–620

Ohmori T, Murata M, Motoyoshi F (1998) Characterization of disease resistance gene-like sequences in near-isogenic lines of tomato. Theor Appl Genet 96: 331–338

Pan Q, Liu YS, Budai-Hadrian O, Sela M, Carmel-Goren L, Zamir D, Fluhr R (2000) Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologs in the genomes of two dicotyledons. Tomato and *Arabidopsis*. Genetics 155: 309–322

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

Sanchez AC, Ilag LL, Yang D, Brar DS, Ausubel F, Khush GS, Yano M, Sasaki T, Li Z, Huang N (1999) Genetic and physical mapping of *xa13*, a recessive bacterial blight resistance gene in rice. Theor Appl Genet 98: 1022–1028

Seah S, Spielmeyer W, Jahier J, Sivasithamparam K, Lagudah ES (2000) Resistance gene analogs within an introgressed chromosomal segment derived from *Triticum ventricosum* that confers resistance to nematode and rust pathogens in wheat. Mol Plant Microbe Interact 13: 334–341

Shen KA, Meyers BC, Islam-Faridi MN, Chin DB, Stelly DM, Michelmore RW (1998) Resistance gene candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. Mol Plant Microbe Interact 11: 815–823

Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald PC (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. Science 270: 1804–1806

Song WY, Pi LY, Wang GL, Gardner J, Holsten T, Ronald PC (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

Tao Q, Zhao H, Qiu L, Hong G (1994) Construction of a full bacterial artificial chromosome (BAC) library of *Oryza sativa* genome. Cell Res 4: 127–133

Tomkins JP, Yu Y, Miller-Smith H, Frisch DA, Woo SS, Wing RA (1999) A bacterial artificial chromosome library for sugarcane. Theor Appl Genet 99: 419–424

Wang GL, Holsten TE, Song WY, Wang HP, Ronald PC (1995) Construction of a rice bacterial artificial chromosome library and identification of clones linked to the *Xa21* disease resistance locus. Plant J 7: 525–533

Wang GL, Ruan DL, Song WY, Sideris S, Chen LL, Pi LY, Zhang SP, Zhang Z, Fauquet C, Gaut BS, Whalen MC, Ronald PC (1998) *Xa21D* encondes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell 10: 765–779

Wang W, Zhou Y, Jiang G, Ma B, Chen X, Zhang Q, Zhu L, Zhai W (2000) Fine mapping of the rice bacterial blight resistance gene *Xa4* and its co-segregation marker. Chin Sci Bull 19: 1779–1784

Woo SS, Jiang J, Gill BS, Paterson AH, Wing RA (1994) Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. Nucleic Acids Res 22: 4922–4931

Xiao J, Fulton T, McCouch S, Tanksley S, Kishimoto N, Ohsawa R, Ukai Y, Saito A (1992) Progress in integration of the molecular maps of rice. Rice Genet Newslett 9: 124–128

Yang D, Parco A, Nandi S, Subudhi P, Zhu YG, Wang GL, 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

Yang D, Sanchez A, Khush GS, Zhu Y, Huang N (1998) Construction of a BAC contig containing the *xa5* locus in rice. Theor Appl Genet 97: 1120–1124

Yoshimura S, Nelson R, Yoshimura A (1992) RFLP mapping of the bacterial blight resistance gene *Xa3* and *Xa4*. Rice Genet Newslett 9: 136–138

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

Yu YG, Buss GR, Maroof MA (1996) Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93: 11751–11756

Zhang HB, Choi S, Woo SS, Li Z, Wing RA (1996) Construction and characterization of two rice bacterial artifical chromosome libraries from the parents of a permanent recombinant inbred mapping population. Mol Breed 2: 11–24

Zhang Q, Lin SC, Zhao BY, Wang CL, Yang WC, Zhou YL, Li DY, Chen CB, Zhu LH (1998) Identification and tagging a new gene for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) from *O. rufipogon*. Rice Genet Newslett 15: 138–142

Zheng KL, Zhuang JY, Wang HR (1998) Performance of resistance gene pyramids to races of rice bacterial blight in Zhejiang Province. Hereditas (Beijing) 20: 4–7