Theor Appl Genet (1996) 93: 117–122

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Identification of a YAC clone carrying the *Xa-1* allele, a bacterial blight resistance gene in rice

Received: 27 September 1995 / Accepted: 3 November 1995

Abstract Map-based cloning methods have been applied for isolation of *Xa-1*, one of the bacterial blight resistance genes in rice. *Xa-1* was previously mapped on chromosome 4 using molecular markers. For positional cloning of *Xa-1*, a high-resolution genetic map was made for the *Xa-1* region using an F_2 population of 402 plants and additional molecular markers. Three restriction fragment length polymorphism (RFLP) markers, *XNpb235*, *XNpb264* and *C600* were found to be linked tightly to *Xa-1*, with no recombinants, and $U08_{750}$ was mapped 1.5 cM from *Xa-1*. The screening of a yeast artificial chromosome (YAC) library using these *Xa-1*-linked RFLP markers resulted in the identification of ten contiguous YAC clones. Among these, one YAC clone, designated Y5212, with an insert of 340 kb, hybridized with all three tightly linked markers. This YAC was confirmed to possess the *Xa-1* allele by mapping the *Xa-1* gene between both end clones of this YAC (Y5212R and Y5212L).

Key words Plant disease resistance · Rice · *Xanthomonas oryzae* pv. *oryzae* · YAC

Communicated by J. W. Snape

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Introduction

Xa-1 is one of the many bacterial blight resistance genes in rice. Bacterial blight is a serious rice disease caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*X. oryzae* pv. *oryzae*). The genetic basis of host resistance to bacterial blight has been studied in great detail, and at least 19 resistance genes have been identified, with some of them mapped by various scientists on the genetic map of rice (Kinoshita 1991). On the basis of the genetic characters and physiological reactions involved in the resistance of *Xa-1*, which is specific to a race of *X. oryzae* pv. *oryzae*, *Xa-1* can be considered to be a dominant (Ogawa and Khush 1989) resistance gene. Linkage analysis of *Xa-1* was first done by Sakaguchi in 1967 who mapped it to chromosome 4. Yoshimura (1993) got the same result by mapping *Xa-1* using restriction fragment length polymorphism (RFLP) markers. However, information on the gene product of *Xa-1* and on the mechanisms of interaction between host resistance and pathogen virulence is still lacking.

In recent years, map-based cloning methods have been applied in the isolation of a few plant resistance genes, such as *Pto* in tomato (Martin et al. 1993) and *RPS2* in *Arabidopsis* (Mindrinos et al. 1994). Cloning of disease resistance genes constitutes an exciting breakthrough for understanding the molecular basis of plant disease resistance. The first step towards map-based cloning would be to discover molecular markers closely linked to the target gene and to determine an accurate position of the target gene on a genetic map. For a reliable detection of the segregation of the phenotype, a clear phenotype conferred by the target gene is required. Since *Xa-1* shows a very clear resistance phenotype, it would be a promising candidate for map-based cloning.

In this paper, we describe the isolation of a yeast artificial chromosome (YAC) clone containing the *Xa-1* allele through high-resolution mapping with RFLP markers. A YAC library constructed from rice cv 'Nipponbare', the standard variety used in the Japanese Rice Genome Re-

search Program (RGP), was screened using *Xa-1*-linked RFLP markers.

The cloning and molecular analysis of *Xa-1* should help in revealing the possible role of this gene during pathogen recognition or in subsequent intracellular events leading to the expression of resistance in rice. Furthermore, the comparative analysis of *Xa-1* and several other previously isolated resistance genes would clarify the similarities and specific structures among resistance genes in different host plants that control resistance to various pathogens. Such an accumulation of information on resistance gene structures and functions would lead to a clearer understanding of defense expression pathways in plants.

Materials and methods

Plant materials

The rice cultivars used in this study are listed in Table 1. 'IR-BB1' derives *Xa-1* from *japonica* cv 'Kogyoku' and has undergone four backcrosses with *indica* cv 'IR24' and eight selfing generations (Ogawa et al. 1988, 1991). 'IR24' is an *indica* cv carrying *Xa-18*, which is not effective against any known Japanese strains of *X. oryzae* pv. *oryzae*.

For studying linkage analysis between *Xa-1* and RFLP markers, we crossed 'IR24' with 'IR-BB1' or 'Kogyoku'. Six F_2 populations derived from the crosses between 'IR24' and 'Kogyoku', and between 'IR24' and 'IR-BB1' (Table 1) were used for bacterial inoculation tests and DNA extraction. Another F_2 population derived from the cross between 'Nipponbare' and 'Kasalath' (Table 1, Kurata et al. 1994) was also used for linkage analysis of RFLP markers and physical mapping of the chromosomal region around *Xa-1* on chromosome 4.

The plants were grown at the experimental farm of the National Institute of Agrobiological Resources in Tsukuba, Japan.

Detection of *Xa-1* phenotype

The segregation of *Xa-1* was examined by testing the six F_2 populations of 'IR24'/'Kogyoku' and 'IR24'/'IR-BB1' crosses (Table 1)

Table 1 Lines and F_2 populations used

Line	Gene(s) involved	Generation (population size)	Cross
IR24	*Xa-18*[c]	–	–
Kogyoku	*Xa-1*	–	–
IR-BB1[a]	*Xa-1*	BC_4F_8	IR24*5/kogyoku
F_2 populations			
	Xa-1	F_2 (98 plants)	IR24/Kogyoku
	Xa-1	F_2 (35 plants)	IR24/Kogyoku
	Xa-1	F_2 (57 plants)	IR24/Kogyoku
	Xa-1	F_2 (50 plants)	IR24/IR-BB1
	Xa-1	F_2 (81 plants)	IR24/IR-BB1
	Xa-1	F_2 (81 plants)	IR24/IR-BB1
		(Total 402 plants)	
	None	F_2 (186 plants)	Nipponbare/ Kasalath[b]

[a] Ogawa et al. [13, 14]
[b] Kurata et al. [6]
[c] *Xa-18* is not effective against any known strains of *Xanthomonas oryzae* pv. *oryzae* in Japan

Fig. 1 Rice leaves inoculated by T7174, the representative strain of Japanese race I of *Xanthomonas oryzae* pv. *oryzae*. 'IR-BB1' is the resistance cultivar possessing the resistance gene *Xa-1*. On the susceptible cultivar, 'IR24', bacterial lesions developed up to 16 cm in size. The photograph was taken at 14 days after inoculation

for their reaction to *X. oryzae* pv. *oryzae* strain T7174, a representative strain of pathogenic race 1 in Japan. For inoculum preparation, the bacterial cells were grown on potato semi-synthetic agar (PSA; Ou 1972) slants for 3 days at 30°C. Inoculum was prepared by suspending the bacterial culture with sterile water to a concentration of about 10^9 cells/ml. Five to six fully expanded leaves of each F_2 plant and parent were inoculated by the clipping method (Kauffman et al. 1973) at 3 months after sowing. Disease reaction was scored visually at 14 days after inoculation and classified into two categories, resistant and susceptible. As shown in Fig. 1, the bacterial lesions elongated less than 1 cm and then stopped on the resistant plants, whereas on the susceptible plants, the lesions were more than 10 cm in length.

Molecular markers

Using an F_2 population of 142 plants derived from the cross between 'Kinmaze' and 'Te-tep' in which the F_2 segregated for *Xa-1* and *Xa-2*, Yoshimura (1993) showed that the RFLP markers *XNpb235*, *XNpb264* and *XNpb267* mapping on chromosome 4 were linked to *Xa-1*. Two RAPD markers $U08_{750}$ and $Y03_{700}$, tightly linked to *Xa-1*, were also obtained by near-isogenic line analysis, and these markers were cloned to be used as RFLP markers (Yoshimura et al. 1995). Tsunematsu *et al.* (1993) also found another RFLP marker, *Q4*, linked to *Xa-1* using recombinant inbred lines segregating for

Xa-1. For the fine mapping of *Xa-1* and subsequent YAC isolation, five genomic DNA markers, Npb235, Npb264, Npb267, $U08_{750}$ and Ky4 (same marker as Q4), were additionally used in this study. Two cDNA markers [C600 and R78 (Kurata et al. 1994)] and two YAC end clones (Y5212L and Y5212R), isolated from a YAC clone Y5212, were also used. The end clones were isolated according to the cassette polymerase chain reaction (PCR) method as described by Umehara *et al.* (1995). In this paper, the name of the mapped locus with the Npb number clone is represented by *XNpb* according to its first designation (Saito et al. 1991). RFLP marker designations are denoted by normal letters, while the corresponding loci are written in italics.

Genetic mapping

Rice genomic DNAs were isolated from fresh leaves according to Dellaporta *et al.* (1983) with a slight modification: 3.65 m*M* sodium hydrogen sulfite was used instead of 10 m*M* mercaptoethanol as a reducing agent in the extraction buffer. Total genomic DNA was digested with the restriction enzymes *Bam*HI, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII or *Pvu*II according to the supplier's instructions, separated by electrophoresis on 0.6% agarose gel and transferred onto nylon membranes. Labeling of the probes and signal detection were done using ECL system (Amersham) according to the supplier's instructions. Chemiluminescence was detected by X-ray film after a 2- to 16-h exposure. Polymorphism was scored in parents and F_2 plants.

Linkage analysis was carried out using the software MAPMAKER/EXP. 3.0 (Lander et al. 1987; Lincoln and Lander 1992), and the recombination values were converted into genetic map distances (centiMorgan; cM) using the Kosambi function (Kosambi 1944).

Physical mapping

A physical map of the *Xa-1* region was constructed by screening a YAC library made from 'Nipponbare' (Umehara et al. 1995) in order to select YAC clones covering the target region. Colony hybridization was done using a total of about 7000 YAC clones in the library dotted on five high-density filters. The six *Xa-1*-linked RFLP markers, Npb235, Npb264, $U08_{750}$, Ky4, C600 and R78 were used as probes. All candidate YAC clones selected by colony hybridization were applied on Southern hybridization analysis to confirm whether those YACs were derived from the marker loci (Umehara et al. 1995). The ECL labeling and detection system was also used for both colony and Southern hybridization. A contig map of YACs was constructed by following the arrangement of the RFLP markers on the linkage map.

Results

Fine genetic mapping of the *Xa-1* gene

For fine mapping of *Xa-1*, large-scale linkage analysis was performed using six F_2 populations, each of which segregated for *Xa-1* (Table 1). Four RFLP markers, Npb235, Npb264, $U08_{750}$ and C600 were scored in these populations. The segregation ratios of all of these markers fitted the expected ratio at the 5% confidence level. Npb267 and Ky4 could not be mapped on these populations, because of no clear polymorphism. The bacterial blight resistant and susceptible phenotypes of all F_2 plants could be clearly scored and the segregation ratio fitted the expected 3:1 ratio for a single dominant gene. The six F_2 populations were integrated for calculation of recombination frequency

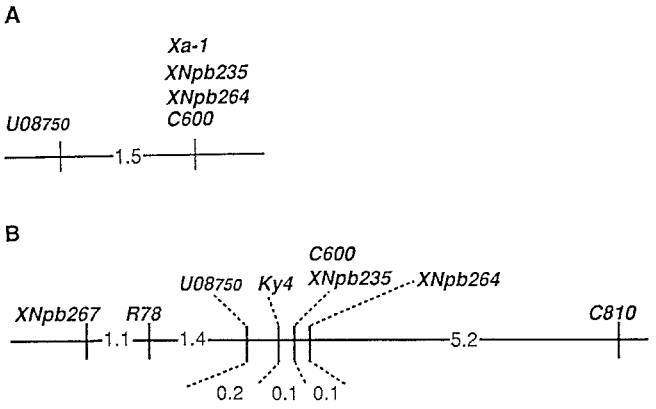

Fig. 2A, B Linkage of *Xa-1* and RFLP markers on chromosome 4. The genetic distances are given in centimorgans. **A** Linkage map around *Xa-1* constructed with 402 F_2 plants derived from six 'IR24'/'IR-BB1' and 'IR24'/'Kogyoku' crosses. **B** Linkage map of the chromosomal region corresponding to *Xa-1*, constructed using 186 F_2 plants derived from the 'Nipponbare'/'Kasalath' cross

since a χ^2 test showed that these populations were identical with respect to genotype segregation. The linkage map is shown in Fig. 2A. *Xa-1* was mapped 1.5 cM away from $U08_{750}$. Even in this large population of 402 plants, no recombinants were observed for *Xa-1*, *XNpb235*, *XNpb264* and *C600*.

To specify the allelism of *Xa-1* on the 'Nipponbare'/'Kasalath' map (Kurata et al. 1994) simultaneously, we also performed linkage analysis by mapping the RFLP markers of Npb235, Npb264, Npb267, Ky4 and $U08_{750}$ on the map. Four out of five *Xa-1*-linked RFLP markers were mapped within a 7.0-cM region between *R78* and *C810* on chromosome 4 of the 'Nipponbare'/'Kasalath' map (Kurata et al. 1994) (Fig. 2B). There was no significant inconsistencies in the gene arrangement and the genetic distances between the markers in the *Xa-1* region of both maps. We thus concluded that these RFLP markers could be used as probes to identify the YAC clones containing the *Xa-1* allele of 'Nipponbare'.

Isolation and analysis of YAC clones

Six RFLP markers, Npb235, Npb264, Ky4, C600, R78 and $U08_{750}$, linked to the *Xa-1* gene were used for screening the YAC library from 'Nipponbare' (Umehara et al. 1995). Two to ten candidate YAC clones were isolated with each of the six RFLP markers by colony hybridization. DNA from each isolated YAC clone was digested by an appropriate enzyme and hybridized with the RFLP marker that was used in the colony hybridization. Examples for Npb264 and C600, which were hybridized with five and three YAC clones respectively, are shown in Fig. 3. Subsequently, ten YAC clones which formed one contiguous stretch were confirmed. Figure 4A shows the YAC contig covering the *Xa-1* region on the 'Nipponbare'/'Kasalath' map. The insert sizes of the ten YACs ranged from 280 kb to 600 kb. The arrangement of ten YACs and the order of

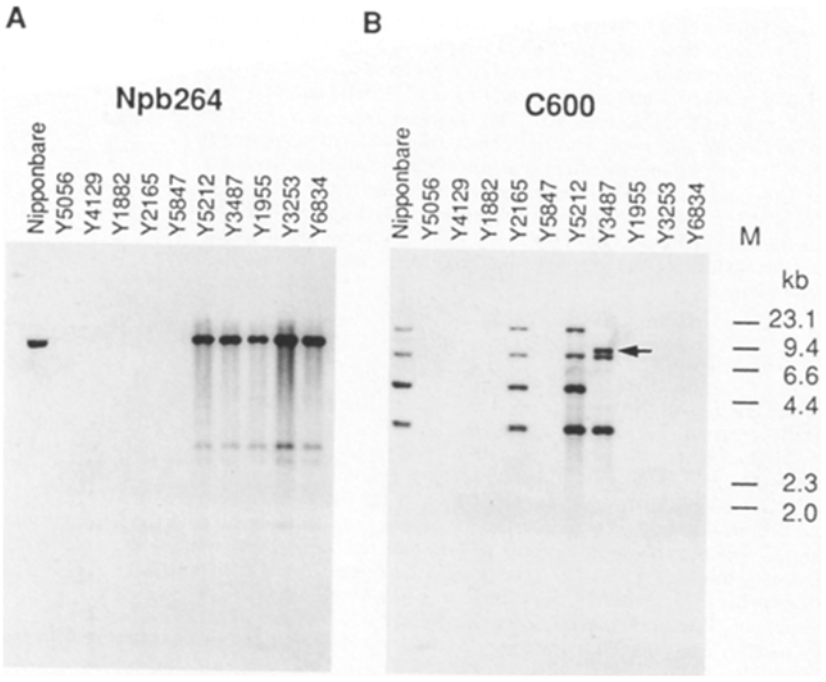

Fig. 3A, B Southern blot analysis of candidate YAC DNAs with RFLP markers Npb264 and C600. YAC DNAs were digested by *Dra*I. **A** Five YAC clones, from Y5212 to Y6834, all possess hybridizing bands with the Npb264 probe of the same fragment length as in 'Nipponbare'. **B** Two YACs, Y2165 and Y5212 have DNA fragments of 4.0, 6.0 and 8.5 kb, which had been mapped as the locus of C600 on the 'Nipponbare'/'Kasalath' map. Y3487 has a 6.0-kb fragment. *M* molecular size marker. *Arrow* shows the fragment that hybridized both with C600 and with the YAC left arm fragment of Y3487

the RFLP markers on them, such as the 0-cM-linked loci of *C600* and *XNpb235*, were located on the physical map by Southern hybridization between these YACs and RFLP markers. Furthermore, C600 hybridized to the same fragment of restriction enzyme-digested YAC DNA that hybridized with the YAC left arm of Y3487, as indicated by an arrow in lane 7 of Fig. 3B. The end fragments of the left arms of Y1882 and Y5056 also hybridized to the fragments that hybridized with Ky4 (data not shown). These results confirm the physical locations of these three YAC ends on the YAC contig, as indicated by the filled squares in Fig. 4A.

Identification of a YAC clone carrying a *Xa-1* allele

Of the ten YACs, Y5212 seemed to be the one that covered the *Xa-1* region (Fig. 4A). To confirm that Y5212 contains the *Xa-1* allele, we isolated both ends of Y5212 by the cassette PCR method (Umehara et al. 1995). From the right and the left ends of Y5212, 750-bp and 950-bp fragments were cloned and designated Y5212R and Y5212L, respectively. These were used for linkage analysis with *Xa-1*.

On the 'IR24'/'Kogyoku' and 'IR-BB1' map, Y5212R and Y5212L were mapped on both sides of *Xa-1* at a distance of 1.7 cM and 0.9 cM, respectively (Fig. 4B). These results showed that the YAC clone Y5212 contains the *Xa-1* allele in its 340-kb insert of 'Nipponbare' DNA. The genetic distance between Y5212R and Y5212L was calculated to be 2.6 cM in this linkage analysis, indicating that 1 cM corresponds to about 130 kb on the integrated 'IR24'/'Kogyoku' and 'IR-BB1' map made by a total of 402 F_2 plants from six crosses.

On the other hand, Y5212R and Y5212L were mapped at a distance of 0.7 cM on the 'Nipponbare'/'Kasalath' map, as shown in Fig. 4A, indicating that 1 cM of this chromosomal region corresponds to 485 kb on the 'Nipponbare'/'Kasalath' map. Though Y5212L cosegregated completely with Ky4, Y5212L was located between $U08_{750}$ and Ky4. Ky4 hybridized with the inside fragment of Y5212 but not with the end fragment of Y5212 in the Southern blot (data not shown). This fact, together with the other data on Npb235 and C600 mentioned earlier, coincides well with the order of DNA markers and the spacing of YAC clones in the physical map (Fig. 4A).

Discussion

We are proceeding to cover the whole rice genome with YAC contigs. To do this, YAC clones have been isolated with RFLP markers and ordered along the high-density molecular map constructed by Kurata et al. (1994). The YAC contigs covering well-defined regions will provide the basis for map-based cloning of genes that have been earlier known only by their phenotypes. However, the construction of YAC libraries for many other cultivars containing the target genes is rather difficult and time-consuming. It would be more practical and convenient to construct cosmid and cDNA libraries using individual cultivars carrying the target genes. Once candidate YAC clones from the standard strain, in our case 'Nipponbare', have been selected by the closely linked DNA markers to the target gene, corresponding cosmid and cDNA clones of gene-donor cultivars could be isolated from those libraries using the candidate YAC clones as probes. We have now constructed cosmid and cDNA libraries from the rice variety 'IR-BB1', which contains the *Xa-1* gene. Y5212 will be

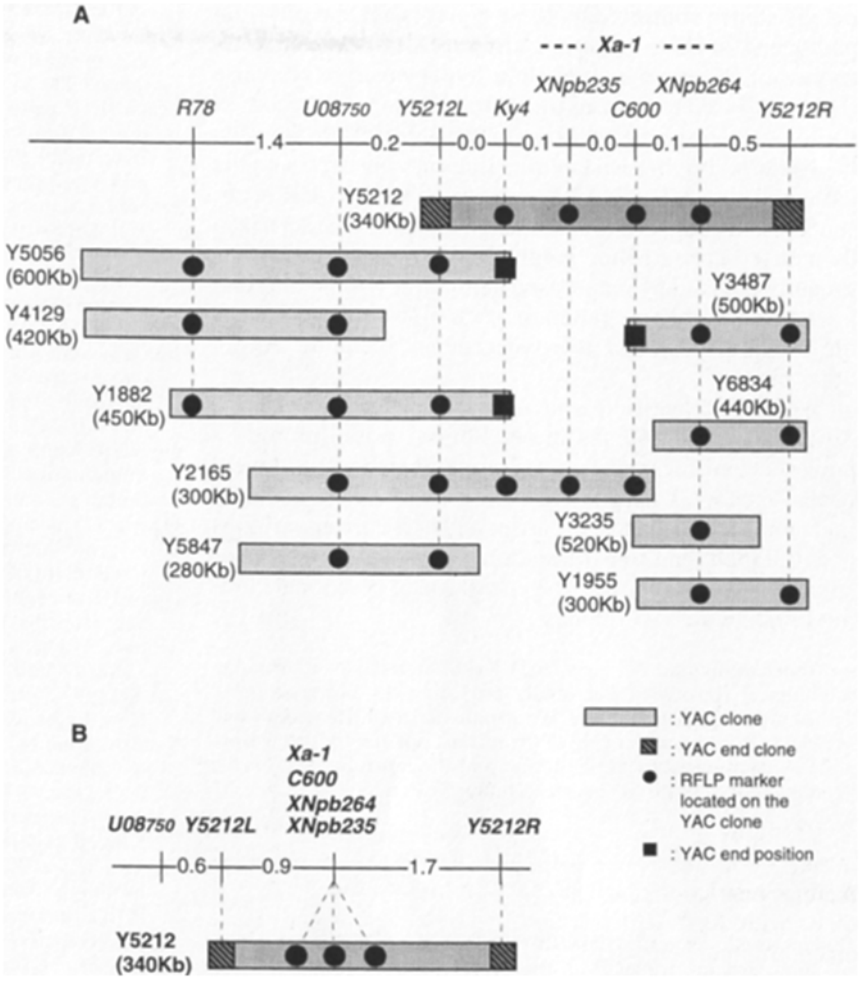

Fig. 4 A, B Arrangement of RFLP markers and YACs in a contig covering the *Xa-1* region. **A** 'Nipponbare'/'Kasalath' map. **B** Combined mapping of a YAC clone Y5212, and the RFLP markers on the linkage map derived from the six 'IR24'/'IR-BB1' and 'IR24'/'Kogyoku' crosses

used as a hybridization probe to select corresponding cosmid and cDNA clones.

In this study, linkage analysis was performed using the same RFLP markers in two different F_2 populations: the F_2 population derived from a cross between 'Nipponbare' and 'Kasalath' which was used for the construction of a high-density molecular map (Kurata et al. 1994), and the F_2 populations derived from the 'IR24'/'Kogyoku' and 'IR24'/'IR-BB1' crosses. As shown in Fig. 2, the gene arrangement and the genetic distances between the markers around the *Xa-1* locus were consistent between the two RFLP linkage maps, indicating that there were no drastic chromosomal rearrangements between 'Kogyoku', 'IR24', 'Nipponbare' and 'Kasalath'.

On the other hand, almost all of the expressed sequences (cDNA clones) located on the 'Nipponbare'/'Kasalath' map (Kurata et al. 1994) hybridized to both parents, though only 0.1%, 1 cDNA clone (R887) out of 883, could hybridize only to 'Nipponbare' genomic DNA and not to 'Kasalath' DNA (These Southern image files can be accessed on the World Wide Web using the URL: http://www.staff.or.jp/rgpdata/ricegmap.html).

All these data allow us to assume that the YAC clone Y5212, even if developed from 'Nipponbare' DNA, possesses an allele of *Xa-1*. On the basis of this assumption, Y5212 is now being used as a probe to screen for cosmid clones of 'IR-BB1' and to isolate candidate cDNA clones for *Xa-1*.

In the 'Nipponbare'/'Kasalath' cross, a 1-cM genetic distance was estimated to be about a 240-kb physical distance on average; however, this can vary from 120 to 1000 kb [data from the mapping of YAC end clones (Kurata et al. 1994)]. In the present study, in judging the genetic distances between the YAC end clones, Y5212R and Y5212L, 1 cM corresponded to 485 kb on the 'Nipponbare'/'Kasalath' cross and 130 kb on the 'IR24'/'Kogyoku' or 'IR-BB1' cross, the latter two having a total of two times more plants than the 'Nipponbare'/'Kasalath' cross. The physical distance on the 'IR24'/'Kogyoku' or IR-BB1 map with 402 F_2 plants was about four times smaller than the 'Nipponbare'/'Kasalath' map of 186 plants on this region. This could be due to the different parents of the crosses affecting the recombination frequency in some chromosomal regions.

Recently, several disease resistance genes such as *Pto* (Martin et al. 1993) and *Cf9* (Jones et al. 1994) in tomato, *N* in tobacco (Whitham et al. 1994) and *RPS2* in *Arabidopsis* (Mindrinos et al. 1994), have been isolated. Although

the resistance conferred by these genes is against different pathogens, such as bacteria and viruses, the resistance response of these genes include a hypersensitive response (HR) that is characterized by necrotic lesions at the site of pathogen invasion. However, in the interaction between the bacterial blight resistant rice lines having *Xa-1* and the pathogenic race T7174 of *X. oryzae* pv. *oryzae*, HR is absent (Leach et al. 1989) and the resistance is characterized by a difference in lesion length (Fig. 1). After *Xa-1* is ultimately isolated by map-based cloning, it will be interesting to see whether the function of *Xa-1* is similar to that of the previously cloned resistance genes inducing HR in other plants.

We have identified one rice YAC clone carrying a *Xa-1* allele. YAC clones in our library should provide a powerful tool for map-based cloning of rice phenotypical genes. This work needs to be followed by cDNA and cosmid clone analysis of both carrier and non-carrier varieties of *Xa-1*, including transformation assays. Our results constitute the first step toward a general use of positional cloning in rice with YAC clones.

Acknowledgments We thank Dr. H. Kaku, National Institute of Agrobiological Resources, for kindly providing the bacterial strain T7174 of *X. oryzae* pv. *oryzae*. We also thank Drs. I. Havukkala and M. Mohan for critical reading of the manuscript and for discussion. S. Y. was supported by the Fellowship of the Japan Society for the Promotion of Science for Japanese Junior Scientists.

References

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JD (1994) Isolation of the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* by transposon tagging. Science 266:789–793

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 (1991) Report of the committee on gene symbolization, nomenclature and linkage groups. Rice Genet Newsl 8:2–37

Kosambi DD (1944) The estimation of map distances 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 SY, 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 ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300 kilobase interval genetic map of rice including 883 expressed sequences. Nat Genet 8:365–372

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

Leach JE, Roberts PD, Guo A, Barton-Willis P (1989) Multiplication of *Xanthomonas campestris* pv. *oryzae* in rice leaves. In: Bacterial blight of rice. International Rice Research Institute, Manila, Philippines, pp 43–53

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

Martin GB, Brommonschenkel SH, Chunwonges J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, Tanksley SD (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science 262:1432–1436

Mindrinos M, Katagiri F, Yu GL, Ausubel FM (1994) The *A. thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78: 1089–1099

Ogawa T, Khush GS (1989) Major genes for resistance to bacterial blight in rice. In: Bacterial blight of rice. International Rice Research Institute, Manila, Philippines, pp 177–192

Ogawa T, Yamamoto T, Khush GS, Mew TW, Kaku H (1988) Near-isogenic lines as international differentials for resistance to bacterial blight of rice. Rice Genet Newsl 5:106–107

Ogawa T, Yamamoto T, Khush GS, Mew TW (1991) Breeding of near-isogenic lines of rice with single genes for resistance to bacterial blight pathogen (*Xanthomonas campestris* pv. *oryzae*). Jpn J Breed 41:523–529

Ou SH (1972) Rice diseases. Commonwealth Mycology Institute, Kew, England

Saito A, Yano M, Kishimoto N, Nakagahra M, Yoshimura A, Saito K, Kuhara S, Ukai Y, Kawase M, Nagamine T, Yoshimura S, Ideta O, Ohsawa R, Hayano Y, Iwata N, Sugiura M (1991) Linkage map of restriction fragment length polymorphism loci in rice. Jpn J Breed 41:665–670

Sakaguchi S (1967) Linkage studies on the resistance to bacterial leaf blight, *Xanthomonas oryzae* (Uyeda et Ishiyama) DOWSON, in rice (in Japanese with English summary). Bull Natl Inst Agric Sci Ser D 16:1–18

Tsunematsu H, Hasegawa H, Yoshimura S, Yoshimura A, Iwata N (1993) Construction of an RFLP/RAPD linkage map by using recombinant inbred lines. Rice Genet Newsl 10:89–90

Umehara Y, Inagaki A, Tanou H, Yasukochi Y, Nagamura Y, Saji S, Otsuki Y, Fujimura T, Kurata N, Minobe Y (1995) Construction and characterization of rice YAC libraries for physical mapping. Mol Breed 1:79–89

Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, Baker B (1994) The product of the tobacco mosaic virus resistance gene N: similarity to Toll and Interleukin-1 receptor. Cell 78: 1101–1115

Yoshimura S (1993) Genetic analysis of bacterial blight resistance genes in rice by using molecular markers. Bull Inst Trop Agric Kyushu Univ 16:1–63

Yoshimura S, Yoshimura A, Nelson RJ, Mew TW, Iwata N (1995) Tagging *Xa-1*, the bacterial blight resistance gene in rice, by using RAPD markers. Jpn J Breed 45:81–85