Theor Appl Genet (1998) 97: 507–514

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Isolation and FISH mapping of Yeast Artificial Chromosomes (YACs) encompassing an allele of the *Gm2* gene for gall midge resistance in rice

Received: 15 December 1997 / Accepted: 5 March 1998

Abstract Ten yeast artificial chromosomes (YACs) spanning the *Gm2* locus have been isolated by screening high-density filters containing a total of approximately 7000 YAC (representing six genome equivalents) clones derived from a *japonica* rice, Nipponbare. The screening was done with five RFLP markers flanking a gall midge resistance gene, *Gm2*, which was previously mapped onto chromosome 4 of rice. This gene confers resistance to biotype 1 and 2 of gall midge (*Orseolia oryzae*), a major insect pest of rice in South and Southeast Asia. The RFLP markers RG214, RG329 and F8 hybridized with YAC Y2165. Two overlapping YAC clones (Y5212 and Y2165) were identified by Southern hybridization, with *Gm2*-flanking RFLP markers, and their inserts isolated. The purified YACs and RFLP markers flanking *Gm2* were labeled and physically mapped by the fluorescence *in situ* hybridization (FISH) technique. All of them mapped to the long arm of chromosome 4 of the resistant variety of rice, 'Phalguna', confirming the previous RFLP mapping data.

Communicated by J. W. Snape

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Key words Map-based cloning · Positional cloning · Fluorescence *in situ* hybridization · Disease resistance · *Orseolia oryzae*

Introduction

Gall midge (*Orseolia oryzae*) is a major dipteran insect pest of rice in India, China, Africa and Southeast Asia. Five biotypes of this insect are found in India (Kalode and Bentur 1989; Nair and Ambika Devi 1994). Although infestation by this insect does not kill the plant, rice yields are considerably reduced. The insect causes deformation of the growing tips into 'onion tip' or 'silver shoot' which otherwise would have matured into a fertile panicle. Currently, many resistance genes which confer resistance to the different biotypes of gall midge have been identified in rice (Chaudhary et al. 1986; Srivastava et al. 1994) . However, the exact nature of the gall midge resistance genes and their product(s) are still unknown. Genetic studies of gall midge resistance indicate that in most cases the resistance is governed by a single dominant gene (Chaudhary et al. 1986; Srivastava et al. 1994; Mohan et al. 1994).

The *Gm2* gene, which confers resistance against biotypes 1 and 2, has been previously mapped onto chromosome 4 of rice (Mohan et al. 1994). Marker-assisted selection of *Gm2* has also been successfully demonstrated (Nair et al. 1995). Recently, polymerase chain reaction (PCR)-based markers linked to *Gm4t*, another gall midge resistance gene in *indica* rice variety 'Abhaya' (Nair et al. 1996), have been developed. It is known that *Gm2* and *Gm4t* are non-allelic (Mohan et al. 1997b), and it has also been demonstrated that two of the other known genes conferring resistance to different biotypes of gall midge are non-allelic (Sahu et al. 1990; Srivastava et al. 1994).

Many resistance genes have been recently isolated from different plants based either on map position,

transposon tagging or the use of genome-specific satellite markers and chromosomal breakpoint analysis (see reviews Mohan et al. 1997a; Hammond-Kosack and Jones, 1997; Cai et al. 1997). The advent of yeast artificial chromosomes (YACs) (Burke et al. 1987) has provided a system that allows long-range coverage of genomic DNA in a cloned form (Koike et al. 1997; Kurata et al. 1997). In plants, yeast artificial chromosome and bacterial artificial chromosome (BAC) cloning have been applied to isolate large regions of DNA encompassing a number of agronomically important genes involved in conferring resistance to particular pests and pathogens: *Xa1* (Yoshimura et al. 1996) and *Xa21* in rice (Song et al. 1995), *Pto* (Martin et al. 1993) and *l2C* in tomato (Ori et al. 1997), *RPS*2 in *Arabidopsis thaliana* (Mindrinos et al. 1994; Bent et al. 1994), and $Hs1^{pat-1}$ in *Beta vulgaris* (Cai et al. 1997).

Molecular cytological methods represented by *in situ* hybridization (ISH) have developed rapidly in the past few years for use in plants (Ambros et al. 1986; Fukui et al. 1987; Dong and Quick 1995; Jiang et al. 1995; Pedersen et al. 1997). The ability to detect the hybridization signals of a low-copy DNA sequence of small size is an important step towards the physical mapping of plant genomes. Rice chromosomes are small and while it is difficult to use these techniques the 45S rDNA loci of rice were successfully detected by [^{125}I]-labeled rDNA (Fukui et al. 1987). A method for rice ISH was first reported in 1990 using biotinylated rDNA probes, and a locus was detected on rice chromosome 9 of *japonica* rice (Fukui 1990; Iijima et al. 1991). Fukui et al. (1994) detected a variability in rDNA loci among rice species by means of fluorescence *in situ* hybridization (FISH). FISH has also been used to map 5S rDNA in *indica* and *japonica* rice (Song and Gustafson 1993; Kamisugi et al. 1994).

FISH mapping of YACs (Y5212 and Y2165) encompassing the *Gm2* locus and the flanking restriction fragment length polymorphism (RFLP) markers will demonstrate the chromosomal location of genomic clones containing *Gm2*. In an attempt to clone the *Gm2* gene we report here the identification and the isolation of a YAC clone encompassing the *Gm2* locus and its physical mapping onto chromosome 4 of rice using FISH.

Materials and methods

RFLP mapping of *Gm2*-flanking markers using the 'Nipponbare' × 'Kasalath' population

RFLP markers flanking *Gm2*, identified in a previous study using a mapping population derived from an *indica* × *indica* cross (Mohan et al. 1994), were mapped in another population from a cross between a *japonica* ('Nipponbare') and an *indica* ('Kasalath') variety (Kurata et al. 1994) using a mapping population consisting of 186 F_2 plants.

YAC library screening and preparation of DNAs from YACs

A high-density rice YAC library was prepared using genomic DNA from *japonica* variety 'Nipponbare' by Umehara et al. (1995). The library was on five filters and consisted of approximately 7000 clones representing six genome equivalents. The average insert size in this library was 350 kb. This library was screened with RFLP markers flanking *Gm2* gene using a non-radioactive labeling kit (ECL) from Amersham as described earlier (Umehara et al. 1995, 1996). Putative YAC clones homologous to RFLP markers flanking the *Gm2* gene were selected using a conventional colony hybridization procedure (Umehara et al. 1995). DNAs from YACs were isolated as described earlier (Umehara et al. 1996).

Preparation of RFLP probes and Southern hybridization of YACs

Fifty nanograms of genomic (insert) DNA was labeled with α-[^{32}P]-dCTP (1.11×10^{14} Bq/mmol, Amersham) using the random primer method of Feinberg and Vogelstein (1983). The homology of RFLP markers flanking the *Gm2* gene with the YAC clones was confirmed by Southern hybridization. Total DNA from YAC clones (8 μg) was digested with *Dra*I (3 U/μg) at 37°C overnight. Restriction fragments were separated by gel electrophoresis through 0.8% agarose in 1 × TBE at 2 V/cm overnight. The gel was stained with ethidium bromide, photographed and capillary-transferred onto a nylon membrane.

*Dra*I-restricted blots were hybridized, and post-hybridization washes were as described earlier (Mohan et al. 1994). Membranes were exposed to Kodak XAR film for autoradiography.

Preparation of yeast plugs and YAC DNA purification

Agarose plugs containing the yeast clones were prepared as described earlier (Umehara et al. 1995). About 100 plugs were made from the yeast strains containing the YAC Y5212 (340-kb insert).

YAC DNA was isolated from agarose plugs by pulsed field gel electrophoresis (PFGE) using the method of Vaudin et al. (1995) with modifications. The PFGE conditions for YAC Y5212 were as follows: run time, 32 h; initial switch time, 22.65 s; final switch time, 27.63 s. The electrophoresis was performed at 6 V/cm and at 100% ramping with an included angle of 120°. The YAC band excised after the first round of PFGE was aligned vertically on a 4% GTG Nusieve (FMC, USA) agarose gel and subjected to gel elctrophoresis at 100 V for 18–20 h. The stacked DNA was cut out, extracted and quantitated (Vaudin et al. 1995).

PCR amplification from YACs using primers from RFLP markers flanking the *Gm2* gene

YAC DNAs (200 ng) were used as templates in PCR amplification reactions. Primers from RFLP markers (RG214, RG329, F8 and F10) flanking the *Gm2* gene were developed as described earlier (Williams et al. 1991; Nair et al. 1995). The sequences of the primers used for PCR amplification of the RG476 locus are as follows:

Forward – 5′-TGCAGAGACATTTGCACTGA-3′ and
Reverse – 5′-GGTTGCACTCTTAGCTTTGA-3′.

PCR amplification conditions were the same as described earlier (Nair et al. 1996). The PCR products were separated on a 1% agarose gel and photographed.

Chromosome preparation for fluorescence *in situ* hybridization (FISH)

About 1 cm of the root tips from 1-week-old diploid plants of *indica* rice, 'Phalguna', which contains the *Gm2* allele, were excised and fixed immediately in a fixative (ethanol: acetic acid, 3:1) without pretreatment. Chromosome samples were prepared by the enzymatic maceration/air-drying (EMA) method with minor modifications (Fukui 1996). All the chromosomes at the prometaphase stage were identified based on their condensation pattern (Fukui and Iijima 1991, 1992) prior to FISH.

Labeling and hybridization of YACs and RFLP markers for FISH analysis

The purified YAC clone, Y5212 (Yoshimura et al. 1996) and a mixture of *Gm2*-flanking RFLP markers (approx. 100 ng of each RFLP marker – RG214, RG329, RG476, and F8) were labeled separately with biotin-dUTP (Boehringer Mannheim) by PCR labeling using random primers. The detailed procedure for PCR labeling has been described previously (Kamisugi and Fukui 1995). As the insert size of the YAC clone was 340 kb, it contained many repeated sequences. The addition of total genomic DNA of rice (50 times more than the probe DNAs) as the blocking DNA reduced the background noise/hybridization. The hybridization cocktail consisted of 100–200 ng of biotinylated YAC DNA (Y5212) or RFLP markers (genomic DNA), 50% formamide, 10% (v/v) dextran sulphate, 500 ng sheared and denatured salmon sperm DNA, 10 μg sonicated and denatured total rice genomic DNA in 70 μl of 2 × SSC per slide. An aliquot of 15 μl was used per slide. The hybridization was carried out as described earlier (Kamisugi and Fukui 1995).

The FISH method followed the protocol described by Ohmido and Fukui (1996). The hybridization signal was detected by avidin-FITC conjugate (1% fluorescein isothiocyanate, Vector Lab). Fluorescent signals were enhanced by a secondary immunological reaction with biotinylated anti-avidin (1%, Vector Lab, USA) and fluorescein-avidin conjugate. To detect the hybridization signals on the rice chromosome, two layers of fluorescein avidin DCS (Vector Lab., USA) and one layer of biotinylated anti-avidin D (Vector Lab., USA) were alternatively applied in a blocking solution at 37°C for 60 min. Chromosomes were counterstained with propidium iodide (PI; 50 μg/ml in phosphate buffer, pH 6.8, 12.5 mg/ml *p*-phenylendiamine dihydrochloride in 90% glycerol). Green fluorescent signals of FITC and red fluorescent chromosomes with PI were independently detected using a fluorescence microscope with suitable filter sets (B10 and G15, Axioplan, Zeiss). Both images were separately captured using a cooled CCD camera (PXL1400, Photometrics), and the images were composed by imaging software (IP-Lab, Photometrics).

Results

RFLP mapping of *Gm2*-linked markers in the 'Nipponbare' × 'Kasalath' population

Previous mapping studies using a recombinant inbred population derived from a 'ARC 6650' × 'Phalguna' cross have shown that RFLP markers RG214, RG329, RG476, F8 and F10 are linked to the *Gm2* gene (Mohan et al. 1994). In this study, mapping of these RFLP markers was carried out in the 'Nipponbare' × 'Kasalath' population with the idea of finding the relative linkage position of these markers on the high-density map developed by Kurata et al. (1994). We were able to map only three markers (RG214, RG329 and F8) which were flanking the *Gm2* gene on either side (Mohan et al. 1994). These markers in the 'Phalguna' × 'ARC 6650' population were found to be tightly linked to end clones of a YAC (left and right end of Y5212) and other RFLP markers, G235, G264, V16, V17, on the rice map (Kurata et al. 1994). This was an indication that the allele of the *Gm2* gene in the *japonica* variety 'Nipponbare' is encompassed by YAC Y5212. In fact, all the markers mapped to the same location on the map. The other two markers, RG476 and F10, could not be mapped because of a lack of RFLP between the parents 'Nipponbare' and 'Kasalath'.

Screening of a YAC library with RFLP markers linked to *Gm2*

Of the more than 20 YAC clones found to hybridize to the RFLP markers 10 YACs, shown earlier to form a contiguous stretch of DNA (see Yoshimura et al. 1996), were chosen for further analysis.

Southern hybridization of putative YACs with RFLP markers flanking the *Gm2* gene

The *Dra*I-restricted YAC DNAs hybridized to RFLP markers flanking the *Gm2* gene. As shown in Fig. 1, RG214 hybridized to two *Dra*I fragments of 5 kb and (faintly to) 1.7 kb in 'Nipponbare' as well as in the YACs, Y2165, Y5847, Y1882, Y4129 and Y5056. This marker did not hybridize to the other YAC clones. RG329 hybridized to three *Dra*I fragments in the 'Nipponbare' genome. Of these three fragments, the 5-kb fragment is present in the YACs Y2165, Y5847, Y1882, Y4129 and Y5056. The presence of the other two RG329-hybridizing fragments, seen only in 'Nipponbare', suggests the presence of RG329 homologous sequences elsewhere in the 'Nipponbare' genome even though they have been found to be single copy in the *indica* variety 'Phalguna' (Mohan et al. 1994), which has resistance to gall midge. It was interesting to note that both RG214 and RG329 hybridized to a 5-kb *Dra*I fragment in Nipponbare and the YACs to which these two probes hybridized. RG214 and RG329 were originally mapped on one side of the *Gm2* gene, whereas F8 and F10 were mapped on the other side. RG476 hybridized to a doublet of approximately 3.6 and 3.4 kb in 'Nipponbare', Y3487 and Y6834. This doublet is due to an internal *Dra*I site in RG476 (data not shown). F8 hybridized to three *Dra*I fragments of 6.0, 4.6 and 3.3 kb in 'Nipponbare'. These three fragments are present in Y2165 and Y5212, while the 4.6-kb fragment is missing in Y3487. However, F8 did not hybridize to any of the other YACs selected for this study (Fig. 1). One of the RFLP markers, F10, although tightly linked to *Gm2* in our previous studies (see Mohan et al. 1994;

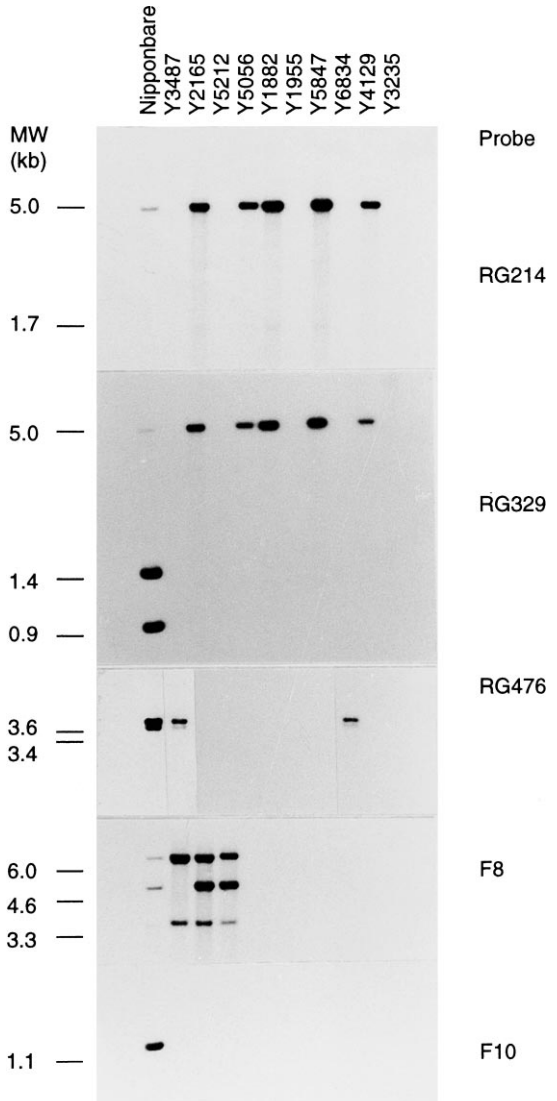

Fig. 1 Southern hybridization of 'Nipponbare' and YAC DNAs restricted with *Dra*I with RFLP markers flanking the *Gm2* gene. Probe numbers are on the *right*

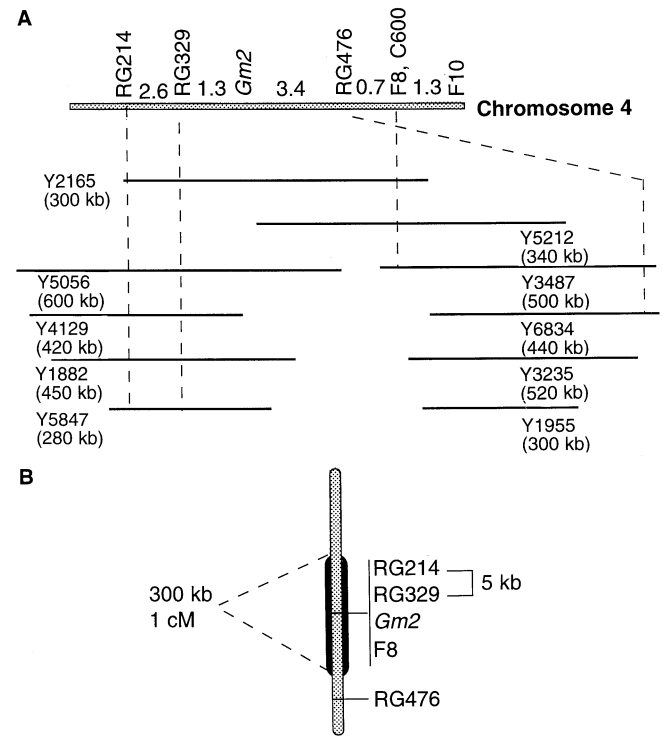

Fig. 2 **A** YAC contig encompassing the allele of the *Gm2* gene on chromosome 4 in 'Nipponbare'. *Shaded bar* represents a portion of the genetic map of chromosome 4 showing RFLP markers flanking the *Gm2* gene (Mohan et al. 1994). Distances on the shaded bar are in centiMorgans (cM). *Unshaded bars* represent YAC clones. *Dotted lines* connect YACs which are Southern positive with specific *Gm2* linked RFLP markers. **B** Fine genetic map showing the position of *Gm2* linked markers on chromosome 4. *Darkly shaded bar* represents the position of YAC Y2165. *Numbers* on the *left* show genetic (cM) and physical (kb) distances in this region of chromosome 4. *Gm2*-linked RFLP markers are on the *right*. The distance between RG214 and RG329 is about 5 kb

Nair et al. 1995), did not hybridize to any of the selected YACs although it hybridized to a 1.1-kb *Dra*I fragment in 'Nipponbare'. On the basis of the above data we have developed a YAC contig in 'Nipponbare' for the region encompassing the allele of *Gm2* gene (Fig. 2A). Based on Southern hybridization data we have also been able to develop a fine genetic map of this region (Fig. 2B).

PCR amplification from YACs using primers specific to RFLP markers linked to *Gm2*

Specific reverse and forward primers synthesized for each of the RFLP markers RG214, RG329, RG476, F8 and F10 were used in a PCR reaction to observe whether these loci, as defined by the RFLP probes, could be amplified from the YACs which form a part of the contig. The results obtained from PCR amplification of the YACs with primers specific to *Gm2* linked markers further corroborated the results obtained on Southern hybridization. While RG214-specific primers amplified 'Nipponbare', Y2165, Y5847, Y1882, Y4129 and Y5056, RG476 primers amplified only 'Nipponbare', Y3487 and Y6834 DNAs. However, with RG214 primers, a doublet was observed on amplification of the YACs, while with 'Nipponbare' and the positive control only the 1.4-kb fragment was amplified. F10 primers amplified the 'Nipponbare' genomic DNA, but not any of the YAC DNAs. The above primers did not amplify other YACs (Fig. 3), including those from unrelated regions of the genome (Y4219, Y4418, Y5350 and Y5940). RG329 and F8 specific primers did not amplify the 'Nipponbare' genome and hence did not amplify any of the YACs.

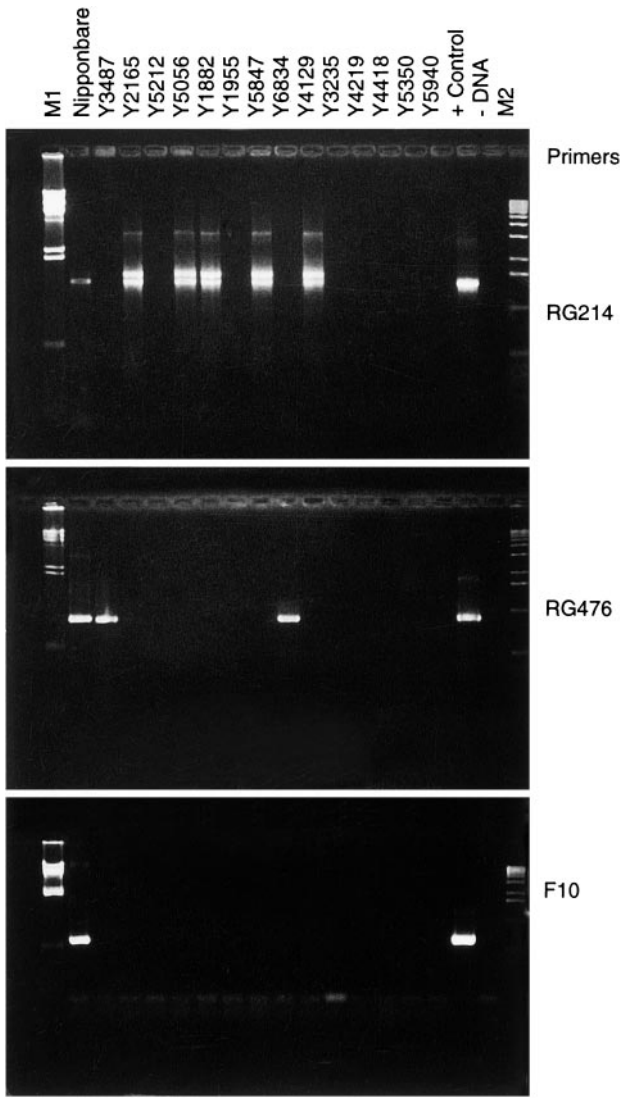

Fig. 3 PCR amplification of 'Nipponbare' and YAC DNAs using primers specific to RFLP markers RG214 (1.4 kb), RG476 (0.8 kb) and F10 (0.6 kb) linked to *Gm2*. *+ Control* is positive plasmid DNA control for the insert being amplified; *− DNA* is minus DNA. *M1* (lambda *Hin*dIII) and *M2* (1-kb ladder) are molecular-weight markers

Fluorescence *in situ* hybridization (FISH) with YAC Y5212 and *Gm2*-linked RFLP markers

Figure 4A and B shows the fluorescence signals occurring at the sub-terminal region of a pair of rice chromosomes among the 12 pairs of rice chromosomes. The signals were found on both chromatids of chromosome 4, indicating that the signals were genuine. The chromosomes with fluorescent signals were identified as rice chromosome 4, by a previous inspection of the chromosomal spread by phase contrast microscopy prior to FISH where chromosome 4 shows a characteristic uneven condensation pattern. As the short arm is completely condensed and the long arm is condensed only in the proximal region with a dispersed tail (see Fukui and Iijima 1991), unambiguous identification of the chromosome could be made. Figure 4C and D depicts the fluorescent signals from RFLP markers linked to the *Gm2* gene. Using all the four *Gm2*-linked biotin-labeled RFLP markers simultaneously in the FISH experiment we obtained fluorescent signals on both homologues of chromosome 4.

Discussion

We have previously mapped *Gm2* in a population derived from an *indica* × *indica* cross (Mohan et al. 1994). In the present, study we have now mapped *Gm2* linked RFLP markers between the left and right ends of a YAC Y5212 and other RFLP markers, G235, G264, V16, V17, on the rice map (Kurata et al. 1994). However 'Nipponbare' does not carry the *Gm2* gene, and it is necessary to construct cosmid and cDNA libraries from 'Phalguna', which does carry the *Gm2* gene. Once candidate YACs carrying an allele of *Gm2* are identified in 'Nipponbare', corresponding cosmid or cDNA clones can be isolated from these libraries from the resistant variety 'Phalguna'.

It has been shown that on average the genetic distance of 1 cM in the 'Nipponbare' × 'Kasalath' population corresponds to a physical distance of around 300 kb (Yoshimura et al. 1996). However, this can vary from 120 kb to 1000 kb (Kurata et al. 1994) depending on recombination events in different chromosomal regions. The genetic distance between *Gm2* flanking markers from RG214 to F10 (Mohan et al. 1994) in the 'ARC 6650' × 'Phalguna' population where 40 recombinant inbred lines were used was found to be 9.3 cM. When these flanking markers (RG214 and F8) were mapped on the 'Nipponbare' × 'Kasalath' population the distance between these markers was considerably less. Furthermore, when these *Gm2*-linked markers were used to screen the YAC library from 'Nipponbare', all except RG476 hybridized to 1 YAC clone (Y2165). Y2165 is 300 kb long, which indicates that *Gm2*-linked markers are closely spaced and are in all probability within 1 cM (Fig. 2B).

We also have indirect evidence to show that the *Gm2* gene is actually much closer to the F8 and F10 markers (less than 4.1 cM) and (5.4 cM) than shown by our previous mapping study (Mohan et al. 1994). This was evident from our tagging study (Nair et al. 1995) in which we developed resistance- and susceptible-specific PCR-based markers for *Gm2*. As very few false positives or false negatives were observed during the PCR-based screening, it seems that these markers are more tightly linked than shown by our mapping data (Mohan et al. 1994). Besides, RG476, which was shown to be closer to *Gm2* than to F8 and F10 in the ARC 6650 × 'Phalguna' population, was found to be

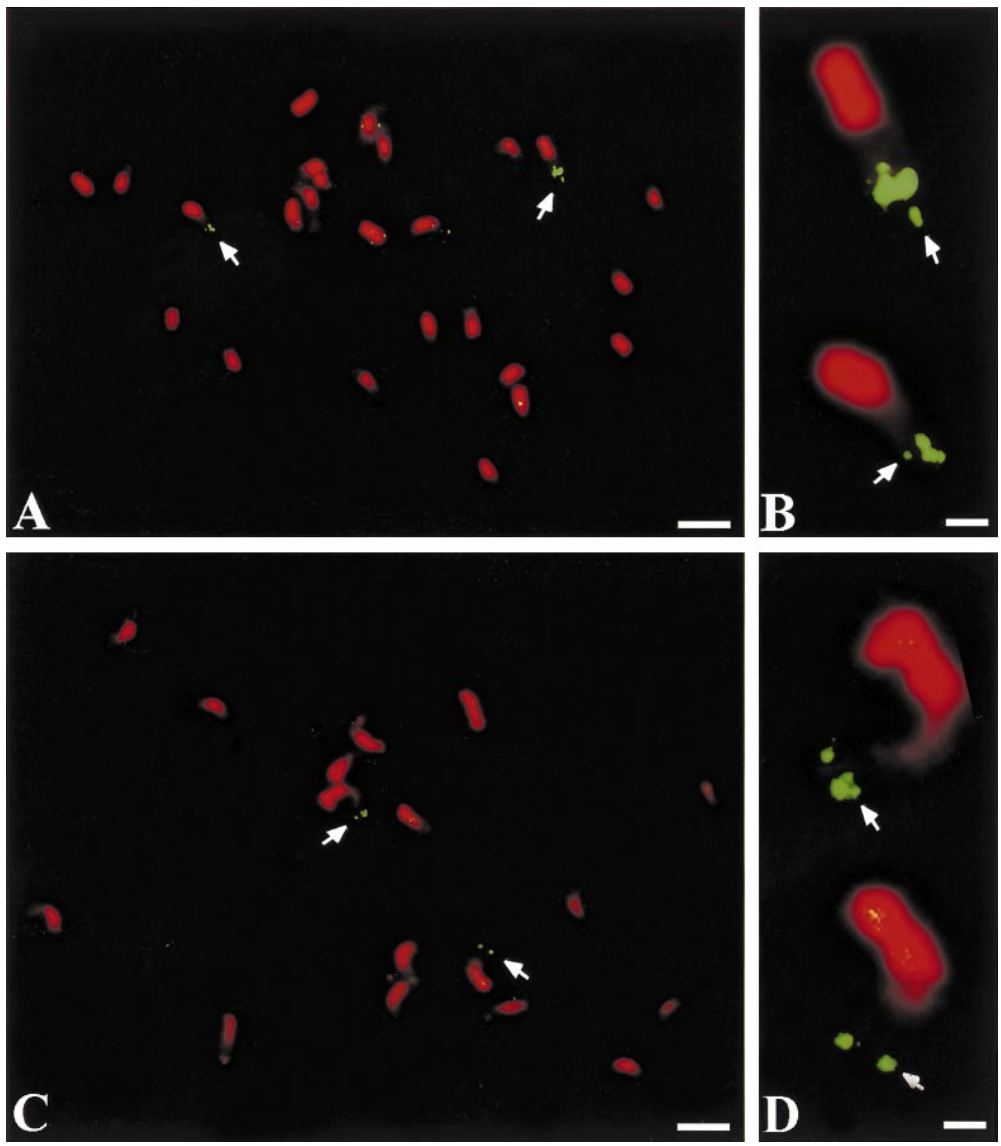

Fig. 4 Fluorescence *in situ* hybridization (FISH) on 'Phalguna' pro-metaphase chromosomes with YAC Y5212 (**A, B**) and mixture of *Gm2*-linked RFLP markers (RG214, RG329, RG476 and F8) (**C, D**). Green fluorescent hybridization signals (arrows) are visible only at the distal end of the long arm of chromosome 4. **B** and **D** are magnifications of a set of chromosome 4 from **A** and **C**, respectively. Bar = 5 μm (for **A, C**) and 3 μm (for **B, D**)

hybridizing to altogether different YAC clones, Y3487 and Y6834, at a distance of about 500 kb away in the 'Nipponbare' × 'Kasalath' population as shown by YAC hybridization experiments. This could be as the result of the populations arising out of crosses between different parents, thereby affecting the recombination frequency in this region of the chromosome. Similarly, it was quite interesting to note that both RG214 and RG329 hybridized to a fragment of the same size in 'Nipponbare' and the YAC clones (Fig. 1), suggesting that in all likelihood they are only 5 kb apart in the 'Nipponbare' genome.

It is becoming apparent from various reports that resistance genes are linked and located in clusters (Kaloshian et al. 1995; Dickinson et al. 1993; McMullen and Simcox 1995; Century et al. 1995; Ellis et al. 1995; Salmeron et al. 1996). It is also interesting to note that the allele of the *Gm2* gene which confers resistance to an insect pest, gall midge biotype 1 and 2, and the *Xa*1 gene which confers resistance to a bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* in rice are linked as they were found to be located on the same YAC Y2165. The pattern of hybridization of F8 to 'Nipponbare' and the different YAC DNAs was similar but not identical to that of C600 (data not shown), a marker tightly linked to *Xa*1. Both F8 and C600 hybridized to three *Dra*I fragments in 'Nipponbare', Y2165 and Y5212, and one of these three fragments was missing in Y3487. In addition to these fragments, C600 also hybridizes to a larger fragment (Yoshimura et al. 1996). This suggests that F8 and C600 are found close to each other on the 'Nipponbare' genome. While *Xa1* is linked to C600 (Yoshimura et al. 1996), earlier work in our lab places *Gm2* close to F8 (Nair et al. 1995); therefore, *Gm2*

is probably located very close to the bacterial blight resistance gene *Xa1*.

The technique of mixing and labeling many small clones linked to the *Gm2* gene provided an excellent approach to overcome the difficulty of *in situ* hybridization mapping using single- and low-copy sequences in rice. As the rice genome contains a large percentage of single-copy DNA (McCouch et al. 1988), this method may contribute to successful application of small and single-copy genomic clones in FISH analysis. Since all these markers mapped to the long arm of chromosome 4, this along with the results of FISH analysis of *Gm2*-linked RFLP markers and homologous YAC clone confirmed the previous RFLP mapping data (Mohan et al. 1994). The procedure of combining RFLP hybridization data with FISH mapping of YAC clones and *Gm2*-linked RFLP markers verifies the accuracy of the map in the region of the *Gm2* gene.

Certain differences were observed between the genetic map (Mohan et al. 1994) based on the 'Phalguna' × 'ARC6650' population and the YAC contig map constructed from the 'Nipponbare' YAC library. While the distance between RG214 and RG329 is 1.3 cM on the genetic map (Mohan et al. 1994), Southern hybridization data suggests that they may not be more than 5 kb apart in the 'Nipponbare' genome. The positions of RG476 and F8 have also flipped around in the YAC contig map as compared to the genetic map. While F10 and F8 are placed close together on the genetic map, F10 is not present in the region covered by the contig in 'Nipponbare'. Finally, while the distance between RG214 and F10 is 9.3 cM (Mohan et al. 1994) on the genetic map, the YAC contig map suggests a distance of not more than 800 kb between the two *Gm2* flanking markers – RG214 and F8/RG476.

To study the structural organization of the *Gm2* gene further, it would be necessary to isolate the entire gene. YACs Y2165 and Y3487, which encompass an allele of the *Gm2* gene, are being used to screen cosmid and cDNA libraries from the resistant variety 'Phalguna'. A 'Phalguna' cosmid contig covering Y2165 and Y3487 is under preparation. Once the cosmids carrying *Gm2* are identified, complementation experiments involving the transformation of susceptible varieties with these cosmids can be carried out. The cosmids containing the putative gene could also be used to screen the cDNA library from the resistant variety 'Phalguna'. The identification, isolation and FISH mapping of Y2165 and *Gm2*-linked RFLP markers in this study is the first and an important step of map-based gene cloning of *Gm2* gene in rice.

Acknowledgements We thank Prof. K. K. Tewari, Director, ICGEB, for encouragement and support and Dr. K. Hasegawa, Managing Director, STAFF Institute, for facilities. We are thankful to Dr. J. Peacock, CSIRO, Australia for his suggestions and critical evaluation of the manuscript. This work was partially supported by funds from the Rockefeller Foundation, Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan and Japan Racing Association (JRA).

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