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Fine physical mapping of the rice stripe resistance gene locus, *Stvb-i*

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Abstract The *Stvb-i* gene confers stripe disease resistance to rice. For positional cloning, we constructed a physical map spanning 1.8-cM distance between flanking markers, consisting of 18 bacterial artificial chromosome (BAC) clones, around the *Stvb-i* locus on rice chromosome 11. The 18 clones were isolated by screening a BAC library derived from a *japonica* cultivar, Shimokita, with three *Stvb-i*-linked RFLP markers and *Dra*I-digested DNAs of a yeast artificial chromosome (YAC) clone. The results of Southern hybridization and restriction enzyme analyses indicated that these BAC clones are contiguous and cover about a 700-kb region containing the *Stvb-i* allele. Utilizing end and internal fragments of the BAC insert DNAs, 33 molecular markers were generated within a small chromosomal region including the *Stvb-i* locus. Genotyping analysis with these markers for a resistant cultivar and four nearby recombinants selected from 120 F_2 individuals indicated that *Stvb-i* is contained within an approximately 286-kb region covered with two overlapping BAC clones.

Keywords Rice · Stripe disease resistance gene · *Stvb-i* · BAC · Physical map

Introduction

Rice stripe virus (RSV) transmitted by the small brown planthopper (*Laodelphax striatellus*) is the pathogen of stripe disease, which causes damage to rice crops in Asia, particularly Korea, China and Japan. As a means of controlling the disease, an incompletely dominant resistance gene, *Stvb-i* (Washio et al. 1968a, b), was introduced in the 1960s into a *japonica* cultivar from *indica* cultivar 'Modan' to develop a stripe disease-resistant cultivar, St. No. 1. Several resistant cultivars were subsequently developed by using 'St. No. 1' as a donor parent of stripe disease resistance. Although these resistant cultivars have been widely cultivated during the last 30 years, a breakdown of stripe resistance has not been observed. Therefore, it is thought that *Stvb-i* constitutes a durable resistance to RSV.

However, the location of the *Stvb-i* locus has remained unclear for a long time. The main reason for this is that bioassay of stripe resistance involves some difficulties. RSV can not be uniformly inoculated into test plants because the only method is to use RSV-infective insects. Additionally, the expression of *Stvb-i* appears to be quantitative rather than qualitative. Therefore, a population of a line or cultivar is needed to evaluate stripe resistance. By means of linkage analysis with molecular markers, *Stvb-i* was eventually located on rice chromosome 11, and four closely linked restriction fragment length polymorphism (RFLP) markers were found (Hayano-Saito et al. 1998).

Recently, the advent of genome libraries with the yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC) has enabled the physical mapping and positional cloning of plant disease resistance genes. Several resistance genes have been isolated from different plants based on information on their chromosomal position (Martin et al. 1993; Mindrinos et al. 1994; Ori et al. 1997; Warren et al. 1998; Milligan et al. 1998; Baker et al. 1997). With respect to positional cloning of important genes in rice, high-resolutional genetic maps and BAC and YAC libraries have been developed, and two bacterial blight disease resistance genes, *Xa1* (Yoshimura et al. 1998) and *Xa21* (Song et al. 1995), have been isolated.

In the study reported here, we constructed a contig covering the *Stvb-i* region utilizing a BAC library

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(Nakamura et al. 1997) and developed new molecular markers from these BAC DNAs. A detailed description of the *Stvb-i* region with the physical map and genotyping analysis using BAC-derived molecular markers will provide a concrete guide and valuable information for map-based cloning.

Materials and methods

Plant materials

One *indica* cultivar, Modan, its resistant progenies (St. No. 1, Tsukinohikari, Asanohikari and Musashikogane) and five *japonica* susceptible cultivars (Norin No. 8, Koshihikari, Koganebare, Aichinokaori and Nipponbare) were used for the development of molecular markers in the *Stvb-i* region. In order to specify the *Stvb-i* region on the physical map, we used resistant cultivar Musashikogane and four F_2 individuals (F_2-10, F_2-14, F_2-38 and F_2-71) between Koshihikari and Asanohikari. Their resistance reactions have been described in our previous report (Hayano-Saito et al. 1998). They are the recombinants within a 1.8-cM segment harboring *Stvb-i*, between XNpb220 and XNpb254/257.

BAC and YAC clones

Five high-density replica filters and BAC clones of a rice BAC library derived from a *japonica* rice cultivar Shimokita (Nakamura et al. 1997) were used. Each membrane containing 3072 BAC clones is equivalent to about the size of one genome rice (450 Mb). A YAC clone, Y4366, which is positive for XNpb257 (Tanoue et al. 1997), in a rice YAC library of *japonica* cultivar Nipponbare (Umehara et al. 1995) was provided by the Rice Genome Research Program (RGP) of Japan.

Construction of a BAC contig

The screening of the BAC libraries and the DNA preparation were done as described by Nakamura *et al*. (1997). All colony and Southern hybridizations were performed using the ECL direct labeling and detection system (Amersham-Pharmacia) according to the manufacturer's instructions. For screening the BAC library, we used four *Stvb-i* -linked RFLP markers (XNpb220, ST10, XNpb254 and XNpb257) and a YAC clone (Y4366). The preparation and the estimation of the size of the YAC insert DNA were done as described by Umehara et al. (1995). In the labeling of the Y4366 insert, 700 ng of the insert DNA (680 kb in size) was digested by *Dra*I, and the fragment mixture was subsequently used as a probe for colony hybridization of the BAC replica filters. DNAs of the selected BAC clones were digested with *Hin*dIII and blotted on a nylon membrane. The mutual positional relationships of the selected BAC clones were investigated by their patterns of agarose gel electrophoresis [0.8% (w/v) agarose gel in 1× TAE buffer) after *Hin*dIII or *Eco*RI digestion and then by hybridization to their blots with the end fragments of the BAC insert DNA. End fragments were amplified by the thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR) method (Liu and Whitter 1995). To estimate the sizes of the BAC inserts, we applied *Not*I-digested DNAs on pulse-field gel electrophoresis [PFGE, 1.0% (w/v) agarose gel in 0.5× TBE]. PFGE was performed by using CHEF mapper (Bio-Rad) with a linearly ramped pulse time of 2–10 s at 6 V/cm for 14 h.

Table 1 BAC clones screened with the *Stvb-i*-linked markers

Probe	BAC clone	Size (kb)
XNpb220	51C19	130
	104A 3	63
	105B 4	165
	116E11	101
	221D 1	116
ST10	18E 4	96
	18E 5	96
	36G 1	172
	123A 6[b]	245
	220B 5	160
XNpb254	218A12[c]	134
XNpb257	None	
Y4366[a]	40D 7	162
	56F 2	177
	82A 8	175
	82E12	144
	88C12	146
	88D 1	146
	123A 6[b]	245
	218A12[c]	134
	219H 2	125

[a] Mixed probe consisting of *Dra*I-digested fragments of YAC clone Y4366
[b] Positive clone with ST10 and Y4366
[c] Positive clone with XNpb254 and Y4366

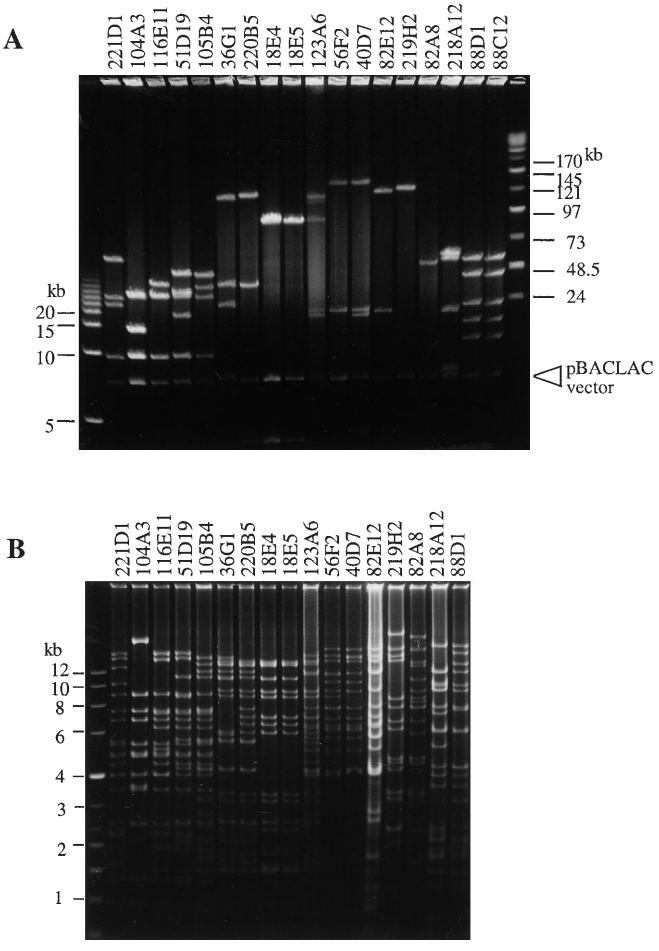


Fig. 1 A, B Restriction enzyme analysis of the 18 BAC clones constituting the contig. **A** *Not*I fragments were resolved by PFGE at a pulse time of 2–10 s at 6 V/cm for 14 h. **B** *Eco*RI fragments were resolved by agarose gel electrophoresis (0.6% (w/v) agarose gel in 1× TAE). Numbers on the *left* and *right* indicate molecular weights in kilobase pairs (kb). Electrophoresis was done at 0.7 V/cm for 16 h

Development of BAC-derived markers

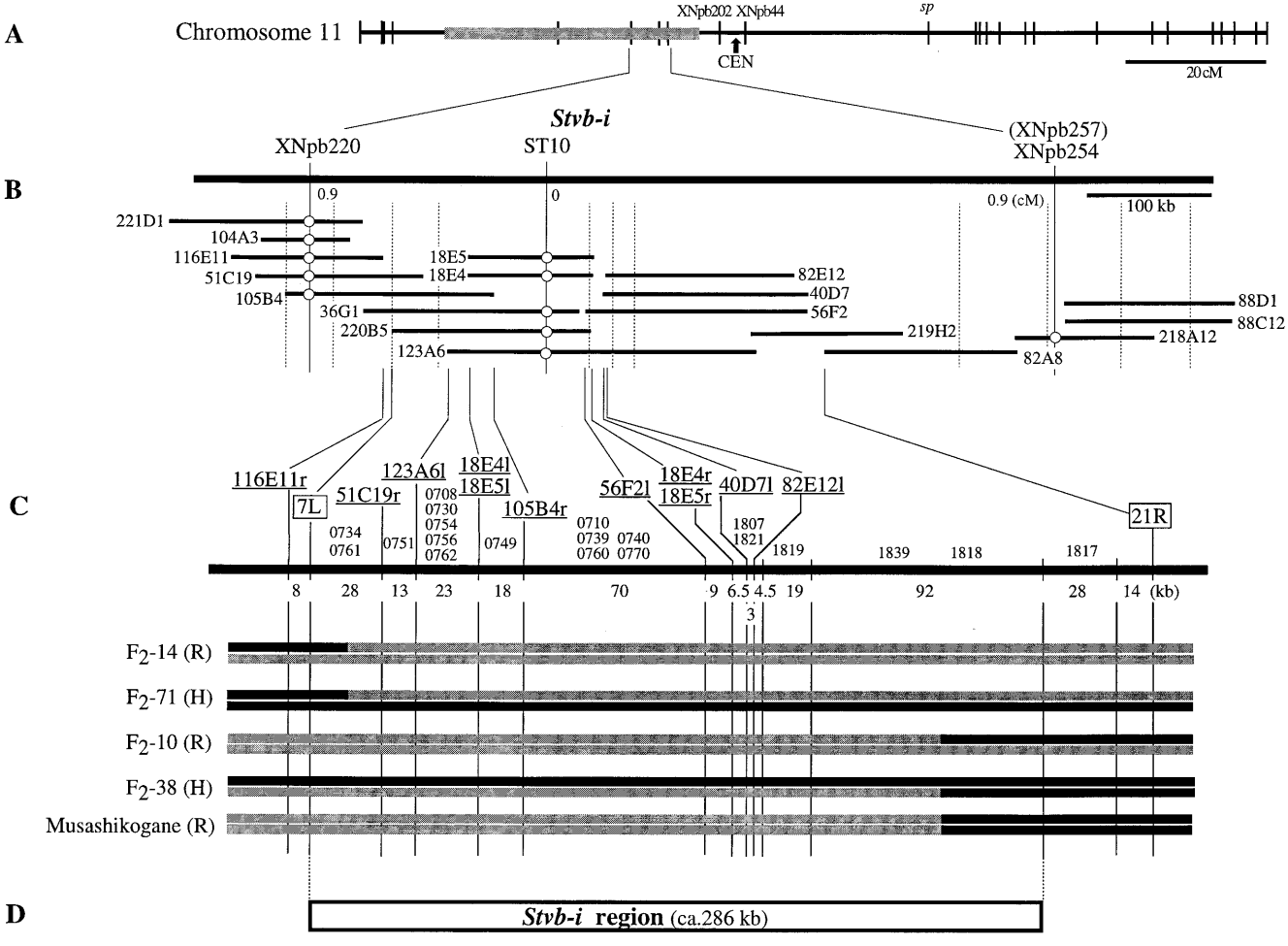
BAC end fragments were cloned using the TA cloning kit (Strategene). BAC DNA fragments digested with *Hin*dIII were subcloned into plasmid vector pUC118. Cloned fragments that showed RFLP between stripe resistant and susceptible cultivars were used for genotyping analysis. Genotyping analysis for Musashikogane and four F_2 recombinants of the Koshihikari/Asanohikari cross was done as described in our previous report (Hayano-Saito et al. 1998). The position of the sub-clone on the physical map was estimated by hybridizations of the blots of the *Hin*dIII- or *Not*I-digested BAC DNAs. BAC end clones given no RFLP were sequenced using a Dye Terminator Cycle Sequencing kit (Perkin Elmer) and an Applied Biosystems 310A genetic analyzer, and sequence-tagged site (STS) markers were generated.

Results

Construction of a BAC contig in the *Stvb-i* region

Positive BAC clones of colony hybridization with the four RFLP markers around *Stvb-i* are shown in Table 1. Five BAC clones each were selected by XNpb220 and ST10, respectively. Although XNpb254 and XNpb257 were mapped at the same position as in our previous study (Hayano-Saito et al. 1998), only 1 BAC clone, 218A12, was positive for XNpb254, and no clone was positive for XNpb257. Furthermore, we screened the BAC library using YAC clone Y4366, which was positive for XNpb257 (Tanoue et al. 1997), as a probe. Through this screening, 9 positive BAC clones were obtained, including 218A12 and 123A6, which were positive for XNpb254 and ST10, respectively. Southern hybridization indicated that Y4366 does not contain the fragment of ST10. Therefore, it is likely that 123A6 has an insert containing ST10 and a part of Y4366. As a re-

Fig. 2 A BAC contig around the *Stvb-i* gene locus, and the *Stvb-i* region on the physical map. **A** The graphical genotype of chromosome 11 in Asanohikari (Hayano-Saito et al. 1998). *Vertical bars* represent RFLP markers developed by Saito et al. (1991). The morphological marker 'short panicle' (*sp*) is shown for comparison with the classical rice map. CEN Centromere (ftp://ftp.staff.or.jp/pub/geneticmap98/mapimage/chr11. gif). The *gray portion* represents the segment introgressed from Modan. **B** Overlapping BAC clones forming the contig in the *Stvb-i* region. The *horizontal bars* accompanied by a combination of *letters* and *numbers* represent BAC clones. The *broken vertical lines* indicate *Not*I sites. **C** The BAC-derived markers and the graphical genotypes of the four F_2 recombinants and Musashikogane. The *squares* and *underlines* represent STS and RFLP markers, respectively, that were derived from the end fragments of BAC clones. The RFLP markers (prefix 07–/– and 18–/–) were developed by sub-cloning of 220B5 and 40D7. The *gray* and *solid* bars represent chromosome regions derived from *indica* (Modan) and *japonica*, respectively. Stripe resistance reaction is presented in parentheses following the F_2 number or cultivar: *R* resistant homozygous, *H* heterozygous. **D** The *Stvb-i* region on the physical map. The *wide white bar* represents the present maximum limits of the *Stvb-i* region



sult, we obtained a total of 18 positive BAC clones around the *Stvb-i* locus.

The insert sizes of the isolated BAC clones were estimated to be from 95 kb to 250 kb by PFGE of *Not*I-digested fragments (Fig. 1A and Table 1). The insert of 123A6 that hybridized to both ST10 and Y4366 was the longest (about 245 kb). The orientation and overlap of these clones were determined by Southern hybridization with both right- and left- end fragments that were amplified by the TAIL-PCR method from each BAC clone. Analysis of the 18 BAC clones revealed that they were contiguous. Furthermore, the order suggested from the hybridization results was confirmed by restriction enzyme analysis (Fig. 1A,B). Overlapping BAC clones can be recognized with fragments in common. On the basis of the results, a contig of 18 BAC clones was constructed around the *Stvb-i* locus (Fig. 2B). It covers about 620 kb, corresponding to the 1.8-cM interval between XNpb220 and XNpb254 on the genetic map.

The *Stvb-i* region on the physical map

To localize the *Stvb-i* locus on the physical map, we developed molecular markers using BAC DNAs between XNpb220 and XNpb254. Out of 26 BAC end fragments, 11 fragments were converted into RFLP markers. The remaining 15 fragments gave no RFLP, and 2 of those fragments were converted into STS markers (7L and 21R, Fig. 2C). Out of 31 tested sub-clones derived from 2 BAC clones, 220B5 and 40D7, 20 showed polymorphism between stripe resistance and susceptible cultivars. In F_2-14 and F_2-71, the genotypes of 51C19r were different from those of 0734 and 0761, which are in the 28-kb region between 7L and 51C19r. This result indicates that recombination occurred between 51C19r and 0734 (or 0761) in F_2-14 and F_2-71. Similarly, the genotype of 1818 was different from that of 1839 in the three recombinants F_2-10, F_2-38 and Musashikogane, indicating that recombination occurred between these two markers. The two markers are in the same 92-kb *Not*I-fragment of the BAC clone 123A6. Figure 2 C shows the physical map with the new markers and the graphical genotypes of the five tested recombinants. Considering the graphical genotypes and the resistance phenotypes of the recombinants, it is thought that the locus conferring stripe resistance is in this approximately 286-kb region on the physical map, containing the 28-kb and 92-kb fragments (Fig. 2D). Furthermore, it is suggested that this 286-kb *Stvb-i* region is covered with the two overlapping BAC clones 220B5 and 123A6.

Discussion

The BAC library with high-molecular-weight DNA is now established as the standard of a genome library and is convenient for constructing a contig map. However, much effort and time is needed to construct a specific library for each of many target genes. Therefore, we used the rice BAC libraries that have been constructed from a stripe-susceptible *japonica* cultivar Shimokita. Available information on YAC distribution on the rice genome was helpful for making the contig, and only one step from both sides of ST10 and XNpb254 might have bridged these markers (Fig. 2). As a result, we constructed a BAC contig consisting of 18 clones around the *Stvb-i* allele.

Screening of the BAC library using the XNpb257 marker did not reveal any positive BAC clone. On the other hand, this marker hybridizes to several clones from the YAC library, including Y4366 (Tanoue et al. 1997). It is likely that the genome of Shimokita lacks a segment including XNpb257. This is may be because the BAC library was constructed from Shimokita with a small introgressed portion of *indica* or that the BAC library might not contain a clone having an XNpb257 fragment. The 500-kb region covered with 9 BAC clones positive for Y4366, containing an XNpb254 fragment, is about 180-kb shorter than the insert DNA of Y4366. However, XNpb257 has been mapped at the same locus as that of XNpb254 by F_2 analysis using 120 plants (Hayano-Saito et al. 1998). It is likely that XNpb257 is located more on the centromeric side than XNpb254.

Construction of the BAC contig resulted in many molecular markers in a small region including the *Stvb-i* allele. The markers were generated throughout the specific interval and were very useful for specifying the *Stvb-i* region on the physical map. Furthermore, these markers sequenced and converted into STSs will be very useful for isolating the *Stvb-i* gene or for selecting a resistant rice plant in a breeding program. Marker-aided selection (MAS) with the markers linked to the stripe resistance gene can be applied to individual plants. One *Stvb-i* -specific RFLP marker, ST10, has already been converted into an STS marker (unpublished), and we have started MAS for stripe resistance with the STS marker.

The resistance responses of most of the single gene resistances depend on the hypersensitive response (HR) that is characterized by necrotic lesions at the site of pathogen invasion (Baker et al. 1997). HR is also induced by tobacco carrying the tobacco mosaic virus resistance gene, *N*, that was isolated by transposon tagging (Whitham et al. 1994). However, HR is not observed in the plant harboring *Stvb-i*. A small percentage of the population of the resistant variety is infected with RSV in bioassay. Even if it is infected with RSV, the severity of symptoms is less than that of the susceptible plant. This character is rather close to being of a quantitative nature along with incomplete dominance. Therefore, the function of the *Stvb-i* gene product and the mechanisms of the resistance may be different from that of other resistance genes producing HR. The cloning of the *Stvb-i* gene may reveal new mechanisms of plant resistance to virus. The results described here provide the basis for isolation of the *Stvb-i* gene.

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