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

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 F₂ 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 U08750 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

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 F2 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 F2 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 F2 populations of ‘IR24’/‘Kogyoku’ and ‘IR24’/‘IR-BB1’ crosses (Table 1) 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 10⁸ cells/ml. Five to six fully expanded leaves of each F2 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 F2 population of 142 plants derived from the cross between ‘Kinmaze’ and ‘Te-tep’ in which the F2 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 U08729 and Y03700, 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

![Fig. 1](image)