Genetic and physical mapping of *CRb*, a gene conferring resistance to clubroot in Chinese cabbage

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Abstract

Chinese cabbage DH line "CR Shinki" confers complete resistance to race 2, 4 and 8 of *Plasmodiophora brassicae*. To develop molecular markers linked to clubroot resistance (CR) gene, a linkage analysis using AFLP markers was performed in an F_2 population derived from a cross between the donor CR Shinki and the susceptible inbred line 94SK, which segregated into 1RR:2Rr:1rr (resistant/susceptible) ratio. A total of about 12,910 AFLP fragments generated in 237 *Pst* I/*Mse* I primer combination were screened. The result showed that five co-dominant AFLP markers and four AFLP markers linked in coupling linked to the resistance gene *CRb*. A reliable conversion procedure allowed six closely linked AFLP markers to be successfully converted into CAPS and SCAR markers. A genetic map around *CRb* covering a total distance of 6.75 cM was constructed using an F_2 population. Using three nearest surrounded markers, 16 recombinants were detected in a segregating F_3 population (487 individuals). Among them, 11 were between TCR09 and *CRb* and seven between *CRb* and TCR01, respectively. To physically map the locus, the *CRb*-linked markers were used to probe the CR Shinki BAC library. Construction of a contig map around the locus was underway with the aid of KBrH and KBrB.

Keywords: Brassica rapa, clubroot disease, genetic map, physical map
URL: http://www.yplim.info

Introduction

Plants were challenged by numerous pathogens throughout their life cycles. Some of plants displayed resistance while others could be severely infected.. *Plasmodiophora brassicae* Wor., a causal agent of clubroot in Chinese cabbage and other Brassica crops, can infect root tissue and subsequently produced galls. To control clubroot disease, different CR (clubroot resistance) cultivars of Chinese cabbage were developed by introducing CR genes from CR European fodder turnip representing the typical resistance to different pathotypes of *P. brassicae* (Yoshikawa 1981; Toxopeus and Janssen 1975; Buczaki et al. 1975). Further analysis found pathotype-specific host resistance is present in some of them. Introgression of CR genes provided a short period resistance to clubroot, as new pathotypes were appeared. Therefore, breeding new cultivars of Chinese cabbage was most efficient to improve the resistance to clubroot disease.

Several dominant genes (*Crr1*, *Crr2*, *Crr3*, *CRa*, *CRb*) were suggested to control resistance either by single or two complementary genes and were mapped in Chinese cabbage (Matsumoto et al. 1998; Suwabe et al. 2003; Piao et al. 2004; Hirai et al. 2004). Previously, we had mapped the gene *CRb* using simple PCR-based markers (Piao et al. 2004). Due to the use of different plant materials and marker types, it is difficult to confirm whether *CRb* is identical to others.

Although biochemical characters of plants during infection with clubroot were studied in *Arabidopsis* and *Brassica* crops (Butcher et al. 1974, Grsic et al. 1999; Grsic et al. 2000), the mechanism of what and how plants provide resistance to clubroot disease remains in question. The way to understand the molecular basis of the mechanism and pathogen-host interaction is to clone the resistance genes. Because of the lack of detectable products of resistance genes, lots of them were isolated by the strategy of map-based cloning (Martin et al. 1993). This study was focused on the cloning of the *CRb* gene. To achieve this, three approaches were employed including construction of high resolution genetic and physical map around *CRb*.

Results and Discussion

Genetic mapping of the CRb *gene*

An F_2 population derived from a cross between CR Shinki DH line and 94SK was used to tag the clubroot resistance gene named *CRb*. Resistance was previously shown to clubroot and is controlled by a single dominant gene (Piao et al. 2002). The genetic map around *CRb* was constructed (Piao et al. 2004). Briefly, 17 markers were totally identified among 256 (16 *Pst* I × 16 *Mse* I) primer combinations tested. On the basis of analysis in 138 F_2 plants with the four markers linked in coupling and the six co-dominant markers, *CRb* was mapped within an interval of 8.14 cM. The six closely linked AFLP markers (Fig. 1A) were converted into SCAR and CPAS markers. The survey of these markers on 143 F_2 plants indicated that resistant-specific markers were found to be present in most resistant plants but absent from susceptible plants. In particular, recombination was not detected between the two co-dominant markers TCR01 and TCR05 among any of the susceptible plants. Three plants exhibiting recombination between TCR09 and the CRb gene were identified. Recombination between

TCR10 and the *CRb* gene was detected in only two plants. For the co-dominant marker TCR01, eight recombinants were detected. Linkage analysis of the segregation data for these markers using JoinMap 3 indicated that they all mapped to the same linkage group, with a minimum LOD score of 3 (Fig. 1B). A dominant marker, TCR09, and a co-dominant marker, TCR05, were found to closely flank the CRb gene at genetic distances of 0.74 cM and 1.97 cM, respectively. One recombination event was detected between TCR01 and TCR05, which were separated by 0.18 cM. TCR10, TCR08 and TCR02 were located on the same side of CRb as TCR05 and TCR01, at distances of 3.84, 4.67 and 7.8 cM from the gene, respectively.

Homology searches of sequences from the converted AFLP markers against NT using BLASTN indicated that TCR01, TCR02, TCR05, and TCR10 have high similarity to genes on *A. thaliana* chromosome 4 including At4g21040, At4g24560, At4g20150, and At4g22740, respectively. Further, the arrangement of these markers showed co-linearity to the order of the corresponding genes (Fig 1C).

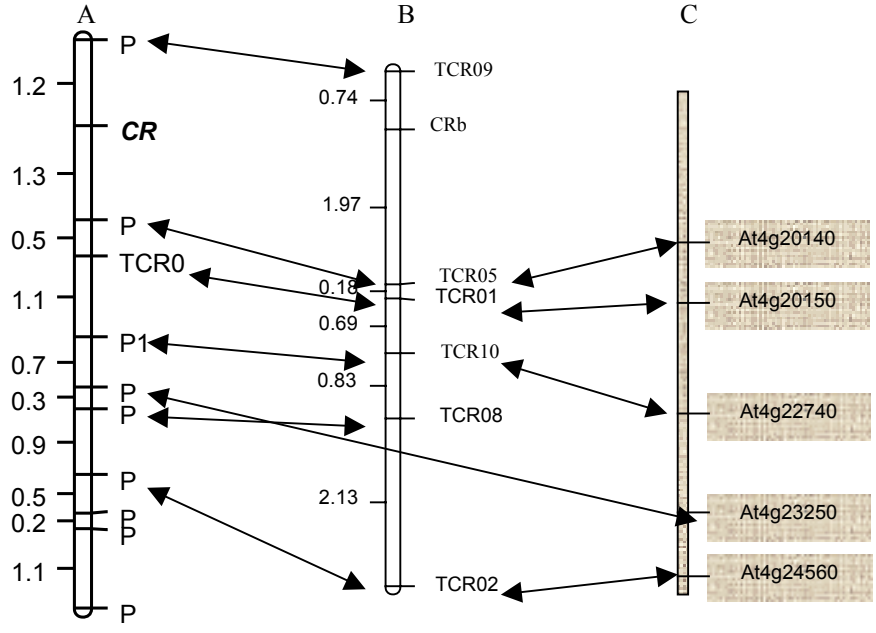

Fig. 1. SCAR and CAPS mapping of the *CRb* gene, and co-linearity between the order of markers and that of the genes from *Arabidopsis* chromosome 4. A, AFLP map of the Crb gene. B, SCAR and CAPS mapping of the *CRb* gene based on the co-dominant and dominant AFLP markers segregating in an F_2 population. Genetic distance located on the left is calculated with Kosambi mapping function. C, Gene orders on *Arabidopsis* chromosome 4. Solid arrows indicates high sequence homology between marker and the gene. Dashed arrow indicates low sequence homology. (source)

High resolution genetic and physical mapping of the CRb *gene*

To facilitate physical mapping of the *CRb* gene, a larger segregating F_3 population consisted of 487 individuals were screened with SCAR marker TCR01, TCR05, and TCR09. Totally, 16 plants with recombination events in the TCR09-TCR05 interval were found. The recombination frequency between TCR09 and TCR05, between TCR09 and *CRb* and between *CRb* and TCR05 were 3.3%, 2.3%, 1.0%, respectively (Table 1). To saturate the map around *CRb*, 256 *Eco*R I/*Mse* I and 240 *Sno* I/*Mse* I AFLP primer combinations were tested in the resistant and susceptible pools. Out of 16 fragments displaying polymorphsim between two bulks in *Eco*R I/*Mse* I primer combinations, 8 were resistant specific fragments. No place marker was found in the TCR09-TCR05 interval on the basis of screening recombinants detected in F_2 population. Twenty eight candidate markers were identified in 240 *Sno* I/*Mse* I. Two were co-dominant markers, 14 linked in coupling, 12 linked in repulsion. Except for 12 markers linked in repulsion, the segregation of others was analyzed on 9 recombinants detected from segregating F_3 population. Eight AFLP markers were closely linked to *CRb* and converted into SCAR markers. These markers plus previously identified markers were used to probe BAC library.

To facilitate physical mapping of the *CRb* gene, BAC libraries were constructed with *B. rapa* inbred line Chiifu and CR shinki inbred line. BAC library of Chiifu was designated as KBrH and KBrB library based on restriction sites for construction. A physical map around *CRb* is constructing although there is still a gap exist.

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