

## **5. Summary**

The resistance gene *RPBI* is located on chromosome 1 of *Arabidopsis thaliana* and confers a dominantly inherited resistance to the obligate biotrophic pathogen *Plasmodiophora brassicae*. The objective of this work was to narrow down the region where the *RPBI* gene had been mapped in preceding studies as far as possible, in order to isolate a DNA fragment carrying the resistance gene, which could then be used for the transformation of susceptible plants. To achieve this, high resolution mapping studies had to be done in the *RPBI*-region.

The first step was the construction of a BAC contig that spans the resistance locus and was used to identify new, closely linked markers. Two BAC clones which span the *RPBI* locus were identified. The published DNA sequence of the smaller one of these two clones, BAC T12O21, was used for further molecular analysis of this region. Since the BAC clones carry DNA of the susceptible *Arabidopsis* ecotype Columbia, a cosmid library of the resistant ecotype Tsu-0 (the ecotype used for building up the mapping population) was created.

The existing mapping population was expanded from 900 to 4230 plants. Two allele-specific PCR markers were established that permitted a quick preselection of plants with recombination events near the *RPBI* locus. Altogether 17 new closely linked RFLP and PCR markers were mapped in a section of 200 kb around the resistance locus. In the last part of the mapping studies some BAC fragments showing cosegregation with the resistance locus in a large section of approximately 29 kb could be mapped. According to these mapping data the *RPBI*-region is a section on chromosome 1 with a reduced number of recombination events (cold spot).

The *RPBI* locus was narrowed down to a region of approximately 71 kb according to the Col sequence. In this section three pseudogenes and 13 coding sequences were located. Six of these 13 candidate genes were placed in the region that showed cosegregation with *RPBI*. The other seven genes were placed in the regions to the left and to the right of this cosegregating region and have to be classified as candidate genes too. One cosmid clone was isolated from the Tsu library that carries three of these candidate genes.

Two of the six genes in the cosegregating section are very probably Col specific, two other genes are duplicated on chromosome 5. None of the 13 coding sequences match known genes or proteins or have similarities to previously identified resistance genes. Therefore these genes were classified as hypothetical or unknown genes in the databases. In cDNA analyses 10 of these 13 coding sequences were confirmed to be expressed in at least one of the two ecotypes Tsu (resistant) and Cvi (susceptible). One gene (CDS9) was exclusively expressed in the resistant ecotype Tsu in both infected and non-infected plants. A high rate of polymorphism with a large number of single-nucleotide-polymorphisms between the individual ecotypes could be detected by sequence comparison of the two ecotypes Tsu and Col or PCR analyses of the four ecotypes Tsu, Cvi, RLD and Col. The high resolution mapping and the molecular analysis of the *RPBI*-region can now serve as the basis for subsequent complementation studies that will be necessary for isolating the *RPBI* gene.