Resistance of *Arabidopsis thaliana* to the Obligate Biotrophic Parasite *Plasmodiophora brassicae*

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Abstract

Two resistance phenotypes to *P. brassicae* have been found in *A. thaliana*. A first resistance phenotype has been detected to the isolate 'e_2' and is polygenically inherited. The second resistance to isolate 'e_3' is caused by the dominant resistance gene *RPB1*. By crossing no influence could be shown for salicylic acid, jasmonic acid and ethylene in the latter resistance reaction. The *RPB1* locus was narrowed down to 71 kb on chromosome 1, where three pseudogenes and 13 coding sequences are located. Six of them showed cosegregation with *RPB1*. None of these sequences have similarities to identified resistance genes or other known genes. Ten coding sequences were expressed, but CDS9 was exclusively expressed in the resistant ecotype Tsu-0.

Keywords: *Plasmodiophora brassicae*; *Arabidopsis thaliana*; resistance

INTRODUCTION

Plasmodiophora brassicae, the causal agent of clubroot, is known to be pathogenic on *Arabidopsis thaliana* (KOCH *et al*. 1991). Different *Arabidopsis* ecotypes showed natural variation and the resistance reactions of ecotypes Tsu-0, Ze-0, Ta-0 to *P. brassicae* isolate 'e_H' and the single-spore-isolate 'e_3' have been characterized by the lack of typical clubroot swellings after infection and by a hypersensitive reaction at the roots (FUCHS & SACRISTÁN 1996; KOBELT *et al*. 2000; KLEWER *et al*. 2001). These three ecotypes are carrying alleles of the resistance gene *RPB1* which is located on chromosome 1 (FUCHS & SACRISTÁN 1996; KOBELT *et al*. 2000). Another resistance reaction to *P. brassicae* single-spore-isolate 'e_2' has been described, which is characterized by a strong reduction of typical clubroot swellings compared to susceptible lines but not by a hypersensitive reaction at the roots (KLEWER *et al*. 2001).

The aims of this study were the characterization of the resistance reactions and the limiting of the *RPB1* region 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.

MATERIALS AND METHODS

Plant material. The ecotypes Ze-0, Tsu-0, Ta-0, Col-0, and Cvi-0 of *A. thaliana* were originally provided by AIS (Frankfurt, Germany) and the mutant lines *ein3-1*, *etr1-1*, *ein4*, *jar1-1*, and *npr1-1* by the Arabidopsis Seed Stock Centres (NASC, Nottingham, UK). The transgenic lines cyc (pcyc1.gus), and ARM1 were provided by G. Gheysen (Ghent, Belgium). *Brassica rapa* L. ssp. *pekinensis* cv. Granaat (ECD-05) was used for propagation of all isolates of *P. brassicae*.

Pathogen material. *Plasmodiophora brassicae* isolate 'e_H', 'e_3' and 'e_2' were described by FUCHS and SACRISTÁN (1996) and KLEWER *et al*. (2001).

Phytopathological analysis. Cultivation and inoculation conditions of *A. thaliana* plants and the assessment of disease symptoms were performed according to KOBELT *et al*. (2000).

Genetic analysis. Crosses with the resistant ecotypes Tsu-0 and Ze-0 and susceptible lines were carried out

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and segregation of F_2-progenies were analysed. For all genetic experiments ecotype Cvi-0 was inoculated as the susceptible control, and only tests in which Cvi-0 was scored 100% susceptible were considered for genetic analysis.

BAC clones and filters. IGF-BAC library filters and BAC clones were kindly provided by the RZPD (Resource Center/Primary Database of the German Human Genome Project, Berlin).

BAC final fragments were either isolated by inverse PCR using T7 or SP6 primers and one of the following restriction enzymes CfoI/RsaI/SacI/HindII/AccI (T7 end) or CfoI/HaeIII/XhoI/ClaI (SP6 end) or final fragments were isolated using specific primers, that were designed based on the sequenced BAC ends. These *RPB1*-region-specific primers were also used for the cDNA PCR analysis according to ARBEITER (2002).

DNA isolation. Plant DNA was isolated according to EDWARDS *et al*. (1991) or to ROGERS and BENDICH (1985).

For RFLP analysis, 3-5 μg plant DNA were digested with the appropriate restriction enzyme (10 units/μg), separated on a 0.8% agarose gel and hybridized with ^{32}P-labelled probes (random priming) or DIG-labelled probes according to the instructions of the manufacturer (Roche Molecular Biochemicals, Mannheim, Germany).

Library constructions were performed using genomic DNA of Tsu-0 for cosmid-library construction according to ARBEITER (2002) and using RNA of roots of Tsu-0 and Cvi-0 and the SMART cDNA substraction kit (Clontech, Palo Alto, USA) according to the instructions of the manufacturer.

Nucleotide sequencing of YAC and BAC final fragments and cloned fragments were performed by Gewerbliche Dienstleistungen in der Molekularbiologie (Rüdersdorf, Germany).

All other standard molecular biology techniques were performed according to SAMBROOK *et al*. (1989).

RESULTS

Genetics of the resistance. The dominant resistance gene *RPB1* is responsible for the resistance phenotype of Tsu-0 to isolate 'e_3'. F_2-populations of crosses of Tsu-0 with susceptible mutant lines *ein3-1*, *etr1-1*, *ein4*, *jar1-1*, and *npr1-1* were scored using isolates 'e_H' and 'e_3' to study the influence of salicylic acid, jasmonic acid and ethylene metabolism. No deviation of the 3:1 segregation in F_2-populations were observed in crosses with these mutant lines, indicating no influence of these compounds to this resistance phenotype. In contrast, screening of subtractive cDNA libraries from infected roots of susceptible and resistant ecotypes indicates the expression of ethylene, jasmonic acid or gibberillin inducible genes during infection, e.g. *A. thaliana* genes MEC 18.3, putative lipase/acylhydrolase, or putative GASA 4.

To study the resistance phenotype of Ze-0 to isolate 'e_2', crosses of this ecotype with susceptible lines were performed. Scoring F_1, F_2, F_3, and F_4 populations after inoculation with isolate 'e_2', the segregation data revealed a complex distribution of resistant and susceptible plants indicating polygenic and recessive inheritance of the resistance phenotype of Ze-0 to isolate 'e_2'.

Mapping of the resistance gene RPB1 in Tsu-0. In order to map *RPB1*, a population of 4230 plants was built up. This mapping population was a first backcross generation (BC_1) from a crossing of the resistant *Arabidopsis* ecotype Tsu-0 with the susceptible ecotype Cvi-0. Two allelspecific PCR markers with low distance to *RPB1* were established, that permitted a quick preselection of plants with recombination events only in the immediate vicinity of *RPB1*. One YAC clone of approximately 800 kb was identified that spans the resistance locus since the final fragments of this clone were mapped to the left and to the right of *RPB1*. Afterwards a second contig consisting of BAC clones was constructed, covering a smaller region around *RPB1*. Both contigs were used to identify new, closely linked markers. Two BAC clones, which span the *RPB1* locus were identified. The published DNA sequence of BAC T12O21, was used for further molecular analysis of this region. 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. Since the BAC clones carry DNA of the susceptible *Arabidopsis* ecotype Col, a cosmid library of the resistant ecotype Tsu-0 was created. This library has a size of approximately 13 000 clones with an average insert size of 16.7 kb corresponding to a 1.7 fold representation of the *Arabidopsis* genome.

The *RPB1* locus was narrowed down to a region of approximately 71 kb according to the Columbia sequence. In this section, i.e. between the two mapped final fragments of BAC T12O21, each at a distance of 0.024 cM from *RPB1*, three pseudogenes and 13 coding sequences were located (Table 1). Six of these 13 candidate genes were placed in the region that showed

Table 1. Organization of the *RPB1* region and cDNA PCR analysis data of the candidate genes (+ detected, – not detected) between the closest mapped left (in the gene T12O21.1) and right (next to the gene T12O21.16) recombination sites in the region around *RPB1*. Markers between the grew-shaded genes showed absolute linkage. The gene lengths, ESTs and putative functions are corresponding to the published sequence of ecotype Columbia

Coding Sequences on BAC clone T12O21 (Columbia Sequence)					cDNA PCR analysis			
Gene	Length (bp) (Start-Stop)	Product	ESTs	Homologies to *Arabidopsis* proteins	Tsu Inf.	Tsu Not inf.	Cvi Inf.	Cvi Not inf.
T12O21.1	584	hypothetical protein	–	–	+	+	+	+
T12O21.2	2184	unknown protein	–	Transmembrane-Protein	+	–	+	+
T12O21.3	1538	unknown protein	1	–	+	–	+	–
T12O21.4	1738	unknown protein	44	Phosphoribulokinase-Precursor	+	–	+	–
T12O21.5	2358	unknown protein	7	Secretory Carrier–Membrane-Protein	+	+	+	+
T12O21.17				Pseudogene				
T12O21.6	1652	hypothetical protein	–	–	–	–	–	–
T12O21.7	1001	hypothetical protein	–	–	+	–	+	–
T12O21.8	516	unknown protein	–	Heatshock – Transcriptionfactor HSF30	–	–	–	–
T12O21.9	2700	hypothetical protein	–	(Myosin)	+	+	–	–
T12O21.10	625	hypothetical protein	–	–	–	–	–	–
T12O21.11				Pseudogene				
T12O21.18				Pseudogene				
T12O21.12	2787	unknown protein	6	RNA – Helicase	+	+	+	+
T12O21.13	522	hypothetical protein	–	–	+	–	+	–
T12O21.14	2388	unknown protein	–	Terpene-Synthase Protein family	–	–	+	–
T12O21.15	526	unknown protein	2	–	+	–	+	–
T12O21.16	3130	unknown protein	2	G-Protein Alpha Subunit	+	+	+	+

cosegregation with *RPB1*. 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 (Table 1). One cosmid clone was isolated from the Tsu-0 library that carries three of these candidate genes. The right final fragment of this cosmid clone, a 1128 bp long fragment without any homology to the Columbia sequence, was also mapped at a distance of 0.024 cM (1 recombinant) on the right side of the resistance locus.

Two of the six genes (CDS6 and CDS8) in the cosegregating section are very probably Columbia specific, two other genes (CDS9 and CDS10) are duplicated on chromosome 5. None of the 13 coding sequences match known genes or proteins or revealed similarities to previously identified resistance genes (Table 1). Therefore these genes were classified as hypothetical or unknown genes in the databases. In cDNA PCR analysis (Table 1) 10 of these 13 coding sequences were confirmed to be expressed in at least one of the two ecotypes Tsu-0 (resistant) and Cvi-0 (susceptible). One gene (CDS9) was exclusively expressed in the resistant ecotype Tsu-0 in both infected and non-infected plants.

DISCUSSION

With high resolution mapping, the region where the resistance locus *RPB1* is located could be narrowed down to a number of 13 candidate genes according to the sequence of the susceptible ecotype Columbia. Therefore an allele of one of these genes or an additional gene in the resistant ecotype Tsu-0 must confer resistance to *Plasmodiophora brassicae*. Further mapping studies seem to be rather pointless because the *RPB1* region is a section with a reduced

number of recombination events. According to data of SCHMIDT *et al*. (1995), the *RPB1* region could be designated as a "cold spot" region. Therefore these mapping data should serve as the basis for subsequent complementation studies that will be necessary for isolating the *RPB1* gene. For such complementation studies, either the cosmid library has to be enlarged in order to isolate more, overlapping cosmid clones for this region or the candidate genes and their up- and downstream regulative elements must be amplified by PCR and cloned for later transformation.

However, crossing with mutant lines revealed no influence of salicylic acid, jasmonic acid and ethylene metabolism to resistance in contrast to many other plant pathogen interactions (GLAZEBROOK 1999). This might point to a new signal transduction chain for this resistance due to *RPB1* but it also implies, that no expression pattern or gene function can be used as a hint to choose between the candidate genes.

Futhermore, genetic analysis revealed an additional polygenic and recessively inherited resistance phenotype of Ze-0 to *P. brassicae* single-spore isolate 'e_2' indicating the usefulness of *A. thaliana* as a model for the mono- and polygenic resistances to *P. brassicae* in *Brassica* crop plants.

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