Theor Appl Genet (1996) 92:281–284

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Molecular mapping of the *Arabidopsis* locus *RPP11* which conditions isolate-specific hypersensitive resistance against downy mildew in ecotype RLD

Received: 4 July 1995 / Accepted: 18 August 1995

Abstract Isolate WELA of the plant pathogenic oomycete fungus *Peronospora parasitica* causes downy mildew in the *Arabidopsis thaliana* ecotypes Weiningen (Wei-0) and La-er, whereas ecotypes RLD and Col-0 are resistant. Genetic crosses between resistant RLD and susceptible Wei-0 showed that resistance was inherited in a simple Mendelian fashion as a monogenic dominant trait. The interactions between different isolates of *P. parasitica* and ecotypes of *A. thaliana* show race-specific variation and fit a gene-for-gene relationship. The *RPP11* resistance gene was mapped by following the co-segregation of the resistance phenotype with RFLP markers in a mapping population of 254 F_3 families derived from RLD × Wei-0 F_2 individuals. Linkage analysis using version 1.9 of the MAPMAKER program placed the *RPP11* resistance locus on chromosome III between marker m249 (two recombinants) and marker g2534 (six recombinants). Markers g2534 and g4117 are on YAC EG7H1. Marker g4117 and one end probe (N5) generated from YAC EG7H1 showed no recombinants. The YAC end probe N5, which was generated by plasmid rescue, was used to screen clones in the Eric Ward YAC library and a YAC was fished (EW19B12) which also hybridised with m249. Thus, a YAC contig has been established over the region where the resistance locus maps. Because the YACs were made with ecotype Columbia DNA it is necessary to isolate the equivalent region from RLD in order to clone the resistance locus. To this end a phage λ-DASH^{TM} genomic library was prepared from RLD and a contig covering the relevant region of the YACs is currently under construction.

Communicated by M. Koornneef

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Key words Resistance gene · *Peronospora parasitica* · *Arabidopsis thaliana* · Molecular markers

Introduction

Downy mildew of *Arabidopsis thaliana* was mentioned by Lindau (1901) and Gaeumann (1918), and is caused by the oomycete fungus *Peronospora parasitica.* The pathosystem was first described in detail by Koch and Slusarenko (1990) who showed that pathotypic variation occurred between host and pathogen and that resistance was associated with a hypersensitive response. Variation in the microscopic and macroscopic phenotypes of resistance in various isolate/ecotype combinations has been characterised and the inheritance of resistance studied in crosses (Holub et al. 1993; Mauch-Mani et al. 1993; Holub et al. 1994). Resistance in *Arabidopsis* to *P. parasitica* is inherited as though it were conditioned by single dominant genes in the host and this is an important pre-requisite for a map-based cloning strategy to isolate the resistance loci.

Arabidopsis has many advantages as a model host for investigating the molecular biology of host-pathogen interactions because of its small genome size and the availability of genetic maps, markers, and YAC-libraries (Chang et al. 1988; Guzmann and Ecker 1988; Nam et al. 1989; Ward and Jen 1990; Grill and Sommerville 1991; Hauge et al. 1993; Lister and Dean 1993; Schmidt and Dean 1993; Jarvis et al. 1994). These features all contribute to successful positional cloning strategies to isolate genes characterised only by the phenotype they confer. Model pathosystems have been developed for interactions of *Arabidopsis* with bacteria and fungi (Davis et al. 1991, Debener et al. 1991; Crute et al. 1993. For reviews see Ausubel et al. 1993; Dangl 1993, Mauch-Mani and Slusarenko 1993; Crute et al. 1994; Staskawicz et al. 1995). The *Arabidopsis* resistance gene *RPS2*, which conditions resistance against *Pseudomonas syringae* pv *tomato* isolates and other pseudomonads containing the avirulence gene *avrRpt2*, has now been

cloned (Bent et al. 1994; Mindrinos et al. 1994) and the map positions of four genes recognizing *P. parasitica* isolates (*RPP*) have been published (Parker et al. 1993; Tör et al. 1994). In the present paper we describe the RFLP-mapping of the race-specific resistance gene *RPP11* of *A. thaliana* to the WELA isolate of *P. parasitica*.

Materials and methods

Plant material and crosses

Seeds of *A. thaliana* ecotypes RLD and Wei-0 were surface-sterilized with bleach (2.5%) for 15 min and washed several times with sterile tap water before sowing into growing compost mixed with perlite. The seeds were kept for 5 days at 4 °C before being transferred into a growth chamber with a photoperiod of 8 h light (approximately 150 μE m^{-2} s^{-1}, 23 °C) and 16 h dark (18 °C). Plants for crosses were sown singly into small pots to facilitate handling whereas plants for phenotype determination were sown into flats at approximatly 2 seeds cm^{-2}.

Infection with *P. parasitica* isolate WELA

Plants were inoculated with *P. parasitica* isolate WELA 3 weeks after sowing, which corresponded to 2-weeks growth in the climate chamber. At this stage the plants had fully developed cotyledons and emerging primary leaves. Inoculum production and the inoculation of the seedlings was performed as described by Mauch-Mani and Slusarenko (1994).

Determination of infection phenotype

Whole seedlings were stained with alcoholic trypan blue solution (Keogh et al. 1980) and cleared in chloral hydrate (2.5 g ml^{-1}) for about 24 h. Whole seedlings were then mounted in chloral hydrate and viewed under a microscope with bright-field optics. The resistance phenotype of F_1, F_2 and backcrosses was first determined using a dissecting microscope to score for the presence or absence of asexual sporulation of the fungus 1 week after infection. One leaf from each plant was then dissected away, cleared in chloral hydrate, and examined microscopically to confirm the macroscopically determined phenotype.

The reactions of the F_3 plant families were divided into three categories. They were scored as resistant when from a subset of 40 seeds all the plants showed a hypersensitive reaction at the location of the attempted fungal penetration. A family was scored as susceptible when all the tested plants were colonized by the fungus and sexual, as well as asexual, spores could be found by 1 week after inoculation. Heterozygote families segregated for resistance and susceptibility as expected.

Extraction and RFLP analysis of DNA from F_3 families

Isolation of total DNA was as described by Dolferus (1991). For RFLP analysis 1–5 μg of DNA was digested with the appropriate restriction enzyme to reveal a polymorphism between RLD and Wei-0 for the probe in question. The digested DNA was separated on 1% agarose gels in TAE buffer (Sambrook et al. 1989) and blotted onto Hybond N membranes (Amersham; Sambrook et al. 1989). The filters were baked for 2 h at 80 °C. The RFLP markers (phage and cosmid markers) were radioactively labelled with ^{32}P-dCTP using a random primed labelling kit (Boehringer). Hybridisation was carried out overnight at 60–65 °C in 10% (w/v) dextransulphate, 1 M NaCl and 1% SDS. Filters were washed in 2 × SSC, 1% SDS, 2 × 45 min at 65 °C and 0.2 × SSC, 0.1% SDS, 2 × 45 min at 65 °C. The filters were exposed to X-ray film for 1 day at −80 °C with intensifying screens.

Linkage analysis was performed with the mapmaker program (Lander and Green 1987; Lander et al. 1987) using a LOD score of 3.0.

RFLP phage, cosmid markers and the E.W. YAC library were kindly provided by the Arabidopsis Biological Resource Center at Ohio State University.

Cosmid and phage propagation and DNA extraction was done according to the methods of Sambrook et al. 1989.

The yeast DNA extraction of the clones EG7H1, EW19B12, EW12D3, the plasmid rescue, and the screening of the YAC library was done as described in Gibson and Sommerville (1992).

Results

Genetics of resistance

In the cross Weiningen × RLD, 100 tested F_1 plants all showed a resistant phenotype when inoculated with the WELA isolate of *P. parasitica*. Testing of an F_2 population of a cross between Weiningen and RLD gave a segregation of 38 resistant to 12 susceptible plants (χ^2 $3:1 = 0.014$, $P > 0.05$). In a representative backcross [Wei-0 × (Wei-0 × RLD)] resistance segregated eight susceptible plants to six resistant (χ^2 $1:1 = 0.28$, $P > 0.05$). These results indicate that resistance in RLD is inherited as though it were conditioned by a single dominant gene. Since these ratios were conserved in reciprocal crosses there is no evidence for maternal effects on the inheritance of resistance in this host-isolate combination. The resistance locus in question was designated *RPP11* (Recognition of *Peronospora parasitica*) by Holub et al. (1994) in a comprehensive survey of the genetics of the interaction between *P. parasitica* isolates and *Arabidopsis* ecotypes.

Mapping of the resistance locus

Fine mapping of the resistance locus was performed by following co-segregation of the susceptible phenotype with chromosome-3 RFLP probes in a total of 254 F_3 families. Phage and cosmid probes needed to be tested empirically with various restriction enzymes to determine whether they highlighted an RFLP between the RLD and Wei-0 ecotypes. This information is summarised in Table 1.

The resistance locus mapped between the phage marker m249 and the cosmid marker g2534 (Fig. 1). Two recombinants were found between *RPP11* and marker m249, and six recombinants with marker g2534. No recombinants were found with marker g4117 located between m249 and g2534. The two markers g2534 and g4117 both map to the YAC EG7H1 (Hwang et al. 1991) and the plasmid-rescued end-fragment from this YAC also showed no recombinants. A *Bam*H1/*Nde*1 digest was used to remove YAC DNA from the end-fragment of EG7H1 and this was used as a probe to isolate an overlapping YAC EW19B12, which closes the gap between the marker m249 and the YAC EG7HI. The

Table 1 Probes and restrictrion enzymes used to highlight polymorphisms between RLD and Wei-0 ecotypes. H = *Hin*dIII, RI = *Eco*RI, RV = *Eco*RV, X = *Xba*I

Chromosome	Probe	Restriction enzyme	Map distance (cM)
I	m241	RI	
	m219	X	9.6
	m402	RI	30.8
	m213	RI	53.1
	m305	RI	12.3
	m421	RI	5.6
	m315	RI	6.5
	m453	RI	12.5
	m532	H	12.3
II	m605	RI	
	m283	RI	22
	m429	X	21.1
	m336	H	0
III	m583	H	
	m560	RI	37.9
	m433	X	16.2
	m249	RI	37.8
	N5	RI	0.4
	g4117	RI	0
	g2534	RI	1.5
	m409	RV	4.6
	g4014	H	4.3
	m457	RV	11
	g4125	RV	2.1
	m424	RI	15
IV	m506	RI	
	m518	RI	22.5
	m600	RV	36.7
V	m217	RI	
	m224	X	7
	m247	RI	31.6
	m422	RI	4.9
	m225	X	16.7

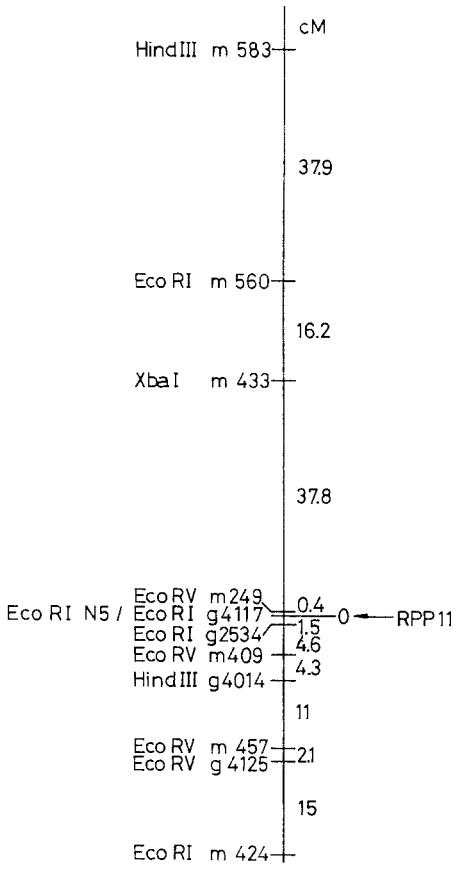

Fig. 1 Fine mapping of *RPP11* on chromosome III in a population of 254 F_3 families

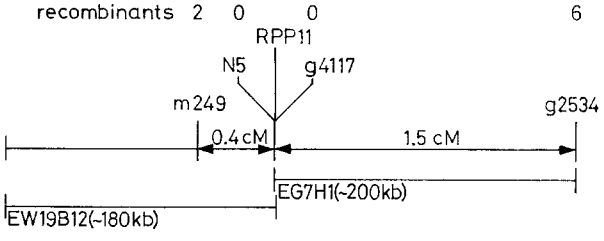

Fig. 2 Physial map of the region on chromosome III around *RPP11*. The YAC, phage and cosmid clones are as described in the text

genomic organisation of the phage and cosmid markers, YACs, and the end-fragment of the YAC EG7HI is shown in Fig. 2. The YAC DNAs were isolated and used to fish cross-hybridizing phage clones from our RLD genomic library and these are currently being ordered on the physical map, and clones which potentially carry the *RPP11* gene are being subcloned into a plant transformation vector for functional testing in transgenic plants.

Discussion

The isolation and characterization of the *RPP11* gene from *Arabidopsis* will add to the list of already cloned resistance genes and increase the composite picture of their common features. Other resistance genes effective against *Peronospora* isolates with different avirulence gene specificities (*RPP1*, *RPP2*, *RPP4* and *RPP5*) are currently being, or have been (*RPP5*, Jane Parker, personal communication), cloned from *Arabidopsis*. This series of resistance genes will be the most comprehensive so far cloned for a particular pathosystem. It will thus be of great interest to see how these genes, which all condition resistance to different races of the same pathogen, relate to each other in structure and function. An interesting feature which has already emerged is that *RPP* genes, although dispersed throughout the genome, often seem to map in clusters. Thus, *RPP1* and *RPP11* map respectively above and below marker m249 on chromosome III and *RPP2*, *RPP4*, *RPP5* all map closely on chromosome IV (Parker et al. 1993; Tör et al. 1994; Joos et al., this work). In *Arabidopsis* only chromosome II has not yet been reported to carry a resistance locus.

Another interesting feature to emerge from studies with the *P. parasitica*/*Arabidopsis* pathosystem is that some resistance genes appear to be dominant, e.g. *RPP11*, whereas others show incomplete dominance, e.g., *RPP5* and *RPP6* (Mauch-Mani et al. 1993; Parker

et al. 1993). This phenomenon has been reported for the *Bremia lactucae*/lettuce pathosystem and the implications for the mechanism of resistance gene function were discussed by Crute and Norwood (1986). It will thus be particularly interesting to see how the structural features of *RPP5*, *RPP11*, and the other *RPP* genes, relate to their biochemical functions and the resistance phenotypes they confer.

Acknowledgements We are grateful to the *Arabidopsis* stock centre at Ohio State, Drs. Grill and Dangl for providing us with RFLP probes and YAC clones. Drs. Crute, Holub, Beynon and Parker are thanked for informative and fruitful discussions. We also thank Alexandra Dolder for technical assistance. This work was supported by the Swiss National Science Foundation's Schwerpunktsprogramm and the Kanton of Zürich.

References

Ausubel FM, Glazebrook J, Greenberg J, Mindrinos M, Yu GL (1993) Analysis of the *Arabidopsis* defence response to *Pseudomonas* pathogens. Adv Mol Genet Plant-Microbe Interact 2:393–403

Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J, Staskawicz BJ (1994) *RPS2* of *Arabidopsis thaliana*: a leucine-rich repeat class of plant disease resistance genes. Science 265:1856–1860

Chang C, bowman JL, DeJohn AW, Lander ES, Meyerowitz EM (1988) Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc Natl Acad Sci USA 85: 6856–6860

Crute IR, Norwood JM (1986) Gene-dosage effects on the relationship between *Bremia lactucae* (downy mildew) and *Lactuca sativa* (lettuce); the relevance to a mechanistic understanding of host-parasite specificity. Physiol Plant Pathol 29:133–145

Crute IR, Holub, EB, Tör M, Brose E, Beynon JL (1993) The identification and mapping of loci in *Arabidopsis thaliana* for recognition of the fungal pathogens: *Peronospora parasitica* (downy mildew) and *Albugo candida* (white blister). Adv Mol Genet Plant-Microbe Interact 2:437–444

Crute I, Beynon J, Dangl J, Holub E, Mauch-Mani B, Slusarenko AJ, Staskawicz B, Ausubel F (1994) Microbial pathogenesis of *Arabidopsis*. In: Meyerowitz EM, Somerville CR (eds) *Arabidopsis*. Cold Spring Harbour Laboratory, Cold Spring Harbor, New York, pp 705–747

Dangl JL (1993) Applications of *Arabidopsis thaliana* to outstanding issues in plant-pathogen interactions. Int Rev Cytol 144:53–83

Davis KR, Schott E, Ausubel FM (1991) Virulence of selected phytopathogenic pseudomonads in *Arabidopsis thaliana*. Mol Plant-Microbe Interact 4:477–488

Debener T, Lehnackers H, Arnold M, Dangl JL (1991) Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. The Plant Jour 1:289–302

Dolferus R (1991) In: Negrutiu I, Gharti-Chhetri G, (eds) Biomethods: a laboratory guide for cellular and molecular plant biology Birkhäuser Verlag, Basel, pp 133–146

Gäumann E (1918) Über die Formen der *Peronospora parasitica* (Pers.) Fries Beitr Bot Zentralbl 35:395–533

Gibson SI, Somerville C (1992) Chromosome walking in *Arabidopsis thaliana* using yeast artifical chromosomes. In: Koncz C, Chua N-H, Schell J (eds) Methods in *Arabidopsis* research. World Scientific Publishing Co, Singapore, pp 119–143

Grill E, Sommerville C (1991). Construction and characterization of a yeast artificial chromosome library of *Arabidopsis* which is suitable for chromosome walking. Mol Gen Genet 226:484–490

Guzman P, Ecker J (1988) Development of large DNA methods in plants: molecular cloning of large segments of *Arabidopsis* and carrot DNA into yeast. Nucleic Acids Res 16:11091–11105

Hauge BM, Hanley SM, Cartinhour S, Cherry JM and Goodman HM, Koornneef M, Stam P, Chang C, Kempin S, Medrano L, Meyerowitz EM (1993) An Integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. The Plant Jour 3:745–754

Holub EB, Crute I, Brose E, Beynon J (1993) Identification and mapping of loci in *Arabidopsis* for resistance to downy mildew and white blister. In: Davis KR, Hammerschmidt R (eds) *Arabidopsis thaliana* as a model for plant-pathogen interactions. APS Press, Minnesota, pp 21–35

Holub EB, Beynon JL, Crute IR (1994) Phenotypic and genotypic characterization of interactions between isolates of *Peronospora parasitica* and accessions of *Arabidopsis thaliana*. Mol Plant Microbe Interact 7:223–239

Hwang I, Kohchi T, Hauge BM, Goodman HM, Schmidt R, Chops G, Dean C, Gibson S, Jba K, Lemieux B, Arondel V, Danhoff L, Somerville C (1991) Identification and map position of YAC clones comprising one-third of the *Arabidopsis* genome. Plant J 1:367–374

Jarvis P, Lister C, Szabo V, Dean C (1994) Integration of CAPS markers into the RFLP map generated using recombinant inbreds of *Arabidopsis thaliana*. Plant Mol Biol 24:685–687

Keogh RC, Deverall BJ, McLeod S (1980) Comparison of histological and physiological responses to *Phakopsora pachyrhizi* in resistant and susceptible soybean. Trans Br Mycol Soç 74:329–333

Koch E, Slusarenko A (1990) *Arabidopdis* is susceptible to infection by a downy mildew. Plant Cell 1:437–445

Lander ES, Green P (1987) Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci USA 84:2363–2367

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newberg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lindau G (1901) Hilfsbuch für das Sammeln parasitischer Pilze. Börnträger Verlag, Berlin

Lister C, Dean C (1993) Recombinant inbred lines for mapping RFLP and phenotypic markers in *Arabidopsis thaliana*. The Plant Jour 4:745–750

Mauch-Mani B, Slusarenko AJ (1993) *Arabidopsis* as a model host for studying host-pathogen interactions. Trends Microbiol 1:265–270

Mauch-Mani B, Slusarenko AJ (1994) Systemic acquired resistance in *Arabidopsis thaliana* induced by a predisposing infection with a pathogenic isolate of *Fusarium oxysporum*. Mol Plant-Microbe Interact 7:378–383

Mauch-Mani B, Croft KPC, Slusarenko AJ (1993) The genetic basis of resistance of *Arabidopsis thaliana* L. Heyhn. to *Peronospora parasitica*. In: Davis KR, Hammerschmidt R (eds) *Arabidopsis thaliana* as a model for plant-pathogen interactions. APS Press, Minnesota, pp 5–20

Mindrinos M, Katagiri F, Yu GL, Ausubel FM (1994) The *Arabidopsis thaliana* disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell 78:1089–1099

Nam HG, Giraudat J, den Boer B, Moonan F, Loos DB, Hauge BM, Goodman HM (1989) Restriction fragment length polymorphism linkage map of *Arabidopsis thaliana*. Plant Cell 1:699–705

Parker JE, Szabo V, Staskawicz BJ, Lister C, Dean C, Daniels MJ, Jones JDG (1993) Phenotypic characterization and molecular mapping of the *Arabidopsis thaliana* locus *RPP5*, determining disease resistance to *Peronospora parasitica*. Plant Jour 4:821–831

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a Laboratory manual, 2nd edn. Cold Spring Harbour Laboratory, Cold Spring Harbor, New York

Schmidt R, Dean C (1993) Towards construction of an overlapping YAC library of the *Arabibopsis thaliana* genome. Genes and Genomes 15:63–69

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JDG (1995) Molecular Genetics of plant disease resistance. Science 268:661–667

Tör M, Holub EB, Brose E, Musker R, Gunn N, Can C, Crute IR, Beynon JL (1994) Map positions of four loci in *Arabidopsis thaliana* associated with isolate-specific recognition of *Peronospora parasitica* (downy mildew). Mol Plant-Microbe Interact 7:214–222

Ward ER, Jen GC (1990) Isolation of single-copy-sequence clones from a yeast artificial chromosome library of randomly sheared *Arabidopsis thaliana* DNA. Plant Mol Biol 14:561–568