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Genetic and physical analysis of a YAC contig spanning the fungal disease resistance locus *Asc* of tomato (*Lycopersicon esculentum*)

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Abstract The *Alternaria* stem canker disease of tomato is caused by the necrotrophic fungal pathogen *Alternaria alternata* f. sp. *lycopersici* (AAL). The fungus produces AAL toxins that kill the plant tissue. Resistance to the fungus segregates as a single locus, called *Asc,* and has been genetically mapped on chromosome 3 of tomato. We describe here the establishment of a 1383-kb YAC contig covering the *Asc* locus and a series of plants selected for recombination events around the *Asc* locus. It was shown that the YAC contig corresponds to a genetic distance of at least 11.2 cM. Thus, the recombination rate in the *Asc* region is six times higher (123 kb/cM) than the average for the tomato genome. Furthermore, the *Asc* locus could be localised to a 91-kb fragment, thus paving the way for the cloning and identification of the *Asc* gene(s) by complementation.

Key words Tomato · *Alternaria* stem canker · Map-based cloning · Physical mapping · Recombination

Introduction

Among plant pathogenic fungi, one distinguishes biotrophs and necrotrophs. Biotrophs enter host tissue and obtain nutrients from living cells, whereas necrotrophs kill the host by producing toxic agents and feeding on the dead cells. Resistance against biotrophs appears to operate by recognition of the fungal avirulence product in a specific gene for gene interaction, which subsequently elicits a defense response (Flor 1971, reviewed by Bent 1996). The structural similarity between resistance genes specific for biotrophs suggests a role in signal transduction pathways leading to programmed cell death and induction of defense gene expression (Staskawicz et al. 1995; Bent 1996). Resistance mechanisms against necrotrophs seem to differ from those triggered by biotrophs. The resistance gene product apparently interferes with the toxic effect of the fungus by altering the affinity of the toxin target (the oat *Vb* gene, Navarre et al. 1995), by eliminating the toxin target (the maize *T-urf* 13 locus, Dewey at al.1988) or by detoxification of the toxin itself (the maize *Hm1* resistance gene; Johal and Briggs 1992).

The *Alternaria* stem canker disease of tomato is caused by the necrotrophic fungus *Alternaria alternata* f. sp. *lycopersici* (AAL). The disease is characterised by dark brown to black cankers on the stem. Leaves develop necrotic areas around the veins and, in later stages, become completely necrotic (van der Biezen et al. 1994a). The AAL toxins produced by the fungus confer the virulence specificity. Resistance to the fungus is based on the presence of the single dominant locus *Asc* (Gilchrist and Gorgan 1976), which behaves as semi-dominant against AAL toxins. Resistance to the fungus and insensitivity to the toxins is characterised by the absence of symptoms on the plant. It has been shown in vitro that AAL toxins inhibit ceramide synthase, an enzyme involved in sphingolipid biosynthesis (Merrill et al. 1993). The mechanism of resistance and its links with cell death are, however, unknown (Wang et al. 1996).

Because the *Asc* gene and its product have not yet been identified, a genetic approach was employed in order to isolate the gene. Targeted and non-targeted transposon tagging has been shown to be feasible in tomato (van der Biezen et al. 1996), leading to the isolation of, among others, disease resistance genes (*Cf9*, Jones et al. 1994; *Cf4*, Takken et al. 1998). However, no

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transposon insertion has been found in *Asc* (the allele conferring insensitivity to AAL toxins) or in *asc* (allele sensitive to AAL toxins) (van der Biezen et al. 1994a; Brandwagt et al. 1998). The *Asc* locus has been positioned on an integrated map of chromosome 3 with respect to tightly linked RFLP markers (van der Biezen et al. 1994b, 1995). Tightly linked markers are essential for cloning a gene by map-based cloning (Wing et al. 1994; Zhang et al. 1994; Tanksley et al.1995).

The development of Yeast Artificial Chromosomes (YACs) allows the isolation and cloning of large fragments of DNA from a region of interest in the genome (Burke et al. 1987). It has shown to be an effective tool for isolating genes based on their position on the genetic map in many instances, including disease resistance genes (reviewed by de Wit 1997; Parker and Coleman 1997) in tomato (Martin et al. 1993; Brommonschenkel and Tanksley 1997), rice, Arabidopsis, sugar beet and barley.

Tomato is a genetically well characterised crop, with a relatively small genome (haploid genome content is 950 Mb; Arumuganathan and Earle 1991). Molecular markers have been mapped over the entire genome with an average coverage of one Restriction Fragment Length Polymorphism (RFLP) every 1.2 cM (Tanksley et al. 1992). On average, 1 cM represents approximately 750 kb (Tanksley et al. 1992). However, it has been shown that recombination frequencies can vary depending on the chromosomal area considered. Suppression of recombination has been observed in regions that are close to the centromere (Ganal et al. 1989).

In this paper we describe a study of the *Asc* region on chromosome 3 of tomato. We have analysed physical distances in this region and compared them to genetic distances. Using a preselection scheme based on phenotypic seedling markers, a population of 31 tomato plants was selected as having undergone a recombination event in the 2.1-cM region between the two closest RFLP markers flanking the *Asc* locus. A YAC contig spanning 1383 kb and covering at least 11.2 cM including the *Asc* locus was established. By comparing genetic and physical distances, we show that the *Asc* locus is present at a position on chromosome 3 where the recombination frequency is about six times higher (123 kb/cM) than the average for the tomato genome (750 kb/cM). Furthermore, the *Asc* locus was delimited to a region of at most 91 kb on a 310-kb YAC, providing the opportunity to isolate and identify the *Asc* gene(s) by complementation.

Materials and methods

Plant material

The *A. alternata* f. sp. *lycopersici* susceptible (*asc*/*asc*) Tester-3 (*L. esculentum*), which carries the phenotypic seedling markers *bls* (baby lea syndrome; hypocotyl anthocyanin lacking) and *sf* (solanifolia, leaves entire) was selected from an F2 cross between LA1004 and LA1182 (obtained from Dr C. Rick, Tomato Genetics Stock Center, UC Davis, Calif.). The T3 genotype was *bls*/*bls, asc*/*asc, sf*/*sf*. Seeds of *Lycopersicon pennellii* (LA716,G1.1611) were obtained from Dr. P. Lindhout, CPRO-DLO, Wageningen, The Netherlands. The genotype of *Lycopersicon pennellii* was *Bls*/*Bls, Asc*/*Asc, Sf*/*Sf*.

Plant crosses

Plant crosses were done in the greenhouse using standard emasculation and pollination techniques. Tomato plants were grown in the greenhouse at 20° C, under conditions of 15 klx light and 60% humidity. The F1 interspecific hybrid was made by crossing the susceptible (*asc*/*asc*) *L. esculentum* Tester 3 line with *L. pennellii* as the staminate parent. Recombinant F2 progenies were obtained by self-fertilisation of F1 plants.

Preparation of cell-free culture filtrate (CFCF)

CFCF of *A. alternata* f. sp. *lycopersici* was obtained as described by Gilchrist and Gorgan (1976).

Leaf bioassay for sensitivity to AAL toxins

Young leaflets were cut from the plant and placed in a petri dish (12 cm diameter) containing a filter paper saturated with 3 ml of AAL toxins obtained from the CFCF of the fungus *A. alternata* f. sp. *lycopersici*. Cut leaflets were incubated in a growth chamber under standard conditions (3 klx of constant light, 22° C). Leaves were assessed for the presence or absence of necrosis symptoms after 72 h.

Tomato YAC libraries

The large-insert YAC library (600 kb) was made from a tomato line that is insensitive to AAL toxins and was screened by Keygene (Wageningen), using the molecular markers TG 134 and TG 442. TG134 and TG 442 were partly sequenced using a dye primer sequencing kit and a semi-automated sequencer (Applied Biosystems). The primers TG134D1(5′-CCTGCTCTAATCTTGTGTGACC-3′) and TG134R1 (5′-CTTCACCGAACTCAGCCACC-3′) were used in a PCR screen to obtain YAC134. Primers TG442D1 (5′-AATTAAATCTGCCCCATGTGG-3′) and TG442R1 (5′-GTT AAGCGCACACCTTGTGG-3′) were used in a similar screen to obtain YAC 442.

The 250 kb-insert YAC library was also made from a tomato line that is insensitive to AAL toxins. It was constructed by Bonnema et al. (1996). The YAC 442 left end was isolated (see below) and partly sequenced. Primers TG442ED1 (5′-ATGGGTCTCATAGAGCAGAG-3′) and TG442ER1 (5′-CGGAGGTTGGGCACGTCG-3′) were used in a PCR screen to obtain YAC 442A.

Both libraries were made using the pYAC4 Yeast Artificial Chromosome vector (Burke et al. 1987).

Isolation of YAC end probes

YAC left-end probes were isolated by plasmid rescue: 10 μg of total yeast DNA was digested with 40 units of *Nde*I restriction enzyme. Digested DNA was selfligated in a total volume of 400 μl overnight at 16° C. Ligated DNA was introduced into competent *E. coli* DH5α by electroporation (Bio Rad Pulse Controller). Colonies were selected on ampicillin-containing LB plates (10 g/l Tryptone, 5 g/l yeast extract, 10 g/l NaCl, 16 g/l Bacto-agar, 10 μg/ml ampicillin).

Right ends were isolated by anchor PCR (Roux and Dhaanarajan 1990). Total yeast DNA was digested with *Hin*cII, *Pvu*I and *Eco*RV, generating blunt ends, and was ligated to an anchor primer (5′-AGCACTCTCCAGCCTCTCACCGCA-3′ annealed to 5′-TGCGGACT-3′), and then amplified with the single

primer Y4R (5′-ATATAGGCGCCAGCAACCGCACCTGCTG-TGGCG-3′) based on the pYAC4 right arm sequence. The PCR conditions were: 10 cycles of 92° C for 1 min, 60° C for 1 min, and 72° C for 3 min. A second amplification was done using a nested primer (5′-CGCCCGATCTCAAGATTACG-3′) and the anchor primer (5′-AGCACTCTCCAGCCTCTCACCGCA-3′) for 30 cycles under the same conditions.

RFLP analysis

Plant DNA isolation from the *L. esculentum* T3 line and *L. pennellii*, their F1 hybrids, and the F2 population described above was performed according to Dellaporta et al. (1983). Parental (*L. esculentum* and *L. pennellii*) and F1 plant DNA was digested with various restriction enzymes and fractionated by gel electrophoresis. Gels were blotted according to Southern (1975). Blots containing parental and F2 plant DNA were hybridised to various DNA probes as described by Church and Gilbert (1984)

PFGE analysis of YAC DNA

The yeast strain (AB1380) containing the YAC of interest was grown in 1% (w/v) Yeast extract-2% Peptone-2% Glucose liquid medium (YPD) for 48 h at 30° C with agitation. Yeast cells were then embedded in agarose plugs containing 0.75% insert agarose (Biozym), 10 mM TRIS-HCl pH 7.2, 20 mM NaCl, and 50 mM EDTA, and lysed using 1 mg/ml lyticase for 2 h at 37° C. Proteins were removed by incubating the plugs in a buffer containing 100 mM EDTA, 1% sodium lauryl sarcosine, 1 mg/ml proteinase K at 50° C overnight. The plugs were washed three times in 20 mM TRIS-HCl pH 8.0 and 50 mM EDTA for 1 h each at room temperature.

YAC DNA to be digested was prepared from cells embedded in agarose microbeads instead of agarose plugs. Microbeads were preincubated in adequate amounts of restriction enzyme buffer overnight before digestion for 24 h in fresh buffer. Complete digestions were performed overnight on 50 µl of microbeads with 100 units of one of the following restriction enzymes: *Ksp*I, *Not*I, *Sal*I, *Sfi*I and *Sma*I. Partial digestions were done using several enzyme dilutions.

Digested and undigested DNA was separated by pulsed field gel electrophoresis (PFGE) using a contour-clamped homogeneous electrophoresis (CHEF) apparatus (Bio Rad) in 0.8% LMP (low melting point) agarose containing 0.5× TBE (45 mM TRIS-borate, 50 mM EDTA pH 8). Running conditions were optimised for separation of 20- to 500-kb fragments: an initial switching time of 20 s and a final switching time of 50 s at 6 V/cm. Total running time was 26 h. DNA in the gel was nicked using UV light, denatured in 0.5 M NaOH, 1.5 M NaCl and capillary blotted onto Hybond membranes in 0.4 M NaOH overnight. Blots were then hybridised with various probes (Church and Gilbert 1984).

Subcloning of YAC 442A

Total yeast DNA containing YAC442A was digested with *Bam*HI and subcloned into pGem3Z (Promega) according to Sambrook et al. (1989).

Thirty clones selected on ampicillin plates containing on average 1-3 kb inserts were randomly picked and used as probes for hybridisation to Southern blots containing YAC 442A and yeast DNA without the YAC (*Saccharomyces cerevisiae* strain AB 1380).

Results

Generation of plants that have undergone recombination events around the *Asc* locus

A population of plants was selected for recombination events on both sides of the *Asc* locus in order to obtain a fine-scale map of molecular markers around the *Asc* locus. For this, the Tester 3 tomato line *L. esculentum* containing the phenotypic markers *bls* and *sf* was crossed with *L. pennellii*. F1 progeny plants were self-pollinated to obtain F2 plants that might have undergone recombination. Some 3266 F2 seeds were sown and screened for segregation of the phenotypic markers *bls* and *sf* on the seedling level. A total of 1123 plants were selected based on the expression of *bls* (718) or *sf* (405). These 1123 plants were screened by a leaf assay to select for plants sensitive to AAL toxins. From these 1123 plants, 216 plants were preselected in total; 107 plants with the *bls* phenotype (homozygous *bls*) that were sensitive to AAL toxins and 109 plants with the *sf* phenotype (homozygous *sf*) and sensitive for AAL toxins. These 216 preselected plants were screened by RFLP analysis by Southern analysis using the RFLP markers TG 134 and TG 442 as probes. In total 31 plants were selected: 12 of these had undergone a recombination event between TG 134 and *asc* and 19

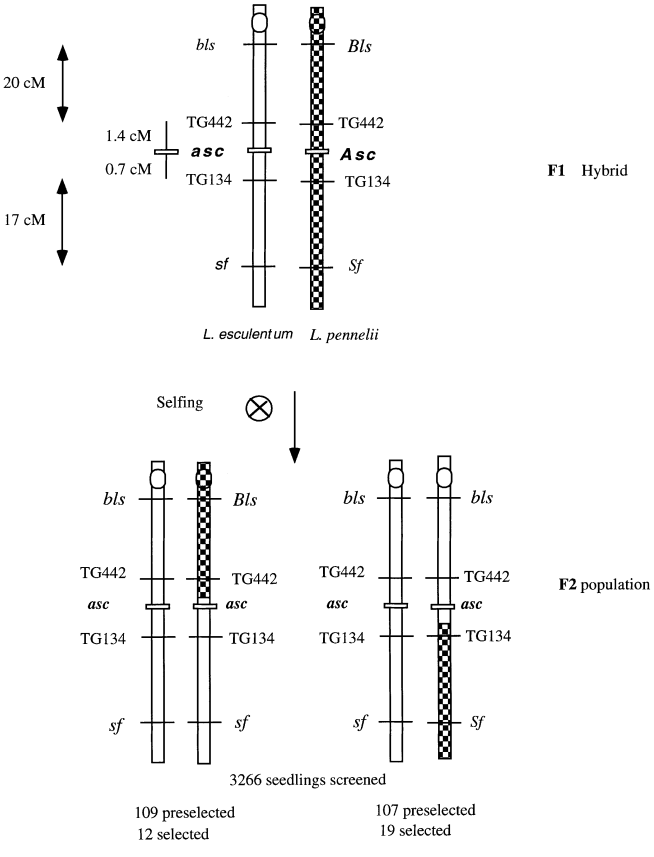


Fig. 1 Selection for recombination events in tomato chromosome 3. The F1 hybrid was obtained by crossing the *L. esculentum* T3 tester line with *L. pennellii*. The F1 plant was self-fertilised and 216 plants were preselected: 107 homozygous *bls* that were sensitive to AAL toxins and 109 homozygous *sf* plants sensitive to AAL toxins. Based on Southern analysis, 12 plants were selected that had undergone a recombination event between *asc* and TG 442, and 19 plants were selected that had a recombination event between *asc* and TG 134. Distances in cM are taken from the integrated map of chromosome 3 of tomato (van der Biezen et al. 1994b)

plants had recombination events between TG 442 and *asc* (Fig. 1).

Establishment of a YAC contig

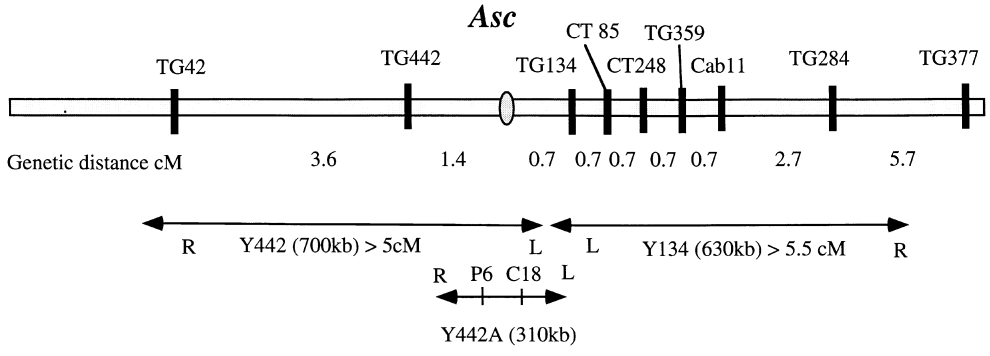
A YAC library containing large inserts (600 kb) was screened in order to have an optimal chance of recovering the *Asc* locus. RFLP markers TG 134 and TG 442, which are 2.1 cM apart, were partly sequenced. This sequence information was then used to identify YACs encompassing the region. YAC 442 (700 kb) and YAC 134 (630 kb) were isolated.

End probes from both YACs were isolated by plasmid rescue (left end) or by a PCR-based method (right ends). The Y442L left-end probe was mapped using the 31 plants that had a recombination event around the *Asc* locus. Because the Y442L end probe mapped on the TG 134 side it was concluded that Y442 spans the *Asc* locus (Fig. 2). The Y134L end probe contained repetitive sequences and could not be mapped on the genetic map. Cross-hybridisation experiments using Y442L with YAC 134 and Y134L end probes with YAC 442 showed that the two YACs did not overlap. The two right-end probes Y134R and Y442R were shown to be repetitive and thus could not be mapped (Fig. 2).

Southern analysis revealed that YAC 442 contained TG 42 in addition to TG 442. YAC 442 thus contains *Asc* and therefore covers a minimum of 5.0 cM (Fig. 2). YAC 134 includes CT 85, CT 248, TG 359, Cab 11, TG 284, thus covering a region of a minimum of 5.5 cM. In order to focus on smaller fragments, a second YAC library was screened containing smaller inserts (on average 250 kb). From that library, an additional YAC clone, YAC 442A (310 kb) was obtained using primers designed from the Y442L end DNA sequence. End probes from Y442A, Y442AL and Y442AR were isolated.Y442AR could be mapped in the recombinant population (Fig. 2). Y442AL did not show polymorphism between *L. esculentum and L. pennellii* and thus could not be mapped. However, that Y134L end probe hybridises with YAC 442A and that Y442AL end probe hybridises with YAC 134. It was therefore concluded that YAC 442A overlaps with both Y442 and Y134, completing the YAC contig covering the *Asc* locus. The total YAC contig is thus equivalent to at least 11.2 cM (5 cM + 5.5 cM + 0.7 cM; Fig. 2).

Y442A was sub-cloned in order to generate internal probes. Seven probes were selected because they were present as single-copy DNA in Y442A. The inserts from these seven clones (C2, C18, P5, P6, P7, Y10 and Y35) were used as probes for Southern analysis of *L. pennellii* and *L. esculentum* DNA. Probes that revealed a RFLP between the parental plants were positioned using the F2 recombinant population. Probes C2, C18, P6, P7, Y10, Y35 could be positioned on the genetic map (Fig. 2). P7 turned out to be identical to P6. The P5 probe contained sequences that are repetitive in plant DNA and could therefore not be mapped on the genetic map. P6 was shown to lie closer to *Asc* than the sites of recombination in 11 recombinants out of the 12. On the other side

Fig. 2 YAC contig containing three YACs and spanning 1380 kb (700 kb for Y442, 630 kb for Y134 and a 50-kb gap in between; see Fig. 3). YAC 442 contains the RFLP markers TG 42 and TG 442. YAC 134 contains TG 134, CT 85, CT 248, TG 359, Cab 11, TG 284 but not TG 377. The total contig covers at least 11.2 cM. The restriction enzymes which reveal RFLPs between *L. esculentum* and *L. pennelli* are indicated *under* the RFLP markers. Distances in cM are taken from the integrated map of chromosome 3 of tomato (van der Biezen et al. 1994b)



number of recombinants	TG442 side	12	4	1	0	0	0
	TG134 side	0	0	0	3	5	19
Corresponding RFLP marker		TG442	442AR Y35	P6 P7 C2	C18 Y10	442L	TG134
RFLP *L. Esculentum/ L. Pennelii*		EcoRV	EcoRI BclI EcoRV	EcoRV	EcorV HinfI	EcoRV BclI	EcoRV

of *Asc*, C18 was shown not to have been affected in 16 out of 19 recombinants. These data indicate a tight genetic linkage between these two probes, P6 and C18, and the *Asc* locus.

Physical map of YAC 442A

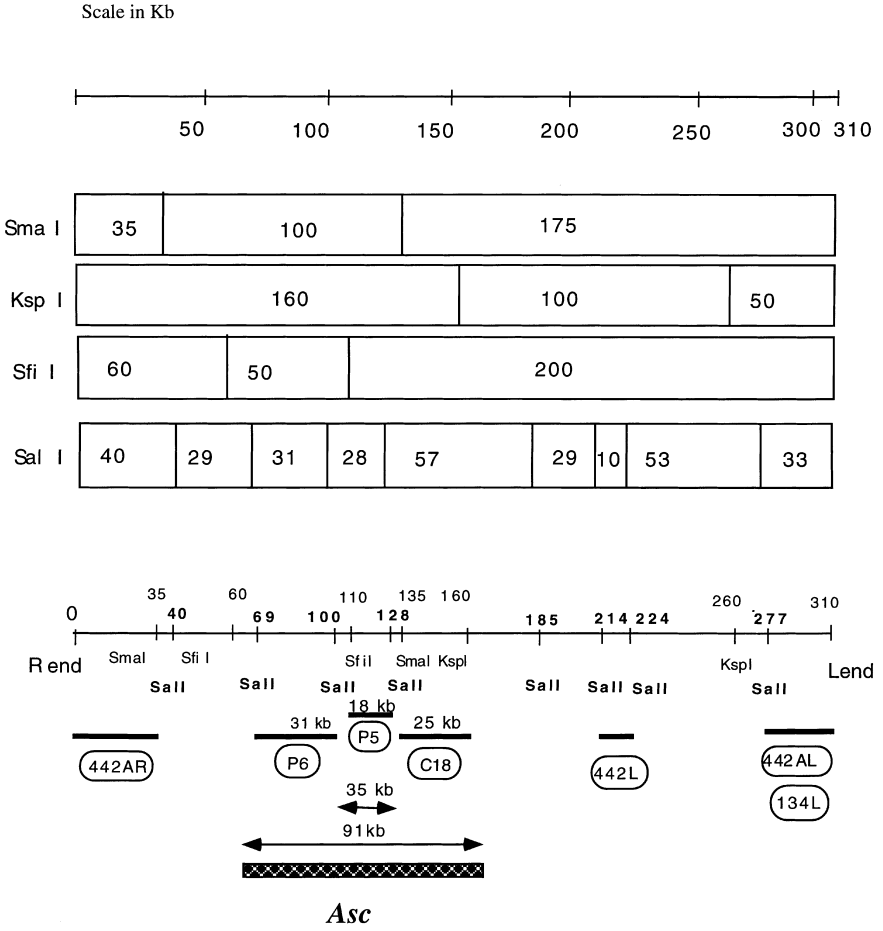
A physical map of YAC 442A was constructed in order to define the physical structure of the region around *Asc* more precisely (Fig. 3).

DNA from YAC 442A was digested using the rare-cutter enzymes *Sfi*I, *Ksp*I, *Sma*I, and *Sal*I to generate large fragments that could be separated by PFGE. Complete and partial digestions were performed. The end probes Y442AR, Y442AL, Y442L, Y134L and the internal probes P6, P5 and C18 were used for Southern analysis of fragments blotted from the gels.

Although it was repetitive in plant DNA, the P5 probe could be physically mapped on YAC 442A. It was positioned on a 18-kb *Sfi*I-*Sal*I fragment between P6 and C18. P6 and C18 hybridise to a common 160-kb *Ksp*I but to different *Sfi*I fragments (50 kb for P6, 200 kb for C18), to different *Sma*I fragments (100 kb for P6 and 175 kb for C18) and different *Sal*I fragments (31 kb for P6 and 57 kb for C18). P6 was thus placed on a 31-kb *Sal*I fragment, and C18 on a 25-kb *Sma*I-*Ksp*I fragment (Fig. 3). Based on the P6 and C18 hybridisation patterns on PFGE Southern blots, the *Asc* locus was delimited to at most a 91-kb *Sal*I-*Ksp*I fragment and a minimum 35-kb *Sal*I- *Sma*I fragment. Thus, four recombination events must have taken place within a region equivalent to a minimum of 35 kb (9 kb per recombinant) and a maximum of 91 kb (23 kb per recombinant).

Positioning of Y442L and Y134L end probes indicated that Y442A covers the 53-kb gap between Y442 and Y134 (Fig. 3). The total contig including the *Asc* locus is thus 1383 kb long. As shown earlier, the genetic distance spanned by the contig is more than 11.2 cM. The average ratio in this region of chromosome three of tomato is therefore less than 1383 kb/11.2 cM = 123 kb/cM.

Fig. 3 Physical map of Y442A was constructed using the rare-cutter restriction enzymes *Sma*I, *Ksp*I, *Sfi*I, and *Sal*I. The P6 and C18 probes which are genetically the closest RFLP probes to the *Asc* locus, map to a common 100-kb *Sma*I fragment and a common 160-kb *Ksp*I fragment. P6 hybridises to a 50-kb *Sfi*I fragment and to a 31-kb *Sal*I fragment. C18 hybridises to a 200-kb *Sfi*I and a 57-kb *Sal*I fragment. Consequently the *Asc* locus must lie within a maximum region of 91 kb and a minimum of 28 kb. The Y442 left end probe maps to a 10-kb *Sal*I fragment, 53 kb away from the Y134 left end probe. Y442 and Y134 are separated at least by a gap of 53 kb



Discussion

Generating plant recombinants

An F2 population of tomato plants that had undergone recombination events around the *Asc* locus was obtained from a cross between *L. pennellii* and the T3 tester line of *L. esculentum*. Morphological markers flanking the locus of interest can be used to preselect for crossover events within the marker interval, thereby reducing the number of plants that must be subjected to molecular analysis (Koornneef et al. 1993; Van Wordragen et al. 1994; Dixon et al. 1995; Liharska et al. 1996).

The use of the available phenotypic markers *sf* and *bls* on both sides of the *Asc* locus allowed us to prescreen for plants having recombination events in the vicinity of *Asc*. This pre-screening reduced the number of plants that had to be screened at the DNA level by a factor of 15.

Out of 216 plants, 31 plants were selected for recombination events between the RFLP markers TG 134 and TG 442, which are 2.1 cM apart (van der Biezen et al. 1994b) giving an average of one recombinant per 0.07 cM in the *Asc* region.

The *Asc* locus, a hot spot for recombination?

A YAC contig was established in order to further characterise the physical structure of the region around the *Asc* locus. YACs were positioned by isolating end probes and using the 31 recombinants and the RFLP markers surrounding the *Asc* locus. For this we used different libraries in order to zoom in from the large fragments in a tomato YAC library with an average insert size of 700 kb to one with 250-kb inserts on average. The total YAC contig spans 1383 kb and is equivalent to at least 11.2 cM. The region around the *Asc* locus is thus a recombinogenic region: 1 cM represents 123 kb – six times lower than the 750 kb/cM average (Tanksley et al. 1992).

Recombination frequencies are not homogeneously distributed over the genome. Hot spots for recombination have been observed in tomato (Gorman et al. 1996). However, due to suppression of recombination in centromeric regions, physical distances can be large for a given genetic distance. Values ranging from 4–16 Mb/cM have been reported for the region of the *Tm2a* locus (chromosome 9; Ganal et al. 1989), which is located close to the centromere. The *I2* disease resistance gene, positioned far from the centromere on chromosome 11 (Tanksley et al. 1992) gives a value of 125 kb/cM, (Ori et al. 1997). We have found a similar ratio for the *Asc* locus (123 kb/cM), indicating that, like *I2*, it is located in a highly recombinogenic area, and confirming that it maps far from the centromere (van der Biezen et al. 1994b).

The physical map of YAC 442A

The 310-kb YAC containing *Asc* narrows down the physical region that must be searched for the *Asc* locus. Two internal probes P6 and C18 from YAC 442A were positioned on opposite sides of *Asc*. On the C18 side still three recombinants remain whereas on the P6 side only one was left. This shows that P6 is genetically closer to *Asc* than C18. Because recombination events are not equally distributed, this does not mean that the *Asc* locus is physically closer to P6 than to C18. The establishment of the long-range physical map of YAC442A allowed us to position the *Asc* locus in a minimum region of 28 kb and a maximum of 91 kb (Fig. 3).

Variation in recombination frequencies around the *Asc* locus

31 recombinants were selected within a 2.1-cM stretch, which, in this region would be equivalent to 2.1×123 kb $= 258.3$ kb**.** This yields an average of one recombination event every 8.2 kb.

YAC 442A is 310 kb long and contains the crossover points of 13 recombinants, which is equivalent to one recombinant every 24 kb. YAC442A thus contains, on average, three times fewer recombination points per unit physical distance compared to the whole area covered by the contig (1380 kb). This confirms that recombination events do not occur at regular intervals even within a small area. This could be related the number of genes present on the DNA fragment. It has been shown in the *a1-sh2* interval in maize (Civardi et al. 1994) that meiotic recombination events occur at a higher rate where genes are present, confirming the hypothesis that recombination occurs at higher rates in coding than in non-coding sequences. Our observation may indicate that the 310-kb region covered by YAC 442A contains fewer genes per unit distance than does the 1383-kb contig as a whole.

Identifying the *Asc* gene(s)

The region delimited by the P6 and C18 probes is now being narrowed down to smaller fragments (15 kb on average), using lambda clones sub-cloned from Y442A.

Chromosome walking with lambda clones derived from YAC 442A and from tomato genomic libraries is now in progress, starting from both P6 and C18. Identification of lambda clones that overlap the region containing the *Asc* locus will provide candidates for complementation tests. Complementation tests will be essential for demonstrating that the *Asc* gene is active in conferring resistance.

Asc behaves as semi-dominant locus when tested on the basis of sensitivity to the AAL toxins (van der Biezen et al. 1994a). We therefore intend to test whether clones derived from a resistant genome library can complement tomato plants for sensitivity to AAL

toxins or vice versa. As in the case of other plant disease resistance genes cloned (reviewed by Bent 1996) genomic DNA and cDNA sequence analysis might provide information about the putative disease resistance gene function.

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