Theor Appl Genet (1999) 99:1068–1074

A.W. van Heusden · M. Koornneef
R.E. Voorrips · W. Brüggemann · G. Pet
R. Vrielink-van Ginkel · X.Chen · P. Lindhout

Three QTLs from *Lycopersicon peruvianum* confer a high level of resistance to *Clavibacter michiganensis* ssp. *michiganensis*

Received: 26 February 1999 / Accepted: 12 March 1999

Abstract *Lycopersicon peruvianum* LA2157 originates from 1650 m above sea level and harbours several beneficial traits for cultivated tomatoes such as cold tolerance, nematode resistance and resistance to bacterial canker (*Clavibacter michiganensis* ssp. *michiganensis*). In order to identify quantitative trait loci (QTLs) for bacterial canker resistance, a QTL mapping approach was carried out in an F_2 population derived from the interspecific F_1 between *Lycopersicon esculentum* cv Solentos and *L. peruvianum* LA2157. Three QTLs for resistance mapped to chromosomes 5, 7 and 9 respectively. The resistance loci were additive and co-dominant with the QTL on chromosome 7 explaining the largest part of the variation for resistance in the F_2 population. The combination of this QTL with either of the other two QTLs conferred a resistance similar to the level in the resistant parent *L. peruvianum.* Some RFLP markers flanking this QTL on chromosome 7 were converted into SCAR markers allowing efficient marker-assisted selection of plants with high resistance to bacterial canker.

Key words Bacterial canker · *L. peruvianum* · Restriction fragment length polymorphism (RFLP) · Sequence characterised amplified region (SCAR) · Quantitative trait locus (QTL)

Communicated by F. Salamini

This publication is dedicated to Gerard Pet (†)

A.W. van Heusden (✉) · R.E. Voorrips · G. Pet
R. Vrielink-van Ginkel · P. Lindhout
Department of Vegetable and Fruit Crops,
DLO-Centre for Plant Breeding and Reproduction Research
(CPRO-DLO), PO Box 16 NL-6700AA Wageningen,
The Netherlands
e-mail: A.W.vanheusden@cpro.dlo.nl
Fax: +31 317 418094

M. Koornneef
Laboratory of Genetics, Wageningen Agricultural University,
Dreijenlaan 2 NL-6703 HA Wageningen, The Netherlands

W. Brüggemann
Department of Botany, J.W. Goethe-University,
POB 11 19 32, D-60054 Frankfurt/Main, Germany

X. Chen
Institute of Vegetables and Flowers,
Chinese Academy of Agricultural Sciences,
100081, Beijing, China

Present addresses:
P. Lindhout, Laboratory of Plant Breeding,
Graduate School for Experimental Plant Sciences,
Wageningen Agricultural University, PO Box 386,
NL-6700AJ Wageningen, The Netherlands

X. Chen, Max-Planck-Institut für Zuchtungsforschung,
Carl-von-Linne-Weg 10, D-50829 Cologne, Germany

Introduction

Bacterial canker, caused by *Clavibacter michiganensis* ssp. *michiganensis* (Smith) Davies et al., is a wilt disease in tomato (*Lycopersicon esculentum* Mill.) that occurs mainly in subtropical and tropical areas and can cause serious yield losses (Strider 1969; Emmatty and John 1973; Chang et al. 1992). This disease has also been reported in moderate climates. *C. michiganensis* is transmitted through seed and can survive in the soil for at least 2 years (Bryan 1930; Thyr 1968). Resistance has also been found in several wild relatives of tomato (Thyr 1968, 1969, Laterrot et al. 1978; Stamova et al. 1985) . Though the resistance levels in these sources were quite high, the presumed polygenic inheritance and unreliable disease tests have limited the introgression of these resistances into commercial cultivars. In preliminary experiments (Lindhout, unpublished results) five accessions of *Lycopersicon peruvianum* were identified as sources with high resistance levels. The resistance was characterised by a lack of wilt symptoms and a limited growth of bacteria. The bacteria could still spread and multiply in resistant plants but seeds of heavily inoculated plants remained completely free of bacteria.

Genetic analysis of this resistance trait was hampered by the incompatibility of the *L. peruvianum* accessions and the cultivated tomato (Hogenboom 1973) . However, as in *L. peruvianum* a large variation for resistance to *C.*

michiganensis has been observed, a genetic analysis was executed with intraspecific BC_1 populations within *L. peruvianum* (Van Ooijen et al. 1994; Sandbrink et al. 1995) . By using a QTL mapping approach, five possible QTLs were identified for resistance to *C. michiganensis* ssp. *michiganensis*. The resistant accessions of *L. peruvianum* are morphologically very distinct from *L. esculentum* and the structure, size and density of vascular bundles and other internal factors might influence the pathogenesis of *C. michiganensis* ssp. *michiganensis*. In order to identify resistance genes in *L. peruvianum* that are also effective in a *L. esculentum* background, a genetic analysis should be carried out with a segregating population derived from an interspecific cross. A segregating F_2 population obtained from an interspecific F_1 between the susceptible *L. esculentum* cv Solentos and the resistant *L. peruvianum* LA2157 was used to investigate the inheritance of resistance to *C. michiganensis* with the use of restriction fragment length polymorphisms (RFLPs).

Materials and methods

Plant material

Embryo rescue was used to obtain an F_1 progeny of the interspecific cross between the susceptible *L. esculentum* cv Solentos (De Ruiter Seeds) x *L. peruvianum* LA2157 (Brüggemann et al. 1996). The single fertile F_1 plant which was obtained yielded hundreds of selfed seeds, allowing us to raise an F_2 population of 324 plants.

Disease test

The bacterial strain *C. michiganensis* Cm 542, known to be highly aggressive (Van den Bulk et al. 1991), was used to inoculate young F_2 plants at the sixth leaf stage by injecting the stem with 10 µl of a bacterial suspension (10^7 /ml) and simultaneously dissecting the top with a scalpel dipped in the same bacterial suspension (Sandbrink et al. 1995) . Wilting symptoms of the infected plants were recorded at 20, 22, 27, 32, 39, 46, 54, 61, 68, 75, 82, 90, 96 and 104 days after inoculation according to the following disease scale: 0 = no symptoms; 1 = one leaflet wilted; 2 = two leaflets wilted; 3 = some leaves partially wilted; 4 = most leaves wilted.

DNA isolation and RFLP analysis

Before inoculation cuttings were made of all plants. These cuttings were used for collecting leaf material. Genomic DNA was isolated from leaf material as previously described (Van der Beek et al. 1992) . Five micograms of DNA were digested with restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Hae*III, and *Xba*I) fractionated on a 0.8% agarose gel, blotted onto Genescreen$^+$ (Dupont, Nemours) membranes and hybridised with radioactively labelled probes (see Table 1). Washing and exposure procedures were performed as previously described (Van Ooijen et al. 1994).

RAPD analysis

Bulked genomic DNA was used as a template for PCR amplification. A single 10-mer oligonucleotide primer (Operon Technologies) was employed in each PCR amplification. The reaction mixture (25 µl) consisted of 1.5 µl of DNA (30 ng), 2.5 µl of Super*Taq* buffer (10x), 0.25 µl of mixed dNTP (10 mM), 0.25 µl of primer (100 ng/µl), 0.2 µl of Super*Taq* enzyme (5 U/µl), completed with MQ water. The reaction solution was overlaid with one drop of mineral oil. Amplification was performed in an Omnigene Thermocycler for 45 cycles of 1 min. at 92° C, 2 min. at 35° C, and 2 min. at 72° C followed by a final extension step of 10 min at 72° C. Amplification products were separated by electrophoresis in a 1.5% agarose gel with 1 × TBE buffer (0.089 M Tris; 0.089 M boric acid; 0.002 M EDTA, pH 8) at 90 V and subsequently visualised with ethidium bromide and UV light.

SCAR-markers

In order to convert RFLP markers into SCAR (sequence characterised amplified regions) markers, the RFLP-probes (Tanksley et al. 1992) were sequenced in both directions using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer). Prior to sequencing, the DNA was purified using the Geneclean II kit (Bio 101 Inc.) followed by a phenol/chloroform extraction. Primers were chosen using PRIMER version 0.5 software (Whitehead Institute for Biomedical Research). With these primers DNA from *L. esculentum* cv Solentos and from *L. peruvianum* LA2157 was amplified. These equally sized amplification products were purified and sequenced. New primers were designed to exploit the differences between the two parental sequences. PCR reactions were carried out in a total volume of 50 µl containing 60 ng of genomic DNA, 100 ng of each primer in 10 mM TRIS-HCl, pH 9.0, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatine, 0.1% Triton X-100, 0.2 mM dNTPs and 0.5 units of *Taq* DNA polymerase (Supertaq, SphaeroQ, the Netherlands). After a denaturation step of 2 min and 30 s at 92°C, a further 30–35 cycles were carried out under the following conditions: 1 min denaturation at 92°C, 1 min annealing (for Tm see Table 5) and a 2-min extension at 72°C, with a 5-min extension at 72°C for the final cycle.

Linkage analysis

Marker data were analysed using JoinMap$^{2.0}$ (Stam and Van Ooijen 1995) . Grouping of the markers was initially done with a minimum LOD-score of 3.0. Unlinked markers were assigned to linkage groups according to two criteria: their highest LOD values for linkage with other markers and the resulting linkage groups corresponding with known chromosome maps (Tanksley et al. 1992) . After grouping markers no LOD threshold was set for mapping markers within one group. The recombination threshold was set at 0.49 and the Kosambi mapping function was used to convert recombination data to map distances. For data analysis the non-parametric rank sum test of Kruskal-Wallis was used (see for example Lehmann 1975) . This test measures the association between marker genotype and disease index and is therefore not affected by distorted segregation ratios (Van Ooijen et al. 1994) .

Results

Genetic linkage map

A genetic linkage map was generated from segregation data of an F_2 population derived from the cross *L. esculentum* cv Solentos x *L. peruvianum* LA2157 using 51 RFLP markers and 324 plants. This map will be referred to as the esc x per map. The markers were chosen to obtain an even distribution over the genome (Table 1). Only one RFLP-marker was placed in another linkage group as compared with the map of *L. esculentum* x *L. pennellii*, hereafter referred to as the esc x pen map (Tanksley et al. 1992) . This marker, TG133, was strongly linked to loci of chromosome 8 and not to those of

Table 1 Genetic linkage map of 51 RFLP markers determined in a segregating population of 324 plants from the cross *L. esculentum* x *L. peruvianum*. Map positions were calculated with JoinMap with the Kosambi mapping function. The chromosome numbers are indicated above the linkage groups, the distances from the top marker are in cM

1		2		3		4		5		6	
CT233	0	TG145	0	TG40	0	TG49	0	TG623	0	TG178	0
TG24	29	TG34	39	TG130	25	TG339	19	TG363	48	TG118	7
TG184	30	TG154	53			TG287	38	TG23	58	TG153	9
TG51	43					TG62	54	TG185	82	TG162	36
TG59	96									TG215	75
TG19	114										
TG281	153										

7		8		9		10		11		12	
TG438	0	TG176	0	TG254	0	TG230	0	CD17	0	TG180	0
TG149	49	TG133	24	TG223	23	TG148	20	TG286	38	TG565	61
TG166	60	TG41	29	TG35	58	TG103	39	CT65	54		
TG61	68	TG282	45	TG144	64	CT20	71				
CT52	87	TG160	77								
TG418	98										
TG342	105										

chromosome 2. The total map distances between the markers were nearly identical in the esc x per map (877 cM) compared to the esc x pen map (879 cM). However, three markers (TG342, TG418 and CT52) at the distal end of the long arm of chromosome 7, that co-segregated completely in the esc x pen population could be separated in the esc x per map, spanning a region of 18 cM. In comparison with the esc x pen map there no obvious condensed regions on the esc x per map were identified.

Distorted segregation

Twenty eight out of the 51 markers showed segregation patterns different from the expected 1:2:1 ratio at $P<0.05$. Nineteen of these markers were skewed in the direction of a surplus of homozygous *L. peruvianum* (PP) loci and five in the direction of homozygous *L. esculentum* (EE) loci, whereas the remaining four were skewed because of an unexpectedly high or low number of heterozygotes. Distorted segregation was not randomly distributed over the chromosomes: significant deviations from the expected 1:2:1 due to a surplus of *L. peruvianum* alleles were observed for markers on chromosomes 2, 4, 7 and 8, while markers on chromosome 6 showed a distorted segregation due to more *L. esculentum* alleles (TG162, TG286, TG178 and TG118) or to less heterozygotes (EP) than expected (TG215). The average percentage heterozygous per plant in the F_2 population was 50 with a standard error of 11.1 and a range from 12 to 81%. The average percentage EE was 20±9.0% (range 2–46%) and of PP 30±10.8% (range 4–64%). The ratio of parental alleles, E:P, in the F_2 population was 46:54.

Identification of quantitative trait loci (QTLs) for resistance to *C. michiganensis* ssp. *michiganensis* (*Cm*)

An F_2 population of 324 plants was raised, inoculated and evaluated for disease symptoms together with 24 plants of each parent. All 24 plants of the susceptible *L. esculentum* parent were scored in class 4 (most leaves wilted). More variation was observed in the 24 *L. peruvianum* plants: 1 plant in class 3, 11 plants in class 2, 5 plants in class 1 and 7 plants in class 0. For all 324 plants of the F_2 population and the parent plants the disease symptoms were scored on 14 different dates. The highest score ever recorded for each individual F_2 plant on one of these dates was used for the initial linkage analysis. The level of disease symptoms and the segregation of 51 RFLP markers were analysed by using the Kruskal-Wallis test statistic and three QTLs were identified on chromosomes 5, 7 and 9 respectively (Fig. 1, Table 2). Four of the markers on chromosome 7 (TG61, CT52, TG418 and TG342) gave a significant effect but the highest score was found with TG61. All resistance alleles originated from the resistant parent *L. peruvianum.* None of the alleles was dominant and all QTL effects were additive (Table 3).

The combined presence of two out of three QTLs for resistance further reduces the level of disease symptoms (Table 3). Combining the QTLs on chromosomes 7 and 9 reduced the average wilting after infection almost to the level of the resistant parent. The average increase in wilt symptoms over time for both parents, and the F_2 individuals classified according to the genotype of TG61 on chromosome 7, showed that the effects of the resistant alleles were rather stable (Fig. 2 a). The effects of two other QTLs were similar (data not shown). Figure 2 b shows the increase in the average disease level of genotypes selected for two QTLs (on chromosomes 7 and 9). Similar disease curves, although with slightly smaller differences, were observed for all genotypes with two out of the three QTLs (data not shown). There was a strong negative correlation between the number of *L. pe-*

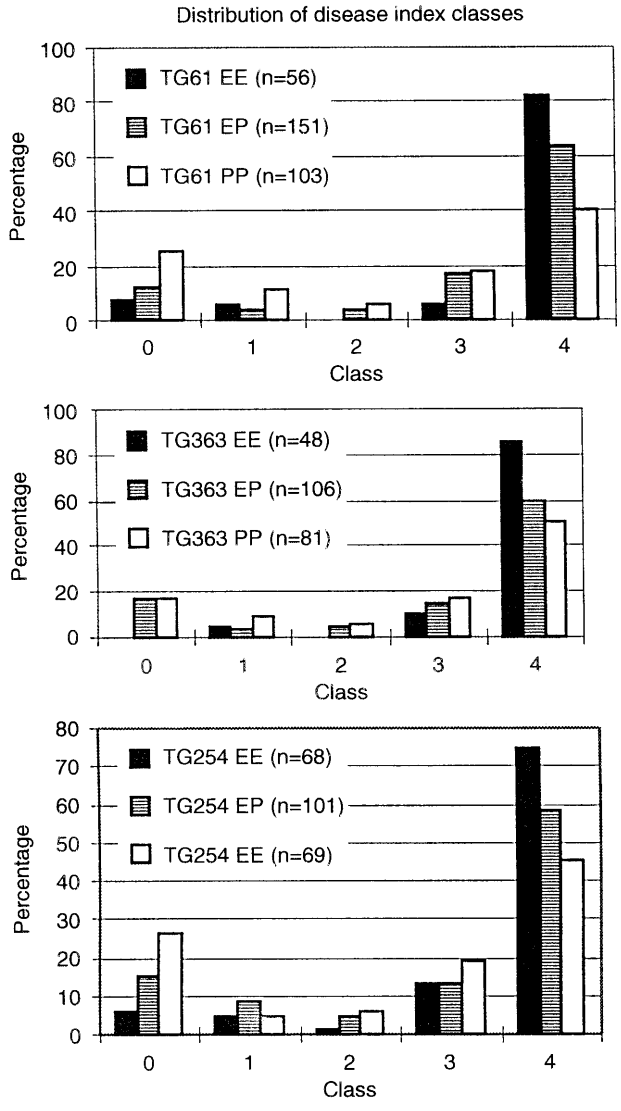

Fig. 1 Frequency distribution of genotypes in the F_2 of the *L. esculentum* x *L. peruvianum* cross based on the disease index classes of the three loci with a significant effect on resistance to *Cm*. TG61 (chromosome 7), TG363 (chromosome 4), and TG254 (chromosome 9); EE = homozygous *L. esculentum*, EP = heterozygous, PP =homozygous *L. peruvianum*

Table 2 Chromosomes (A) and markers (B) associated with disease resistance level. Number of plants (C) and significance levels (D)

A	B	C	D
5	TG363	235	0.0005
7	TG149	311	0.01
7	TG166	311	0.001
7	TG61	311	0.0001
7	TG342	300	0.0001
9	TG254	307	0.005

ruvianum resistance alleles and the average wilt symptoms (Table 4): all 16 plants with only one resistance allele showed wilting of all leaves (a maximum disease score of 4.0), while all 45 plants with five or six resistance alleles had only one or two wilted leaflets (a disease score equal to or lower than 2.0).

Table 3 For two QTLs the F_2 population has been divided into nine possible classes, the average disease rating in every class has been calculated and the number of plants is given between brackets.EE(n): *L. esculentum* homozygotes; EP(n) heterozygotes and PP(n) *L. peruvianum* homozygotes. The two parents homozygous are for the three loci

Marker			
TG61	EE(n)	EP(n)	PP(n)
TG363			
EE(n)	4.0(5)	3.8(34)	3.6(8)
EP(n)	3.8(16)	3.0(49)	2.6(40)
PP(n)	2.6(17)	3.1(34)	2.4(29)
TG61	EE(n)	EP(n)	PP(n)
TG254			
EE(n)	3.6(11)	3.2(27)	3.8(17)
EP(n)	3.5(40)	3.2(79)	2.4(27)
PP(n)	3.5(15)	2.3(62)	1.7(25)
TG363	EE(n)	EP(n)	PP(n)
TG254			
EE(n)	3.9(8)	3.8(30)	3.4(90)
EP(n)	3.3(27)	3.1(53)	2.1(23)
PP(n)	3.6(21)	2.5(42)	2.3(18)
L. esculentum	4.0(24)		
L. peruvianum			1.3(24)

Additional markers in the TG61–TG342 interval on chromosome 7

The most prominent QTL was located in the TG61–TG342 region on chromosome 7 that spans 14 cM in the esc x pen map, with only one known marker (CD62) in this interval. Marker TG342 was part of a cluster of 11 co-segregating markers. To identify more markers in this region, random amplified polymorphic DNAs (RAPDs) were used in combination with bulked segregant analyses (BSA; Michelmore et al. 1991).One bulk consisted of ten plants with a disease index of 0 and homozygous for *L. peruvianum*-alleles TG61 and TG342; the other bulk consisted of ten plants with a disease index of 4 and homozygous for *L. esculentum* alleles at these loci. A total of 286 10-mer primers (Operon Technologies, Alameda, Calif.) were used revealing more than 1000 RAPDs, but no additional markers in the target interval were found. With two primers (OPF07; 5'-CCGATATCCC-3', OPU13; 5'-GGCTGGTTCC-3') a bulk-specific amplification product was identified (OPF07–450 and OPU13–900). To determine genetic distances, segregation of these markers was evaluated for a selection of 50–70 F_2 plants. No recombinants were found between TG61 and OPF07–450 while the other marker was located proximal to TG61, i.e. outside the interval of interest.

Bonnema et al. (1997) showed a large difference in recombination frequency in a region on chromosome 1 between the esc x per and the esc x pen maps. In a similar way we tried to unravel the TG342 cluster on chromosome 7 of the esc x pen map by analysing the genetic distances between the markers of this cluster present on the esc x per map. Three RFLP-probes from the TG342 cluster (TG418, CT52 and TG113) were selected and

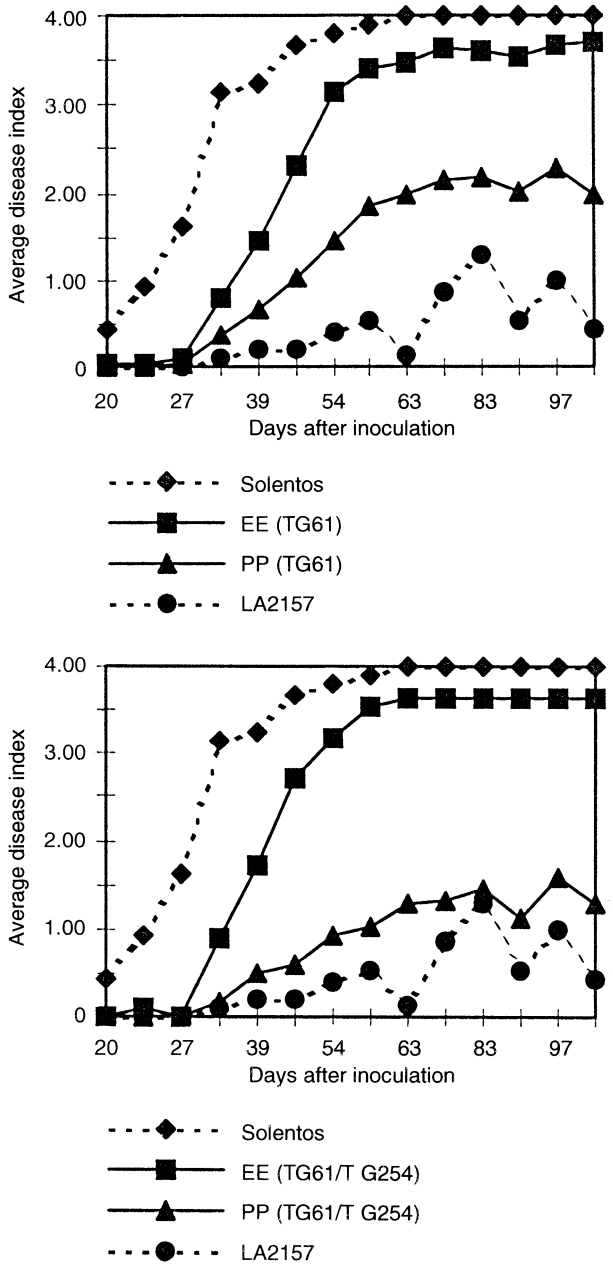

Fig. 2 Average disease ratings of genotypes in the F_2 of the *L. esculentum* x *L. peruvianum* cross as measured on 13 different dates after inoculation. *The upper panel* shows parental values and the three different groups of the F_2 based on the TG 61 genotype(EE = homozygous *esculentum*, EP = heterozygous and PP = homozygous *peruvianum*). The *lower panel* shows the progress of the disease in time of groups of F_2 plants classified according the combination of two QTLs (TG254 and TG61). The average disease rating is calculated for those F_2 plants that were homozygous for both loci

converted into SCAR-markers. After sequencing, two primers were designed for each probe aimed at amplifying fragments in the range of 400–500 bp (Table 5). With these primer combinations, equally sized products of *L. esculentum* cv Solentos and *L. peruvianum* LA2157 were amplified and sequenced. Many differences were found between the "TG418" sequences of Solentos and

Table 4. Average disease scores of plants with 1–6 of the *L. peruvianum* alleles (P) at the three QTLs for resistance to *C. michiganensis*

Number of P-alleles	Average disease rating(n)
0	–(0)
1	4.0(16)
2	3.5(50)
3	3.4(75)
4	2.9(48)
5	2.0(38)
6	1.6(7)

LA2157: in Solentos a 17-bp deletion, a 50-bp insertion and 14 single base-pair changes were identified. Subsequently, two primer sets were designed aimed at specifically exploiting these differences; one for a *L. esculentum*-specific SCAR (107 bp) and the other for a *L. peruvianum* specific SCAR (159 bp) (Table 5). There were substantially less differences between the Solentos and *L. peruvianum* fragments generated with the "CT52" and the "TG113" primers. Comparison of the "CT52" sequences gave 99.3% homology but the three base-pair differences were sufficient to design specific primers. No specific primers could be successfully designed to explore the two base-pair differences in the "TG113" sequences (99.5% homology). The specific primer sets of TG418 and CT52 were used to map these SCARs (Fig. 3). These two SCAR markers mapped at 7 cM and 18 cM proximal to the TG342 locus in the esc x per map, while they were at the same locus in the esc x pen map (Fig. 3). In this region of 37 cM on chromosome 7 it was not possible to find the marker with the closest linkage to the resistance gene, because no obvious differences in resistance levels were found. The SCAR-markers of TG342 and TG61 are being used to develop nearly-isogenic Lines (NILs) for this region of chromosome 7 of *L. peruvianum*.

Discussion

The present research aimed at identifying QTLs conferring resistance to *C. michiganensis* ssp. *michiganensis*. A genetic map was generated based on the interspecific F_2 of *L. esculentum* x *L. peruvianum*. The 877-cM map covers a large part of the total genome while the map distances were similar to those of the known esc x pen map (Tanksley et al. 1992) . Both in this and in earlier studies (Bonnema et al. 1997) markers were identified with no recombination in the esc x pen map that spanned more than 10 cM in the esc x per map. Suppression of recombination might be caused by an inversion in the corresponding chromosomal regions in *L. pennellii*. The fact that the overall map lengths are similar, indicates that there might be genomic regions in the esc x per map with reduced recombination compared to that in the esc x pen map. To find these regions it is necessary to study the segregation of many more than 51 markers in the esc x per F_2 population and compare them with the same markers in an esc x pen F_2 population of the same size.

Table 5. A. Three primer combinations based on the sequence of the original three RFLP-probes. B. Allele-specific primer combinations for the two RFLP markers TG418 and CT52 designed to exploit the differences in the sequences of *L.esculentum* and *L. peruvianum*

A RFLP probe	Primers sequences	TM (°C)	PCR product (base-pairs)
TG418	F:5′-atggctccaatatgcctgac-3′ R:5-cactccacccctttcttccta-3′	60	500–550
CT52	F:5′-atcgaattcttgtctgaggagg-3′ R:5′-tccctcggaaaatataccgt-3′	55	440
TG113	F:5′-ttcaagaccaaacacccttttt-3′ R:5′-ttagtccaatgggctaagtgt-3′	55	393

B SCAR marker			PCR product) (base-pairs)
TG418Esc	F:5′-caggtgttgtaactgagagcg-3′ R:5′-cactccacccctttcttccta-3′	55	107
TG418Per	F:5′-gagctcattaataatcatacaga-3′ R:5′-cactccacccctttcttccta-3′	55	159
CT52Esc	F:5′-cccatctttcatgccttcg-3′ R:5′-tccctcggaaaatataccgt-3′	10 cycli 62 25 cycli 55	303
CT52Per	F:5′ttcccatctttcatgccttca-3′ R:5′gccttaattttcagccaaccc-3′	10 cycli 62 25 cycli 55	98

The observed distorted segregation of markers in interspecific populations in *Lycopersicon* towards a surplus of alleles from the wild relative has been reported before (De Vicente et al. 1993; Goldman et al. 1995; Maliepaard et al. 1995; Paran et al. 1995). The skewed segregating markers were not randomly distributed over the genome, but were concentrated on chromosomes 2, 4, 7 and 8, with a distortion in the direction of a surplus of *L. peruvianum* alleles. Remarkably, chromosome 7 showed severe skewed segregation ratios in many interspecific *Lycopersicon* populations. This suggests a reduced fitness of *L. esculentum* alleles on chromosome 7 or a reduced transmission of gametes with such alleles.

With a large population and 51 RFLP markers three QTLs were identified with a substantial influence on resistance to *C. michiganensis* ssp. *michiganensis*. These QTLs were located on chromosomes 5, 7 and 9; a combination of any two of them conferred a high level of resistance. Although the density of the markers on the map does not exclude the possibility that some QTLs are not detected (especially on chromosomes 3 and 12) we believe this is not very likely because of the high degree of differences in resistance level already explained . The effect was largest for the QTL on chromosome 7. In a *L. peruvianum* intraspecific BC_1 population Sandbrink et al. (1995) identified five loci with significant effects on chromosomes 1, 6, 7, 8, and 10 involved in resistance to this pathogen. Several reasons may explain the differences between the results of these two studies. Both the number of analysed plants and the threshold values were higher in the present study, reducing the chance of detecting ghost QTLs. Minor QTLs that were observed in the intraspecific cross may reflect genetic differences between the susceptible *L. peruvianum* accession (LA2172) in the first experiment and the susceptible *L. esculentum* in our experiment; in both studies the same resistant parent was used. For instance, the QTL on chromosome 10 was not detected in our experiment because this QTL originated from the susceptible *L. peruvianum* of the intraspecific population. In addition, bacterial canker is a wilting disease and wilting is dependent on plant physiology and morphology. Conceivably the genes involved in physiology and morphology might also influence resistance. This may explain why there is so little overlap between the results of the intra- and inter-specific mapping studies. The QTL on chromosome 7 close to TG61 had a substantial influence on resistance and there were also indications for a QTL in the same region in the study with the intraspecific population. This QTL might be the most important resistance gene. Because the influence on resistance is almost equally high over the complete fragment of 37 cM, the possibility of more QTLs in this region cannot be excluded, especially because disease resistance genes are known to occur in clusters on some of the chromosomes (Hammond Kosack and Jones 1997) . A bacterial wilt resistance gene (Danesh et al. 1994) is known to be located on chromosome 7 exactly in the same region as our bacterial canker resistance locus. Further molecular and genetic analyses of these QTLs may give insight into a possible similarity or common ancestry.

After identifying the QTL on chromosome 7 we were interested in finding more markers in this region and in the determination of the exact order of these markers. However, bulked segregant analysis and the screening of many 10-mer primers did not result in any additional marker in the gap between TG342 and TG61. On the esc x pen map the distance between the two markers is 14 cM and only one marker is present in this interval where 15 would be expected where markers to be equally dispersed over the genome. This suggests a distorted relation between genetic and physical distance, which might be due to a hotspot of recombination. Since recombination between TG61 and TG342 was higher in the esc x

per cross than in the esc x pen cross (37 cM compared with 14 cM) we expected that the RFLP cluster near TG342 in the esc x pen map might show some recombination in the esc x per population. For this reason we successfully converted two markers from this cluster into SCARs. It is surprising that the two remotely related *Lycopersicon* species, *L esculentum* and *L. peruvianum*, showed more than 99% DNA sequence homology.

It is essential for map-based cloning strategies to find markers linked as closely as possible to a resistance gene and also to minimise linkage drag in a molecular marker-assisted breeding program. Additional research, such as the fine mapping of the resistance loci and the re-confirmation of the resistance in progeny of those F_2 plants with two *L. peruvianum* QTLs in an otherwise purely *L. esculentum* genetic background, is necessary. For this purpose we are developing nearly-isogenic lines (NILs) by backcrossing with tomato and selecting for molecular markers flanking the two regions with the desired resistance loci. The presence of only a few loci in *L. peruvianum* LA2157 that confer resistance to *C. michiganensis* ssp. *michiganensis*, and the identification of the chromosomal regions harbouring these loci, opens the way for a successful and fast introgression of the resistance in tomato with the use of molecular markers and without the need of laborious disease tests.

Acknowledgements Parts of the research were co-sponsored by a group of eight Dutch tomato breeding companies. The authors thank Dr. A.P.M. Den Nijs for critically reading this manuscript and Peter Stové and Ria Jongerius for their support with the RFLP analyses.

References

Bonnema G, Schipper D, Heusden AW van, Zabel P, Lindhout P (1997) Tomato chromosome 1: high-resolution genetic and physical mapping of the short arm in an interspecific *Lycopersicon esculentum* × *L. peruvianum* cross. Mol Gen Genet 253: 455–462

Brüggemann W, Linger P, Wenner A. Koornneef M (1996) Improvement of post-chilling phtosynthesis in tomato by sexual hybridisation with a *Lycopersicon peruvianum* line from elevated altitude. Adv Hort Sci 10: 215–218

Bryan MK (1930) Studies on bacterial canker of tomato. J Agric Res 41: 825–851

Chang RJ, Ries SM, Pataky JK (1992) Reductions in yield of processing tomatoes and incidence of bacterial canker. Plant Dis 76: 805–809

Danesh D, Aarons S, McGill GE, Young ND (1994) Genetic dissection of oligogenic resistance to bacterial wilt in tomato. Mol Plant Microbe Interact 7: 464–471

De Vicente MC, Tanksley SD, De Vicente MC (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 585–596

Emmatty DA, John CA (1973) Comparison of yield loss to bacterial canker of tomato in a resistant and a susceptible variety. Plant Dis Rep 57: 787–788

Goldman IL, Paran I, Zamir D (1995) Quantitative trait locus analysis of a recombinant inbred line population derived from a *Lycopersicon esculentum* × *Lycopersicon cheesmanii* cross. Theor Appl Genet 90: 7–8

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annual Rev Plant Physiol Plant Mol Biol 48: 575–607

Hogenboom NG (1973) A model for incongruity in intimate partner relationships. Euphytica 22: 219–233

Laterrot H, Brand R, Daunay MC (1978) La résistance à *Corynebacterium michiganensis* chez la tomate. Ann Amélor Plant 28: 579–591

Lehmann EL (1975) Nonparametrics. McGraw-Hill, New York

Maliepaard C, Bas N, Heusden Sv, Kos J, Pet G, Verkerk R, Vrielink R, Zabel P, Lindhout P (1995) Mapping of QTLs for glandular trichome densities and *Trialeurodes vaporariorum* (greenhouse whitefly) resistance in an F_2 from *Lycopersicon esculentum* × *Lycopersicon hirsutum* f. *glabratum*. Heredity 75: 425–433

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88: 9828–9832

Paran I, Goldman I, Tanksley SD, Zamir D (1995) Recombinant inbred lines for genetic mapping in tomato. Theor Appl Genet 90: 542–548

Sandbrink JM, Ooijen JWv, Purimahua CC, Vrielink M, Verkerk P, Zabel P, Lindhout P (1995) Localization of genes for bacterial canker resistance in *Lycopersicon peruvianum* using RFLPs. Theor Appl Genet 90: 444–450

Stam P, Van Ooijen JW (1995) JoinMap version 2.0:software for the calculation of genetic linkage maps.

Stamova L, Iordanov M, Yordanov M (1985) New source of resistance to *Corynebacterium michiganense* (E. F. Sm.) H. L. Jens. Rasteniev"dni Nauki 22: 67–70

Strider DL (1969) Bacterial canker of tomato caused by *Corynebacterium michiganense* - - a literature review and bibliography. North Carolina Agric Exp Station, Tech Bull: 1–110

Tanksley SD, Ganal MW, Prince JP, Vicente MCd, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND, De Vicente MC (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141–1160

Thyr BD (1968) Resistance to *Corynebacterium michiganense* measured in six *Lycopersicon* accessions. Phytopathology 61: 972–974

Thyr BD (1969) Additional sources of resistance to bacterial canker of tomato (*Corynebacterium michiganense*). Plant Dis Rep 53: 234–237

Van den Bulk RW, Jansen J, Lindhout WH, Loffler HJM (1991) Screening of tomato somaclones for resistance to bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*). Plant Breed 107: 190–196

Van der Beek JG, Verkerk R, Zabel P, Lindhout P (1992) Mapping strategy for resistance genes in tomato based on RFLPs between cultivars: *Cf9* (resistance to *Cladosporium fulvum*) on chromosome 1. Theor Appl Genet 84: 106–112

Van Ooijen JW, Sandbrink JM, Vrielink M, Verkerk R, Zabel P, Lindhout P (1994) An RFLP linkage map of *Lycopersicon peruvianum*. Theor Appl Genet 89: 1007–1013