L. PERUVIANUM AS A SOURCE FOR RESISTANCE TO *CLAVIBACTER MICHIGANENSIS* SSP. *MICHIGANENSIS* IN TOMATO

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Abstract

Twenty three accessions of *L. peruvianum* were screened for resistance to *Clavibacter michiganensis* ssp. *michiganensis* (bacterial canker). Resistance was detected in five accessions. One of these, *L. peruvianum* LA2157 was crossed with a susceptible accession of *L. peruvianum* and with the cultivated tomato. Evaluation of the segregation of the progenies for disease resistance and of RFLP markers identified several regions that are involved in bacterial canker resistance. Two resistance loci cross explained almost completely the difference in resistance between *L. esculentum* and *L. peruvianum* LA2157 and offers good potentials for breeding tomato for bacterial canker resistance.

Key words: Bacterial Canker, *L. esculentum*, Restriction Fragment Polymorphisms (RFLPs), Quantitative Trait Loci, Marker Assisted Breeding.

1. Introduction

Bacterial canker, caused by *Clavibacter michiganensis* ssp. *michiganensis* (Smith) Davies et al., is a wilt disease in tomato (*Lycopersicon esculentum*, Mill.) that occurs worldwide, mainly in subtropical and tropical areas and can cause serious yield losses (Chang et al., 1992; Emmatty et al., 1973; Strider, 1969). *C. michiganensis* is transmitted through seed and can survive in the soil for at least two years (Bryan, 1930; Thyr, 1968). In several wild relatives of tomato resistance has been characterized (Laterrot et al., 1978; Stamova et al., 1985; Thyr, 1968,1969).

For a successful introgression of resistance to *C. michiganensis* ssp. *michiganensis* from a wild relative into the cultivated tomato several conditions have to be met, such as a source of resistance to the disease, a way to introduce the resistance and a reliable selection method. The cultivated tomato is very susceptible to bacterial canker and only partial resistance has been found in related species. Because *L. peruvianum* is known to have a wide genetic variation and was not yet exhaustively tested for resistance to bacterial canker this wild relative was chosen to screen for resistance. Molecular markers for bacterial canker resistance were also investigated.

2. Description of experiments

2.1 Variation for resistance in *L. peruvianum*

The highly aggressive *C. michiganensis* strain Cm 542 was used for inoculation in all experiments. Bacteria were injected in the stem between the cotyledons and the first true leaf of plants grown to the sixth true leaf stage (Löffler et al., 1989). Simultaneously the top of the plant was cut off above the fourth true leaf with a scalpel dipped in inoculum. Wilt symptoms of the infected plants were scored regularly according to a disease scale: 0 = plant healthy; 1 = one leaflet wilted; 2 = two leaflets wilted; 3 = some leaves partially wilted; 4 = most leaves wilted. Lindhout et al. (in preparation) tested 23 different *L. peruvianum* accessions. Some of unknown origin (CPRO56137, CPRO56138, CPRO56139, CPRO56140, CPRO61280, CPRO63103 and CPRO74457), some from the Plant Introduction Station, Iowa, USA (PI126926, PI126928, PI127829, PI127832, PI128650, PI128653 and PI251306) and the remaining from the collection of C.A. Rick, Davis, California, USA (LA374, LA385, LA1708, LA2172, LA2157, LA2326, LA2333, LA2334 and LA2338). Five accessions did not show clear wilt symptoms at any time after inoculation (LA385, LA2157, LA2334, LA2338 and PI127829). Plants with disease index of 0 or 1 were scored as healthy and plants with disease grade ≥2 as diseased. Even after re-inoculation no symptoms were found in the resistant accessions for at least three subsequent months.

2.2 Characterization of resistance in the five resistant *L. peruvianum* accessions

The distribution of bacteria in inoculated plants was determined according to the following procedure: Four weeks after sowing, all plants were inoculated with *C. michiganensis*. Three, six and eleven weeks after inoculation, the main stem of one plant per genotype was divided into three equal parts. From each part a segment was surface sterilized and cut into 2 to 3 mm thick slices. Numbers of recovered living bacteria were estimated by colony counts. In all accessions bacteria could be collected from stem segments. However, large differences in numbers of bacteria were detected in different parts of the stem of the resistant *L. peruvianum* accessions. In 40% of the stem segments of the resistant accessions over 10^9 bacteria were detected while in 20% of the segments no bacteria at all were found. In the remaining stem segments intermediate numbers of bacteria were present. In the susceptible Moneymaker and *L. peruvianum* LA1708 more than 10^9 bacteria were detected in all stem segments. Apparently, bacteria moved and multiplied at a lower rate in resistant than in susceptible genotypes. There seems to be a more or less irregular distribution of bacteria within the resistant plants. The bacteria seem to move through the vascular system and colonize at random positions. When the bacterial growth or a compound excreted by the bacteria reaches a certain level, water transport through the vascular tissue may be inhibited, resulting in wilting and eventually in necrosis.

Seed transmissibility of *C. michiganensis* in one resistant *L. peruvianum* accession (LA2334) was investigated. All seed samples from inoculated flowers on inoculated plants of *L. peruvianum* LA2334 were free from *C. michiganensis* bacteria.

2.3 Genetic analysis

The inheritance of the disease resistance was studied in segregating populations of a backcross within the *L. peruvianum* species (Van Ooijen et al., 1994; Sandbrink et al., 1995) as well as in the segregating F_2 offspring of an interspecific cross of *L. esculentum* x *L. peruvianum* LA2157 (Van Heusden et al., in preparation). These progenies were tested for segregation of bacterial canker resistance as well as for the segregation of RFLP loci. Map positions of RFLPs were calculated with JoinMap (Stam, 1993) with a minimum LOD (Logarithm Of Odds) of 3.0 for establishing linkage groups and 0.05 for mapping. LOD is a generally used test statistic for determining linkage. For mapping quantitative trait loci (QTL) the disease evaluation data could not be used for interval mapping as the disease was scored using classes on an ordinal scale. Consequently, QTLs were determined with the nonparametric ranksum test of Kruskal-Wallis for every marker separately (see *e.g.* Lehmann, 1975).

2.3.1 Intraspecific cross

Lindhout et al., (1989) described the inheritance of the resistance of accession LA2157 of *L. peruvianum* using the F_2 of a cross between the resistant *L. peruvianum* LA2157 and the susceptible accession LA2172, as well as the backcrosses to both parents. They showed that the resistance might be caused by two to three recessive, complementary genes. However, RFLP studies on the same populations by Sandbrink et al. (1995) indicated that more loci were involved in the resistance. Chromosomal regions on chromosomes 1, 6, 7, 8 and 10 had a significant association with *C. michiganensis* resistance. The putative resistance genes originated from the resistant parent, except for the gene on chromosome 10 that did originate from the susceptible parent.

2.3.2 Interspecific cross

Embryo rescue using *L. esculentum* cv Solentos (de Ruiter Seeds) made it possible to analyze a segregating F_2 population of the interspecific cross *L. esculentum* cv Solentos x *L. peruvianum* LA2157 (Van Heusden et al., in preparation). The resistance level of 324 plants was recorded at 22, 27, 32, 39, 46, 54, 61, 68, 75, 82, 90, 96 (table 1) and 104 days after inoculation.

Table 1 - Number of plants with a certain disease index 96 days after inoculation.

Disease index	0	1	2	3	4
L. esculentum (cv *Solentos*)	0	0	0	0	24
L. peruvianum (LA2157)	9	6	7	1	0
F_1	not determined				
F_2	49	21	13	46	188

A partial RFLP linkage map was made of 23 RFLPs covering chromosomes 1, 2, 7, 8, 9 and 10. Possible associations of these RFLP-alleles and disease symptoms were calculated with the Kruskal-Wallis test statistic. Two QTLs were identified on chromosome 7 near TG61 and on chromosome 9 near TG254. Both putative resistance genes originated from the resistant parent and the homozygous presence of these genes greatly increased the resistance to *C. michiganensis* (table 2).

Table 2 - The F_2 individuals classified according to the genotypes of TG61 and TG254 and the average disease index of the classes. EE homozygous *L. esculentum*, EP heterozygous and PP homozygous *L. peruvianum*. *L. esculentum* 4.0 ± 0.0(25), *L. peruvianum* 1.2 ± 0.9 (25), F_2-average 2.9 ± 1.5(324). Number of plants between brackets.

	TG61 EE	EP	PP
TG254			
EE	4.0 (7)	3.6 (39)	3.4 (19)
EP	3.7 (22)	3.0 (93)	2.3 (56)
PP	3.8 (5)	2.6 (35)	2.0 (23)

3. Concluding remarks and breeding perspectives

Five of the accessions showed higher levels of resistance than in any accession described sofar. RFLP studies with an intraspecific hybrid (*L. peruvianum* susceptible x resistant) identified five QTLs involved in resistance on chromosome 1, 6, 7, 8 and 10 (Sandbrink et al., 1995). Earlier genetic models based on segregation ratios assumed only two to three genes with recessive inheritance (Lindhout et al., 1989). However, it is always inaccurate to propose genetic models based on only ratios because of frequently occurring segregation distortions (*e.g.* Rick 1963, 1966, 1969; Gadish et al., 1987).

Here we described also the inheritance of resistance in an interspecific hybrid and its offspring. The involvement of only a few resistance loci in this interspecific cross was confirmed by the analysis with molecular markers. Only two QTLs were identified that almost completely explained the difference in resistance between the two parents of the hybrid. The region on chromosome 7 was also identified in the intraspecific cross. The effects on chromosomes 1, 8 and 10 were not observed in the interspecific cross in spite of the fact that the same resistance donor was used. Apparently, the susceptible *L. peruvianum* parent lacked several QTLs for resistance that were present in the susceptible *L. esculentum* parent. This might be due to physiological or morphological differences between the two species as bacteral canker is a wilting disease and wilting is dependent on plant physiology and morphology.

The identification of only two resistance loci makes it feasible to introduce resistance in the cultivated tomato by molecular marker assisted selection. Yet, additional research will be necessary such as the fine mapping of the resistance loci in order to minimize linkage drag and reconfirmation of the resistance in progeny of those F_2 plants with the two *L. peruvianum* QTLs in an otherwise purely *L. esculentum* genetic background. For this purpose we are developing nearly isogenic lines by backcrossing with tomato and selecting for molecular markers flanking the two regions with the two resistance loci.

In case that the resistance of the *L. peruvianum* accessions described above are not strong enough for commercial tomato cultivars Crino et al. (1995) are already trying to pyramide different genes to obtain the highest possible level of resistance. They are using, besides *L. peruvianum* resistance loci, also resistance loci from other wild species such as *L. hirsutum.* Studies on the spread and multiplication of the bacteria in resistant tomato plants will be needed for a better understanding of the mechanisms of resistance.

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.

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