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Mapping genes of *Solanum caripense* involved in resistance to *Phytophthora infestans*, the causal agent of potato late blight

Friederike Trognitz & Bodo Trognitz
ARC Seibersdorf Research GmbH, Biotechnology Department, 2444 Seibersdorf
(E-mail: friederike.trognitz@arcs.ac.at)

ABSTRACT: Reciprocal, bi-parental cross progenies of *Solanum caripense*, a diploid (2n = 2x = 24), self-incompatible, non-tuberizing, wild herb occurring throughout the Andes from Bolivia to Costa Rica, were used for the construction of parental framework linkage maps. Unexpectedly, low levels of polymorphism were encountered. The map of crp-1 comprises in total 287 cM and contains 89 markers in 10 linkage groups, whereas the map of crp-4 spans a genetic distance of 310 cM distributed over 66 markers in 12 linkage groups. Gene-specific markers detecting R gene analogs, a kinase similar to the tomato *Pto* gene, and a homolog of *SGT1* were also mapped. Two QTLs associated with resistance were located on the parental linkage maps by composite interval analysis of marker-trait associations. Within the cytoplasmic background of parent crp-4, a portion of the susceptible progenies carried the marker alleles for resistance at the position of the QTLs. This suggested that resistance may have been abrogated by an additional, independent nuclear factor that interacts with the cytoplasm of parent crp-4 to cause a susceptible phenotype. By single-marker analyses of marker-trait association, three additional marker loci independent of the QTLs were detected that significantly contributed to the resistance phenotype. *S. caripense*, a wild plant that has not been subjected to domestication and breeding should be a valuable source of resistance to late blight.

Key words: Genetic map – late blight – potato – QTL – resistance genes – *Solanum caripense*

Introduction

Late blight, the economically most important disease of potato, caused by the oomycete *Phytophthora infestans*, occurs almost everywhere where potatoes are grown. At around 1850, potato breeders started to introduce resistance from related *Solanum* species. Until present, 11 *R* genes from *S. demissum* have been included into potato breeding stocks. But most of the *R* genes used have been overcome by the pathogen throughout a large geographic area (Müller 1936). New resistance genes, such as *RB* from *S. bulbocastanum* (Song et al. 2003, van der Vossen et al. 2003) have been found in the gene pool of wild *Solanum* species, which are valuable sources for breeding. We detected late blight resistance in the wild *S. caripense*. Although this resistance segregates in the fashion of single dominant factors it proved intact against a wide array of *P. infestans* isolates representing most likely all strains of this pathogen occurring outside of South America (Trognitz 1998a). We report about the development of genetic maps and the localization on these maps of loci involved in the resistance. This work will facilitate eventual positional cloning of the blight resistance gene(s) of *S. caripense*.

Material and methods

Phenotyping of resistance to the late blight

S. caripense seedlings were grown and tested for resistance to late blight as described in Trognitz (1998b). Two parental genotypes, crp-1 and crp-4, were intercrossed reciprocally to produce two unselected cross progenies of 60 individuals each, designated crp-1xcrp-4 for the forward cross and crp-4xcrp-1 for the reverse cross. For the resistance tests using two highly virulent *P. infestans* isolates, either entire pot plants were exposed or lateral leaflets were detached from plants prior to flowering. The criteria percent leaflet area affected (A) and

sporulation intensity (S) were assessed. Typically, resistant plants developed none or very small lesions covering up to 10 % of the total leaflet area and no sporulation, whereas in susceptible interactions, a single lesion covered 60 - 90 % of the leaflet and there was abundant sporulation of the pathogen. Numbers of resistant and susceptible individuals within each cross progeny were compared to ratios of segregation expected under several plausible models of inheritance using a χ^2 test for goodness-of-fit.

DNA extraction and molecular marker techniques
DNA extraction and RFLP analysis were carried out as described in Trognitz et al. (2002). The AFLP procedure was carried out according to Vos et al. (1995), the digestion and ligation was done in one step overnight. For the sequence specific amplification polymorphism (S-SAP) procedure the protocol of Waugh et al. (1997) was followed using primers specific for conserved domains of R genes and a primer for the *Eco*RI adapter. Parental maps were constructed and marker-trait regression calculated in Joinmap v 3.0 (van Oijen & Voorrips 2001). QTL were detected by interval mapping in QTL cartographer (Wang et al. 2001-2003).

Results and discussion

Segregation of the resistance phenotype
We detected binomial distribution of resistant and susceptible cross progenies that indicated inheritance of blight resistance by few dominant genes. In the crp-1xcrp-4 cross the figures could be explained by segregation of two genes. However, the frequency of resistant and susceptible plants in the reverse cross could be explained by this model only under the assumption of an additional, interfering cytoplasmic factor.

Map construction
Of 140 EcoRI/MseI and 28 EcoRI/PstI-specific primer combinations tested, 48 primer combinations were used for construction due to their sufficient levels of polymorphism. For these, on average not more than 4.6 polymorphic bands per primer combination were obtained. Of 26 RFLP probes of known position on the potato map tested in addition, only two were polymorphic in the *S. caripense* crosses and one of these could be placed on the map. To map resistance gene analogs (RGAs), conserved motifs were used for amplification of RGAs from genomic DNA. Fragments amplified with specific primers for the *R1* gene from *S. demissum* were used as a probe and two fragments were polymorphic for parent crp-1. Based on sequence information of known R genes specific primers were designed and used in an S-SAP approach. We detected polymorphism for several *R1* like genes, for a kinase based on the tomato *Pto* gene (Jia et al. 1997), for a TIR-NBS resistance gene based on the "motif 2" sequence (Hammond-Kosack & Jones 1997), and a homolog of the *SGT1* gene involved in resistance signaling. Of several potato SSR markers tested, only one (STM0025-1/EcoR00) was polymorphic and could be mapped. Additional molecular marker techniques (SSR and CAPS-cleaved amplified polymorphic sequence- from potato) failed to detect polymorphic fragments in *S. caripense*. The map of parent crp-1 (Figure 1) consists of 89 markers distributed throughout ten linkage groups, of a total of 128 segregating markers obtained for this parent. This map spans a genetic distance of 287 cM, its markers are spaced on average at 3.2 cM. Several clusters of markers spaced at small genetic distance are apparent, whereas other, frequently distal regions on individual linkage groups present large spaces between adjacent markers. For the crp-4 parent, a total of 94 markers were obtained, 66 of which were sufficiently informative to construct a map of 12 linkage groups covering a total genetic distance of 310 cM (average spacing of markers at 4.6 cM). The total number of 12 linkage groups corresponds to the number of chromosomes in the haploid genome of *S. caripense*. Because of the reduced levels of polymorphism encountered within the mapping populations,

no assignment of the linkage groups to the consensus potato/tomato chromosomes can be made at present. Nonetheless, the maps have been useful for detection of genomic regions associated with late blight resistance.

Detection of marker-trait associations

Single factor analyses of variance were carried out for 89 markers from crp-1 and 66 markers from crp-4 as included on the genetic maps and phenotype values of four resistance criteria (A and S following inoculation with two *P. infestans* isolates), in separate for the crp-1xcrp-4 and crp-4xcrp-1 populations. Within crp-1xcrp-4, two clustered markers from crp-1, E+AAA/M+ACG-319 and E+AAC/M+AAG-188, localized on linkage group crp-1-10 were significantly associated with the resistance phenotype and this position coincided with a QTL, as detected by interval mapping (Figure 2). On the map of crp-4, 11 clustered markers significantly associated with resistance were detected. AFLP marker E+ACT/M+CTG-67 had the largest LOD score in the QTL analysis. When the data for both reciprocal cross populations were combined, the marker-trait associations and QTLs tended to disappear. Close inspection of the data revealed the unexpected presence of marker alleles associated with resistance in twenty individuals of the crp-4xcrp-1 population. When these twenty individuals were omitted from the analysis, the QTLs re-appeared with large LOD scores (as indicated in Figure 2). The most likely hypothesis to account for this phenomenon at present is to assume an additional, independent nuclear genetic factor that would interact with an unknown factor present within the cytoplasm of parent crp-4. Additional crosses and investigations to test this hypothesis are underway.

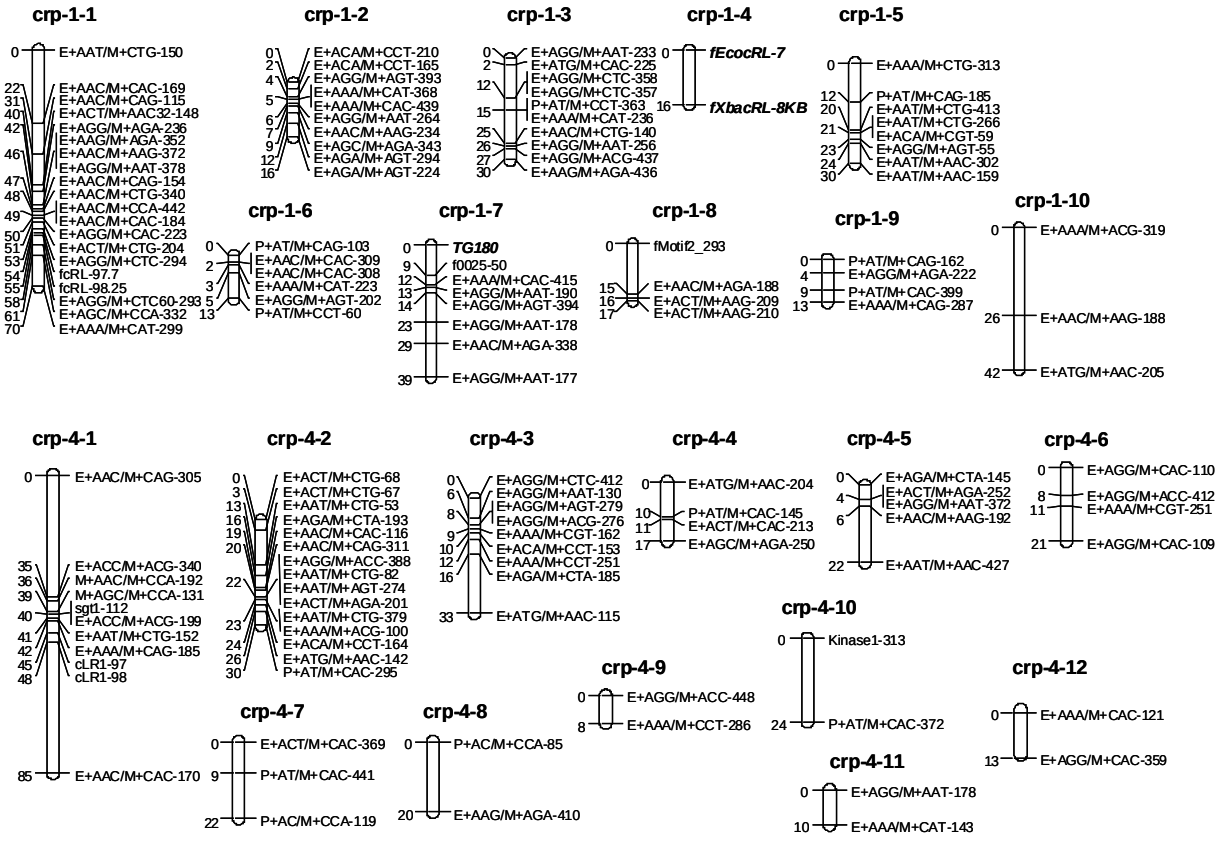

Figure 1. Genetic linkage maps of two *S. caripense* parental genotypes, crp-1 (top) and crp-4 (bottom).

AFLP markers are indicated to the right of a linkage group and genetic distance (cM) to the left. LOD scores are plotted along the genetic distance. Dotted line; LOD score of original data for population crp-1xcrp-4, dashed line; data combined for reciprocal crosses (excluding 20 individuals from population crp-4xcrp-1 with unexpected marker alleles; see text), solid line; population crp-4xcrp-1 (excluding 20 individuals with unexpected marker alleles).

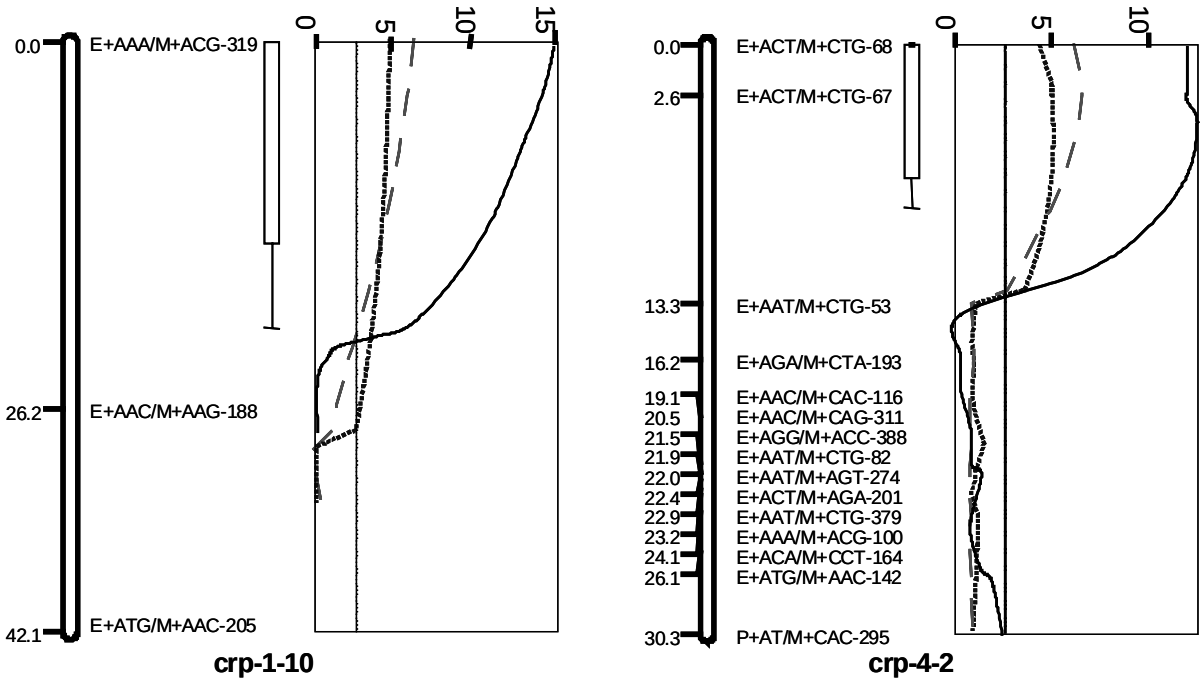

Figure 2. QTL positions as detected by composite interval analysis on the maps of parental genotypes crp-1 and crp-4.

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