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Genetic Mapping of a Wide Spectrum Nematode Resistance Gene (*Hero*) Against *Globodera rostochiensis* in Tomato

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The *Hero* gene confers resistance to a wide spectrum of pathotypes of the potato cyst nematode *Globodera rostochiensis*. This gene has been introgressed from the wild tomato species *Lycopersicon pimpinellifolium* into the cultivated tomato. We have used RFLP and RAPD analysis for the targeted search of the *L. pimpinellifolium* segment. The resistant line LA 1792 contains a single introgressed segment on chromosome 4, which is characterized by three RFLP markers from the high-density RFLP map of tomato. The map position of the *Hero* gene is not equivalent to any of the previously mapped *G. rostochiensis* resistance genes in potato. For the fine mapping of the *Hero* gene in large populations, four additional markers were identified in the introgressed region. After analyzing more than 800 gametes for recombination, we found that one marker is only 0.4 cM away from the *Hero* gene. YAC clones isolated from a region near the *Hero* gene indicate that in this area of the genome, the kb/cM ratio is relatively low (<450 kb/cM) and chromosome walking should be feasible in order to isolate this gene.

The interaction between dominant disease resistance genes and plant pathogens is well explained by the gene-for-gene hypothesis (Flor 1971). The recent isolation of some plant resistance genes indicates that the respective disease resistance genes are part of the signal transduction pathway leading to a hypersensitive response against bacterial, fungal, and viral pathogens (Martin et al. 1993; Mindrinos et al. 1994; Jones et al. 1994; Whitham et al. 1994; Bent et al. 1994). In contrast to this, nothing is known about the mode of action of nematode resistance genes despite their great agricultural importance.

In contrast to *Arabidopsis* (Sijmons et al. 1991), dominant resistance genes against a wide spectrum of pathogenic nematodes can be found in tomato (*Lycopersicon esculentum* Mill.) and its wild relatives (Rick 1986). Some of these genes have been introgressed into commercial cultivars. The most well-known nematode resistance gene is the *Mi* gene, that is located on chromosome 6 of tomato (Messeguer et al. 1991, Ho et al. 1992). This gene has been bred into a very large number of cultivars. Another nematode gene that has been identified in wild tomato species is the *Hero* gene (Ellis and Maxon-Smith 1971) that confers resistance to the cyst nematode *Globodera rostochiensis*. However, due to its low economic importance in tomato-growing areas, this gene has been neglected in tomato breeding. The *Hero* gene was identified in *L. pimpinellifolium* LA 121 and segregation analysis indicated that it is a single dominant gene. Resistance against the potato cyst nematode *G. rostochiensis* is not limited to the wild tomato species *L. pimpinellifolium* since similar resistance is also found in another wild tomato species *L. peruvianum* (Ellis 1968). Further data indicate that the *Hero* gene and the *Mi* gene are not allelic to each other but are distinct genes (Ellis and Maxon-Smith 1971).

In contrast to tomato, *G. rostochiensis* is an extremely important pathogen of the closely related potato (*Solanum tuberosum*) where cyst nematodes cause serious damage in temperate areas of the world (Ross 1986). Five pathotypes (Ro1-5) have been identified based on their ability to infect certain potato clones or hybrids, and resistance genes to one, several or all pathotypes have been identified and used in potato breeding (Ross 1986). The dominant gene *H1* located on chromosome 5 of potato confers resistance to the pathotypes Ro1 and Ro4 (Gebhardt et al. 1993; Pineda et al. 1993). The dominant gene *Gro1*, which is most likely equivalent to the *Fb* gene (Ross 1986), is located on chromosome 7 of potato and confers resistance to basically all pathotypes (Barone et al. 1990). Furthermore, genes responsible for quantitative resistance to the pathotype Ro1 have been located to chromosomes 10 and 11 of potato (Kreike et al. 1993). Thus, due to the almost complete colinearity of the potato and tomato genome (Tanksley et al. 1992), it would be extremely interesting to find out whether similar resistance genes are indeed located at comparable map positions. Once a nematode resistance gene is cloned, it would be also interesting to investigate whether a resistance gene from tomato is also functional in potato after transformation.

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Attempts are in progress to clone some of the *G. rostochiensis* resistance genes in potato via a map-based cloning approach. However, this has been hampered by the complicated genetics of the tetraploid and highly heterozygous potato and the lack of an appropriate large insert library in yeast artificial chromosomes (YACs). In tomato, as a diploid, inbreeding species, genetics is comparatively simple, and all other tools for the cloning of genes via a map-based approach are available as has been demonstrated by the successful cloning of the *Pto* resistance gene (Martin et al. 1993a; Tanksley et al. 1995).

In an attempt to isolate nematode resistance genes by the map-based cloning technique, we report here the genetic mapping of the *Hero* gene of tomato and present data on the feasibility of cloning this gene in the future.

RESULTS

Characterization of the pathotype specificity of the *Hero* gene.

The specificity of the *Hero* gene against the five different pathotypes Ro1-5 has not been determined in detail in the previous studies (Ellis and Maxon-Smith 1971). Thus, to characterize its entire pathotype specificity spectrum, the tomato line LA 1792 with the *Hero* gene and the tomato cultivar Moneymaker have been inoculated with the five different pathotypes of *G. rostochiensis*. The results of one such inoculation experiment are shown in Table 1. The *Hero* gene confers clear resistance against all known pathotypes of *G. rostochiensis*. As controls, we have also tested the susceptible tomato cultivar Ailisa Craig and some tomato lines with the *Mi* resistance gene and these lines (*L. esculentum* cv. Mogeor, *L. peruvianum* PI 128650) are susceptible to *G. rostochiensis* (results not shown) as previously described by Ellis and Maxon-Smith (1971).

Localization of the introgressed segment containing the *Hero* gene.

The *Hero* gene has been introgressed from the closely related wild tomato species *L. pimpinellifolium* into the cultivated tomato line LA 1792. Thus, this introgressed segment should reveal considerable DNA polymorphism when compared to *L. esculentum* DNA (Miller and Tanksley 1990). Since the original recurrent parent for LA 1792 was not available for this study, we have chosen a cultivar that is very similar to LA 1792, namely the cultivar Ailisa Craig, as an *L. esculentum* control lacking the *Hero* gene. In a first attempt to localize the introgressed region, we have used the three lines Ailisa Craig, LA 1792, and LA 121 which is the actual donor of the *Hero* gene for the isolation of RAPD markers as described by Martin et al. (1991). More than 700 different RAPD primers were tested on these lines; however, not a single marker could be associated with the introgressed segment. This indicates that either the introgressed segment is physically quite small and/or the level of polymorphism between these lines is very low.

In a second step, we subsequently evaluated the level of polymorphism between these lines using RFLP markers and hybridization to DNA digested with 6 different restriction enzymes. The data from these experiments (not shown) indicate that with approximately 50% of the hybridization probes used, polymorphism between LA 1792 and LA 121 was detected with, on average, one of six restriction enzymes, whereas LA 1792 and Ailisa Craig were in most cases identical. This indicates that the introgressed segment is probably small. Thus, a directed search for the introgressed segment was started in which RFLP markers from the high-density RFLP map of tomato (Tanksley et al. 1992) were used. Probes were chosen at 3- to 5-cM intervals across the entire tomato genome and hybridized in groups of 5 (Young et al. 1988) onto the three lines. A total of more than 350 probes which were equally spread over all 12 tomato chromosomes have been analyzed in this study.

Table 1. Potato cyst nematode tests on the tomato line LA 1792 and the tomato cultivar Moneymaker with the five pathotypes of *Globodera rostochiensis*

Pathotype	Tomato line	Average cyst number on roots
Ro1	LA 1792	0
	Moneymaker	14.66
Ro2	LA 1792	0.33
	Moneymaker	55.33
Ro3	LA 1792	0.50
	Moneymaker	66.33
Ro4	LA 1792	0.33
	Moneymaker	73.00
Ro5	LA 1792	0
	Moneymaker	56.33

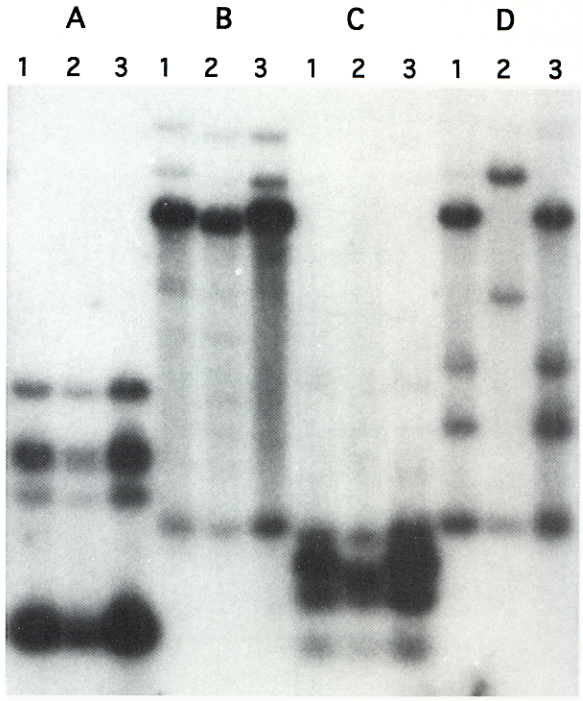

Fig. 1. Detection of the introgressed segment from LA 1792 with the RFLP marker CT 229. DNA from the donor line *Lycopersiocn pimpinellifolium* LA 121 (1), Ailisa Craig (susceptible) (2) and LA 1792 containing the *Hero* gene (3) was digested with the restriction enzymes *Taq*I (A), *Bst*NI (B), *Rsa*I (C), and *Xba*I (D) blotted and hybridized with the marker CT 229. Polymorphism between LA 1792 and LA 121 (resistant and donor) compared to Ailisa Craig (susceptible) are detectable for *Bst*NI, *Rsa*I and *Xba*I demonstrating that CT 229 is located within the introgressed fragment.

Only for the RFLP marker CT 229 on chromosome 4, LA 1792 and LA 121 were identical in their restriction fragment pattern and Ailisa Craig displayed a different restriction fragment pattern (Fig. 1). This indicates a probable introgressed fragment from *L. pimpinellifolium* in this region of the genome. A subsequent analysis with additional markers from chromosome 4 and using 15 to 30 different restriction enzymes revealed that the markers TG 15 and TG 370 which flank CT 229 on the RFLP map of tomato also show a fragment pattern identical to the donor of the *Hero* gene, LA 121. Interestingly, the markers TG 15 and TG 370 reveal polymorphism between LA 1792 and Ailisa Craig with two out of 15 restriction enzymes, while CT 229 shows polymorphism for 12 of the 15 enzymes. No other markers from this region revealed any polymorphism between these lines. Thus, the introgressed region in LA 1792 has, based on the standard map of tomato (Tanksley et al. 1992) a size of approximately 12 cM or less than 1% of the genome.

Isolation of additional markers in the introgressed region on chromosome 4.

Since this region on chromosome 4 of the tomato genome is sparsely populated with RFLP markers and contains one of the largest gaps of the high-density RFLP map of tomato (Tanksley et al. 1992), a search for additional markers in this interval was initiated. For this, pooled samples (Giovannoni et al. 1991; Michelmore et al. 1991) with defined genotypes for CT 229 have been selected from the standard mapping population (*L. esculentum* × *L. pennellii*). Two pools of 6 plants each, one homozygous for the *L. esculentum* allele and one homozygous for the *L. pennellii* allele, have been used for RAPD reactions with 1,000 different decamer primers. Since *L. pennellii* exhibits a much higher level of polymorphism than *L. pimpinellifolium*, the probability of finding markers in this region is much higher with this material (Miller and Tanksley 1990). The analysis revealed three RAPD markers that were linked to CT 229. After excision of the respective fragments, cloning, and using them as hybridization probes, it was found that two of them contain a single copy (HR1 = OPAH-03_{950} and HR2 = OPW-13_{700}) and one of them a repeated DNA sequence (HR3 = OPAB-11_{500}). Mapping of the two single copy sequences onto the standard tomato population indicates that they are indeed derived from this region of the genome (data not shown). While HR1 revealed polymorphism between Ailisa Craig and LA 1792 for at least 7 of the 15 analyzed restriction enzymes, the marker (HR2) did not reveal polymorphism between Ailisa Craig and LA 1792 with 30 restriction enzymes and was thus not useful for fine mapping the *Hero* gene. During the course of other experiments in the laboratory, an additional RFLP marker (H293) was identified as a new anonymous RFLP marker that mapped accidentally to the region. This marker showed a similar mapping position as HR2 and revealed polymorphism between the cultivated tomato and LA 1792. Thus, HR2 was not investigated further. Marker H293 was used instead for fine mapping of the *Hero* gene.

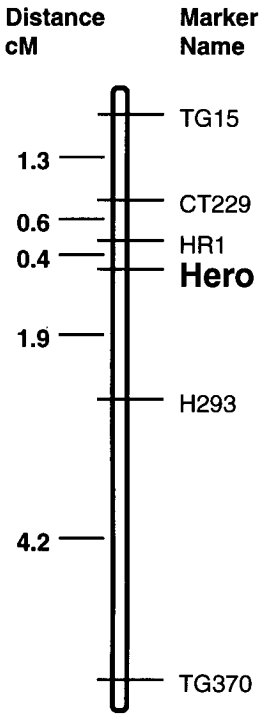

Fig. 2. High-resolution genetic map of the *Hero* gene. The data are based on 844 informative gametes. Genetic distances of the markers are calculated according to MAPMAKER using the Kosambi mapping function.

Fine mapping of the *Hero* gene within the introgressed region.

For the genetic mapping of the *Hero* gene, an F_2 population was generated from a cross between Ailisa Craig and LA 1792. In a first experiment, 86 F_2 individuals were screened with the three RFLP markers TG 15, CT 229, and TG 370 for recombinants in the introgressed region. Nine recombinants were identified. These recombinants, along with 3 to 5 plants from each genotype without a recombination event in this region, were selfed, and 10 F_3 plants each were tested for resistance with the *G. rostochiensis* pathotype Ro 1. From these data it was evident that in this small population, the *Hero* gene cosegregated perfectly with the markers CT 229 and HR1.

Finer mapping of the gene relative to all relevant markers from this region (TG 15, CT 229, HR1, H293, TG 370) was performed in a population of 336 plants from Ailisa Craig × LA 1792. In the same way as above, for resistance testing, all recombinants between the markers TG 15 and TG 370 were allowed to self and F_3 seeds were used for the testing with *G. rostochiensis* pathotype Ro 1. Figure 2 shows the combined results from both progenies (total of 422 F_2 plants representing 844 gametes). In this population the *Hero* gene is 3 recombinants or 0.4 centiMorgan away from the marker HR1.

Table 2. Analysis of yeast artificial chromosomes isolated with the markers CT 229 and hybridized to HR1

YAC clone	Size in kb	Hybridization with	
		CT 229	HR1
YCT229-1	200	+	–
YCT229-2	270	+	+
YCT229-3	280	+	+
YCT229-4	330	+	–
YCT229-5	420	+	+

On the other side of the gene towards the centromere, the marker H293 is 1.9 cM from the gene. The total genetic distance between TG 15 and TG 370 in the population Ailisa Craig × LA 1792 is 8.4 cM. This is approximately 50% shorter than the distance in the standard mapping population (*L. esculentum* × *L. pennellii*) and may be due to the fact that the standard mapping population consists only of 67 F_2 individuals and thus has a higher margin of error.

Isolation of YAC clones with CT 229.

In an initial attempt to isolate yeast artificial chromosomes with large inserts from this region, the marker CT 229 was sequenced from both ends and oligonucleotide primers were designed for PCR amplification of this clone. With these primers, approximately 35,000 YAC clones from tomato (Martin et al. 1992) were screened according to Green and Olson (1990). It was possible to isolate five clones that contain the marker CT 229. Pulsed field gel electrophoresis revealed that the insert size of the individual clones ranged from approximately 200 to 420 kilobases. When these YACs were hybridized to the marker HR1 that is 0.6 cM or five recombinants separated from CT 229, three out of the 5 YACs yielded also a hybridization signal with this marker, indicating that they contain the entire region between these two markers (Table 2). The smallest of the clones bridging the distance between CT 229 and HR1 is 270 kb, which is therefore the maximum distance between these markers. If this value is converted onto a kb/centiMorgan ratio, this results in a maximum distance of approximately 450 kb/cM for this region in the analyzed cross of Ailisa Craig × LA 1792.

DISCUSSION

The *Hero* gene has been introgressed from the wild tomato species *L. pimpinellifolium* into the cultivated tomato. The line LA 1792 carries a relatively small introgressed segment on chromosome 4 which contains the gene. This is most likely a result of the facts that *L. pimpinellifolium* is very closely related to *L. esculentum* and *L. pimpinellifolium* DNA recombines quite readily with *L. esculentum* DNA. Similar results have been found for other resistance genes introgressed from *L. pimpinellifolium* such as *I-2* and *Pto* (Sarfatti et al. 1989; Martin et al. 1993b).

The position of the *Hero* gene on chromosome 4 of tomato indicates that this gene is not allelic with the previously mapped *G. rostochiensis* resistance genes of potato on chromosome 5 and 7 since the linkage groups between potato and tomato are conserved and differ only by the inversion of five chromosome arms (Tanksley et al. 1992). In the case of *H1* (Gebhardt et al. 1993; Pineda et al. 1993), this is not completely unexpected since the *H1* gene of potato confers resistance to a different spectrum of pathotypes (only Ro1 and Ro4). However, the *Gro1* gene of potato is located on chromosome 7 (Barone et al. 1990) and has a similar host spectrum as the *Hero* gene. Two recently mapped quantitative resistance loci for Ro1 are located on chromosomes 10 and 11 and thus are also different genes (Kreike et al. 1993). The same is true for quantitative resistance against the cyst nematode *G. pallida* on chromosome 5 of potato (Kreike et al. 1994). These results indicate that there are numerous genes in the tomato/potato genome that can be responsible for resistance against potato cyst nematodes. Since potato and tomato are very closely related species, it would be interesting to investigate the interrelationship among the nematode resistance genes. Furthermore, with the observation that resistance genes are often members of gene families (Martin et al. 1993; Jones et al. 1994), it is possible that the specificity of the *Hero* gene to the different races of *G. rostochiensis* is due to a cluster of tightly linked resistance genes.

The *Hero* gene is in this respect an extremely interesting gene since it confers resistance against a wide spectrum of *G. rostochiensis* pathotypes. With this feature, the gene belongs to a group of three genes that give wide spectrum nematode resistance in tomato. The other two genes that yield in a similar way a broad spectrum nematode resistance are the *Mi* gene of tomato which provides resistance to many root knot nematodes species such as *Meloidogyne incognita, M. arenaria,* and *M. javanica* (Roberts and Thomason 1986) and a currently unnamed gene that results in resistance against sugar beet cyst nematode (*Heterodera schachtii*) and *Rotylenchulus reniformis* (Rebois et al. 1977; Ganal et al., unpublished results). With these three genes, tomato provides an excellent model system for the study of mechanisms conferring nematode resistance in plants since such resistance genes are not identified in other model species such as, for example, *Arabidopsis thaliana.* Future knowledge of the mode of action of these three genes might reveal a number of steps in the signal cascade leading to the induction of nematode resistance in plants.

The fine mapping of a large segregating population in connection with data from the analysis of yeast artificial chromosomes isolated with CT 229 indicate that the region around the *Hero* gene has a lower kb/cM ratio (<450 kb/cM) than the average of the tomato genome. With this and the fact that a marker has been isolated that is only 0.4 cM away from the *Hero* gene, this resistance gene provides a prime target for the map-based cloning of a nematode resistance gene in a similar way as it has been performed for the *Pseudomonas* resistance gene *Pto* (Martin et al. 1993; Tanksley et al. 1995). Additionally, the fine mapping of the *Hero* gene provides an ideal opportunity to isolate the gene by tagging with closely linked transposons as this has been successfully done for the *Cf9* resistance gene of tomato (Jones et al. 1994).

MATERIALS AND METHODS

Plant material.

All tomato lines described in this paper have been received from the Tomato Genetic Stock Center in Davis, CA. DNA from the standard mapping population of tomato (Tanksley et al. 1992) was extracted from leaves of the original plants maintained at Cornell University, Ithaca, NY.

Nematode testing.

Testing for nematode resistance using the different pathotypes of *G. rostochiensis* was either performed according to standard procedures described by Behringer (1985) or in the following way: Seeds were sown in seed pans and after approximately 10 days the seedlings were transplanted to 8-cm-diameter pots containing compost inoculated with cysts from

the Ro1 pathotype of *G. rostochiensis* with an initial population density of 10 eggs/gram of soil. The plants were grown in a glasshouse at a minimum temperature of 20°C for 12 weeks. After this time, the root balls were examined and the visible number of females was counted. Tests with the different pathotypes were performed in five replicas for each line. Recombinant F_2 plants from the mapping population were selfed and progeny tests with the pathotype Ro1 were performed in most cases on 10 to 15 F_3 plants. The resistance tests were usually conducted in two independent replications.

Genetic mapping and RAPD analysis.

RFLP mapping was performed according to standard techniques using the markers from the high-density map of tomato (Tanksley et al. 1992). The marker scanning experiment was performed with DNA from the lines LA 1792, Ailisa Craig, and LA 121 that was cut with the restriction enzymes *Eco*RI, *Dra*I, *Eco*RV, *Bst*NI, *Hin*dIII, and *Xba*I, separated on 1% agarose gels and blotted. Five PCR-amplified inserts from tomato markers were labeled (25 ng each) simultaneously and hybridized onto these filters. The same lines were used for the RAPD analysis using more than 700 decamer primers (Martin et al. 1991). The search for additional markers near CT 229 was performed with the 1,000 decamer primers that are available from Operon (Alameda, CA) as described by Giovannoni et al. (1991). Genetic distances were calculated using the MAPMAKER program (Lander et al. 1987).

YAC screening.

For the YAC screening, the marker CT 229 was sequenced from both sides according to standard procedures for an ALF automated sequencer (Pharmacia). Primers were designed according to this sequence and used for PCR amplification of the respective locus from the tomato genome (TTGTGAGTGGTGAACTACGGGC and CGGCAATGGTTATGGGAACG with a product size of approximately 375 bp). After confirmation of the amplified product by hybridization, DNA from a YAC library of tomato was screened similar to the procedure described by Green and Olson (1990). First, DNA pools from 380 microtiter plates (96 colonies each) were used for PCR amplification and pools that yielded the expected fragment after gel electrophoresis were identified. Five microliters from the glycerol stock of each well from a positive microtiter plate was transferred to a new microtiter dish and 5 µl of a 1 to 2 mg/ml solution of zymolyase 100T (ICN) were added. After incubation for 30 min at 37°C, PCR reactions were performed in a total volume of 50 µl and the products separated on agarose gels. Individual clones that produced the expected fragments were purified on selective plates and chromosomal DNA was purified. The size of the respective YACs was determined after pulsed field gel electrophoresis, blotting and hybridization with CT 229 and HR1.

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