Theor Appl Genet (1996) 92: 572–576

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RFLP analysis of resistance to Columbia root-knot nematode derived from *Solanum bulbocastanum* in a BC_2 population

Received: 4 November 1995 / Accepted: 11 November 1995

Abstract The mapping of resistance to *Meloidogyne chitwoodi* derived from *Solanum bulbocastanum* is reported. A population suitable for mapping was developed as follows. A somatic hybrid of nematode-resistant *S. bulbocastanum* and cultivated tetraploid potato was produced. This was backcrossed to tetraploid potato, and a single resistant BC_1 was selected and backcrossed again to the same recurrent tetraploid parent. The mapping population consisted of 64 BC_2 progeny scored for restriction fragment length polymorphic (RFLP) markers and 62 of these were evaluated for the reproductive efficiency of race 1 of *M. chitwoodi*. Forty-eight polymorphic RFLP markers, originally derived from tomato and mapped in diploid cultivated potato, were assigned to 12 chromosomes of *S. bulbocastanum*. Of the 62 progeny screened for nematode resistance, 18 were non-hosts and four were poor hosts. The rest were highly susceptible (good hosts). Analysis of the resistance (including non-hosts and poor hosts) as both a qualitative trait and as a meristic trait on which QTL analysis was applied supported the same genetic hypothesis. Genetic control was localized solely to factor(s) lying at one end of chromosome 11. The level of expression of resistance in the *S. bulbocastanum* parent and the resistant portion of the BC_2 was essentially the same. This fact, together with the highly significant LOD scores for one end of the chromosome-11 marker array, supports a genetic model equivalent to monogenic dominant control.

Key words Wild species · Introgression · *Meloidogyne chitwoodi* · Gene mapping · Potato resistance breeding · QTL

Communicated by H. F. Linskens

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Introduction

Molecular markers have expanded the analytical capability of geneticists by providing virtually unlimited polymorphism spanning the entire genome. This is especially useful in the breeding of crops where associations of markers with phenotypes can be utilized in an analytical fashion to select progeny and parents for crossing (Tanksley 1993). The introgression of traits from wild relatives is one area where the slow rate of progress, and the high rate of failure, in trait transfer invites the use of polymorphic molecular markers to analyze donor trait introduction and the nature of its incorporation into the cultivated genome.

Columbia root-knot nematode (*Meloidogyne chitwoodi* Gold) is an important pest of potato (*Solanum tuberosum* L.) in the Pacific Northwest of the United States, and a potentially important pest in the Netherlands (Evans and Trudgill 1992). Several studies have revealed resistance to two of the three races of the nematode in wild tuber-bearing *Solanum* species (Brown et al. 1989, 1991, 1994), while extensive screening in cultivated potato has failed to find resistance (Hoyman 1974; Martin, unpublished). *Solanum bulbocastanum*, a Mexican diploid (2n = 24) which is difficult to cross with cultivated potato, was found to have resistant accessions (Brown et al. 1989). Cultivated tetraploid (2n = 48) *Solanum tuberosum* ssp *tuberosum* and diploid *S. bulbocastanum* were combined by protoplast fusion to produce hexaploid (2n = 72) somatic hybrids (Austin et al. 1993). Nematode-resistant F_1 hybrids were crossed to cultivated tetraploid potato and nematode resistant BC_1 introgressants were verified (Brown et al. 1994, 1995). The present study presents the localization of resistance to race 1 of *M. chitwoodi* on a restriction fragment length polymorphism (RFLP) map of *S. bulbocastanum* chromosomes assigned in a BC_2 progeny population segregating for nematode resistance and polymorphic markers.

Materials and methods

Pedigree

The derivation of the segregating population used to establish polymorphic markers and assemble the RFLP map is shown in Fig. 1. Nematode resistance was detected in several accessions of *S. bulbocastanum* (Brown et al. 1989). A single selected nematode-resistant clone of *S. bulbocastanum* was somatically hybridized with cultivated tetraploid potato 203900 obtained from NRSP-6, Potato Introduction Station, Sturgeon Bay, Wisconsin. A somatic hybrid, designated CBP233, found to be resistant to the nematode (Austin et al. 1993; Brown et al. 1994), was hybridized with a second cultivated tetraploid, A84118.3, obtained from Dr. J. Pavek, USDA/ARS, Aberdeen, Idaho. A single nematode-resistant BC_1 individual, DG 17, was hybridized to A84118.3 again to produce the BC_2 (Brown et al. 1995). Markers were scored and resistance was measured within this population of BC_2 progeny.

DNA extraction and hybridization with probes

Fresh leaves, 20–35 g, from greenhouse-grown plants were macerated in a blender with ice-cold buffer and DNA extracted, according to Bonierbale et al. (1988). Ten micrograms of purified potato nuclear DNA was digested separately with 30–50 units of the restriction enzymes (RE) *Dra*I, *Eco*RV, *Bam*HI, *Pst*I, *Xba*I, *Hin*dIII, or *Eco*RI following manufacturers' recommendations. Digested DNA was run at 80 mA in 0.9% agarose gels for 14–16 h in NEB (neutral electrophoresis buffer, Tris, 10 mM; EDTA, 1 mM; sodium acetate, 1.25 mM; adjusted to pH = 8.1). The gel was de-purinated with 0.25 N HCl (aq) for 10 min and rinsed with distilled water. DNA was transferred to Amersham Hybond-N-plus (RPN.203N) with 0.4 N NaOH (aq) overnight using capillary action. After quick plasmid preparations were performed (Birnboim and Doly 1979; Kraft et al. 1988) plasmids containing the RFLP probes as inserts were oligo-labelled using the Multiprime DNA labelling system (RPN.1601Y, Amersham) with ^{32}P-dCTP. Hybridization was carried out at 65 °C overnight and the filter washed to high stringency starting at 2 × SSC plus 0.1% SDS for 30 min and completed at 0.5 × SSC plus 0.025% SDS.

Mapping

Markers were obtained from Dr. S. D. Tanksley, Department of Biometry and Plant Breeding, Cornell University, Ithaca, New York. These consisted of cDNA clones from the tomato genome previously mapped to locations in a diploid cultivated potato × wild potato F_1 progeny (Tanksley et al. 1992). Markers were selected on the basis of providing coverage of the 12 homologues of potato. This allowed an initial identification of the chromosome(s), and regions thereon, that co-segregate with nematode resistance. As a first step, Southern analysis was performed on batches of genomic DNA of the resistant *S. bulbocastanum* parent and the susceptible cultivated potato parents restricted with different REs, using the cDNA markers as probes. Polymorphism was identified for particular marker × RE combinations, with an emphasis on the size fragments unique to *S. bulbocastanum*.

Linkages and map distances were calculated using the computer program MAPMAKER/EXP (Lander et al. 1987). Determination of LOD scores for quantitative data of nematode resistance was calculated using MAPMAKER/QTL (Paterson et al. 1988).

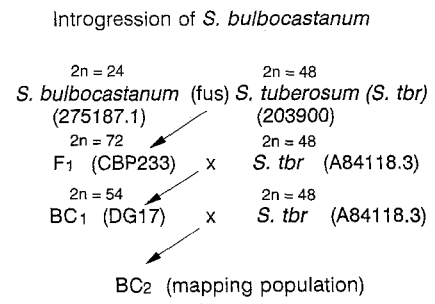

Fig. 1 Derivation of the BC_2 mapping population

Nematode inoculation

Rooted cuttings of 62 BC_2 progeny, the resistant *S. bulbocastanum* and susceptible recurrent parents 203900 and A84118.3, approximately 7 cm tall, were transplanted into plastic pots (10 cm diameter) containing loamy sand (84% sand, 10% silt, and 6% clay) and were fumigated with methyl bromide. The nematode population used in this experiment, WAMc1 (*M. chitwoodi*, race 1), was maintained at the Washington State University-Irrigated Agriculture Research and Extension Center, Prosser, Wash., collection (Pinkerton et al. 1987). Inoculum was derived from single-egg mass cultures, and prepared by collecting eggs after shaking infested roots of tomato (*Lycopersicon esculentum* Mill cv Columbia) in 0.5% NaOCl (Hussey and Barker 1973). At transplanting, an aliquot containing 5000 eggs was pipetted into depressions in the soil around the base of the plants of five replicated pots arranged in a completely randomized design. Potato (cv Russet Burbank), tomato (cv Columbia), alfalfa (*Medicago sativa* L., cv Thor), and wheat (*Triticum aestivum* L., cv Gaines) were included as experimental entries. Potato and tomato, suitable hosts for *M. chitwoodi*, were used as susceptible standards. Alfalfa, a non-host for race 1, was included to detect cross-contamination by race 2 of *M. chitwoodi*, for which it is a suitable host; while wheat, a non-host for *M. hapla*, was used to check against this possible contaminant. Plants were grown with regular watering and fertilization at 24 ± 3 °C for 55 days, and eggs were extracted and counted (Hussey and Barker 1973).

Chromosome counts

Root tips for chromosome counts were taken from cuttings rooted in vermiculite. Root tips measuring 3 mm in length were immersed in distilled water at 1 °C for 24 h. They were then placed in fixative (3:1, 95% ethanol: glacial acetic acid) for 24 h and transferred to 70% ethanol for long-term storage at 4 °C. Root tips were placed in 1 N HCl (aq) at 55 °C for 8 min, washed with tap water, placed in a drop of acetocarmine (45% acetic acid with 2% w/v carmine), macerated, and heated gently over an alcohol flame before and after a cover slip was placed over the tissue which was smeared by tapping the coverslip sharply with the eraser tip of a pencil. Chromosome counts were made at 1000 ×.

Results

Development of the map

The RFLP markers had been previously selected to provide broad coverage of the linkage groups. Forty-eight markers were placed on 12 linkage groups (Fig. 2). Four markers were not associated with any groups and two others were closely linked to each other but did not lie on any of the established linkage groups. Four of the forty eight markers were established in linkage groups that differed from their assignment in the diploid potato linkage map (Tanksley et al. 1992). The marker TG20 changed from chromosome 7 to 1; TG176 from chromosome 8 to 1; TG71 from chromosome 1 to 4; and TG275 from chromosome 6

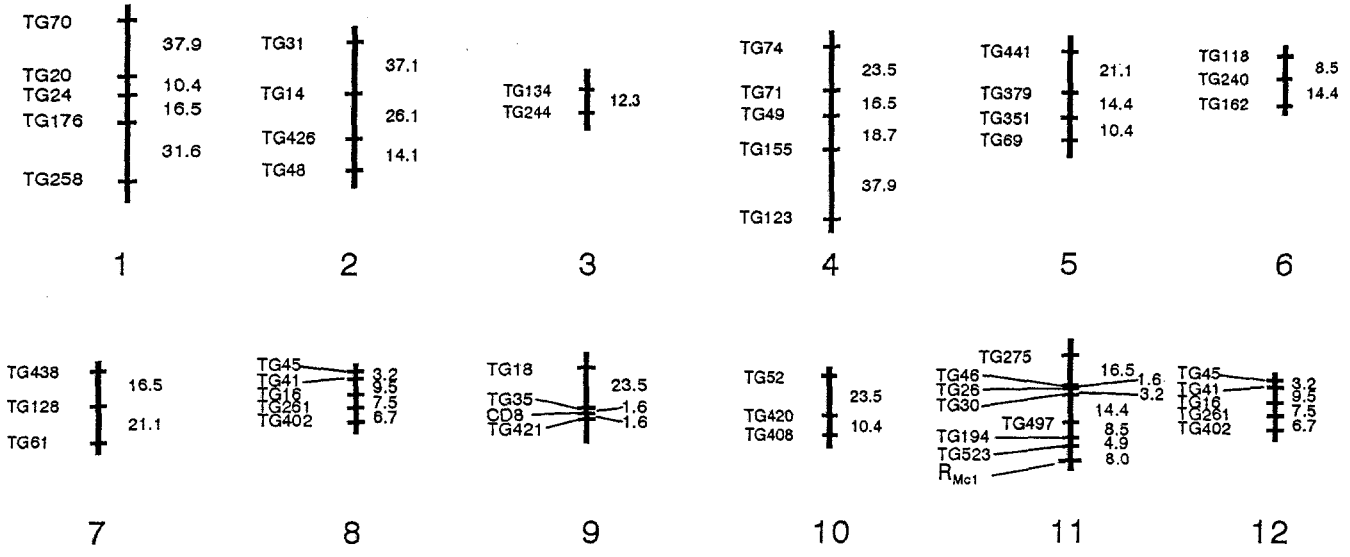

Fig. 2 RFLP map of *S. bulbocastanum* with the assignment of the putative *M. chitwoodi* resistance locus, R_{Mc1}, to chromosome 11

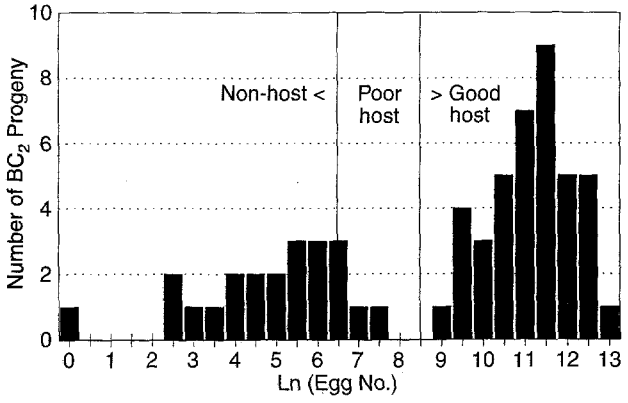

Fig. 3 Distribution of Ln (no. eggs) for 62 BC_2 progeny. Host suitability regions are indicated

to 11. This might indicate a disruption of synteny between *S. bulbocastanum* (Series Bulbocastana) and cultivated potato (Series Tuberosa; *S. bulbocastanum* has in fact been found to be a genetic outlier in several studies of genetic relationship (Bonierbale et al. 1990; Debener et al. 1990). The homosequentiality between *S. bulbocastanum* and cultivated potato and/or tomato is, however, sufficient to serve as a guide for the selection of markers.

The F_1 somatic hybrid was determined to have a 2n = 72 constitution. The BC_1 parent selected as the parent of BC_2 had 2n = 54.

Pattern of segregation of nematode resistance

Reproductive efficiency (R_f=final population/initial population) depends on the fecundity of a proportion of the primary inoculum and the intermediate generations that successfully infest the host and reach reproductive maturity. Reproductive efficiency is one measure of resistance of a host crop to species of *Meloidogyne* (Oostenbrink 1966; Sasser et al. 1984). This measurement was chosen as the resistance parameter in the present study. Host status is generally divided into three categories on the basis of R_f values as follows: $R_f > 1.0$, suitable host; $0.1 < R_f < 1.0$, poor host; $R_f < 0.1$, non-host (Sasser et al. 1984).

The population of 62 BC_2 individuals separated into three groups. Eighteen progeny were classified as non-hosts ($R_f < 0.1$) and four as poor hosts ($0.1 < R_f < 1.0$), while the remainder were classified as good hosts ($R_f > 1.0$). The distribution of the mean natural logarithm of nematode counts of the progeny is shown in Fig. 3. The data were used as a meristic trait for quantitative trait analysis. For convenience, the graph is divided into the three host suitability categories discussed above.

The treatment of Ln (no. of eggs) as a quantitative trait locus (QTL) is presented in Fig. 4. LOD scores were only significant for certain markers on chromosome 11. Resistance factor(s) appear to be located unambiguously on the left-hand portion of the map of chromosome 11 as depicted in Fig. 4. Resistance was also treated as a discrete trait where BC_2 progeny exhibiting non-host and poor-host responses were classified as resistant. A putative nematode resistance locus, R_{Mc1}, maps 8 cM distal to TG523 (Fig. 2).

The chromosome number of the BC_1 parent was less than would be expected from a totally balanced megasporogensis. Masuelli et al. (1995) examined the microsporogenesis of the hexaploid F_1 hybrid, CBP233, used in the present backcross study and found pollen mother cells exhibiting either regular or completely disrupted meiosis with unequal partitioning of chromosomes to tetrads. While CBP233 was hexaploid, 2n = 72, DG17 had 2n = 54. It would appear that DG17 was a product of irregular meiosis. However, it is likely that the chromosomes lost were those of the *S. tuberosum* genome as DG17 was selected

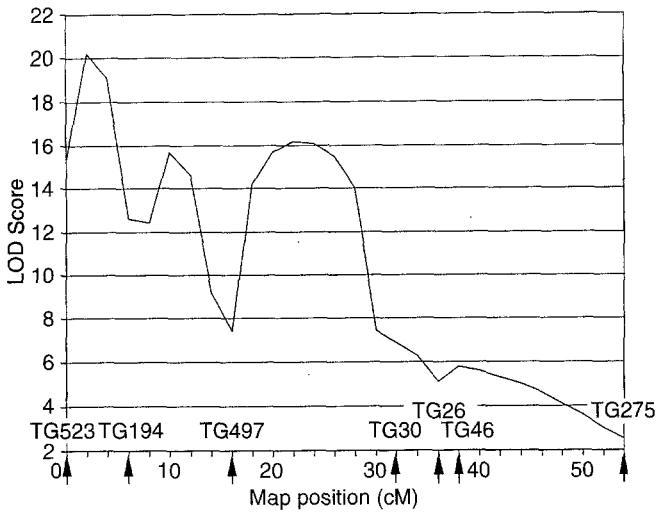

Fig. 4 LOD scores for QTL analysis of Ln (no. eggs) of BC_2. Chromosome 11 was the only linkage group with significant scores

not only because it was resistant, but also because it possessed a full complement of *S. bulbocastanum* markers.

The level of resistance appears to be undiminished between the resistant *S. bulbocastanum* parent and resistant BC_2 segregants. One-third of the progeny are resistant, while the QTL analysis attributes genetic control to one portion of chromosome 11, distal to marker TG523, and to no other location on the map. The model of inheritance is consistent with a completely dominant monogene. However, this analysis cannot exclude the possibility that several linked loci are reponsible for resistance.

All of the somatic hybrids produced from the original fusions were resistant to the nematode. The plants arose from a single callus and may have been derived from a single heterofusion cell (Austin et al. 1993). Four of the 20 BC_1 progeny evaluated were resistant to the nematode (Brown et al. 1995), while 35% of the BC_2 progeny were resistant. The QTL analysis shows an unambiguous attribution of genetic control to a particular region of chromosome 11. Considering that the inheritance is consistent with monogenic dominant genetic control and the segregation pattern in the BC_1 and BC_2 generations, it would appear that the single selected clone of *S. bulbocastanum* was heterozygous for this locus. The polysomic composition of the populations and the deviation from a 1:1 segregation in both the BC_1 and BC_2 generations would not by themselves have permitted such a conclusion. The RFLP map, combined with resistance segregation data, has served, therefore, as a valuable elucidative analytical tool.

Discussion

Potato has been the beneficiary of extensive mapping studies. Bonierbale et al. (1988) and Gebhardt et al. (1989, 1991) described genetic maps of potato and tomato, based on RFLP markers, and the uses to which the maps could be put to enhance breeding efficiency. Debener et al. (1990) and Bonierbale et al. (1990) used RFLPs to determine phylogenetic relationships between distinct species of *Solanum*. This has led to the knowledge that *S. bulbocastanum* is distinct from other species of wild and cultivated potato. Molecular markers have proven useful in the mapping of a number of other traits in cultivated potato. Kreike et al. (1993) reported the association of two regions on chromosomes 10 and 11 with quantitative resistance to *Globodera rostochiensis* pathotype Ro1. Resistance to *G. rostochiensis* (Golden nematode) derived from *S. tuberosum* ssp. *andigena* germ plasm accession CPC 1673 has been mapped to chromosome 5 using RFLP markers, and closely linked markers have been identified (Gebhardt et al. 1993; Pineda et al. 1993). The genetic control of trichome characters originating from *Solanum berthaultii* that provide insect resistance when backcrossed into cultivated potato has been attributed to several different chromosome regions as QTLs (Bonierbale et al. 1994). Molecular mapping of QTLs affecting tuber dormancy has assigned control to several chromosomes of potato with a predominant effect ascribed to chromosome 7 (Freyre et al. 1994).

Mapping of traits has also been important in other crop species. The effect of introgressed chromosome segments of the wild species *Lycopersicon chmielewskii* on the expression of quality traits and yield in cultivated tomato has been determined by Azanza et al. (1994). Significant contributions were attributed to wild donor chromosomes 7 and 10. Random amplified polymorphic DNA (RAPD) markers have been found that are linked to the single gene in *Beta vulgaris*, originating from *Beta patellaris*, controlling resistance to the Beet cyst nematode, *Heterodera schachtii* (Uphoff and Wricke 1992). RAPD-based markers have been identified that flank this gene, $Hs1^{pat-1}$ (Salentjin et al. 1995).

The *Mi* gene, controlling resistance to *Meloidogyne incognita*, derived from *Lycopersicon peruvianum* and introgressed into *L. esculentum*, has been mapped to chromosome 6 of tomato and closely linked markers have been identified (Williamson et al. 1994). The level of homosequentiality between the tomato and potato genomes is high (Gebhardt et al. 1991; Tanksley et al. 1992). It is interesting to ask the question whether the resistance factors described here are related to the *Mi* gene of tomato. The determination that the location of genetic control of resistance to *M. chitwoodi* is located on chromosome 11 in *S. bulbocastanum* argues for an unrelated gene or gene(s) in this wild species rather than the expression of an homoeologous Solanaceous or potato-related "*Mi*" factor. The placement of resistance on a genetic map should facilitate the identification of more closely linked markers that will accelerate the laborious process of selecting resistance to *M. chitwoodi* in segregating potato breeding populations.

Acknowledgements We thank the Washington State Potato Commission for financial support and S. D. Tanksley for generously providing RFLP markers. Mention of a trademark or a proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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