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RAPD linkage map of the genomic region encompassing the root-knot nematode (*Meloidogyne javanica*) resistance locus in carrot

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Abstract Inheritance studies have indicated that resistance to the root-knot nematode (*Meloidogyne javanica*) in carrot inbred line 'Brasilia-1252' is controlled by the action of one or two (duplicated) dominant gene(s) located at a single genomic region (designated the *Mj-1* locus). A systematic search for randomly amplified polymorphic DNA (RAPD) markers linked to *Mj-1* was carried out using bulked segregant analysis (BSA). Altogether 1000 ten-mer primers were screened with 69.1% displaying scorable amplicons. A total of approximately 2400 RAPD bands were examined. Four reproducible markers (OP-$C2_{1700}$, OP-$Q6_{500}$, OP-$U12_{700}$, and OP-$AL15_{500}$) were identified, in coupling-phase linkage, flanking the *Mj-1* region. The genetic distances between RAPD markers and the *Mj-1* locus, estimated using an F_2 progeny of 412 individuals from 'Brasilia 1252' × 'B6274', ranged from 0.8 to 5.7 cM . The two closest flanking markers (OP-$Q6_{500}$ and OP-$AL15_{500}$) encompassed a region of 2.7 cM . The frequency of these RAPD loci was evaluated in 121 accessions of a broad-based carrot germplasm collection. Only five entries (all resistant to *M. javanica* and genetically related to 'Brasilia 1252') exhibited the simultaneous presence of all four markers. An advanced line derived from the same cross, susceptible to *M. javanica* but relatively resistant to another root-knot nematode species (*M. incognita*), did not share three of the closest markers. These results suggest that at least some genes controlling resistance to *M. incognita* and *M. javanica* in 'Brasilia 1252' reside at distinct loci. The low number of markers suggests a reduced amount of genetic divergence between the parental lines at the region surrounding the target locus. Nevertheless, the low rate of recombination indicated these markers could be useful landmarks for positional cloning of the resistance gene(s). These RAPD markers could also be used to increase the *Mj-1* frequency during recurrent selection cycles and in backcrossing programs to minimize 'linkage drag' in elite lines employed for the development of resistant F_1 hybrids.

Key words Bulked segregant analysis · Carrot · *Daucus carota* · Disease resistance · *Meloidogyne javanica* · Root-knot nematode

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Introduction

The root-knot nematode *Meloidogyne javanica* (Treub.) Chitwood is one of the most important soil-borne pathogens affecting carrot (*Daucus carota* L.) production in tropical and subtropical regions of the world (Peterson and Simon 1986; Stein and Nothnagel 1995). *M. javanica* infection often causes substantial economic losses due to tap-root galling (Huang and Charchar 1982). Nematode damage at infection sites may induce secondary development resulting in forked or ramified roots (Huang 1986). Sources of near-immunity resistance have been reported in carrot (Huang et al. 1986; Simon et al. 1997). The open-pollinated cultivar 'Brasilia' and inbred lines derived from this population are the most promising sources of resistance to *M. javanica* identified so far (Charchar et al. 1982; Charchar and Vieira 1995; Simon et al. 1997). Inheritance studies indicated that resistance to *M. javanica* in the inbred line 'Brasi-

lia-1252' is conditioned by the action of one (or two duplicated, linked) dominant factor(s). This genomic region has been tentatively designated as the *Mj-1* locus (Simon et al. 1999).

A positional (map-based) approach is one of the currently available strategies for isolation of the *M. javanica* resistance gene(s) in carrot. One of the prerequisites for positional gene cloning is to saturate, with a large collection of genetic markers, the target genomic region controlling the trait of interest (Paran and Michelmore 1993). Bulked segregant analysis (BSA) in combination with randomly amplified polymorphic DNA (RAPD) (Williams et al. 1990) offers one of the simplest methods for generating genetic markers in regions near to a particular locus within complex genomes (Michelmore et al. 1991). In addition, the screening for linked markers can be performed in as early as the F_2 generation of controlled crosses with no need for the development of near-isogenic lines (Paterson 1996). The use of F_2 progeny in map construction is important for the genetic analysis of a biennial and outcrossing species such as carrot, where inbreeding depression precludes the efficient development of recombinant inbred lines. Furthermore, with BSA, a larger number of primers can be screened in relatively short periods of time (Michelmore et al. 1991). Analytical procedures employing RAPD-BSA have been used as a routine to identify molecular markers linked to a large number of disease resistance loci in several crops (Michelmore 1995; Mohan et al. 1997). In the present work, we report an extensive search for RAPD markers linked to the *M. javanica* resistance locus and the development of the first genetic linkage map encompassing this genomic region in carrot.

Materials and methods

Mapping population and nematode resistance evaluation

The segregating population employed in the present study was derived from a cross between the *M. javanica* resistant inbred line 'Brasilia-1252' as female parent and the susceptible inbred line 'B6274' as male parent. A single F_1 plant was self-pollinated to produce 442 F_2 individuals that were evaluated for a *M. javanica* reaction under greenhouse conditions at University of California, Riverside, California (Simon et al. 1999). Resistance screening was carried out using individual carrot seedlings cultivated in pots containing sterilized sand. Each plant was infected with a suspension of 30 000 eggs, which were pipetted onto soil depressions around the rosette of the plants 30 days after germination. Sixty days after inoculation, taproots were harvested, the root system was carefully washed free of soil and a root gall rating was assigned where: 0 = no galls; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76–100% of fibrous roots galled. A strong positive correlation was observed between nematode multiplication levels on root tissues and root galling (Simon et al. 1999). Plants were considered as resistant in the 0–1 range and as susceptible in the range 2–4. After evaluation, all F_2 taproots were vernalized (4°C for 40 days) and were then planted in a greenhouse at Madison-Wisconsin. All DNA samples were extracted from lyophilized leaves collected from the F_2 plants originated from vernalized roots. In general, the high nematode infection observed in some F_2 roots did not preclude sample collection. Therefore, 412 leaf samples out 442 F_2 plants were collected and used for the construction of the linkage map.

DNA extraction and DNA pools

DNA was extracted as described by Saghai-Maroof et al. (1984) but with minor modifications (Boiteux et al. 1999). Pulverized samples (approximately 0. 14 g of lyophilized leaves in liquid nitrogen) were dispersed in 15 ml of preheated (65°C) extraction buffer [500 mM Tris pH = 9.0, 1.4 M NaCl, 2% (w/v) CTAB, 0.2% beta-mercaptoethanol, 20 mM ethylenediamine tetraacetic acid – EDTA]. Tubes containing the buffer + leaf tissue mixture were vigorously shaken for a few seconds and incubated at 65°C for 60 min with occasional mixing by gentle rotation. Fifteen microliters of chloroform + isoamyl alcohol (24:1 v/v) were added and the solution was mixed by inversion to form an emulsion that was centrifuged at 11 240 g for 10 min at 4°C. The aqueous phase was removed, and 2/3 vol of ice-cooled isopropanol was added and mixed by gentle inversion. Preparations were centrifuged at 11 240 g for10 min. The pellet was rinsed with 76% ethanol + 10 mM ammonium acetate, allowed to air-dry for up to 15 min and then resuspended in Tris-EDTA (TE) pH = 8.0 buffer. Individual DNA samples were obtained initially from a group of five resistant (R) and a group of five susceptible (S) plants with F_2:F_3 true-breeding families. In addition, only F_2 plants with a score = 0 were included in the R bulk and only susceptible F_2 plants with a score of 3 or 4 were included in the S bulk. DNA pools were prepared by mixing equal amounts of total DNA (20 ng/µl) of selected plants within each phenotypic group.

Polymerase chain reaction and RAPD analysis

One thousand 10-mer primers (kits A to Z and AA to AX from Operon Technologies, Alameda, California, USA) were evaluated in two replications using as template DNA samples from both parents as well as R and S bulks. The first round of evaluation employed *Taq* DNA polymerase (Promega, Madison, Wisconsin, USA). Primers with at least one amplification product (whether polymorphic or not) were tested again with Ampli*Taq* DNA polymerase (Perkin Elmer, Branchburg, New Jersey, USA). The DNA amplification reaction was done with 23 µl of master mix reaction + 2 µl of DNA template. The master mix consisted of 11.32 µl of milliQ water, 2.5 µl of 10 × DNA polymerase buffer, 2.3 µl of $MgCl_2$ (25 mM), 5.7 µl of deoxynucleotide triphosphates (0.5 mM of each), 1 µl of primer (5 mM), and 0.18 µl (5 units/µl) of thermostable DNA polymerase (Boiteux et al. 1999). PCR was carried out with Perkin Elmer Cetus 9600 or 9700 thermocyclers. The reaction mixture (containing the template DNA) was heated at 94°C for 2 min and then subjected to 40 cycles consisting of 30 s at 94°C, 1 min at 36°C, 1.5 min at 72°C, and a final step at 68°C for 10 min. The reaction products were subjected to electrophoresis on 250 ml of agarose (1.0–1.4%) gel supplemented with 2.5 µl of ethidium bromide (5 mg/ml). Electrophoresis was carried out for 4–5 h at 80 V in 1 × TAE buffer (40 mM Tris-acetate pH 7.7, 20 mM sodium acetate, 1 mM EDTA). All amplicons (whether polymporphic or not) with a size equal to or under 2.5 kb were scored using a qualitative scale where: 1 = strong, 2 = medium and 3 = weak intensity of the amplified product. Markers were named by using an alpha-numerical designation. For example, marker $OP\text{-}C2_{1700}$ denotes a marker (amplicon) obtained with the primer Operon C2 with a molecular size of approximately 1700 bp.

Mapping the *Mj-1* locus

PCR reactions with putative polymorphisms between bulks were replicated 2–3 times to check the reproducibility of the amplification profiles. Primers were then evaluated using the parental lines and a random sample of 22 (11 R and 11 S) F_2 individuals. Primers with stable (i.e., reproducible) polymorphic amplicons with evidence of linkage to the *Mj-1* locus were then investigated in more extensive studies with DNA samples of the 412 plants derived from vernalized F_2 roots. Markers with unstable performance were dropped from the analysis. The final marker order and map dis-

tances of the polymorphic amplicons in the F_2 population were obtained using MAPMAKER version 3.0 for IBM-PC (Lander et al. 1987; Lincoln et al. 1992). Linkage groups were first assigned based upon two-point analysis via 'group' command. The most-likely order of the markers was obtained using exhaustive multipoint analysis via 'compare' command and the final maximum likelihood map distances were obtained with 'map' command. Final mapping distances were determined using a logarithm of odds (LOD) threshold of 4.0. Recombination frequencies were transformed to Kosambi distances (Kosambi 1944).

Frequency of the RAPD markers linked to *Mj-1* locus in a germplasm collection

A diverse carrot collection of 121 genetic materials was evaluated (in two replications) for the presence/absence of the polymorphic RAPD markers identified in linkage with *Mj-1* (see Table 1). This germplasm was representative of the major market classes 'Imperator', 'Danvers', 'Chantenay' and 'Nantes' used for fresh market, processing, and the 'baby-carrot' industry in USA, South America, and Europe. For this assay, fresh leaves from field-grown plants collected at Palmyra (Wisconsin) were used as source of DNA. This analysis also included four F_3 lines derived from the cross 'BR-1252' × 'B6274'. Two of these lines were homozygous resistant and two were homozygous susceptible in response to *M. javanica*. One of the *M. javanica* resistant lines was also resistant to *Meloidogyne incognita* (636–12), while the other was more susceptible to *M. incognita* (636–11). One of the *M. javanica* susceptible lines was more resistant to *M. incognita* (638–1), while the other line (638–2) was susceptible to both nematode species. Leaves were stored at –20°C until use. DNA extraction, PCR conditions, and eletrophoretic conditions were identical to those described by Boiteux et al. (1999).

Results and discussion

Sixty two primers, corresponding to a total of 63 bands, displayed amplification profiles with putative polymorphisms between R and S bulks in at least one of the BSA replications. More extensive analyses of these primers were performed with the parental lines and 22 F_2 individuals (11 R and 11 S). However, most of these candidate markers were found to be either pseudo-polymorphisms (observed only during BSA with pooled DNA as a template) or difficult to score reliably due to the presence of faint or inconsistent amplicons. Only four primers displayed amplicons with consistent evidence of linkage to the *Mj-1* locus: OP-C2_{1700} (5'-GTGAGGCGTC-3'), OP-Q6_{500} (5'-GACCGCCTTG-3'), OP-U12_{700} (5'-TCACCAGCCA-3'), and OP-AL15_{500} (5'-AGGGGAC-ACC-3'). The primer OP-C2 displayed an additional amplicon (named OP-C2_{420}) with apparent linkage to the *Mj-1* locus. However, OP-C2_{420} was a very faint band, which prohibited a reliable scoring of this amplicon. Likewise, the primers OP-I6, OP-I8 and OP-Y10 generated markers with evidence of linkage to *Mj-1* (viz., OP-I6_{700}, OP-I8_{500}, and OP-Y10_{1000}). However, the instability of these RAPD markers precluded their reliable placement on a linkage map. Therefore, the final genetic map included only four RAPD markers. A typical amplicon profile using this set of four primers is displayed in Fig. 1. The linkage of these RAPD markers was confirmed with an F_2 population of 412 individuals.

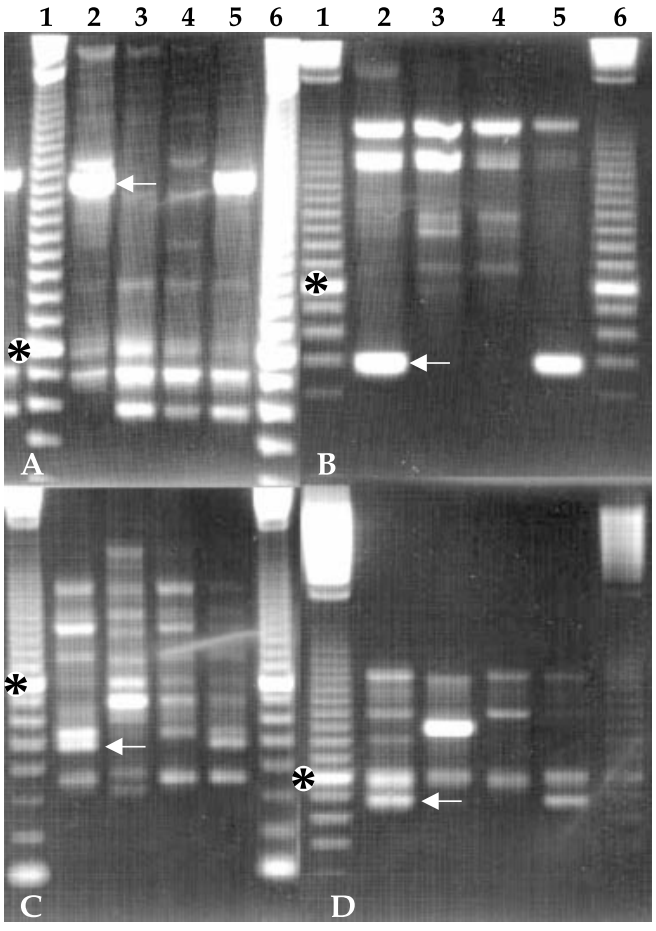

Fig. 1A—D Agarose (1.4%)-gel electrophoresis of RAPD products (amplicons stained with ethidium bromide) where *lanes 1 and 6* = 100-bp size marker, *2* = *M. javanica* resistant parent 'Brasilia 1252', *3* = susceptible parent '6274', *4* = DNA bulk of five susceptible F_2 individuals and *5* = DNA bulk of five resistant F_2 individuals. Panel (**A**) amplicon profile obtained with primer OP-C2 (5'-GTGAGGCGTC-3'); panel (**B**) primer OP-Q6 (5'-GA-CCGCCTTG-3'); panel (**C**) primer OP-AL/(5'-AGGGGACACC-3'); panel (**D**) primer OP-U12 (5'-TCACCAGCCA-3'). *Arrows* indicate polymorphic amplicons observed in RAPD-BSA and F_2 analyses. Size marker is 100-bp ladder. The band with an *asterisk* is the 800-bp size marker

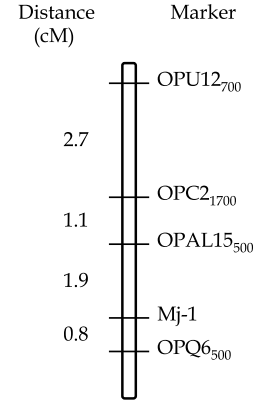

Fig. 2 The RAPD linkage map of the *Mj-1* (*M. javanica* resistance) locus in carrot. The map population was composed by 412 F_2 individuals from a cross between 'Brasilia-1252' (resistant parent) × 'B6274' (susceptible parent). The genetic distance in cM (Kosambi function) between pairs of markers is shown at the left and the RAPD marker designation is shown on the right

Table 1 Phenotypic score of a carrot germplasm collection evaluated for presence (+) or absence (A) of four polymorphic amplicons detected in linkage with the *Mj-1* locus (controlling resistance to *M. javanica*) in the mapping carrot population 'Brasilia –1252' × 'B6274'

Carrot accession	Seed origin	Root type[a]	RAPD markers			
			OP-C2$_{1700}$	OP-Q6$_{500}$	OP-U12$_{700}$	OP-AL15$_{500}$
BR-1252	USDA	C-N	+	+	+	+
6274B	USDA	C-N	A	A	A	A
BR-1091	USDA	C-N	+	+	+	+
Brasilia	EMBRAPA	C-N	+	+	+	+
636–12 (Ri-Rj)[b]	USDA	C-N	+	+	+	+
638–1 (Ri-Sj)	USDA	C-N	A	A	+	A
636–11 (Si-Rj)	USDA	C-N	+	+	+	+
638–2 (Si-Sj)	USDA	C-N	A	A	+	A
493B	USDA	C-N	A	A	A	+
Kuroda	USDA	C-N	+	+	A	+
Savory	USDA	C-N	A	A	A	+
Flakee	USDA	C-N	+	A	A	+
Amsterdam Forcing	USDA	C-N	+	A	A	+
First Class	Asgrow	C-N	+	A	A	+
Premium	Asgrow	C-N	A	A	A	+
Idaho	Bejo	C-N	+	A	A	+
CXC94668	Chriseed	C-N	A	A	A	+
Sunrise	Crookham	C-N	+	A	A	+
XPH97W96	Crookham	C-N	A	A	A	+
975719	Daehnfeldt	C-N	A	A	A	+
975720	Daehnfeldt	C-N	A	A	A	+
975785	Daehnfeldt	C-N	A	A	A	+
975794	Daehnfeldt	C-N	A	+	A	+
Gold Pride	Harris Moran	C-N	+	A	A	+
HMX 5315	Harris Moran	C-N	+	A	A	+
Six-Pak	Harris Moran	C-N	+	A	A	+
Six-PakII	Harris Moran	C-N	+	A	A	+
Six Pence	Harris Moran	C-N	A	A	A	+
Alenka	IV-Lithuania	C-N	+	A	A	+
Khaeskovskaya	IV-Lithuania	C-N	A	A	A	+
RCR2566	Novartis	C-N	A	A	A	+
RCR2567	Novartis	C-N	A	A	A	+
Temptation	Petoseed	C-N	+	A	A	+
Pacific Gold	Sakata	C-N	A	A	A	+
XCR-7239	Sakata	C-N	+	A	A	+
XCR-7240	Sakata	C-N	A	A	A	+
SSC 19098	Shamrock	C-N	A	A	A	+
SSC 19115	Shamrock	C-N	A	A	A	+
Apache	Sunseeds	C-N	+	A	A	+
Choctaw	Sunseeds	C-N	+	A	A	+
Tripleplay 58	Sunseeds	C-N	+	A	A	+
633–4	USDA	C-N	A	A	A	+
738–2	USDA	C-N	+	A	A	+
716–12	USDA	C-N	A	A	A	+
CXC94747	Chriseed	C-N	A	A	A	+
Monique	Crookham	C-N	A	A	A	+
NR.01110	LIH-Lithuania	C-N	+	A	A	+
NR.01163	LIH-Lithuania	C-N	+	A	A	+
NR.01443 A	LIH-Lithuania	C-N	+	A	A	+
Merida	Nunhems	C-N	+	A	A	+
Parano	Nunhems	C-N	+	A	A	+
Starca	Nunhems	C-N	+	A	A	+
RCR 1851-A	Novartis	C-N	+	A	A	+
KXPC-042	Polonica	C-N	+	A	A	+
XCR-7374	Sakata	C-N	A	+	A	+
XCR-7412	Sakata	C-N	A	+	A	+
SSC 19119	Shamrock	C-N	+	A	+	+
SPMO1	Sperling	C-N	+	A	A	+
Bolero	Vilmorin	C-N	+	A	A	+
Concerto	Vilmorin	C-N	+	A	A	+
Maestro	Vilmorin	C-N	+	A	A	+
Presto	Vilmorin	C-N	A	A	A	+
Tempo	Vilmorin	C-N	+	A	A	+
Caro-Pride	Asgrow	C-P	+	A	A	+

Table 1 (continued)

Carrot accession	Seed origin	Root type[a]	RAPD markers			
			OP-C2$_{1700}$	OP-Q6$_{500}$	OP-U12$_{700}$	OP-AL15$_{500}$
XPH18004	Asgrow	C-P	A	A	NE[c]	+
XPH95W94	Crookham	C-P	A	A	A	+
XPH97W99	Crookham	C-P	A	A	A	+
955504	Daehnfeldt	C-P	A	A	A	+
965656	Daehnfeldt	C-P	A	A	A	+
965669	Daehnfeldt	C-P	A	A	A	+
RCR 2566	Novartis	C-P	A	A	A	+
DAU 156	Nunhems	C-P	A	A	A	+
DAU 157	Nunhems	C-P	+	A	A	+
DAU 158	Nunhems	C-P	+	A	A	+
Crispy	Petoseed	C-P	+	A	A	+
Enterprise	Petoseed	C-P	+	A	A	+
Vita-Treat	Petoseed	C-P	+	A	A	+
Grower's Choice	Polonica	C-P	A	A	A	+
KXPC-054	Polonica	C-P	A	A	A	+
KXPC-055	Polonica	C-P	A	A	A	+
KXPC-080	Polonica	C-P	A	A	A	+
REX-190	Sakata	C-P	A	A	A	+
XCR-7240	Sakata	C-P	+	A	A	+
XCR-7248	Sakata	C-P	NE	A	A	+
Primecut 59	Sunseeds	C-P	+	A	A	+
Tripleplay 58	Sunseeds	C-P	+	A	A	+
SRC 3284	Sunseeds	C-P	+	A	A	+
Nantaise	PI 193504	C-N	+	A	A	+
London Torg	PI 205998	C-N	+	A	A	+
Amager #23	PI 225866	C-N	+	A	A	+
Amesterdam #378	PI 225867	C-N	+	A	+	A
Chantenay Red Core	PI 225868	C-N	+	A	+	+
Nantes #20	PI 225870	C-N	A	A	+	+
Touchon # 26	PI 225872	C-N	+	A	A	+
Cape Market	PI 232073	C-N	+	A	A	+
Oxheart	PI 234621	C-N	+	A	A	+
Nantesa	PI 249535	C-N	+	A	+	+
St. Valerio	PI 261614	C-N	+	A	A	NE
Nantejska	USDA	C-N	+	A	+	+
Heritage	Asgrow	Pr	+	A	A	+
XPH97W28	Crookham	Pr	A	A	A	+
Svelia	LIH-Lithuania	Pr	+	A	A	+
RCR1851-A	Novartis	Pr	+	A	A	+
Enterprise	Peto	Pr	+	A	A	+
Kamila	Plantico	Pr	A	A	A	+
Napa	Seedway	Pr	A	A	A	NE
Nevis	Seedway	Pr	+	A	A	+
Neal	Seedway	Pr	+	A	A	+
W276A × WAY273C	UW-Madison	Pr	A	A	A	+
W279A × W276B	UW-Madison	Pr	A	A	A	+
W276A × W280B	UW-Madison	Pr	A	A	A	+
XPH3910	Asgrow	Pr	+	A	A	+
XPH18085	Asgrow	Pr	+	A	A	+
SDC 1443	Campbell	Pr	+	A	A	+
SDC 1682	Campbell	Pr	+	A	A	+
SDC 1742	Campbell	Pr	+	A	A	NE
Goliath	Petoseed	Pr	+	A	A	+
Carson	Seedway	Pr	+	A	A	+
(8532 × 5238) × 186C	USDA	Pr	A	A	A	+
(9304 × 2254) × 186C	USDA	Pr	+	A	A	NE
Danvers 126	NK	Pr	A	A	+	NE

[a] Root type: C-N = Cello-Nantes, C-P = cut and peel; Pr = processing-type

[b] '638–1', '638–2', '636–11', and '636–12' are F_3 lines derived from 'BR-1252 × 'B6274'. The designations Ri and Rj = resistant to *M. incognita* and *M. javanica*, respectively. The designations Si and Sj = susceptible to *M. incognita* and *M. javanica*, respectively

[c] NE = not evaluated

The linkage relationships of these markers and their genetic distances to the *Mj-1* locus are shown in Fig. 2. Their genetic distance to *Mj-1* ranged from 0.8 to 5.7 cM. These dominant markers were found in a coupling configuration and flanking the locus (Fig. 2). Theoretically, it would be possible to detect markers also in repulsion-phase linkage with these DNA bulks since both were composed of F_2:F_3 individuals with genotypes completely classified for the target locus. Some amplicons were indeed found associated with the susceptible bulk but did not provide evidence for repulsion-phase linkage.

The closest amplicon was OP-$Q6_{500,}$ located 0.8 cM from the *Mj-1* locus. This amplicon is most likely the best candidate for marker-assisted selection because of its distance from the *Mj-1* locus and its band intensity (rating 1 to 2). Additionally, scoring is efficient since it has a very distinct electrophoretic pattern without comigrating bands (Fig. 1, panel B). The other nonpolymorphic, slow-migrating amplicons in the profile of the OP-Q6 primer can be used as an internal control to verify whether or not the RAPD reaction was properly performed. It is interesting to note that only nine of the carrot entries had amplicons similar to OP-$Q6_{500}$ in their genomes (Table 1). The marker OP-$U12_{700}$ was also relatively easy to score. It is the fastest migrating band (intensity score = 2) whose amplicon profile is not complex (Fig. 1, panel D). However, OP-$U12_{700}$ is the most distant marker scored (5.7 cM away from the locus).

Not all markers were as easy to score. OP-$AL15_{500}$, for example, has a comigrating band at about 550 bp (Fig. 1, panel C). This characteristic, along with its weak intensity (score = 2 to 3), will limit the genetic use of this marker. Moreover, the presence of bands similar to OP-$AL15_{500}$ in a large number of accessions will limit its usefulness in marker-assisted selection. Likewise, a large number of accessions had similar OP-$C2_{1700}$ profiles, which were also difficult to score. Plants without this marker may have one or two (fainter) bands with similar electrophoretic mobility (Fig. 1, panel A). It has not been determined if one of these bands is an alternate OP-$C2_{1700}$ allele. If PCR is conducted under less-stringent conditions (e.g., primer annealing temperatures below34°C) a brighter band with similar electrophoretic behavior can be observed.

An overall effect of the type of DNA polymerase on the number of amplicons was not systematically evaluated in our experiments. However, our results indicated that Ampli*Taq* DNA polymerase was able to produce more readily scorable amplicons than displayed by *Taq* DNA polymerase. It is important to note that no remarkable differences were observed between enzymes when only amplicons with strong to medium intensity were taken into account. This observation reinforces the notion that one of the major factors associated with the reproducibility of a given RAPD marker is its amplification intensity (Skroch and Nienhuis 1995). The ability to generate more-complex amplicon profiles (even consisting of weak band intensities) may, however, be useful especially in high-resolution local mapping. In our case, two of the polymorphic amplicons (OP-$AL15_{500}$ and OP-$U12_{700}$) linked to the *Mj-1* locus were scored as being of medium-to-weak intensity (Fig. 1, panels C and D). Even though some of these markers may not be useful directly as stable RAPD markers their sequence information can still be used to produce more stable markers. RAPDs with low band intensity can be isolated from the gel, sequence characterized, and converted into a more-stable class of markers. In this context, the development of SCAR primers from these RAPD markers is one applicable strategy to improve both stability and specificity (Paran and Michelmore 1993). A similar approach applies with the putative markers OP-$C2_{420}$ and OP-$I6_{700}$, OP-$I8_{500}$ and OP-$Y10_{1000}$, for which a reliable placement on the map (as RAPD markers) was not possible.

As discussed by Michelmore et al. (1991), the success of the RAPD-BSA approach for the identification of molecular markers will depend on the amount of genetic divergence between the parental lines at the target locus. In our survey, we were able to detect only four reliable amplicons in the vicinity of the *Mj-1* region. These results suggest that this locus is a novel mutation and/or it resides in an evolutionary constrained region of the carrot genome. Nevertheless, the relative tight linkage of these markers to the *Mj-1* locus (Fig. 2) indicates that they might have merit for marker-assisted selection (MAS) for increasing resistance-associated alleles in populations derived from 'Brasilia 1252'. They can also be used to accelerate recovery in backcrossing programs of the recurrent genotype, while minimizing the 'linkage drag' associated with the source of resistance. In fact, the resistant cultivar ('Brasilia') has some undesirable characteristics including precocious bolting, green shoulder, and low-to-intermediate root carotene content (Padua et al. 1984; Giordano 1991). The linkage relationships of these undesirable traits with *Mj-1* locus have still to be determined.

The usefulness of these markers to assist the transfer of resistance into different genetic backgrounds was estimated by analyzing a large number of carrot accessions (Table 1). The results demonstrated that, with few exceptions, this collection of markers (as a group) is readily applicable for linkage analysis and marker-assisted selection (MAS) in 'Brasilia 1252'–derived germplasm (Table 1). In fact, only five accessions (all derived from 'Brasilia 1252' and resistant to *M. javanica*) had the simultaneous presence of all markers in their genomes (Table 1). Therefore, one (or more) of these RAPD markers may be valuable in MAS for the incorporation of resistance into currently available elite carrot germplasm. It is interesting to note that OP-$AL15_{500}$ and OP-$C2_{1700}$ occurred in 114 out of 118 and 71 out of 120 entries, respectively, while OP-$Q6_{500}$ and OP-$U12_{700}$ occurred only in 9 out of 121 and 14 out of 120 entries, respectively. It seems unlikely that the high incidence of the first two markers is due to recombination around the

Mj-1 locus, especially in view of the low incidence of the more-distant marker (OP-U12$_{700}$). Another plausible explanation is that OP-AL15$_{500}$ and OP-C2$_{1700}$ are duplicated elsewhere in the carrot genome and the susceptible parent used for mapping ('B6274') happens to lack both of these RAPD amplicons. The development of SCAR markers for these RAPD loci, in conjunction with a more extensive carrot mapping using distinct genetic backgrounds, could clarify the genetic nature of this observation. Although few of the entries listed in Table 1 have been evaluated for *M. javanica* resistance, its occurrence is very rare (Roberts et al., unpublished). Therefore, it would be important to determine whether the entries bearing RAPD amplicons associated with the *Mj-1* locus are, in fact, resistant.

The original 'Brasilia' population is also source of resistance against another important root-knot nematode, *M. incognita* (Charchar and Vieira 1994). Our analysis using F_3 lines derived from a similar genetic background (and segregating for the reaction against both nematodes) provided insights regarding the organization of the root-knot nematode resistance genes in this cultivar. The F_3 lines '638–1' (moderately resistant to *M. incognita* but susceptible to *M. javanica*) and '638–2' (susceptible to both pathogens) did not share three of the *Mj-1* markers (OP-C2$_{1700}$, OP-Q6$_{500}$, and OP-AL15$_{500}$). On the other hand, the lines '636–11' (susceptible to *M. incognita* and resistant to *M. javanica*) and '636–12' (resistant to both pathogens) displayed all the polymorphic RAPD markers. Therefore, all four lines had in common only the most-distant marker (OPU12$_{700}$). These preliminary results suggest that genes controlling resistance to *M. incognita* and *M. javanica* reside at distinct loci, which may be located relatively close together in the same linkage group as indicated by the overlapping of OPU12$_{700}$.

The high number of scorable amplicons (approximately 2400) obtained with RAPD-BSA in our assay can be employed for subsequent general linkage mapping work to generate new landmarks throughout the carrot genome. The population employed in the present study is well-suited for this purpose since both parental lines also display a large amount of phenotypic diversity for several traits of agronomic interest (Padua et al. 1984; Giordano 1991). In addition, the present study was able to identify a large number of primers with poor or no amplification (309 out 1000). This database can be used in future RAPD mapping work as a guideline to select particular sub-sets of genetically more informative primers thus increasing the genome scanning efficiency in this carrot population.

The isolation of the first two genes conferring resistance to plant parasitic nematodes was achieved by using a positional cloning approach (Cai et al. 1997; Milligan et al. 1998). However, a major requirement before initiating any map-based cloning project is the identification of a large collection of markers spanning the target locus. This 'chromosome landing' approach has been successfully employed for disease resistance gene isolation in near-saturated genomic regions in *Arabidopsis thaliana* and tomato (Young and Phillips 1994; Tanksley et al. 1995). Development of dense molecular maps employing isozyme, RAPD, RFLP, and AFLP markers are under construction using diverse carrot populations (Westphal and Wricke 1991; Schulz et al. 1994; Vivek and Simon 1999). These maps include the genetic location of important loci controlling root quality and other phenotypic traits of interest for carrot breeding and genetics (Vivek and Simon 1999). The work reported here is, however, the first systematic attempt to map a locus conditioning disease resistance in carrot.

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