Theor Appl Genet (1999) 99: 115–122

Z.-X. Lu · K. Sossey-Alaoui · G. L. Reighard
Wm. V. Baird · A. G. Abbott

Development and characterization of a codominant marker linked to root-knot nematode resistance, and its application to peach rootstock breeding

Received: 4 January 1999 / Accepted: 4 January 1999

Abstract The presence of a codominant AFLP marker, EAA/MCAT10, correlates with the primary source of resistance to root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) in rootstock cultivars of peach [*Prunus persica* (L.) Batsch]. Two allelic DNA fragments of this AFLP marker were cloned, sequenced and converted to sequence tagged sites (STS). Four nucleotide differences (i.e. one addition and three substitutions) were observed between the two clones. Furthermore, there was a diagnostic *Sau*3 AI cleavage site (GATC) in the large fragment that was absent from the small fragment (GTTC at this site). The applicability of this STS marker system to peach germplasm improvement was evaluated: genomic DNAs of cross parents (i.e. 'Lovell' and 'Nemared'), four F_1 hybrids (K62-67, K62-68, P101-40 and P101-41) and two F_2 populations (from K62-68 and P101-41), as well as DNA from a test panel of 18 rootstock cultivars or selections, were PCR-amplified with the Mij3F/Mij1R primer pair and then digested with *Sau*3 AI. The banding patterns showed that the EAA/MCAT10 STS markers can clearly distinguish the three genotypes – homozygous resistant, heterozygous resistant and homozygous susceptible – in the 'Lovell' × 'Nemared' cross. In addition, results from the rootstock survey were consistent with each rootstock's phenotypic response to nematode infection, except for 'Okinawa', 'Flordaguard' and 'Yunnan' where root-knot resistance may have arisen independently. Therefore, the EAA/MCAT10 STS markers will be a useful tool to initiate marker assisted selection studies in peach rootstock breeding for root-knot nematode resistance.

Key words Amplified fragment length polymorphism Nematode resistance · *Meloidogyne incognita* · *Meloidogyne javanica* · *Prunus persica*

Technical contribution no. 4416 of the South Carolina Experiment Station, Clemson University

Communicated by M. A. S. Maroof

Z.-X. Lu · G. L. Reighard (✉) · Wm. V. Baird
Department of Horticulture, Clemson University, Clemson, SC 29634-0375, USA
Fax: +1 864 656 4960
E-mail: grghrd@clemson.edu

K. Sossey-Alaoui · A. G. Abbott
Department of Biological Sciences, Clemson University, Clemson, SC 29634-1901, USA

Introduction

Molecular markers are of interest to plant geneticists and breeders as a source of new genetic information on plant genomes and for use in trait selection. Marker-assisted selection (MAS) dictates that selection of one or more traits of interest be conducted indirectly by selecting for markers linked to the trait(s) (Melchinger 1990). MAS is most efficient when selection for the marker is convenient and there is tight linkage between the marker and the trait of interest (Kelly 1995). Markers for disease resistance offer the additional advantage of permitting selection for resistance in the absence of the pathogen(s) (Mehlenbacher 1995).

Amplified fragment length polymorphism (AFLP) analysis is a relatively new advancement in DNA-based marker technology, which rests on the selective polymerase chain reaction (PCR) amplification of restriction fragments from a total digest of genomic DNA (Vos et al. 1995). This marker system combines the strengths of restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNAs (RAPDs), and offers a reproducible and comparatively cost-effective approach to detect DNA polymorphism, develop genetic maps or generate DNA fingerprints in

species with large genomes and/or low genetic diversity (Ballvora et al. 1995; Cervera et al. 1996; Cnops et al. 1996; Meksem et al. 1995; Voorrips et al. 1997).

Compared to major field crops, much less effort is devoted to perennial fruit or nut crop improvement. Breeding programs for many fruit crops have been underway for only a short time relative to the length of the breeding cycle (Mehlenbacher 1995). The long generation time of fruit trees greatly limits the amount of genetic improvement per year, and their large size makes each increment of improvement expensive. Fruit tree breeding is made even more difficult by changes that occur during the transition from juvenility to maturity and the use of separate scion and rootstock to achieve commercial production (Baird et al. 1996). Therefore, methods to improve selection efficiency in fruit tree breeding would be of considerable value, and the application of MAS appears to be a powerful approach to overcome many limitations.

Peach [*Prunus persica* (L.) Batsch] is a member of the Rosaceae, which contains many important fruit, nut and ornamental species. Currently, research efforts in the United States and Europe are focused on developing maps for peach geneticists and breeders to tag fruit quality, tree architecture and pest/disease resistance traits of economic importance for peach production or on developing a core *Prunus* map to provide 'generic' landmarks and identify phylogenetically conserved islands of chromosome homology among species (Arús et al. 1994; Chaparro et al. 1994; Dirlewanger and Bodo 1994; Foolad et al. 1995; Lu et al. 1998b; Rajapakse et al. 1995; Stockinger et al. 1996; Viruel et al. 1995). Thus, practical applications of molecular markers for genetic linkage mapping promise to make peach a model for the identification and isolation of agriculturally important genes in perennial tree fruit species (Baird et al. 1996). Although the identification of RAPD markers linked to the *Ma1* gene in Myrobalan plum has been reported (Dirlewanger et al. 1996; Salesses et al. 1998), no sequence-characterized (SCAR) or sequence-tagged site (STS) markers for use in MAS have been reported for any *Prunus* species.

Root-knot nematodes (*Meloidogyne* spp.) are serious pathogens in agriculture (Fassuliotis 1987; Sasser 1980). Genetic studies of resistance to root-knot nematodes in different crops show that inheritance patterns vary from simple to complex. In most cases, resistance behaves as either a single dominant or two dominant gene(s), such as the *Mi* gene in tomato (*Lycopersicon esculentum* Mill.) (Gilbert and McGuire 1956; Ho et al. 1992; Klein-Lankhorst et al. 1991), the *Mae* and *Mag* genes in peanut (*Arachis hypogaea* L.) (Garcia et al. 1996) or the *Ma1* and *Ma2* genes in Myrobalan plum (*P. cerasifera* L.) (Esmenjaud et al. 1996, 1997; Lecouls et al. 1997).

Peach rootstocks are susceptible to two species of root-knot nematodes [i.e *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood] in the southeastern United States (Nyczepir 1991). Some cultivars or selections, such as 'Nemaguard', 'Nemared', 'Flordaguard', 'Okinawa' etc., show various levels of resistance (Layne 1987; Lu et al. 1996; Okie et al. 1994; Ramming and Tanner 1983; Scorza and Sherman 1996; Sharpe et al. 1969; Sherman et al. 1991). Lu et al. (1998a) proposed a two-gene model for resistance to root-knot nematodes. Genetic analysis indicated that resistance to *M. incognita* and resistance to *M. javanica* are controlled by two dominant genes (*Mi* or *Mij*; and *Mj* or *Mij*, respectively), where the 'shared' gene (*Mij*) may be required for resistance to both species (Lu 1997; Lu et al. 1998a). This model was derived as a best fit for an analysis of several F_2 progenies segregating for resistance to *M. incognita* and *M. javanica*, however, it does not rule out the possibility of a model for each species having a single dominant gene with segregation distortion (Lownsbery and Thomson 1959). Further genetic studies are needed to determine the true genetic nature of resistance to root-knot nematodes in peaches.

It would be very useful to introgress root-knot nematode resistance genes into elite rootstock germplasm. However, the extended juvenile stage of peach impedes such progress by traditional methods. Also, it is difficult to observe and select resistance traits (i.e. phenotypes) of a rootstock after grafting and planting. Obviously, the application of MAS can greatly improve the efficiency of peach breeding for resistance to root-knot nematodes. In a previous mapping study (Lu et al. 1998b), a codominant AFLP marker (EAA/MCAT10) was found to be tightly linked to the putative *Mij* locus. The objective of the study reported here was to demonstrate the practical application of an AFLP-derived STS marker for germplasm screening and breeding resistance to root-knot nematodes in peach.

Materials and methods

Two peach rootstocks, 'Lovell' and 'Nemared', were used as parents by D. W. Ramming at the USDA-ARS, Horticultural Crops Research Laboratory (Fresno, Calif.) to produce four F_1 hybrids: K62-67, K62-68, P101-40, and P101-41. 'Lovell', a widely used rootstock originating as a chance peach seedling from California in 1882, is homozygous susceptible to *M. incognita* and *M. javanica*. In contrast, 'Nemared', a newly released rootstock originating from a F_3 seedling of 'Nemaguard' crossed with a red leaf selection, is homozygous resistant to these two root-knot nematodes (Ramming and Tanner 1983). F_2 populations were produced from K62-68 and from P101-41 (55 and 100 individuals, respectively) by T. G. Beckman at the USDA-ARS, Fruit and Tree Nut Research Laboratory (Byron, Ga) in 1995. The F_2 self-pollinated seeds were stratified at 4°C for 2 months, then the germinating seeds were planted in 12-cm-diameter plastic pots filled with approximately 1 200 cm^3 sand/vermiculite medium (50 : 50 v/v) and the subsequent seedlings were grown in the greenhouse. Bioassays were conducted to measure nematode resistance by scoring each F_2 phenotype for the number of galls and for the number of egg masses produced by each nematode species. Esterase isoenzyme analysis was used to identify the nematode species in dual infections (Lu 1997).

Young leaves (< 4 weeks old) were collected from the two parents, the four F_1 hybrids and each seedling of the two F_2 families. Similarly, young leaves (< 6 weeks old) were collected in 1994 from 12 other peach rootstock cultivars or selections (i.e. 'Nemaguard', SL1089, SL1090, 'Okinawa', 'Flordaguard', 'Higama', 'Yunnan', 'Halford', GF305, 'Rubira', 'Harrow Blood' and 'Montclar') at Clemson University's Sandhill Research and Education Center (Columbia, S.C.) or the USDA-ARS, Fruit and Tree Nut Research Laboratory. All leaf samples were refrigerated during transportation to the laboratory and subsequently stored at − 80°C until processed. Genomic DNA was isolated from the leaf samples using a modified CTAB method (Eldredge et al. 1992). DNA concentrations were measured using a minifluorometer (Hoefer Scientific, San Francisco, Calif.). Working solutions of genomic DNA at 100 ng/µl in TE buffer (pH 8.0) were prepared for AFLP analysis and/or the subsequent PCR reaction. AFLP core reagent and starter primer kits were purchased from Life Technologies (Gibco BRL, Gathersburg, Md.), and AFLP analysis was performed as described by the manufacturer or modified by Lu et al. (1998b).

Based on previous identification of the AFLP EAA/MCAT10 (Lu et al. 1998b), the region containing the DNA fragments was cut from the dry acrylamide gel and re-hydrated in 100 µl of distilled H_2O for 1 h at 4°C to extract the DNA template. The AFLP fragments were reamplified using the same primers (i.e. *Eco*RI + AA adapter and *Mse*I + CAT adapter) as in the original AFLP analysis in a total volume of 25 µl containing 10 µl template solution, 1.5 U *Taq* DNA polymerase (Promega, Madison, Wis.), 5 m*M* $MgCl_2$ and 200 µ*M* dNTP nucleotides (Perkin-Elmer/Cetus, Norwalk, Conn.) in reaction buffer [10 m*M* TRIS-HCl (pH 8.3), 50 m*M* KCl]. The amplification was performed in an automated thermal cycler (model 480, Perkin-Elmer/Cetus) programmed for 35 cycles of 1 min at 94°C, 1 min at 56°C and 2 min at 72°C. An aliquot of the amplified products was resolved by agarose gel electrophoresis to confirm the expected fragment sizes of the EAA/MCAT10 codominant markers. pGEM DNA size standards (Promega) were used to estimate fragment sizes.

The amplification reaction solution was extracted using phenol/chloroform, and the DNA precipitated with ethanol and resuspended in 10 µl TE buffer. These amplification products were cloned into the pGEM vector using a T/A cloning kit (Clontech, Palo Alto, Calif.), following the manufacturer's recommendations. Sequencing of both strands of the clones (10 each) was performed using a Dye Terminator Cycle DNA Sequencing Kit (Perkin-Elmer/Cetus), and these were analyzed on an ABI 377 automated sequencer (PE Applied Biosystems, Foster City, Calif.). Six 20-mer oligonucleotide primers were designed and synthesized according to the sequence of the EAA/MCAT10 marker clones (Fig. 2).

Amplifications of the EAA/MCAT10 marker in the K62-68 and the P101-41 families and in other peach rootstock cultivars or selections were each carried out in a total volume of 25 µl containing 5 µl genomic DNA template, 0.5 µ*M* of each primer pair, 1.5 U of *Taq* DNA polymerase (Promega), 5 m*M* $MgCl_2$, and 200 µ*M* dNTP nucleotides (Perkin-Elmer/Cetus) in reaction buffer [10 m*M* TRIS-HCl (pH 8.3), 50 m*M* KCl]. The amplification was performed as described above. Then, 10 µl of the reaction solution was transferred to a new 0.5-ml tube and digested with 5 U of the *Sau*3 AI restriction enzyme (Promega) as recommended by the manufacturer. The digested products were sized-fractionated by electrophoresis on 1.5% agarose gels, and the DNA fragments were visualized by ethidium bromide staining. pGEM DNA markers (Promega) were used to estimate fragment sizes. Data for the EAA/MCAT10 marker in both K62-68 and P101-41 families and other peach rootstocks were scored as a codominant marker and analyzed using chi-square tests from the statistical analysis system (SAS Institute, Cary, N.C.).

Results

Molecular and genetic characteristics of the AFLP EAA/MCAT10 marker

As reported earlier (Lu et al. 1998b), the EAA/MCAT10 marker was scored as two adjacent amplified DNA fragments in AFLP analysis (i.e., 217 bp and 216 bp) (Fig. 1). EAA/MCAT10 correlated well with the resistance or susceptibility phenotypes of the parents ('Lovell' and 'Nemared'), the F_1 hybrid and 55 F_2 progeny in the K62-68 family. For example, plants homozygous susceptible to *M. incognita* and *M. javanica* display the larger DNA fragment, while plants

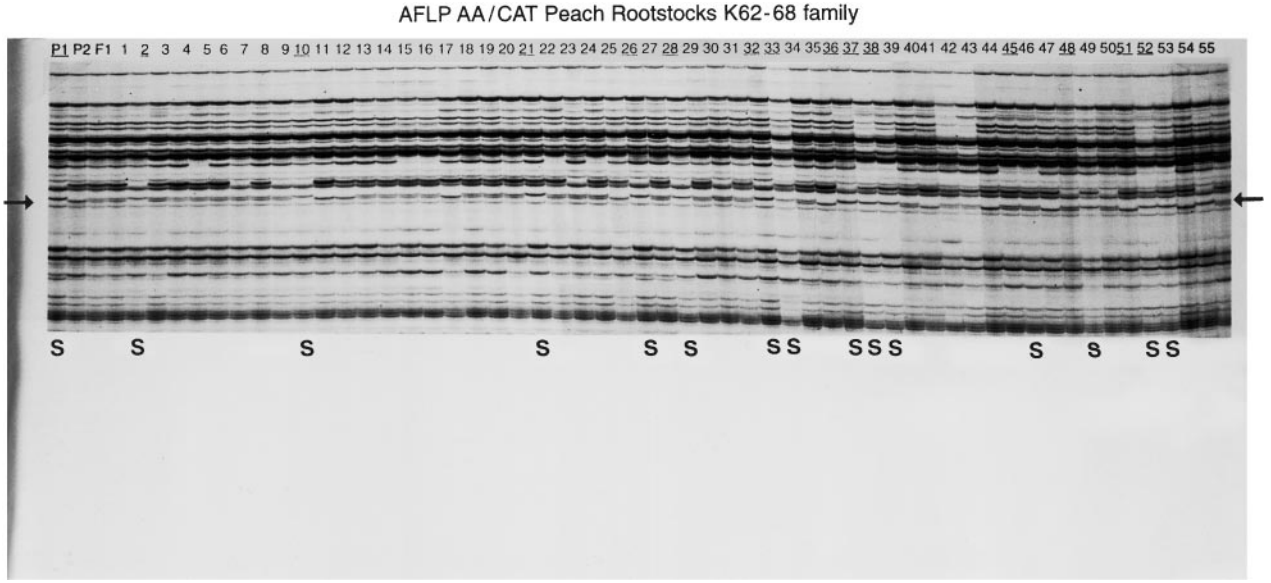

Fig. 1 DNA banding patterns of the codominant AFLP marker EAA/MCAT10 (*arrows*) in an analysis of individuals from the K62-68 family. *Lane 1* (*P1*) the susceptible (S) parent, 'Lovell', *lane 2* (*P2*) the resistant (R) parent, 'Nemared', *lane 3* the F_1 hybrid (H). The segregating F_2 individuals are in *lanes* 1-55. Susceptible individuals are *underlined* and indicated by an *s below* each *lane*

homozygous resistant to *M. incognita* and *M. javanica* display the smaller DNA fragment. Furthermore, plants heterozygous resistant to *M. incognita* and *M. javanica* (i.e., the F_1 hybrid and approximately one-half of the segregating F_2 progeny) displayed both DNA fragments.

Cloning of the two AFLP EAA/MCAT10 marker bands was carried out, and sequencing revealed four nucleotide differences (i.e. one addition and three substitutions) between the DNA sequences of the fragments (Fig. 2). These were: (1) the larger fragment had an additional adenine (A) at position 12; (2) the larger fragment had guanine (G) at position 69, whereas the smaller fragment had A; (3) the larger fragment had A at position 108, whereas the smaller fragment had thymine (T); and (4) the larger fragment had A at position 174, whereas the smaller fragment had G. Restriction site analysis showed that the larger fragment contained a *Sau*3 AI recognition sequence (GATC) at the 107th–110th position, which was absent at this position in the smaller fragment (Fig. 2). BLAST server nucleic acid sequence database searches failed to reveal significant sequence similarity of this marker's sequences to other characterized sequences.

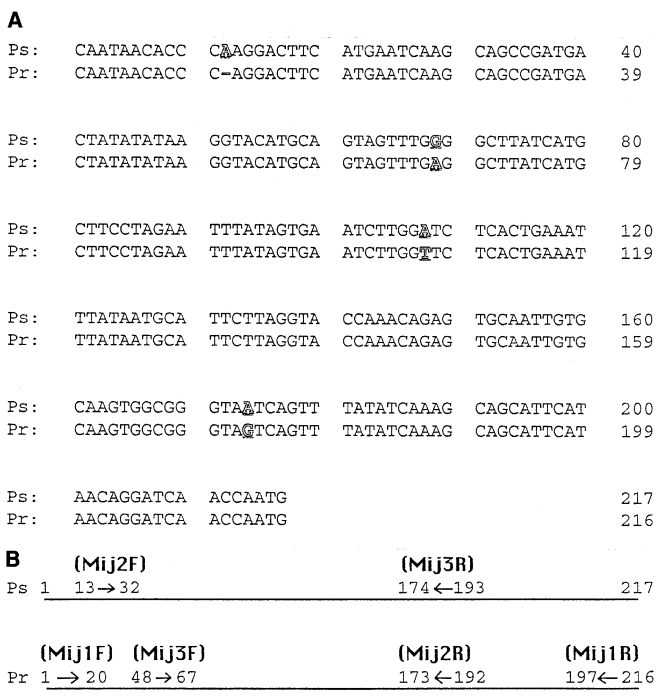

Fig. 2A, B DNA sequences of the EAA/MCAT10 alleles (**A**), and six PCR primer sequences (**B**) used for amplifying peach rootstock genomic DNAs. Four nucleotide differences are indicated (i.e., one addition/deletion, and three substitutions). *Ps* indicates the sequence obtained from the susceptible parent (Lovell), and *Pr* indicates the sequence obtained from the resistant parent (Nemared)

Conversion of the EAA/MCAT10 marker to a PCR-based marker

Six PCR primers (i.e. Mij1F, Mij2F, Mij3F, Mij1R, Mij2R and Mij3R), and therefore nine possible primer combinations, were used to amplify the EAA/MCAT10 locus using genomic DNAs from both the K62-68 and P101-41 F_2 families as template. DNA amplification with Mij1F/Mij1R, Mij1F/Mij2R or Mij1F/Mij3R was not as consistent as that with the other six primer combinations. Mij2R and Mij3R were originally designed to amplify only resistant genotypes or susceptible genotypes, respectively, based upon a single nucleotide difference at their 3′-ends. However, results showed that Mij2R could amplify susceptible genotypes; and that Mij3R could amplify resistant genotypes (in combinations with Mij2F or Mij3F). Therefore, among the nine possible primer combinations, Mij2F/Mij1R and Mij3F/Mij1R were best suited for PCR amplification of peach genomic DNAs.

With the Mij2F/Mij1R or Mij3F/Mij1R primer combination, one major DNA band was amplified from each individual of the K62-68 and the P101-41 families (Fig. 3). In heterozygous resistant genotypes, this band actually contained two DNA fragments of equal size (i.e. 170 bp = 217 − 47; = 216 − 46; Fig. 2). However, the two fragments could be distinguished by digesting the PCR amplification products with *Sau*3 AI. For example, after amplification with Mij3F/Mij1R and the *Sau*3 AI digestion, susceptible genotypes displayed two smaller DNA fragments (i.e. 60 and 110 bp), homozygous resistant genotypes displayed only one

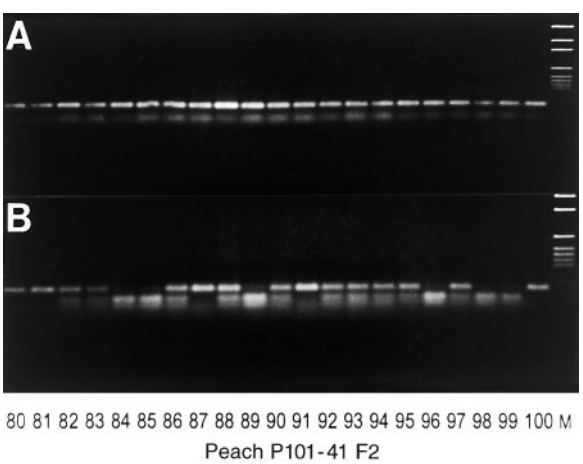

Fig. 3A, B Comparison of DNA banding patterns before (**A**) and after (**B**) *Sau*3 AI digestion of the PCR products amplified by the Mij3F/Mij1R primer combination. Twenty-one F_2 individuals from the P101-41 family are shown in this example. The DNA patterns in *lanes 1-4* (tree nos. 80-83), 7-9 (nos. 86-88), 11-16 (nos. 90-95), 18 (no. 97) and *21* (no. 100) are from plants displaying the resistance (R) phenotype. The DNA patterns in lanes *5* and *6* (tree nos. 84 and 85), 10 (no. 89), 17 (no. 96) and 19 and 20 (nos. 98 and 99) are from plants displaying the susceptible (S) phenotype. *M* indicates the lane containing the pGEM molecular size markers

DNA fragment (i.e. 170 bp), and heterozygous resistant genotypes had three fragments; both the single large undigested DNA fragment and the two smaller fragments produced by digestion (Fig. 3).

Application of the EAA/MCAT10 markers in peach F_2 populations

The F_2 progeny of K62-68 were examined with the PCR-based amplification primers Mij3F/Mij1R. The DNA banding patterns of PCR amplification products following *Sau*3 AI digestion were consistent with the AFLP EAA/MCAT10 codominant marker and correlated well with the nematode response phenotype of each plant. Interestingly, two recombined genotypes (i.e. K62-68-45 and K62-68-54) between the EAA/MCAT10 and the *Mij* loci were confirmed with the Mij3F/Mij1R primers (Fig. 4). The results indicated that the 55 F_2 genotypes consist of nine homozygous resistant, 31 heterozygous resistant, 13 homozygous susceptible, one recombined resistant (two small fragments; Fig. 4, lane 20) and one recombined susceptible (three fragments, Fig. 4 lane 19). Statistical analysis with the chi-square test revealed that the observed segregation ratio fit the 1:2:1 for a codominant marker ($P \leq 0.05$).

One hundred F_2 individuals of the P101-41 family were examined with the Mij3F/Mij1R primer combination. The DNA banding patterns of PCR amplification products after *Sau*3 AI digestion (Fig. 3) correlated well with the known resistant or susceptible phenotypes, which were previously evaluated in an inheritance study under greenhouse conditions (Lu et al. 1998a).

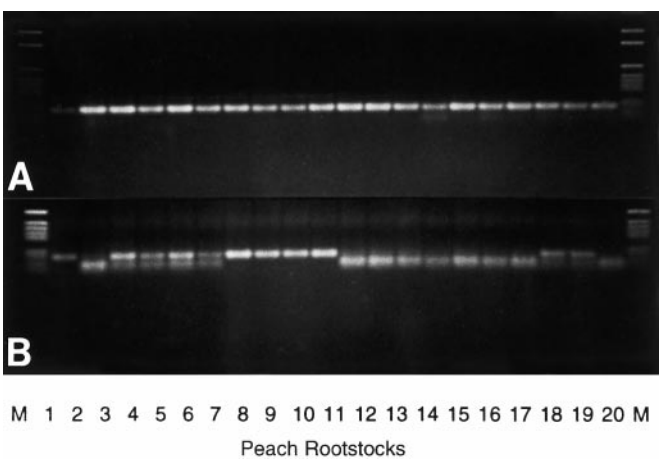

Fig. 4A, B Comparison of DNA banding patterns from peach rootstocks before (**A**) and after (**B**) *Sau*3 AI digestion of the PCR products amplified by the Mij3F/Mij1R primer combination. *Lane 1* 'Nemared', *2* 'Lovell', *3* K62-67, *4* K62-68, *5* P101-40, *6* P101-41, *7* 'Nemaguard', *8* SL1089, *9* SL1090, *10* 'Yunnan', *11* 'Halford', *12* GF 305, *13* 'Okinawa', *14* 'Flordaguard', *15* 'Rubira' *16* 'Harrow Blood', *17* 'Montclar', *18* 'Higama', *19* K62-68–45, *20* K62-68-54. *M* indicates the lane containing the pGEM molecular size markers

Three recombined genotypes (i.e. P101-41-71, P101-41-79 and P101-41-84) between the EAA/MCAT10 marker and the *Mij* locus were detected. Overall, results showed that the 100 F_2 genotypes consisted of 26 homozygous resistant, 54 heterozygous resistant, 17 homozygous susceptible and the three recombined resistant. As with the K62-68 family, statistical analysis of the P101-41 family with the chi-square test ($P \leq 0.05$) revealed that the observed segregation ratio fit the expected 1:2:1 for a codominant marker.

Verification of the EAA/MCAT10 marker in peach rootstock germplasm

In order to evaluate the broad applicability of the EAA/MCAT10 marker for screening and breeding peach rootstocks for resistance to root-knot nematodes, a total of 18 cultivars or selections were tested with the Mij3F/Mij1R primer combination. As anticipated, DNA banding patterns of the PCR amplification products following *Sau*3 AI digestion and size fractionation on agarose gels were basically consistent with the known resistance or susceptibility phenotypes of the rootstocks (Fig. 4). For example, 'Nemaguard', 'Nemared', SL1089 and SL1090, which are all resistant to *M. incognita* and *M. javanica*, displayed a single major undigested DNA fragment of 170 bp in size. On the other hand, 'Lovell', 'Halford', GF305, 'Rubira', 'Harrow Blood' and 'Montclar', all susceptible to *M. incognita* and *M. javanica*, displayed only the two smaller digested DNA fragments of 110 bp and 60 bp. Four F_1 selections (i.e. K62-67, K62-68, P101-40, and P101-41), all heterozygous resistant to *M. incognita* and *M. javanica*, displayed both the large, undigested amplification product and the two smaller, digested fragments. 'Higama' displayed the same DNA amplification banding pattern as the four F_1 selections. Therefore, 'Higama' is interpreted as a heterozygous resistant genotype. Three inconsistent amplification patterns were obtained. The DNA banding patterns of 'Okinawa' and 'Flordaguard', both considered resistant to *M. incognita* and *M. javanica*, showed only the two smaller DNA fragments following digestion with *Sau*3 AI (Fig. 4). This banding pattern was unexpected for peach rootstocks resistant to both root-knot nematode species. Additionally, 'Yunnan' displayed a banding pattern that suggests it is homozygous for the resistance allele of *Mij*. This is inconsistent with it being susceptible to *M. javanica*.

Discussion

Basic PCR techniques are sensitive to changes in reaction conditions (Weeden et al. 1992; Yang et al. 1997). As such, it is difficult to determine if the absence of an

amplification product(s) is due to the genetic nature of the sample or due to unfavorable reaction conditions. Dominant molecular markers, such as RAPDs or AFLPs, are less informative for map development and MAS than are codominant markers (e.g. RFLPs or STS) because heterozygotes can not be identified. Furthermore, AFLP analysis is a relatively complicated procedure, requiring highly specialized gel technologies. Thus, it is not well suited for large-scale screening of breeding materials in germplasm improvement programs. Therefore, it is desirable to convert AFLP markers to more stable and reliable codominant STS markers for ease and specificity of analysis. For example, such conversions were successfully applied in tomato (Williamson et al. 1994) and potato (Meksem et al. 1995). Therefore, conversion of the AFLP marker linked to the *Mij* locus to a STS marker and addition of a restriction enzyme digestion step was undertaken. The strategy used in this study was first to produce amplified PCR products from each genomic DNA, then to detect the polymorphism by *Sau*3 A I digestion. EAA/MCAT10 is a codominant marker in AFLP analysis by virtue of a single nucleotide length difference (i.e. 216 bp vs. 217 bp), which makes detection problematic. After conversion to a PCR-based STS marker, EAA/MCAT10 retains its codominant nature following amplification and subsequent enzyme digestion, and the variation in fragment lengths (i.e. 170 bp vs. 110 bp and 60 bp) is more easily detected in agarose gels. The relative ease of using this marker in MAS is expected to facilitate peach rootstock breeding for resistance to root-knot nematodes.

MAS can be a powerful tool to overcome some of the limitations or disadvantages of traditional breeding methods, especially for perennial tree fruit species (e.g. long generation time and large plant size) (Mehlenbacher 1995). MAS efficiency is greatly improved when selection for the molecular marker(s) is more rapid and convenient than traditional selection for the trait (Kelly 1995). The EAA/MCAT10 STS marker developed and characterized in this study appears very informative for MAS. EAA/MCAT10 is a codominant marker and clearly distinguishes the three genotypes: homozygous resistant (RR), heterozygous resistant (Rr), and homozygous susceptible (rr) to root-knot nematodes. Therefore, 75% of the F_2 seedlings with Rr and rr genotypes, or 50% of the backcross (B_1) seedlings with the Rr genotype could be immediately culled according to DNA banding patterns following specific PCR amplification and *Sau*3 AI digestion. Genomic DNAs can be isolated from leaves of young F_2 or B_1 seedlings, and DNA banding patterns of the EAA/MCAT10 marker can be determined quickly. The efficiency of MAS also depends on the degree of genetic linkage between the marker and the trait of interest (Melchinger 1990). In this study, the frequency of recombination between the EAA/MCAT10 marker and the *Mij* locus in the K62-68 family (55 individuals) was 0.036, and in the P101-41 family (100 individuals) the recombination frequency was 0.030. These results showed the genetic distance to be consistent between the two F_2 populations derived from the cross of 'Lovell' × 'Nemared', and that this codominant marker is tightly linked to the *Mij* locus (approx. 3.2 cM). Therefore, MAS with this STS marker has the potential to greatly reduce the time, space and cost required for maintaining and evaluating large segregating populations in peach breeding programs.

The DNA banding patterns of the EAA/MCAT10 marker agree well with the resistance or susceptibility phenotypes of peach rootstock germplasm examined in this study. The susceptible rootstocks (e.g. 'Lovell', 'Halford', GF305, 'Rubira', 'Harrow Blood' and 'Montclar') all had the two small, restriction-digested DNA fragments. Therefore, their genotypes should be homozygous recessive (*mijmij*) for the "shared" root-knot nematode response locus, *Mij*. This supports findings from previous evaluations of resistance to root-knot nematodes under greenhouse and field conditions (Layne 1987; Scorza and Sherman 1996). 'Nemaguard', 'Nemared', SL1089, SL1090, all resistant to *M. incognita* and *M. javanica*, display the single large, non-restrictable DNA fragment, and therefore their genotypes should be homozygous dominant (*MijMij*) at the *Mij* locus. This is in agreement with their putative pedigrees, since 'Nemared' is a F_3 seedling of 'Nemaguard' (Ramming and Tanner 1983), and since SL1089 and SL1090 are offspring of the BY520-9 selection, originally derived from 'Nemaguard' (Okie et al. 1994).

Two resistant genotypes ('Okinawa' and 'Flordaguard') unexpectedly displayed the homozygous susceptible DNA banding pattern. Similarly, a susceptible genotype ('Yunnan') displayed the homozygous *Mij*-resistant banding pattern. Since the EAA/MCAT10 marker and the *Mij* locus are tightly linked, it is unlikely, though possible ($P \leq 0.001$), that both 'Okinawa' and 'Flordaguard' are homozygous recombined resistant genotypes. Similarly, this could be argued for the case of susceptibility to *M. javanica* in 'Yunnan'. Alternatively, these data may suggest that the source of resistance in these genotypes is different than that of 'Nemaguard' and that the resistance source(s) do not possess the EAA/MCAT10 polymorphism. 'Nemaguard' and 'Okinawa' are two major sources for genetic resistance to root-knot nematodes in peach. 'Nemaguard' originated from a commercial seed lot mislabeled *Prunus davidiana* (Okie et al. 1985), and it may be a hybrid of *P. persica* and *P. davidiana*. 'Okinawa' originated from chance peach seedlings first introduced from Japan to Florida (Sharpe et al. 1969). Therefore, it appears that, based on DNA banding patterns of the EAA/MCAT10 marker, the genetic source of resistance to root-knot nematodes could be different between 'Nemaguard' and 'Okinawa'. Furthermore, 'Flordaguard', an F_3 seedling of 'Okinawa' (Sherman et al. 1991), had the same DNA banding

pattern as 'Okinawa', consistent with both rootstocks carrying the same resistance locus/loci. A similar argument could also be made for the 'Yunnan' genotype, a peach introduction from China.

Therefore, the EAA/MCAT10 marker system may not be suitable to conduct MAS in segregating populations originating or derived from 'Okinawa' and 'Yunnan' resistance sources. Alternatively, because rootstock cultivars are seed-propagated, our source of 'Okinawa' and 'Yunnan' may be rare recombinants. Further studies on comparing segregation patterns of resistance to *M. incognita* and/or *M. javanica* in both 'Nemaguard'-, 'Okinawa'- and 'Yunnan'-derived populations are necessary both to verify the hypothesized genotypes as well as to fully appreciate the usefulness of EAA/MCAT10 in breeding peach rootstocks for resistance to root-knot nematodes.

Acknowledgments We thank Drs. D. W. Ramming (USDA-ARS, Fresno, Calif.) and T. G. Beckman (USDA-ARS, Byron, Ga.) for supplying the parents of the cross and the F_1 hybrids used in this study; and Bryon Sosinski for expert technical assistance and for reviewing the original manuscript. This work was supported by grants from the USDA-NRICGP (Plant Genome; 93373008818) to WVB and AGA, and from the USDA-CSREES (Special Grants, 97341263984) to GLR. All experiments conformed to the laws and guidelines for scientific research in the United States.

References

Arús P, Messeguer R, Viruel M, Tobutt K, Direwanger E, Santi F, Quarta R, Ritter E (1994) The European *Prunus* mapping project. Progress in the almond linkage map. Euphytica 77:97–101

Baird WV, Ballard RE, Rajapakse S, Abbott AG (1996) Progress in *Prunus* mapping and application of molecular markers to germplasm improvement. HortScience 31:1099–1106

Ballvora A, Hesselbach J, Niewohner J, Leister D, Salamini F, Debhardt C (1995) Marker enrichment and high-resolution map of the segment of potato chromosome VII harbouring the nematode resistance gene *Gro1*. Mol Gen Genet 249:82–90

Cervera MT, Gusmao J, Steenackers M, Peleman J, Storme V, Broeck AV, Montagu MV, Boerjan W (1996) Identification of AFLP molecular markers for resistance against *Melampsora larici-populina* in *Populus*. Theor Appl Genet 93:733–737

Chaparro JX, Werner DJ, O'Malley D, Sederoff RR (1994) Targeted mapping and linkage analysis of morphological, isoenzyme, and RAPD markers in peach. Theor Appl Genet 87:805–815

Cnops G, Boer BD, Gerats A, Montagu MV, Lijsebettens MV (1996) Chromosome landing at the *Arabidopsis TORNADO1* locus using an AFLP-based strategy. Mol Gen Genet 253:32–41

Dirlewanger E, Bodo C (1994) Molecular genetic mapping of peach. Euphytica 77:101–103

Dirlewanger E, Lecouls A, Salesses G, Esmenjaud D (1996) Detection of molecular markers linked to resistance to the root-knot nematode *Meloidogyne arenaria* in Myrobolan plum (*Prunus cerasifera* Ehr.)(abstr.). In: Plant Genome IV. w47 (http://www.intl-pag.org/pag/4/abstracts/w47.html)

Eldredge L, Ballard R, Baird V, Abbott A, Morgens P, Callahan A, Scorza R, Monet R (1992) Application of RFLP analysis to genetic linkage mapping in peaches. HortScience 27:160–163

Esmenjaud D, Minot JC, Voisin R, Bonnet A, Salesses G (1996) Inheritance of resistance to the root-knot nematode *Meloidogyne arenaria* in Myrobalan plum. Theor Appl Genet 92:873–879

Esmenjaud D, Minot JC, Voisin R, Pinochet J, Simard MH, Salesses G (1997) Differential response to root-knot nematodes in *Prunus* species and correlative genetic implications. J Nematol 29:370–380

Fassuliotis G (1987) Genetic basis of plant resistance to nematodes. In: Veech JA, Dickson DW (ed) Vistas on nematology. Society of Nematologists, Hyattsville, Md., pp 364–371

Foolad MR, Arulsekar S, Becerra V, Bliss FA (1995) A genetic linkage map of *Prunus* based on an interspecific cross between peach and almond. Theor Appl Genet 91:262–269

Garcia GM, Stalker HT, Shroeder E, Kochert G (1996) Identification of RAPD, SCAR, and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. Genome 39:836–845

Gilbert JC, McGuire DC (1956) Inheritance of resistance to severe root-knot from *Meloidogyne incognita* in commercial type tomatoes. Proc Am Soc Hortic Sci 63:437–442

Ho J-Y, Weide R, Ma HM, van Wordragen MF, Lambert KN, Koornneef M, Zabel P, Williamson VM (1992) The root-knot nematode resistance gene (*Mi*) in tomato: construction of a molecular linkage map and identification of dominant cDNA markers in resistant genotypes. Plant J 2:971–982

Kelly JD (1995) Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogens. HortScience 30:461–465

Klein-Lankhorst R, Rietveld P, Machiels D, Verkerk R, Weide R, Gebhardt C, Koornneef M, Zabel P (1991) RFLP markers linked to the root knot nematode resistance gene *Mi* in tomato. Theor Appl Genet 81:661–667

Layne REC (1987) Peach rootstocks. In: Rom RC, Carlson RF (ed.) Rootstocks for fruit crops. John Wiley & Sons, New York, pp 185–216

Lecouls A, Salesses G, Minot J, Voisin R, Bonnet A, Esmenjaud D (1997) Spectrum of the *Ma* genes for resistance to *Meloidogyne* spp. in Myrobalan plum. Theor Appl Genet 95:1325–1334

Lownsbery BF, Thomson IJ (1959) Progress in nematology related to to horticulture. Proc Am Soc Hortic Sci 74:730–746

Lu Z-X (1997) Genetic study of resistance to root-knot nematodes (*Meloidogyne* spp.) in peach rootstocks. PhD thesis, Clemson University, Clemson, S.C.

Lu Z-X, Reighard GL, Baird WV, Abbott AG, Rajapakse S (1996) Identification of peach rootstock cultivars by RAPD Markers. HortScience 31:127–129

Lu Z-X, Reighard GL, Nyczepir AP, Beckman TG, Ramming, DW (1998a) Inheritance of resistance to root-knot nematodes in peach rootstocks. Acta Hortic 465:111–116

Lu Z-X, Sosinski B, Reighard GL, Baird WV, Abbott AG (1998b) Construction of a genetic linkage map and identification of AFLP markers for resistance to root-knot nematodes in peach rootstocks. Genome 41:199–207

Mehlenbacher SA (1995) Classical and molecular approaches to breeding fruit and nut crops for disease resistance. HortScience 30:466–477

Meksem K, Leister D, Peleman J, Zabeau M, Salamini F, Gebhardt C (1995) A high-resolution map of the vicinity of the *R1* locus on chromosome V of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81

Melchinger AE (1990) Use of molecular markers in breeding for oligogenic disease resistance. Plant Breed 104:1–19

Nyczepir AP (1991) Nematode management strategies in stone fruits in the United States. J Nematol 23:334–341

Okie WR, Ramming DW, Scorza R (1985) Peach, nectarine, and other stone fruit breeding by the USDA in the last two decades. HortScience 20:633–641

Okie WR, Beckman TG, Nyczepir AP, Reighard GL, Newall J, Zehr EI (1994) BY520–9, a peach rootstock for the southeastern United States that increases scion longevity. HortScience 29:705–706

Rajapakse S, Belthoff LE, He G, Estager AE, Scorza R, Verde I, Ballard RE, Baird WV, Callahan A, Monet R, Abbott AG (1995)

Genetic linkage mapping in peach using morphological, RFLP and RAPD markers. Theor Appl Genet 90 : 503–510

Ramming DW, Tanner O (1983) Nemared peach rootstock. HortScience 18 : 376

Salesses G, Dirlewanger E, Bonnet A, Lecouls A, Esmenjaud D (1998) Interspecific hybridization and rootstock breeding in peach. Acta Hortic 465 : 209–217.

Sasser JN (1980) Root-knot nematodes: a global menace to crop production. Plant Dis 64 : 36–41

Scorza R, Sherman WB (1996) Peaches. In : Janick J, Moore JN (eds) Fruit breeding, vol 1: tree and tropical fruits. John Wiley & Sons, New York, pp 325–440

Sharpe RH, Hesse CO, Lownsbery BF, Perry VG, Hansen CJ (1969) Breeding peaches for root-knot nematode resistance. J Amer Soc Hort Sci 94 : 209–212

Sherman WB, Lyrene PM, Sharpe RH (1991) Flordaguard peach rootstocks. HortScience 26 : 427–428

Stockinger EJ, Mulinix CA, Long CM, Brettin TS, Iezzoni AF (1996) A linkage map of sweet cherry based on RAPD analysis of a microspore-derived callus culture population. J Hered 87 : 214–218

Viruel MA, Messeguer R, de Vicente MC, Garcia-Mas J, Puidomènech P, Vargas F, Arús P (1995) A linkage map with RFLP and isozyme markers for almond. Theor Appl Genet 91 : 964–971

Voorrips RE, Jongerious MC, Kanne HJ (1997) Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracae* by means of RFLP and AFLP markers. Theor Appl Genet 94 : 75–82

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP : a new technique for DNA fingerprinting. Nucleic Acids Res 23 : 4407–4414

Weeden NF, Timmerman GM, Hemmat M, Kneen B, Lodhi M (1992) Inheritance and reliability of RAPD markers. Crop Sci Soc Am, Am Soc Hortic Sci, and Am Genet Assoc, Minneapolis, Minn.

Williamson VM, Ho J-Y, Wu FF, Miller N, Kaloshian I (1994) A PCR-based marker tightly linked to the nematode resistance gene, *Mi*, in tomato. Theor Appl Genet 87 : 757–763

Yang H-Y, Korban SS, Kruger J, Schmidt H (1997) A randomly amplified polymorphic DNA (RAPD) marker tightly linked to the scab-resistance gene *Vf* in apple. J Am Soc Hortic Sci 122 : 47–52