Euphytica (2015) 206:393–399
DOI 10.1007/s10681-015-1489-x

Development of a SNP-based CAPS assay for the *Me1* gene conferring resistance to root knot nematode in pepper

Ali Tevfik Uncu · Ibrahim Celik · Zubeyir Devran · Ercan Ozkaynak · Anne Frary · Amy Frary · Sami Doganlar

Received: 6 May 2015 / Accepted: 3 June 2015 / Published online: 12 June 2015

Abstract Root knot nematodes (*Meloidogyne* spp.) are significant agricultural pests on many crops, including pepper (*Capsicum annuum*). Host plant resistance offers the most sustainable means of controlling this pest. A cluster of genes on chromosome 9 confers resistance, with *Me1* providing protection against three nematode species: *M. incognita*, *M. javanica* and *M. arenaria*. We describe the development of a codominant CAPS marker located 1.13 cM away from the *Me1* gene. This marker should be useful for marker assisted selection of nematode resistance in pepper breeding programs.

Keywords *Capsicum annuum* · Marker assisted selection · *Me* resistance genes · *Meloidogyne* spp.

A. T. Uncu · I. Celik · A. Frary · S. Doganlar (✉)
Department of Molecular Biology and Genetics, Izmir Institute of Technology, Urla, 35430 Izmir, Turkey
e-mail: samidoganlar@iyte.edu.tr

Z. Devran
Department of Plant Protection, Faculty of Agriculture, Akdeniz University, Yerleske, 07058 Antalya, Turkey

E. Ozkaynak
Yuksel Seeds, Ltd., Kiziltoprak, 07300 Antalya, Turkey

A. Frary
Department of Biological Sciences, Mount Holyoke College, South Hadley, MA 01075, USA

Introduction

Root knot nematodes (*Meloidogyne* spp.) are obligate parasites that infect plant roots and induce the development of characteristic galls (root knots) (Williamson 1999). Nutrient losses and damage inflicted by the nematodes reduce root growth in infected plants, compromising shoot growth and photosynthetic capacity (Trudgill and Phillips 1997). More than 98 *Meloidogyne* species have been identified (Jones et al. 2013). Three of these species (*M. incognita*, *M. javanica*, *M. arenaria*) are widely distributed, have broad host ranges and cause significant yield losses in a range of crop species (Sasser 1980). While soil fumigation has been one means of controlling these pests, the use of many nematicides has been curtailed due to environmental and health concerns. Host plant resistance therefore offers a more sustainable and ecologically sensitive solution to this serious agricultural pest which is especially damaging to solanaceous crops, including tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*).

Root knot nematode resistance in pepper was first described by Hare (1956) and attributed to the dominant gene *N*. Subsequently several other dominant resistance (R) genes, the *Me* and *Mech* genes, were identified and characterized, some conferring species-specific immunity and others imparting resistance to a wide range of root knot nematode species (Hendy et al. 1983, 1985; Djian-Caporalino et al.

1999). Using inbred lines derived from three *C. annuum* accessions (PI322719, PI201234, CM334) of separate genetic and geographical origin, Djian-Caporalino et al. (2007) fine-mapped six of the *Me* genes (*Me1*, *Me3*, *Me4*, *Me7, Mech1, Mech2*) to a 28 cM region of pepper chromosome 9. In the process they concluded that the *Bs2* gene for resistance to bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) is also in this region. The *N* gene has recently been mapped to this same cluster (Fazari et al. 2012).

Introgression of *Me1* and *Me3* is a particular aim of pepper breeding programs because each of these genes provides resistance to the three major species of root knot nematodes: *M incognita, M. arenaria* and *M. javanica*. In the process of mapping the *Me* genes, Djian-Caporalino et al. (2007) developed several *Me*-specific markers. While these PCR-based markers reportedly showed promise in the breeding lines tested by Djian-Caporalino et al. (2007), we found that the majority of these markers were either not polymorphic or did not co-segregate with nematode resistance in an F_2 population generated from the cross *C. annuum* cv. AZN-1 (susceptible line from Turkey) × *C. annuum* cv. PM217 (resistant inbred derived from PI 201234 and carrying *Me1*). However, the *Me1* specific marker (SCAR-CD) developed by Djian-Caporalino et al. (2007) was linked to the gene in our population; but, as a dominant marker, it is not ideal for marker-assisted selection. To meet this need, we developed a tightly-linked codominant marker that would help us identify both homozygous and heterozygous resistant individuals in our PM217-derived pepper population.

Materials and methods

Plant material

A F_2 population segregating for root knot nematode resistance was generated by selfing F_1 hybrids from a cross of *C. annuum* cv. AZN-1 (susceptible line from Yuksel Seeds, Ltd, Turkey) × *C. annuum* cv. PM217 (resistant inbred line derived from PI201234). 100 individuals from the F_2 population were screened for nematode resistance against two isolates (D5 and G3) of *M. incognita* race 2 and genotyped with SSR and SNP markers in order to map the *Me1* gene.

Nematode culture

Two isolates (D5 and G3) of *M. incognita* race 2 previously identified by Devran and Sögüt (2009, 2011) were maintained on the susceptible fresh market tomato F_1 line Tueza (Multi Tohum, Antalya, Turkey). Tomato seeds were germinated in steam-sterilized sandy soil in seed trays, and 2-week-old seedlings were transplanted singly into 250 ml plastic pots and grown to the fourth true leaf stage prior to inoculation. Egg masses were collected from infected roots using a small needle and hatched at room temperature (25 °C). Juveniles were counted with a microscope and then used to inoculate the tomato plants. Plants were grown in a 25 °C growth chamber and egg masses were harvested 8 weeks after inoculation.

Screening for nematode resistance in the F_2 population

Pepper seeds were germinated in seedling trays containing steam-sterilized sandy soil. Two-week old seedlings were transplanted individually to 250 ml plastic pots and grown under controlled conditions of 25 °C and 65 % relative humidity. Egg masses from two isolates (D5 and G3) of *M. incognita* race 2 were collected from infected tomato roots using a small needle and then hatched at room temperature (25 °C). Second stage juveniles (J2) were counted under a microscope. Each F_2 plant was inoculated with a suspension of 1000 J2s at the fourth true leaf stage. Plants were evaluated for nematode resistance 8 weeks after inoculation. Inoculated plants were uprooted and roots were rinsed under tap water. Egg masses were assessed on a 0–5 scale according to Hadisoeganda and Sasser (1982) as follows: 0 = no egg masses (resistance), 1 = 1–2 egg masses (resistance), 2 = 3–10 egg masses, 3 = 11–30 egg masses, 4 = 31–100 egg masses and 5 = more than 100 egg masses per root system.

Genotyping the F_2 population with chromosome 9 markers

DNA was isolated from frozen leaf tissue (200 mg/sample) of the parental genotypes and 200 F_2 individuals using a CTAB method (Doyle and Doyle 1990). DNA from 100 F_2 individuals inoculated with the *M. incognita* race 2 isolate D5 was used to amplify

the *Me*-specific markers SSCP-B54, SSCP-B322, SCAR-B94, SCAR-CD and CAPS-F4R4 according to Djian-Caporalino et al. (2007). Chromosome 9 SSR markers HPMS 1-3, HPMS 1-117, HPMS 2-41, HPMSE 102 (Lee et al. 2004), GPMS171 and GPMS172 (Ben-Chaim et al. 2006; Yi et al. 2006) were also tested. SSR fragments were amplified in 20 μL reaction mixtures containing 1× PCR buffer, 1.5 mM $MgCl^2$, 0.25 mM of each deoxyribonucleotide triphosphate (Promega Corp.), 1 U *Taq* polymerase, 0.25 μM of each primer, and 5 ng template DNA. Thermal cycling conditions consisted of one cycle of initial denaturation for 10 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, with a final extension step of 10 min at 72 °C. SSR fragments were separated and visualized with a Qiaxcel advanced capillary electrophoresis system (Qiagen, Valencia, CA, USA) using the Qiaxcel DNA High Resolution Kit (Qiagen). The high resolution run method OM800 was applied with a sample injection time of 10 s. QIAxcel ScreenGel Software (Qiagen) was used for visualizing the SSR fragments. The molecular sizes of the SSR fragments were determined using the QX DNA Size Marker 50–800 bp, v2.0 along with the QX Alignment Marker 15 bp/1000 bp (Qiagen).

The parental genotypes, *C. annuum* cv. AZN-1 and *C. annuum* cv. PM217, were screened for the polymorphisms of SNP markers on pepper chromosome 9 by TraitGenetics GmbH (Germany) using a KASP™ (LGC Genomics, Middlesex, UK) genotyping platform. A total of 73 SNPs that are proprietary to TraitGenetics GmbH were used to screen polymorphisms between the parental genotypes. The F_2 individuals were then genotyped for the polymorphic SNP loci.

Mapping nematode resistance and development of a CAPS assay linked with the *Me1* gene

JoinMap 4.0 (Van Ooijen 2006) was used with the Kosambi mapping function (Kosambi 1944) to locate the *Me1* gene relative to the pepper chromosome 9 SNP and SSR markers. The map positions of the SSR markers were used as anchors to select putative COSII markers linked with the *Me1* gene from the COSII genetic map of chromosome 9 (Wu et al. 2009).

COSII marker C2At2g06530 was amplified from *C. annuum* cv. AZN-1 and *C. annuum* cv. PM217 using the primers: P-F: AAGGTGTCTCCCTCAGAATTCAG, P-R: ATCTGTCCCATTGCCTTTGTAAC (Jung et al. 2010). PCR reaction mixtures and thermal cycling conditions were the same as described above for the SSR fragment amplifications. Five replicates of each PCR reaction were performed and the resulting products were combined before purification with the DNA Clean & Concentrator-5 Kit (Zymo Research, Irvine, CA, USA). Purified PCR products were then sequenced via dye-terminator sequencing, using GenomeLab DTCS Quick Start Kit (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions. The thermal cycling conditions for the sequencing reactions consisted of 30 cycles of 96 °C 20 s, 50 °C 20 s, 60 °C 4 min. The reaction mixture for each amplicon was then purified using ZR DNA Sequencing Clean-up Kit (Zymo Research), resuspended in 30 μL of sample loading solution (Beckman Coulter), and run on a Beckman CEQ8800 capillary electrophoresis device using the LFR-b method (injection voltage 2.0 kV for 10–15 s, separation temperature 57 °C, separation voltage 6.0 kV, separation time 60 min). Sequence peaks were visualized with the 'Sequencing' module of Beckman CEQ 8800 software version 8.0 (Beckman Coulter). Sequence alignment and SNP identification were performed using the 'Investigator' module of CEQ 8800 (Beckman Coulter) software. The sequences were then analyzed with the NEBcutter Version 2.0 software (http://tools.neb.com/NEBcutter2/) (New England Biolabs Inc., Ipswich, MA) to identify the allelic variants of each SNP.

SNPs with alleles altering restriction enzyme recognition motifs were selected to develop a restriction digestion-based genotyping assay (CAPS assay). Inner primers flanking the SNP of interest were designed using the computer program Primer3 (Rozen and Skaletsky 2000). These inner primers (C2At2g06530-CAPS-F: TTGGTGCTGTAAGGGACTAAA, C2At2g06530-CAPS-R: TCTTAATCAATCATTCACACAGCA) were then used to amplify PCR products from the F_2 population and parental lines. PCR reaction mixtures and thermal cycling conditions were the same as described above for the SSR fragment amplifications. PCR products were directly subjected to restriction digestion without any purification step. Digestion reaction mixtures contained 20 μL of PCR product, 1 μL of *Hpy*CH4IV restriction enzyme (New England Biolabs Inc.), and 2.5 μL of CutSmart

reaction buffer (New England Biolabs Inc.) in a total volume of 25 μL. Reaction mixtures were incubated at 37 °C for 6 h. The digestion fragments were separated using a Qiaxcel DNA High Resolution Kit and the Qiaxcel Advanced capillary electrophoresis system (Qiagen). The high resolution run method OM800 was applied with a sample injection time of 15 s. QIAxcel ScreenGel Software (Qiagen) was used for visualizing the digestion profiles. The molecular sizes of the fragments were determined using the QX DNA Size Marker 25–500 bp, v2.0 along with the QX Alignment Marker 15 bp/600 bp (Qiagen).

Physical mapping of C2At2g06530

The pepper genome (Pepper.v.1.55) (http://pepperge nome.snu.ac.kr/download.php) was searched using the nucleotide sequences of *Bs2* (Tai et al. 1999) (Pubmed accession number: AF202179.1), C2At2g0 6530 (https://www.gabipd.org/database/) and N-SCA R-315 (Wang et al. 2009) using BLAST software (Altschul et al. 1990).

Results and discussion

Phenotypic characterization of the F_2 population for resistance against *M. incognita*

The F_2 population generated from the cross *C. annuum* cv. AZN-1 (susceptible line from Turkey) × *C. annuum* cv. PM217 was tested for resistance to *M. incognita* race 2. PM217 is an inbred line derived from the Central American accession PI 201234, it carries the closely-linked R genes *Me1* and *Mech1* (Berthou et al. 2003). The *C. annuum* cv. AZN-1, displayed a susceptible phenotype when inoculated with both *M. incognita* isolates, as expected. The number of observed egg masses was more than 100. PM217 was resistant to both *M. incognita* isolates. Up to six egg masses (0–6) were counted for the resistant inbred line PM217. F_2 plants displayed variable number of egg masses on their roots at 8 weeks post-inoculation. Based on this criterion, 78 % of the 100 F_2 plants were resistant to *M. incognita* race 2 isolate D5. In a separate test, 73 % of the 100 F_2 individuals were resistant to isolate G3. These results indicated that the resistance gene was inherited as a dominant trait with the classical Mendelian segregation ratio of 3:1. This segregation ratio was confirmed by a Chi square goodness of fit test ($p = 0.45$ for isolate D5 and $p = 0.68$ for isolate G3). Thus, as expected, the *Me1* gene derived from PI201234 conferred dominant resistance to race 2 of *M. incognita*.

Testing the *Me*-specific markers in our population

We tested the five *Me*-specific markers (Djian-Caporalino et al. 2007) in 100 F_2 individuals inoculated with race 2 isolate D5. SSCP-B322, SCAR-B94 and CAPS-F4R4 were not polymorphic in our population and therefore could not be mapped. SSCP-B54 mapped to chromosome 9 however at a distance of 17.1 cM from the resistance gene, indicating that this marker was unsuitable for screening our population for *Me1*. This finding was not unexpected given two considerations. Firstly, SSCP-B54 was reported to be closely linked to *Me3-Me4* and *Me7-Mech1* and not *Me1*. Secondly, the *Me* cluster in pepper is large and estimated to span some 28 cM (Djian-Caporalino et al. 2007). In contrast to the situation with SSCP-B54, SCAR-CD mapped in close proximity to *Me1* in our population (1.9 cM away). While SCAR-CD could be used to identify nematode resistant individuals, as a dominant marker, it does not provide for the most efficient selection. In contrast, a closely-linked codominant marker would allow the identification of all three *Me1* genotypes and thereby provide the best means of screening breeding populations. Thus our goal was to develop a codominant PCR-based marker that co-segregates with the *M. incognita* resistance gene *Me1*.

Mapping *M. incognita* resistance and development of a CAPS assay linked with the *Me1* gene

Six SSR markers spanning the genetic map of chromosome 9 including HPMS 1-3, HPMS 1-117, HPMS 2-41, HPMSE 102 (Lee et al. 2004), GPMS171 and GPMS172 (Ben-Chaim et al. 2006; Yi et al. 2006) were assayed on the F_2 population. None of these markers was in close linkage with nematode resistance, and all mapped further away from *Me1* than SSCP-B54 (i.e. >17.1 cM) (Fig. 1). As a result, it was necessary to screen chromosome 9 of the parental genotypes for polymorphic SNPs. A total of 73 SNPs were tested (Fig. 1a), and of the 70 that yielded products, 13 (19 %) were polymorphic. The F_2

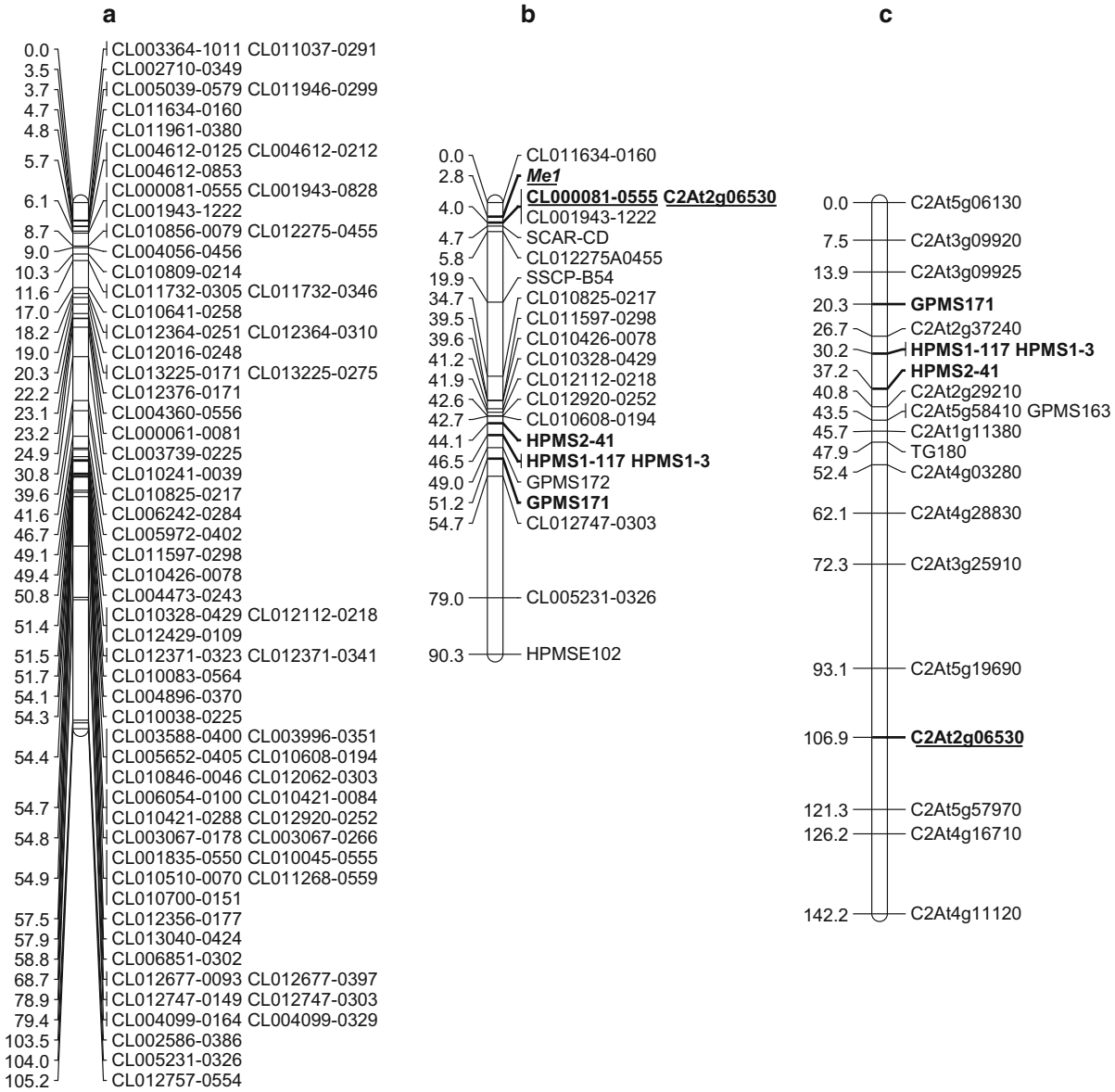

Fig. 1 **a** Genetic linkage map of 73 SNP markers on pepper chromosome 9. **b** Genetic linkage map displaying the location of the *Me1* gene relative to SCAR, SNP, SSR and COSII markers, **c** COSII linkage map of chromosome 9 from Wu et al. (2009). SSR markers common to our analysis and Wu et al. (2009) are boldface. Markers closest to *Me1* are underlined

population was then genotyped for these 13 SNP loci. SNP marker CL000081-0555 displayed the closest linkage with the *Me1* gene, 1.13 cM away. A second SNP marker also displayed close linkage with *M. incognita* resistance, mapping 1.22 cM away from the *Me1* gene.

Because the SNP sequence information is proprietary, it was not possible to use CL000081-0555 to develop a PCR-based assay for *Me1* resistance. Four of the SSR markers we screened (GPMS 171, HPMS 1-117, HPMS 1-3 and HPMS 2-41) had been localized on the COSII pepper map (Fig. 1c; Wu et al. 2009). Therefore we used their positions to predict the location of the *Me1* gene on the COSII map as follows. HPMS 1-117 and HPMS 1-3 were identified as co-segregating loci both in the COSII map and in

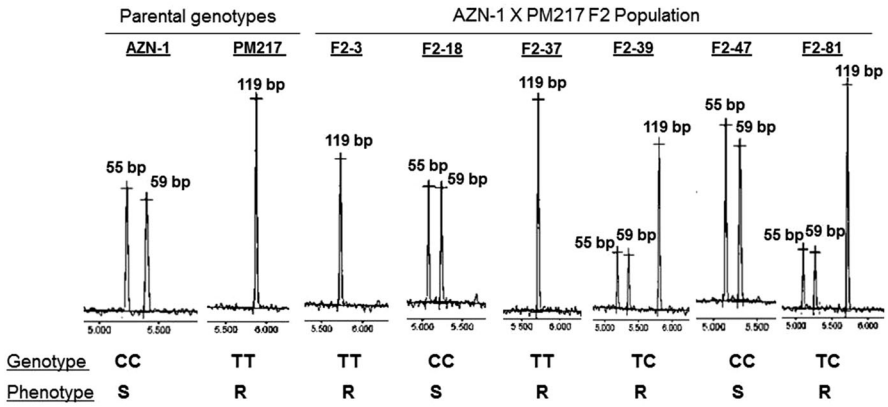

Fig. 2 Electropherograms illustrating parental and selected F_2 genotypes for the *Me1* CAPS assay. Root knot nematode resistance phenotypes are also given for each line: *R* resistant, *S* susceptible

our linkage analysis. The distance between GPMS 171 and these two co-segregating loci was 4.7 cM on our linkage map; whereas, the COSII map displayed a genetic distance of 9.9 cM for the same interval. Similarly, the genetic distance between GPMS 171 and HPMS 2-41 was 7 cM in our analysis, approximately half of the distance displayed in the COSII map (16.9 cM). Because *Me1* was located 41.3 cM and 48.3 away from HPMS 2-41 and GPMS 171, respectively, we extrapolated that *Me1* should lie approximately 80–95 cM away from these two markers on the COSII map. Consequently, we hypothesized that the 28 cM region flanked by COSII markers C2At5g 19690 and C2At5g57970 harbored the *Me1* gene. Physical mapping of the region using DNA sequences specific to COSII marker C2At2g06530 (which is positioned in the center of this putative *Me1* region), the *N* gene (N-SCAR-315; Wang et al. 2009) and *Bs2* (Djian-Caporalino et al. 2007) revealed that *Bs2*, C2At2g06530 and N-SCAR-315 were positioned at 249, 250 and 251 Mb, respectively, on chromosome 9. Because the *Me*, *Bs2* (Djian-Caporalino et al. 2007) and *N* (Fazari et al. 2012) genes reside in the same genomic region, this result provided independent evidence that C2At2g06530 was probably the closest COSII marker to *Me1*.

Comparison of the DNA sequences of C2At2g06530 in the two parental genotypes uncovered a SNP locus amenable to restriction digestion-based genotyping. The susceptible parent (cv. AZN-1) was homozygous for the C allele in the SNP locus and therefore was homozygous for an intact *Hpy*CH4IV restriction site. In contrast, the donor parent for the nematode resistance allele (cv. PM217) was homozygous for the T allele at the locus, which disrupted the recognition motif of the restriction enzyme. Accordingly, a CAPS assay was developed and applied to both race 2-tested F_2 populations (Fig. 2). The SNP marker genotype predicted nematode resistance/susceptibility phenotype for *M. incognita* with 99 % accuracy. Linkage analysis revealed co-segregation of the C2At2g06530 COSII SNP locus with SNP CL000081-0555.

Thus we have developed a CAPS assay for a SNP marker 1.13 cM away from the *Me1* gene. This closely linked codominant marker should be useful for marker assisted selection of root knot nematode resistance.

Acknowledgments This study was supported by Grant 319.STZ.2002/1 from the Republic of Turkey Ministry of Science, Industry and Technology with contributions from Yuksel Seeds, Ltd.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Ben-Chaim A, Borovsky Y, Falise M, Mazourek M, Kang BC, Paran I, Jahn M (2006) QTL analysis for capsaicinoid content in *Capsicum*. Theor Appl Genet 113:1481–1490

Berthou F, Pallaoix A, Mugniery D (2003) Characterisation of virulence in populations of *Meloidogyne chitwoodi* and evidence for a resistance gene in pepper *Capsicum annuum* L. line PM 217. Nematology 5:383–390

Devran Z, Söğüt MA (2009) Distribution and identification of root-knot nematodes from Turkey. J Nematology 41:128–133

Devran Z, Söğüt MA (2011) Characterizing races *of Meloidogyne incognita*, *M. javanica* and *M. arenaria* in the West Mediterranean region of Turkey. Crop Prot 30:451–455

Djian-Caporalino C, Pijarowski L, Januel A, Lefebvre V, Daubeze A, Palloix A, Dalmasso A, Abad P (1999) Spectrum of resistance to root-knot nematodes and inheritance of heat-stable resistance in pepper (*Capsicum annuum* L.). Theor Appl Genet 99:496–502

Djian-Caporalino C, Fazari A, Arguel MJ, Vernie T, Vandecasteele C, Faure I, Brunoud G, Pijarowski L, Palloix A, Lefebvre V, Abad P (2007) Root-knot nematode (*Meloidogyne spp.*) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome. Theor Appl Genet 114:473–486

Doyle JJ, Doyle JE (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Fazari A, Palloix A, Wang LH, Hua MY, Sage-Palloix AM, Zhang BX, Djian-Caporalino C (2012) The root-knot nematode resistance *N*-gene co-localizes in the *Me*-genes cluster on the pepper (*Capsicum annuum* L.) P9 chromosome. Plant Breeding 131:665–673

Hadisoeganda WW, Sasser JN (1982) Resistance of tomato, bean, southern pea, and garden pea cultivars to rootknot nematodes based on host suitability. Plant Dis 66:145–150

Hare WW (1956) Resistance in pepper to *Meloidogyne incognita acrita*. Phytopathology 46:98–104

Hendy H, Pochard E, Dalmasso A (1983) Identification de deux nouvelles souches de résistance aux nématodes du genre *Meloidogyne* chez le piment, *Capsicum annuum* L. CR Séances Acad Agric Fr 69:817–822

Hendy H, Pochard E, Dalmasso A (1985) Transmission héréditaire de la résistance aux *Meloidogyne* portée par deux lignées de *Capsicum annuum*: études de descendances d'homozygotes issues d'androgénèse. Agronomie 5:93–100

Jones JT, Haegeman A, Danchın EGJ, Gaur HS, Helder J, Jones MGK, Kıkuchı T, Manzanılla-López R, Palomares-Rıus JE, Wesemael WML, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol 14:946–961

Jung JK, Park SW, Liu WY, Kang BC (2010) Discovery of single nucleotide polymorphism in *Capsicum* and SNP markers for cultivar identification. Euphytica 175:91–107

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugenics 12:172–175

Lee JM, Nahm SH, Kim YM, Kim BD (2004) Characterization and molecular genetic mapping of microsatellite loci in pepper. Theor Appl Genet 108:619–627

Rozen S, Skaletsky H (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Methods in molecular biology: bioinformatics methods and protocols. Humana Press, Totowa, NJ, pp 365–386

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

Tai T, Dahlbeck D, Stall RE, Peleman JB, Staskawicz BJ (1999) High-resolution genetic and physical mapping of the region containing the *Bs2* resistance gene of pepper. Theor Appl Genet 99:1201–1206

Trudgill DL, Phillips MS (1997) Nematode population dynamics, threshold levels and estimation of crop losses. In: Maqbool MA (ed) Plant nematode problems and their control in the near east region, (FAO Plant Production and Protection Paper, No.144), Rome

Van Ooijen JW (2006) JoinMap® 4, software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen

Wang LH, Gu XH, Hua MY, Mao SL, Zhang ZH, Peng DL, Zhang BX (2009) A SCAR marker linked to the N gene for resistance to root knot nematodes (*Meloidogyne* spp.) in pepper (*Capsicum annuum* L.). Sci Hortic 122:318–322

Williamson VM (1999) Plant nematode resistance genes. Curr Opin Plant Biol 2:327–331

Wu F, Eannetta NT, Xu Y, Durrett R, Mazourek M, Jahn MM, Tanksley SD (2009) A COSII genetic linkage map of the pepper genome provides a detailed picture of synteny with tomato and new insights into recent chromosome evolution in the genus *Capsicum*. Theor Appl Genet 118:1279–1293

Yi G, Lee JM, Lee S, Choi D, Kim BD (2006) Exploitation of pepper EST-SSRs and an SSR-based linkage map. Theor Appl Genet 114:113–130