Theor Appl Genet (2005) 110: 802–811
DOI 10.1007/s00122-004-1814-6

ORIGINAL PAPER

M. J. Gonzalo · M. Oliver · J. Garcia-Mas · A. Monfort
R. Dolcet-Sanjuan · N. Katzir · P. Arús · A. J. Monforte

Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.)

Received: 5 July 2004 / Accepted: 4 September 2004 / Published online: 8 February 2005

Abstract A set of 118 simple sequence repeat (SSR) markers has been developed in melon from two different sources: genomic libraries (gSSR) and expressed sequence-tag (EST) databases (EST-SSR). Forty-nine percent of the markers showed polymorphism between the 'Piel de Sapo' (PS) and PI161375 melon genotypes used as parents for the mapping populations. Similar polymorphism levels were found in gSSR (51.2%) and EST-SSR (45.5%). Two populations, F_2 and a set of double haploid lines (DHLs), developed from the same parent genotypes were used for map construction. Twenty-three SSRs and 79 restriction fragment length polymorphisms (RFLPs), evenly distributed through the melon genome, were used to anchor the maps of both populations. Ten cucumber SSRs, 41 gSSRs, 16 EST-SSR, three single nucleotide polymorphism (SNP) markers, and the *Nsv* locus were added in the DHL population. The maps developed in the F_2 and DHL populations were co-linear, with similar lengths, except in linkage groups G1, G9, and G10. There was segregation distortion in a higher proportion of markers in the DHL population compared with the F_2, probably caused by selection during the construction of DHLs through in vitro culture. After map merging, a composite genetic map was obtained including 327 transferable markers: 226 RFLPs, 97 SSRs, three SNPs, and the *Nsv* locus. The map length is 1,021 cM, distributed in 12 linkage groups, and map density is 3.11 cM/marker. SSR markers alone cover nearly 80% of the map length. This map is proposed as a basis for a framework melon map to be merged with other maps and as an anchor point for map comparison between species of the Cucurbitaceae family.

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00122-004-1814-6

Communicated by I. Paran

M. J. Gonzalo · M. Oliver · J. Garcia-Mas · A. Monfort
R. Dolcet-Sanjuan · P. Arús · A. J. Monforte (✉)
Laboratori CSIC-IRTA Genètica Molecular Vegetal,
Carretera Cabrils s/n, 08348 Cabrils (Barcelona), Spain
E-mail: Antonio.Monforte@irta.es
Tel.: +34-93-7507511
Fax: +34-93-7533954

N. Katzir
Department of Genetics and Vegetable Crops,
Agricultural Research Organization,
Newe Ya'ar Research Center,
P.O. Box 1021, Ramat Yishay, 30-095, Israel

Present address: M. Oliver
Syngenta Seeds S.A.S, 12 Chemin de l'Hobit,
B.P.27, 31790 Saint Sauveur, France

Introduction

Melon (*Cucumis melo* L.) is a crop of the Cucurbitaceae family, grown in temperate and tropical regions worldwide. Fruits of *C. melo* exhibit a wide range of morphological variation, including fruits from a few grams to several kilograms, climacteric to non-climacteric, oblong to very elongated shapes or high to low flesh sugar content (Kirkbride 1993; Stepansky et al. 1999; Liu et al. 2004; Monforte et al. 2004a). High genetic diversity has been shown in studies using molecular markers (Stepansky et al. 1999; Mliki et al. 2001; Akashi et al. 2002; Monforte et al. 2003).

Even though 162 major genes controlling different aspects of melon biology have been described (Pitrat 2002), most of them have not been mapped. The genetic inheritance of most important agronomic traits is largely unknown due, at least in part, to the fact that full linkage maps have not been available until recently (Oliver et al. 2001; Périn et al. 2002a). With the available maps, quantitative trait loci (QTL) involved in fruit quality traits were detected in three different crosses of European cultivars and exotic Asian accessions (Périn et al. 2002b; Monforte et al. 2004b), providing the first results of the genetic control of important fruit qualities. However, the use of linkage maps in melon breeding programs is still very limited. The map from Périn et al.

(2002a) consists mainly of amplified fragment length polymorphism (AFLP) and intersimple sequence repeat (ISSR) markers. These markers are dominant and cannot be transferred readily to other populations. The map from Oliver et al. (2001) contains a large number of codominant transferable markers, mainly restriction fragment length polymorphisms (RFLPs). These markers have not been widely adopted by the melon research community, because the laborious RFLP procedures are not available to all laboratories.

Microsatellite or simple sequence repeat (SSR) markers are preferable, because they are easy to implement and amenable for high-throughput in most laboratories. SSR markers are highly polymorphic, codominant, and readily transferable among mapping populations.

A set of SSR markers (Katzir et al. 1996; Danin-Poleg et al. 2001; Chiba et al. 2003; Ritschel et al. 2004), produced sufficient polymorphism within melon germplasm, and is useful in map construction (Danin-Poleg et al. 2001; Monforte et al. 2003). A subset of these SSRs has been included in some melon maps (Danin-Poleg et al. 2000, 2002; Oliver et al. 2001; Périn et al. 2002a; Silberstein et al. 2003; Ritschel et al. 2004). Unfortunately, these SSRs cover only part of the melon genome and are not sufficient in bridging between melon maps (Danin-Poleg et al. 2000; Ritschel et al. 2004).

Genome mapping is a continuous process. However, most populations used for melon genome mapping are transient F_2 (Braudracco-Arnas and Pitrat 1996; Oliver et al. 2001; Daning-Poleg et al. 2002; Silberstein et al. 2003), not suitable for long-term mapping projects. Recombinant inbred lines [(RILs) Périn et al. 2002a] and double haploid lines (DHLs) can be propagated by seed and are suitable for such objectives.

In the this study, a new set of SSR markers has been developed to build a framework genetic based mainly in SSRs. Thirteen cucumber SSRs (Fazio et al. 2002), 59 melon SSRs, and three single nucleotide polymorphism (SNP) markers were added to the melon genetic map by merging F_2-based (Oliver et al. 2001) and a newly developed DHL-based maps. The extended genetic map is proposed as a bridge with other melon genetic maps to further develop a reference map containing highly polymorphic and transferable markers.

Material and methods

Plant material and DNA extraction

Two mapping populations (F_2 and DHL) were obtained from the cross between the Korean accession PI 161375 and a 'Piel de sapo' (PS) inbred line from Semillas Fitó S.A., Spain. The 93 F_2 individuals were obtained and maintained in vitro, as described by Oliver et al. (2001). Seventy-seven DHLs were developed by in situ-induced parthenogenesis through pollination with Co^{60} gamma-irradiated pollen, in vitro rescue of parthenogenic embryos, in vitro chromosome-doubling by colchicine treatment, and self-pollination of acclimated plants (Gonzalo 2003), adapting a protocol previously developed for *Dianthus caryophyllus* (Dolcet-Sanjuan et al. 2001). Total DNA was extracted from young leaves of DHL plants, as described by Doyle and Doyle (1990), with minor modifications (Oliver et al. 2001).

RFLPs

RFLPs were obtained as described by Oliver et al. (2001). The prefix of the RFLP indicates the origin of the clones: MG, melon genomic DNA; MC and CM, melon cDNA (Oliver et al. 2001; Baudracco-Arnas and Pitrat 1996); and AEST, *Arabidopsis* cDNA (Paterson et al. 1996).

SSR markers

Two sets of SSR markers were used for map construction. A first set consisted of 39 SSR markers developed previously, including 22 melon and three cucumber SSRs from Danin-Poleg et al. (2001) and 13 cucumber SSR markers, labeled with prefix CSW-, from Fazio et al. (2002) (Table 1). A second set of SSR markers consisted of newly developed markers from two different sources: genomic libraries and database sequences.

Simple-sequence repeat markers from genomic libraries (gSSR) were developed at Newe Ya'ar Research Center, according to Danin-Poleg et al. (2001). Clones

Table 1 List of simple sequence repeat (*SSR*) markers from melon and cucumber used for map construction and developed previously as indicated in the source

Melon SSR	Source[a]	Cucumber SSR	Source[a]
CMACC146	1	CSAT425[b]	1
CMAG59	1	CSCCT571	1
CMAT141	1	CSGA057	1
CMAT35	1	CSWCT01	2
CMCAA145	1	CSWCT10	2
CMCT505	1	CSWCT11	2
CMGA104	1	CSWCT12	2
CMGA108	1	CSWCT16	2
CMGA128	1	CSWCT18B	2
CMGA15	1	CSWCT22A	2
CMGA165	1	CSWCT2[b]	2
CMGA172	1	CSWCTT02	2
CMTA134a	1	CSWGAT01	2
CMCT134b	1	CSWTA02	2
CMGA36[a]	1	CSWCT03B	2
CMTA170a	1		
CMTAA166	1		
CMTC123	1		
CMTC13	1		
CMTC160b	1		
CMTC168	1		
CMTC47	1		

[a] *1* Danin-Poleg et al. 2001, *2* Fazzio et al. 2002
[b] The primers amplified two loci named with the suffixes -a and -b

containing SSR motifs from small-insert genomic libraries were selected by hybridizing filters containing their DNA, with the primer $(CT)_{10}$ end-labeled with [$\gamma-^{32}$P]-ATP (Katzir et al. 1996; Danin-Poleg et al. 2001). Positive clones were selected for DNA sequencing. These SSRs were labeled with the prefixes CMTCN-, CMCTN-, CMGAN-, CMAGN-, CMATN-, and CMTAAN-. SSR markers from expressed sequence-tag database sequences (EST-SSR) were obtained from the GenBank database and EST sequences developed by IRTA analyzed using the SPUTNIK software (http://espressosoftware.com/pages/sputnik.jsp). Only microsatellites with dinucleotide repeats longer than 10 or the equivalent length in nucleotides with tri-, tetra-, or pentanucleotide motifs were considered for primer design using Primer 3 (Rozen and Skaletsky 2000). EST-SSRs were labeled with the prefix TJ-. The SSRs CMCTN-86, CMATN-89, CMCAN-90, and CMATN-101 were also obtained from public EST databases at Newe Ya'ar Research Center.

Temperature-gradient PCR in a PTC-200 Thermocycler (MJ Research, Waltham, Mass., USA) was performed to obtain the optimum annealing temperature for each primer pair. PCR reactions were performed in a final volume of 15 µl with 1× *Taq* buffer [20 mM $(NH_4)SO_4$, 75 mM Tris-HCl (pH 8.8), 0.01% (v/v) Tween 20], 2 mM $MgCl_2$, 166 µM dNTPs, 0.6 µM each primer, 1 U *Taq* DNA polymerase (PE Applied Biosystems), and 60 ng DNA. The cycling conditions were as follows: an initial cycle at 94°C for 1 min, followed by 35 cycles at 94°C, 30 s, 40–60°C, 30 s and 72°C, 1 min, and a final cycle at 72°C for 5 min. Amplification products were visualized with UV light after electrophoresis in 2% agarose gels with 1× TBE (0.9 M Tris-borate, 0.002 M EDTA, pH 8.0), stained with ethidium bromide.

Tests of SSR polymorphism between PS and PI 161375 and genotyping of the polymorphic SSR markers were performed by standard polyacrylamide gel electrophoresis separation of [^{33}P]-labeled amplification fragments or by analysis of IRD-800-labeled amplicons in a LI-COR IR^2 (LI-COR, Nebraska, USA) automatic sequencer. PCR reactions with radioactive labeling were as described above, except that the final dCTP concentration was 2 µM, and 0.1 µl [$\alpha-^{33}$P]dCTP (3,000 Ci/mmol) was also added to the reaction. Cycling conditions were also as above. Labeled fragments were separated in 6% denaturing polyacrylamide gels in 1× TBE buffer and 7.5 M urea at 60 W. Gels were dried and exposed to X-ray films. For LI-COR analysis, microsatellites were amplified as above, except that 2 pmol of each primer (one labeled with IRD-800) was used. Five microliters of loading buffer (95% formamide, 20 mM EDTA, 0,05% bromophenol blue, 0.05% xylene cyanol) were added to the PCR mix, samples were denatured at 100°C for 5 min, and 0.8 µl was loaded onto a LI-COR IR^2 sequencer using 25-cm plates with 6% acrylamide in 1× TBE and 7.5 M urea. Electrophoresis was performed at 1500 V, 35 mA, and 31 W at 50°C until the PCR products were visible. The molecular weight of each microsatellite band was estimated by comparing its migration with the IRD-labeled STR molecular size marker (LI-COR). Polymorphic SSR markers were used to genotype the DHL population as described above.

SNPs

SNPs for the ESTs EST5.5, EST1.41 and EST1.11 were detected in the DHL population with cleaved-amplified polymorphic sequence assays or with the ABI Prism SNaPshot ddNTP Primer extension kit (Applied Biosystems, Foster City, Calif., USA), as described by Morales et al. (2004).

Linkage analysis and map construction

Segregation distortion at each marker locus was tested against the expected ratios (1:2:1 for F_2 and 1:1 for DHL) using a chi-square test with JoinMap, version 3.0 (van Ooijen and Voorrips 2001). Distortion was declared significant at $P < 0.005$. Loci with a distortion between $P < 0.05$ and $P > 0.005$ and linked to a loci with significant segregation distortion at $P < 0.005$ were used to define the confidence interval of the position of the putative locus causing the distortion.

Linkage maps were constructed independently in each population with MAPMAKER, version 3.0 (Lander et al. 1987). Markers were associated with the *group* command with LOD > 3.0. Markers within groups were ordered using the *order* command with LOD > 3.0 and were considered as the framework for each linkage group. Markers that could not be resolved were located using a LOD > 2.0. Distances were calculated with the Kosambi function (Kosambi 1944). The remaining markers were then located with the *try* command.

F_2 and DHL maps were compared based on the positions of common markers. A framework marker order was set, taking into account those marker groups that maintained the same order in the two populations, using a LOD > 2 in each individual map. Total map length was compared by a paired *t*-test using the longest distance between common markers within each linkage group. Heterogeneity tests of recombination rates within linkage groups were performed with JoinMap and were declared statistically significant at $P < 0.005$. The composite map was obtained after merging the individual maps using the framework marker order as fixed order with JoinMap. Maps were drawn with MapChart, version 2.1 (Voorrips 2002).

Results

Development of SSR markers and polymorphism

Eighty-five primer pairs flanking SSR motifs were designed from positive genomic clones after screening

Table 2 Primer sequences, motifs, and expected product size for genomic SSRs

SSR designation	Sense primer 5′ to 3′ (top) Antisense primer 5′ to 3′ (bottom)	Core repeat motif and number of repeats	Expected size (bp)
CMTCN1	CCCTTCATTTTCATCATCC GAAGACGGCAAATTGAGCT	$(TC)_{15}$	155
CMCTN2	CTGAAAGCAGTTTGTGTCGA AAAGAAGGAAGAGGCTGAGA	$(CT)_{12}$	172
CMGAN3	GTTAAAGGCTATGGTATAGAAC AGAATAAGGTCCACATAAGG	$(GA)_{10}$	203
CMCTN4	AAAACAAAAGCTCTCCACGA CTTTCCTTTATTATGCCTACG	$(CT)_{21}(CT)_{23}$	126
CMCTN5	CACCTTAAAGTTTAGCCCC AAAAATGCAATGAACTGAGCGC	$(CT)_7AT(CT)_{15}(AT)_8(AC)_6$	211
CMTCN6	GCATTGCTCGATCAGTTTTAC ACTCCGTCAAGATCCCAAAA	$(TC)_2TT(TC)_{16}$	151
CMCTN7	AATGACACTGCCCACATTCT AGGTTTTTCAATGGAGGGGA	$(CT)_{20}$	130
CMTCN8	CCTCCGCCACATATTACAAT TTCATCTTGACACGTAAGAG	$(TC)_{19}N_8(TAA)_6$	164
CMTCN9	CCCCCATATTCATCAAAACT CTTCCTTTTTTTCACACCCT	$(TC)_{11}$	207
CMGAN12	TTTTTGTCGTTATATGAGGG GTTGCATAATGCTAATTTGG	$(GA)_{21}$	179
CMTCN14	TATATTGGCTTTGGCTCTCG GATTCGTTATCTCGACCAAC	$(TC)_{18}$	165
CMTCN18	ACCAATCCATCACTCTCACT GAAAGAATGGGGGAAAAGAG	$(TC)_{11}N_{24}(TC)_{17}(TA)_{12}$	172
CMCTN19	GAATGATTGGAGCAACCAGT GCTTTTTGAATTTGTGCAGGG	$(CT)_{12}$	103
CMGAN21	GCTGTAAAACGAAACGGAGA CGATCTTCTTTATTCTTCGCC	$(GA)_{10}$	135
CMATN22	CGGCAATCATCTTATCTTTC AAGATTGAAGTGGGAAAATG	$(AC)_7(AT)_8$	166
CMGAN24	TAACTATGATGAAGAGTT TGCCAAACTATAATCACACTA	$(TA)_4(GA)_7$	182
CMGAN25	AGCCAATGTGAAGGATGAACA TGCAATTAGCCTCTTCTCTA	$(GA)_9$	164
CMTCN30	GGAGGGAAAGGAAAGAGAGA GGCAAGAAGATGGCAAAGAT	$(TC)_{13}$	193
CMAGN32	CAGATTAGAAGAAAAAGAGG AGCAGACAGCATATAAAGCT	$(AG)_{14}$	169
CMAGN33	CTGTCTGCTATTCTCCACTTGG TGTATGCCACGTAGCGAAAC	$(AG)_2AA(AG)_8TT(GA)_3$	122
CMCTN35	CCAATAATGTAATCGTCTTGG GTTCCAAACTTTCTACCAATCA	$(CT)_{10}$	186
CMCTN38	TAAAACACTCTCGTGACTCC GATCTGAGGTTGAAGCAAAG	$(CT)_{15}N_5(CT)C(CT)_3$	141
CMTCN41	CCCCAAGATTCGTATTAATC TGGTAGTAGAGATGATATAC	$(TC)_{12}$	129
CMAGN45	CCCACAAGAGAGAGAGAGAG GTGTGACAGGTAGATTGTTGG	$(AG)_{14}$	100
CMTCN50	TCTACTTCCATGAATCCATC TAGAATGGTTAGGAAACCCT	$(TC)_{16}(TA)_{12}$	134
CMAGN52	CCACCAACATAACACACAAC CTCTCACACTGTTGGGAAGA	$(AG)_{18}$	135
CMCTN53	CCACATTTGATGGAAATCTT CATTTTATAGCTTATCTTCCG	$(CT)_{16}$	149
CMTCN56	CTTTTCTCTTCTTCTATTCTC ATCCAAAAGGAATCGGAAAG	$(TC)_{11}$	96
CMAGN61	GGAGACACAAGGAATATGTG ATAACAAAGGGGCATAACAC	$(AG)_4AA(AG)_8$	142
CMTCN62	AAGATCGCCTCTATCACAG ATTTGTACTCCCAACGCATC	$(TC)_{15}$	145
CMCTN65	TTAGGTGTATTTGATCTC AATTTTATGGCTCAAGGTTC	$(TC)_{18}$	119
CMTCN66	CTCCGATCAATTTTACATCT GAATAAACTTGGTGTCCAAC	$(TC)_{17}$	127
CMTCN67	TCTCTTACAACTCTTTGTCG GGTTCAAGGATTCATCGTTG	$(TC)_{12}$	145

Table 2 (Contd.)

SSR designation	Sense primer 5′ to 3′ (top) Antisense primer 5′ to 3′ (bottom)	Core repeat motif and number of repeats	Expected size (bp)
CMAGN68	GGAAGGAAATTAGCATGCAC GCCACTCTGTCTTTCTTCC	$(AG)_{20}$	190
CMCTN71	TCAATTTTTGCCAAACAAGC CAAGGACACAGATTTAATAC	$(CT)_{11}$	160
CMAGN73	ATCCAACTCGACCAAGAAAC CAGCTCTACAACAACATCTC	$(AG)_{19}$	130
CMAGN75	TGGGTTTTCTTCTACTACTG TGCTTTTACTCTCATTCAAC	$(AG)_{18}$	157
CMAGN79	CTTCACTAAAACTACAAGAG TTCCAACTTATTCATCCCAC	$(AG)_{13}$	153
CMGAN80	ATATTGATTGCTGGGAAAGG CTTTTTTGGCTTTATTGGGTC	$(GA)_2TG(GA)_{14}TCT(CCTTT)_6$	159
CMGAN92	GAGAGAGAGAGAGAGATG GGTTGGGTACTCCGAGTTA	$(GA)_8$	140
CMGAN94	GAGAGAGAGAGAGAGATCTAAAC GTCATGTCCGGTTATCTTGT	$(GA)_8$	167
CMTAAN100	CGAATCTCCGGAACAGACAC CCGTCTACAAGCGTGACTGTC	$(TAA)_9N_8(GA)_9(AT)_3$	175

genomic libraries with oligonucleotide probes (gSSR). Two hundred and twenty-eight ESTs containing 330 SSR motifs (EST-SSR) were identified from a total of 1,262 melon ESTs (a proportion of 18% of EST with SSR motifs), and primers pairs were designed to flank 33 selected EST-SSRs. The dinucleotide motif TC/GA was the most common in the gSSRs, whereas trinucleotide and higher order motifs were common in the EST-SSRs: 52% of EST-SSRs had trinucleotide motifs and 9% had higher order motifs.

Amplification products were in general within the expected size range. A total of 57 (49.6%) of the 118 SSRs studied were polymorphic, with similar polymorphism levels found in gSSRs (51.2%, Table 2) and EST-SSRs (45.5%, Table 3) markers.

Fifty-five primer pairs amplified a single reproducible polymorphic locus and two, CMTCN66 and TJ12, amplified two polymorphic loci each, giving a total of 59 new polymorphic SSR loci. All polymorphic SSR loci showed codominant inheritance in the F_2 population.

F_2 linkage map

Seventeen newly developed melon gSSRs and nine cucumber SSRs (CSW-) were added to the 261 marker (235 RFLPs and 26 SSRs) data set of Oliver et al. (2001), giving a total of 287 mapped loci. The map spans a total genetic distance of 1,240 cM in 12 linkage groups (G1–G12), an average of 103 cM per linkage group, ranging from 56 cM (G9) to 155 cM (G12), a marker density of 4.3 cM/marker, and longest gap of 32 cM in G6 between markers CSWCT11 and MC294. Only six markers (2.1%) localized in G1 showed significant segregation distortion ($P < 0.005$) from the expected Mendelian ratios (Fig. 1), caused by an excess of individuals in the heterozygous genotypic class and a lack of individuals in the homozygous genotypic class for PI 161375 alleles (Oliver et al. 2001).

Double haploid line map

A total of 173 polymorphic markers were used to construct the genetic map with the DHL population. Twenty-three previously designed SSRs (Katzir et al. 1996; Danin-Poleg et al. 2001) and 79 RFLPs were selected from the F_2 map to evenly cover the melon genome and to provide anchor points between both maps. Ten cucumber SSRs (Fazio et al. 2002), 41 gSSRs, 16 EST-SSR, three SNPs (Morales et al. 2004), and the *Nsv* locus (Morales et al. 2002) were included in the previous data set. Markers were grouped in 12 linkage groups. The map spanned 1,223 cM, with an average of 102 cM per linkage group, ranging from 82.9 cM to 154 cM. Map density was 7 cM/marker, and the maximum gap was 42 cM between markers MC279 and MC134 on G6. Twenty-two markers (12%) showed significant segregation distortion ($P < 0.005$). Fourteen of them, with an excess of PS alleles, were localized on G5, G6, G8, and G12 (Fig. 1). The remaining markers, with an excess of PI 161375 alleles, were localized on G1 and G3.

Map comparison and map merging

The marker order was the same in both F_2- and DHL-based maps. Full linkage maps from both populations can be obtained from the journal Web site as Electronic Supplementary Material (S1 to S12) or by request to the authors. Map lengths were not statistically different ($P > 0.2$), although significant heterogeneity ($P < 0.005$) was detected between some marker pairs, such as CSGA057-CMGA59 on G1, CSWCT01-CM101b on

Table 3 Primer sequences, motifs, and expected product size for EST-SSRs. SSRs with a TJ- prefix were obtained from local EST databases. The GenBank accession number, organism, and significance (*P*-value) of the best sequence similarity matching the melon ESTs in the GenBank, using BLASTX, are also given. For the rest, SSRs were obtained directly from the sequence of the indicated GenBank accession

SSR	Sense primer 5′ to 3′ (top) Antisense primer 5′ to 3′ (bottom)	Core motif and number of repeats	Expected size (bp)	GenBank accession number and gene description of the best BLASTX match	Organism	*P*-value
TJ2	GAGGAATCCGAGACCACAAC GCCAAGTGTGTGTTGGAAAA	$(CAT)_8$	152	None		
TJ3	TGGGCCTACGCTACAAACTT AGCAGCACAAAAGCACTTCA	$(GA)_{11}$	158	P08222 Chlorophyll a–b-binding protein of LHCII type I	*Cucumis sativus*	9.00E-71
TJ10	ACGAGGAAAACGCAAAATCA TGAACGTGGACGACATTTTT	$(CTT)_5(CT)_3$	117	Q96473 vacuolar ATP synthase 16-kDa proteolipid subunit	*Kalanchoe daigremontiana*	2.00E-51
TJ12	TCCACCCAAACAGTTCTTGA CCAATGGAGTTTTCCCAATG	$(CTT)_9$	139	AB027000 PCAS-1 mRNA for beta-cyanoalanine synthase	*Solanum tuberosum*	1.00E-70
TJ24	AAACACGGGCTTGAAGAAAA CCCAGAAGGTGAGAGAGACCT	$(CTT)_{18}$	165	AAM67211 serine-rich protein	*Arabidopsis thaliana*	2.00E-14
TJ26	GGAGATTGGTGCTGTCCTTC CAAAACGCAAATTGACCAAA	$(TC)_{11}$	133	Q9LZ71 hypothetical 30.4-kDa protein	*Mus musculus*	1.40E-10
TJ27	AAGCGGAACAAGCTCATCTC CAAAAGCATCAATTGCTTGAA	$(TCT)_8$	171	P93472 cell elongation protein diminuto	*Oncorhynchus tschawytscha*	4.20E-15
TJ29	AGCCTAAGCCACCGATTTTT TTCCCAAGTGGGGTTATGAG	$(TC)_{10}$	137	NP192386 putative ABC transporter	*A.thaliana*	3.00E-22
TJ30	TTAGGGAAGGCAATCAATCG AGGAGGAGGGAATGCTTTGT	$(CAATC)_7$	178	Q9LY04 hypothetical 25.1-kDa protein	*A. thaliana*	5.60E-06
TJ31	GAGGCCTCCTCAGCTCTACA AGCCCATTAGCACAAGCTGA	$(GA)_{13}$	196	Q9LZ71 hypothetical 67.3-kDa protein	*A. thaliana*	1.10E-05
TJ33	GGAAGCTTCATTTGGGTCTATG GGAGGAGGATGAAGATGAAGC	(AAG)10	196	O65684 hypothetical 9.4-kDa protein	*A. thaliana*	8.60E-10
CMCTN86	GTGACAGTTATCAAGGATGC AAGGGAATGCATGTGGAC	$(CT)_{21}$	184	AAB91462 ADP-glucose pyrophosphorylase small subunit	*Cucumis melo*	
CMATN89	CACTACCTTAAAACAGAATTG GGACAATTTAGGGAGGATC	$(AT)_{13}A(AT)2$	138	AF249912 Galactinol synthase (*GAS1*) gene	*C. melo*	
CMCAN90	CTAACGCTGACCCAACTCTC GTGGTTGTGAGTTATGAGGAG	$(TA)_5TC$ $(CA)_5T(AC)_2$	130	AB032936 CMe-ACS2 gene for 1-aminocyclopropane-1-carboxylate synthase	*C. melo*	
CMATN101	GCTTGTCTTTGTGTTTGC GAGAACAAGACTCCTTAATCC	$(TA)_3AA(TA)_2C(AT)_7$	167	X95551 CMACO1 *C. melo* ACC oxidase gene	*C. melo*	

G9, and CM47-MC219 on G10, with higher recombination rates in the DHL population in all cases.

After map merging, a composite genetic map was obtained including 327 markers: 226 RFLPs, 97 SSRs, three SNPs, and the *Nsv* locus (Fig. 1). The position of nine RFLPs could not be resolved after map merging. Map length was 1,021 cM, slightly smaller than the F_2 and DHL maps. Comparison between individual and merged maps can be obtained as Electronic Supplementary Material or by request to the authors. Map density was 3.11 cM/marker, with a maximum gap of 30 cM on G12 between markers MC42 and MC224.

Both gSSRs and EST-SSRs were evenly distributed throughout the melon genome, with an average of 8 SSRs per LG, ranging from 4 (G7) to 12 (G2 and G4). The new SSRs contributed to reducing the gap length between codominant markers, e.g., TJ2 on G1 and also expanded some linkage groups, such as CMTCN56 on G1. Assuming that each SSR marker has a window of 10 cM on each side of its position, the set of SSRs used in the current map covers 795 cM, 78% of the length of the composite map.

Fig. 1 Composite linkage map of melon (*Cucumis melo* L.) after merging the F_2 and double haploid line (DHL) maps. Linkage groups are represented by *vertical double lines* and labeled G1–G12. Loci are listed to the *right*, and recombination distances (in centiMorgans) to the *left*, of each linkage group. Loci in *boldface* define the framework of each linkage group. *Underlined* loci were scored in both populations; those in *italics* were scored only in the DHL population and the remainder only in the F_2 population. Loci in *brackets* could not be mapped after map merging; these markers are in their most likely map positions, according to those calculated with the individual populations. *Boxes* on the *left* of the linkage groups indicate regions with distorted segregation ($P < 0.005$) in the population indicated. *Solid boxes* indicate excess of 'Piel de Sapo' alleles, *white boxes* excess of PI 161375 alleles, and *dashed boxes* excess of heterozygous genotypic class and lack of PI 161375 homozygous. The confidence interval of the position of the putative locus involved in the distorted segregation is shown with *bars*, including markers with distortion at $P < 0.05$

Discussion

SSR characterization

The polymorphism for SSR markers between the parent genotypes of the mapping populations was high (49%) regardless of their origin, confirming that both genomic libraries and EST sequences provide useful SSR markers and that the PS × PI 161375 cross is a highly polymorphic one. The isolation of SSR sequences from genomic libraries is tedious and labor intensive. EST sequences in public databases are useful source for searching for SSR sequences. EST-SSRs are associated with transcribed genes that can be used as candidate genes.

A large number of melon ESTs (18%) in this study contained SSR sequences. This proportion of ESTs containing SSRs is high in comparison with these found in other crop species, including rice (1.6%, Temnykh et al. 2000), grape (2.5%, Scott et al. 2000), and barley (7.5%, Thiel et al. 2003). However, only 16 EST-SSR (1.27%) of the ESTs surveyed could be mapped in the population, implying that tens of thousands of ESTs should be screened to obtain a map with several hundred EST-SSRs.

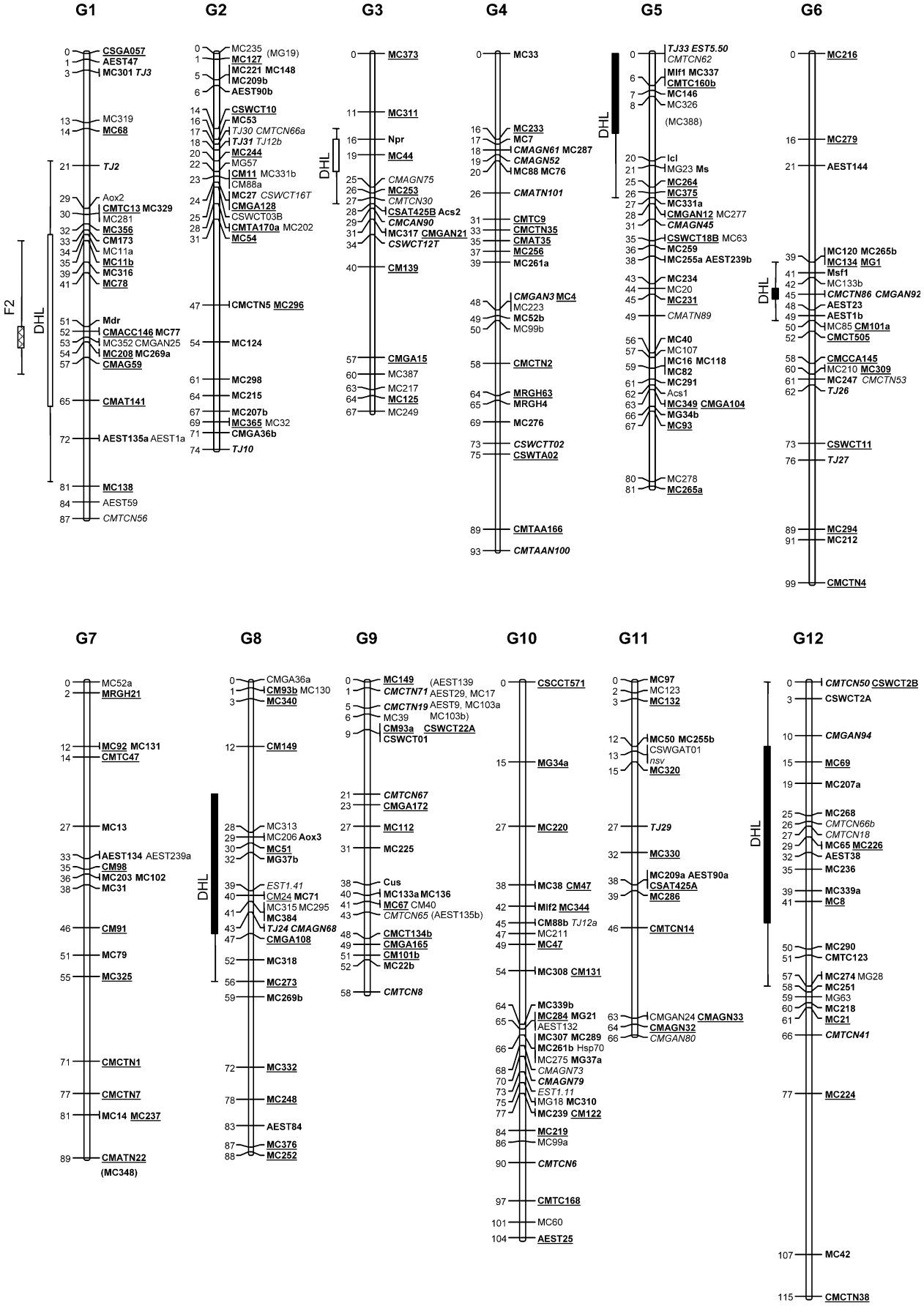
Most of the gSSRs had CT/GA repeats, confirming the high efficiency of screening with CT/GA probes to isolate SSRs in melon (Danin-Poleg et al. 2001). Trimeric SSRs (CAT, CTT, TCT, AAG) were common among EST-SSRs, as found in other plant species (Temnykh et al. 2000; Chin 1996; Thiel et al. 2003). This difference may be due to the suppression of non-trimeric SSRs that could cause frameshift mutations in coding regions (Metzgar et al. 2000). EST-SSR markers showed similar levels of polymorphism to gSSRs among the parent genotypes (45% vs 51% respectively), although it is well documented that EST-SSRs are less polymorphic than gSSRs (Cho et al. 2000; Thiel et al. 2003; Eujayl et al. 2002). Cho et al. (2000) found that G/C-rich polytrinucleotides had the lowest values of genetic variability, suggesting a different variability potential depending on the trinucleotide motif. Differences in the trinucleotide motif may explain the higher polymorphism observed in this set of melon EST-SSR markers, as the trimeric EST-SSRs isolated in melon were rich in A/T (CAT, CTT, TCT, AAG motifs), whereas in the previous reports, the majority of trimeric EST-SSRs were rich in G/C (CGC, AGG, and ACG motifs).

Melon map

Markers evenly distributed in the F_2 map (Oliver et al. 2001) were used to transfer a basic map framework to the DHL population. The maps developed in the F_2 and DHL populations were co-linear. Map lengths were also very similar except in three regions on G1, G9, and G10. The two populations were generated from the same hybrid, although the DHL map reflected only recombination events in the female gamete. Differences in recombination rates between female and male gametes (Plomion and O'Malley 1996; Wang et al. 1995), sampling errors, or selection during the in vitro processes may be the reasons for the recombination differences between the two maps in some genomic regions.

A higher proportion of markers in the DHL population showed segregation distortion compared with the F_2, as it has been extensively reported in other species (Mannien 2000; Chani et al. 2002; Guzy-Wróbelska and Szarejko 2003). Some of this distortion may be caused by selection during the steps needed to construct DHLs through in vitro culture (Dufour et al. 2001). Markers with distortion only in the DHL population are candidates to be linked to genes selected during DHL construction.

The differences in segregation distortion and local recombination rates between populations did not produce any significant difference between the maps. These


G1
0 CSGA057
1 AEST47
3 MC301 TJ3
13 MC319
14 MC68
21 TJ2
29 Aox2
30 CMTC13 MC329
MC281
32 MC356
33 CM173
34 MC11a
35 MC11b
39 MC316
41 MC78
51 Mdr
52 CMACC146 MC77
53 MC352 CMGAN25
54 MC208 MC269a
57 CMAG59
65 CMAT141
72 AEST135a AEST1a
81 MC138
84 AEST59
87 CMTCN56
F2
DHL
G2
0 MC235 (MG19)
1 MC127
5 MC221 MC148
MC209b
6 AEST90b
14 CSWCT10
16 MC53
17 TJ30 CMTCN66a
18 TJ31 TJ12b
20 MC244
22 MG57
23 CM11 MC331b
CM88a
24 MC27 CSWCT16T
CMGA128
25 CSWCT03B
28 CMTA170a MC202
31 MC54
47 CMCTN5 MC296
54 MC124
61 MC298
64 MC215
67 MC207b
69 MC365 MC32
71 CMGA36b
74 TJ10
G3
0 MC373
11 MC311
16 Npr
19 MC44
DHL
25 CMAGN75
26 MC253
27 CMTCN30
28 CSAT425B Acs2
29 CMCAN90
31 MC317 CMGAN21
34 CSWCT12T
40 CM139
57 CMGA15
60 MC387
63 MC217
64 MC125
67 MC249
G4
0 MC33
16 MC233
17 MC7
18 CMAGN61 MC287
19 CMAGN52
20 MC88 MC76
26 CMATN101
31 CMTC9
33 CMCTN35
35 CMAT35
37 MC256
39 MC261a
48 CMGAN3 MC4
MC223
49 MC52b
50 MC99b
58 CMCTN2
64 MRGH63
65 MRGH4
69 MC276
73 CSWCTT02
75 CSWTA02
89 CMTAA166
93 CMTAAN100
G5
0 TJ33 EST5.50
CMTCN62
6 Mlf1 MC337
CMTC160b
7 MC146
8 MC326
(MC388)
DHL
20 Icl
21 MG23 Ms
25 MC264
26 MC375
27 MC331a
28 CMGAN12 MC277
31 CMAGN45
35 CSWCT18B MC63
36 MC259
38 MC255a AEST239b
43 MC234
44 MC20
45 MC231
49 CMATN89
56 MC40
57 MC107
59 MC16 MC118
MC82
61 MC291
62 Acs1
63 MC349 CMGA104
66 MG34b
67 MC93
80 MC278
81 MC265a
G6
0 MC216
16 MC279
21 AEST144
39 MC120 MC265b
MC134 MG1
41 Msf1
42 MC133b
DHL
45 CMCTN86 CMGAN92
48 AEST23
49 AEST1b
50 MC85 CM101a
52 CMCT505
58 CMCCA145
60 MC210 MC309
61 MC247 CMCTN53
62 TJ26
73 CSWCT11
76 TJ27
89 MC294
91 MC212
99 CMCTN4
G7
0 MC52a
2 MRGH21
12 MC92 MC131
14 CMTC47
27 MC13
33 AEST134 AEST239a
35 CM98
36 MC203 MC102
38 MC31
46 CM91
51 MC79
55 MC325
71 CMCTN1
77 CMCTN7
81 MC14 MC237
89 CMATN22
(MC348)
G8
0 CMGA36a
1 CM93b MC130
3 MC340
12 CM149
DHL
28 MC313
29 MC206 Aox3
30 MC51
32 MG37b
39 EST1.41
40 CM24 MC71
41 MC315 MC295
MC384
43 TJ24 CMAGN68
47 CMGA108
52 MC318
56 MC273
59 MC269b
72 MC332
78 MC248
83 AEST84
87 MC376
88 MC252
G9
0 MC149 (AEST139
1 CMCTN71 AEST29, MC17
5 CMCTN19 AEST9, MC103a
6 MC39 MC103b)
9 CM93a CSWCT22A
CSWCT01
21 CMTCN67
23 CMGA172
27 MC112
31 MC225
38 Cus
40 MC133a MC136
41 MC67 CM40
43 CMTCN65 (AEST135b)
48 CMCT134b
49 CMGA165
51 CM101b
52 MC22b
58 CMTCN8
G10
0 CSCCT571
15 MG34a
27 MC220
38 MC38 CM47
42 Mlf2 MC344
45 CM88b TJ12a
47 MC211
49 MC47
54 MC308 CM131
64 MC339b
65 MC284 MG21
AEST132
66 MC307 MC289
MC261b Hsp70
MC275 MG37a
68 CMAGN73
70 CMAGN79
73 EST1.11
75 MG18 MC310
77 MC239 CM122
84 MC219
86 MC99a
90 CMTCN6
97 CMTC168
101 MC60
104 AEST25
G11
0 MC97
2 MC123
3 MC132
12 MC50 MC255b
CSWGAT01
13 nsv
15 MC320
27 TJ29
32 MC330
38 MC209a AEST90a
CSAT425A
39 MC286
46 CMTCN14
63 CMGAN24 CMAGN33
64 CMAGN32
66 CMAGN80
G12
0 CMTCN50 CSWCT2B
3 CSWCT2A
10 CMAGN94
15 MC69
19 MC207a
DHL
25 MC268
26 CMTCN66b
27 CMTCN18
29 MC65 MC226
32 AEST38
35 MC236
39 MC339a
41 MC8
50 MC290
51 CMTC123
57 MC274 MG28
58 MC251
59 MG63
60 MC218
61 MC21
66 CMTCN41
77 MC224
107 MC42
115 CMCTN38

results confirm the validity of the previous map (Oliver et al. 2001) and the suitability of the DHL population as the base population to saturate the melon genetic map.

A map was constructed exclusively with RFLP, SSR, and SNP markers that are typically co-dominant, and highly or moderately transferable after merging F_2- and DHL-based maps. The merged map was slightly shorter than individual maps (1,021 cM for the merged map vs 1,240 cM for the F_2 map and 1,223 cM for the DHL map). Individual maps were built with MAPMAKER, whereas JoinMap was used to build the composite map. MAPMAKER usually produces larger linkage groups than JoinMap, even when using the same data set and the same settings (Liebhard et al. 2003). These differences may be explained by differences in the algorithms used by these softwares. JoinMap uses map distances to build the map and calculate the chi-square for the goodness of fit of the constructed map, whereas MAPMAKER uses recombinant frequencies for adjacent intervals, assuming no interference, to calculated likelihoods to search the best order and then recombination frequencies are translated into mapping distances (Stam 1993). Therefore, when the Kosambi mapping function is used, some degree of discrepancy may occur when using these softwares. Nevertheless, the discrepancy observed in this study was minor and we think that it was not relevant. Thus, 59 newly developed melon SSRs, 13 previously developed cucumber SSRs, and three SNPs were added to the original melon map, filling some gaps and increasing the map density. SSRs were apparently distributed randomly throughout the melon genome as has been observed in other plant species such as rice (Temnykh et al. 2000) or *Prunus* (Aranzana et al. 2003). The association of SSR sequences with centromeric regions, observed in species such as tomato (Bryan et al. 1997; Areshchenkova and Ganal 1999), was not observed.

Most previous melon maps have been constructed with a large proportion of dominant anonymous markers (Wang et al. 1997; Périn et al. 2002a; Silberstein et al. 2003), making full map comparisons and map merging not possible. The set of co-dominant molecular markers used here covers the whole melon genome. Interestingly, SSR markers alone cover nearly 80% of the map. A selected sample of these SSRs can be used as bridges with other published maps to build a melon consensus maps.

Framework maps based on SSRs and RFLPs could be used for genetic analysis in other melon populations, allowing the comparison of the positions of major genes and QTLs what will help to improve our knowledge about the genetic control of important fruit quality traits. Finally, given that some of these SSRs are also transferable to cucumber (Danin-Poleg et al. 2000), *Cucurbita pepo* (Paris et al. 2003) and other *Cucumis* species (Garcia-Mas et al. 2004), they could also be used as anchor points for map comparison between species of the Cucurbitaceae family.

Acknowledgements The authors thank V. Alfaro and N. Galofré for technical assistance. This work was funded in part by grants for projects AGL2000-0360 and AGL2003-09175-C02-01 from The Spanish Ministry of Science and Technology and by the Semillas Fitó-Institut de Recerca i Tecnologia Agroalimentàries (IRTA) joint programme. A.J.M. was supported by a contract from Instituto Nacional de Investigaciones Agrarias (INIA). M.J.G. was supported by a fellowship from Institut de Recerca I Tecnologia Agroalimentàries (IRTA). The experiments presented here comply with current Spanish law.

References

Akashi Y, Fukunda N, Wako T, Masuda M, Kato K (2002) Genetic variation and phylogenetic relationships in East and South Asian melons, *Cucumis melo* L., based on the analysis of five isozymes. Euphytica 125:385–396

Aranzana MJ, Pineda A, Cosson P, Dirlewanger E, Ascasibar J, Cipriani G, Ryder CD, Testolin R, Abbott A, King GJ, Iezzoni AF, Arús P (2003) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor Appl Genet 106:819–825

Areshchenkova T, Ganal MW (1999) Long tomato microsatellites are predominantly associated with centromeric regions. Genome 42:536–544

Baudracco-Arnas S, Pitrat M (1996) A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor Appl Genet 93:57–64

Bryan GJ, Collins AJ, Stephenson P, Orry A, Smith JB, Gale MD (1997) Isolation and characterisation of microsatellites from hexaploid bread wheat. Theor Appl Genet 94:557–563

Chani E, Ashkenazi V, Hillel J, Veilleux E (2002) Microsatellite marker analysis of an anther-derived potato family: skewed segregation and gene-centromere mapping. Genome 45:236–242

Chiba N, Suwabe K, Nunome T, Hirai M (2003) Development of microsatellite markers in melon (*Cucumis melo* L.) and their application to major cucurbit crops. Breed Sci 53:21–27

Chin ECL (1996) Maize simple repetitive DNA sequences: abundance and allele variation. Genome 39:866–873

Cho YG, Ishii T, Temnykh S, Chen X, Lipovich L, McCouch SR, Parl WD, Ayres N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:713–722

Danin-Poleg Y, Reis N, Baudracco-Arnas S, Pitrat M, Staub JE, Oliver M, Arús P, de Vicente CM, Katzir N (2000) Simple sequence repeats in *Cucumis* mapping and map merging. Genome 43:963–974

Danin-Poleg Y, Reis N, Tzuri G, Katzir N (2001) Development and characterisation of microsatellite markers in *Cucumis*. Theor Appl Genet 102:61–72

Danin-Poleg Y, Tadmor Y, Tzuri G, Reis N, Hirschberg J, Katzir N (2002) Construction of a genetic map of melon with molecular markers and horticultural traits, and localization of genes associated with ZYMV resistance. Euphytica 125:373–384

Dolcet-Sanjuan R, Claveria E, Llauradó M, Ortigosa A, Arús P (2001) Carnation (*Dianthus caryophyllus* L.) dihaploid lines resistant to *Fusarium oxysporum* f. sp. *Dianthi*. Acta Hortic 560:141–144

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

Dufour P, Johnsson C, Antoine-Michard S, Cheng R, Murigneux A, Beckert M (2001) Segregation distortion at marker loci: variation during microspore embryogenesis in maize. Theor Appl Genet 102:993–1001

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W (2002) Isolation of EST-derived microsatellite markers for genotyping the A and B genomes of wheat Theor Appl Genet 104:399–407

Fazio G, Staub JE, Chung SM (2002) Development and characterization of PCR markers in cucumber (*Cucumis sativus* L.). J Am Soc Hortic Sci 127:545–557

Garcia-Mas J, Monforte AJ, Arús P (2004) Phylogenetic relationships among *Cucumis* species based on the ribosomal internal transcribed spacer sequence and microsatellite markers. Plant Syst Evol 248:191–204

Gonzalo MJ (2003) Generación, caracterización molecular y evaluación morfológica de una población de líneas dihaploides en melón (*Cucumis melo* L.). PhD Dissertation, Universiad de Lleida

Guzy-Wróbelska J, Szarejko I (2003) Molecular and agronomic evaluation of wheat doubled haploid lines obtained through maize pollination and anther culture methods. Plant Breed 122:305–313

Katzir N, Danin-Poleg Y, Tzuri G, Karchi A, Lavi U, Cregan PB (1996) Length polymorphisms and homologies of microsatellites in several *Cucurbitaceae* species. Theor Appl Genet 93:1282–1290

Kirkbride JH (1993) Biosystematic monograph of the genus *Cucumis* (Cucurbitaceae). Parkway, Boone

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

Lander ES, Green P, Abrahamson J, Barlow A, Daley M, Lincoln S, Newburg L (1987) MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Liebhard R, Koller B, Gianfranceschi L, Gessler C (2003) Creating a saturated reference map for the apple (*Malus* × *domestica* Borkh.) genome. Theor Appl Genet 106:1497–1508

Liu L, Kakihara F, Kato M (2004) Characterization of six varieties of *Cucumis melo* L. based on morphological and physiological characters, including shelf-life of fruit. Euphytica 135:305–313

Manninen OM (2000). Associations between anther-culture response and molecular markers on chromosomes 2H, 3H and 4H of barley (*Hordeum vulgare* L.). Theor Appl Genet 100:57–62

Metzgar D, Bytof J, Wills C (2000) Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Res 10:72–80

Mliki A, Staub JE, Zhangyong S, Ghorbel A (2001) Genetic diversity in melon (*Cucumis melo* L.): an evaluation of African germplasm. Genet Res Crop Evol 48:587–597

Monforte AJ, Garcia-Mas J, Arús P (2003) Genetic variability in melon based on microsatellite variation. Plant Breed 122:153–157

Monforte AJ, Eduardo I, Abad S, Arús P (2004a) Inheritance mode of fruit traits in melon. Heterosis for fruit shape and its correlation with genetic distance. Euphytica (in press)

Monforte AJ, Oliver M, Gonzalo MJ, Álvarez JM, Dolçet-Sanjuan R, Arús P (2004b) Identification of quantitative trait loci involved in fruit quality traits in melon. Theor Appl Genet 108:750–758

Morales M, Luís-Arteaga M, María Álvarez J, Dolcet-Sanjuan R, Monfort A, Arús P, Garcia-Mas J (2002) Marker saturation of the region flanking the gene NSV conferring resistance to the melon necrotic spot carmovirus (MNSV) in melon. J Am Soc Hortic Sci 127:540–544

Morales M, Roig E, Monforte AJ, Arús P, Garcia-Mas J (2004) Single-nucleotide polymorphisms detected in expressed sequence tags of melon (*Cucumis melo* L.). Genome 47:352–360

Oliver M, Garcia-Mas J, Cardús M, Pueyo N, López-Sesé AI, Arroyo M, Gómez-Paniagua H, Arús P, De Vicente MC (2001) Construction of a reference linkage map for melon. Genome 44:836–845

Ooijen JW van, Voorrips RE (2001) JoinMap 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Paris HS, Yonash N, Portnoy V, Mozes-Daube N, Tzuri G, Katzir N (2003) Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. Theor Appl Genet 106:971–978

Paterson AH, Lan TH, Reischmann KP, Chang C, Lin SC, Burow MD, Kowalski SP, Katsar CS, DelMonte TA, Feldmann KA, Schertz KF, Wendel JF (1996) Toward a unified genetic map of higher plants, transcending the monocot–dicot divergence. Nat Genet 14:380–382

Périn C, Hagen LS, de Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002a) A reference map for *Cucumis melo* based on two recombinant inbred line populations. Theor Appl Genet 104:1017–1034

Périn C, Hagen LS, Giovinazzo N, Besombes, D, Dogimont C, Pitrat M (2002b) Genetic control of fruit shape acts prior to anthesis in melon (*Cucumis melo* L.). Mol Gen Genomics 266:933–941

Pitrat M (2002) 2002 melon gene list. http://www.umresearch.umd.edu/CGC/genes.htm

Plomion C, O'Malley DM (1996) Recombination rate differences for pollen parents and seed parents in *Pinus pinaster*. Heredity 77:341–350

Ritschel PS, Lins TCL, Tristan RL, Buso GSC, Buso JA, Ferreira ME (2004) Development of microsatellite markers from an enriched genomic library for genetic analysis of melon (*Cucumis melo* L.). BMC Plant Biol 4:9

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

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSRs derived from grape ESTs. Theor Appl Genet 100:723–726

Silberstein L, Kovalski I, Brotman Y, Perin C, Dogimont C, Pitrat M, Klingler J, Thompson G, Portnoy V, Katzir N, Perl-Treves R (2003) Linkage map of *Cucumis melo* including phenotypic traits and sequence-characterized genes. Genome 46:761–773

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Stepansky A, Kovalski I, Perl-Treves R (1999) Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. Plant Syst Evol 217:313–332

Temnykh S, Park WD, Ayres N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:697–712

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development and characterization of gene-derived SSR markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106:411–422

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Wang G, Hyne V, Chao S, Henry Y, Buyser J, Gale MD, Snape JW (1995) A comparison of male and female recombination frequency in wheat using RFLP maps of homoeologous group 6 and 7 chromosomes. Theor Appl Genet 91:744–746

Wang YH, Thomas CE, Dean RA (1997) A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers. Theor Appl Genet 95:791–798