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ORIGINAL PAPER

Bin mapping of genomic and EST-derived SSRs in melon (*Cucumis melo* L.)

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Abstract We report the development of 158 primer pairs flanking SSR motifs in genomic (gSSR) and EST (EST-SSR) melon sequences, all yielding polymorphic bands in melon germplasm, except one that was polymorphic only in *Cucurbita* species. A similar polymorphism level was found among EST-SSRs and gSSRs, between dimeric and trimeric EST-SSRs, and between EST-SSRs placed in the open reading frame or any of the 5′- or 3′-untranslated regions. Correlation between SSR length and polymorphism was only found for dinucleotide EST-SSRs located within the untranslated regions, but not for trinucleotide EST-SSRs. Transferability of EST-SSRs to *Cucurbita* species was assayed and 12.7% of the primer pairs amplified at least in one species, although only 5.4% were polymorphic. A set of 14 double haploid lines from the cross between the cultivar "Piel de Sapo" and the accession PI161375 were selected for the bin mapping approach in melon. One hundred and twenty-one SSR markers were newly mapped. The position of 46 SSR loci was also verified by genotyping the complete population. A final bin-map was constructed including 80 RFLPs, 212 SSRs, 3 SNPs and the Nsv locus, distributed in 122 bins with an average bin length of 10.2 cM and a maximum bin length of 33 cM. Map density was 4.2 cM/marker or 5.9 cM/SSR.

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Introduction

Microsatellites or simple-sequence repeats (SSRs) are short tandem repeats from 1 to 6 nucleotides, being the most variable types of DNA sequences in the genome. SSR-derived markers are hypervariable, multiallelic, often codominant, evenly distributed through the genome, highly reproducible and they can be used as anchor points for molecular linkage group comparisons and map merging (Gonzalo et al. 2005). For these reasons, SSR markers are widely used for applications such as fingerprinting, cultivar identification, hybrid seed purity testing, linkage map construction, gene tagging and genetic diversity studies and evolution (Varshney et al. 2005). SSR markers can be designed from genomic (gSSRs, Panaud et al. 1995) or expressed sequence tags (EST, also denominated EST-SSRs) sequences. EST-SSRs have several advantages: (i) development costs are relatively inexpensive; (ii) they are related to genes, being functional markers that can be used as candidate genes to study their association with phenotypic variation, (iii) the flanking sequences are more likely to be conserved among close or distant species than those derived from genomic sequences, as they are under functional constraint, making their use as markers for comparative mapping easier. On

the other hand, they typically exhibit lower polymorphism than gSSRs (Cho et al. 2000). EST-SSRs have been developed in many plant species, e.g., cotton (Han et al. 2004), *Festuca* sp. (Saha et al. 2004), grapevine (Rossetto et al. 2002), *Medicago* ssp. (Eujayl et al. 2004), sugarcane (Cordeiro et al. 2001), spruce (Rungis et al. 2004), barley (Pillen et al. 2000), rice (Temnykh et al. 2000) and wheat (Eujayl et al. 2001).

Melon (*Cucumis melo* L.) is an economically important crop of the Cucurbitaceae family grown in temperate and tropical climates worldwide. This family includes other crops such as cucumber (*C. sativus* L.), watermelon (*Citrullus lanatus* L.) and several species of the genus *Cucurbita*, such as *C. pepo* L., *C. maxima* D., and *C. moschata* D. Recent melon genomics initiatives include the Spanish Melon Genomics Project MELOGEN (http://www.melogen.upv.es) and the Cucurbit Genomics Resource (CGR, http://cucurbit.bti.cornell.edu), both of them now integrated in the International Cucurbit Genomics Initiative (ICuGI, http://www.icugi.org/). A collection of over 30,000 melon ESTs, clustered into 16,637 non-redundant sequences or unigenes, were developed in the MELOGEN project from which 1,052 potential SSRs were identified (Gonzalez-Ibeas et al. 2007).

Several melon maps have been constructed to date (Baudracco-Arnas and Pitrat 1996; Wang et al. 1997; Liou et al. 1998; Brotman et al. 2000; Oliver et al. 2001; Danin-Poleg et al. 2000; Périn et al. 2002; Gonzalo et al. 2005). The first melon map composed mainly of co-dominant markers was developed by Oliver et al. (2001), based in an F_2 population from a cross between two genetically distant accessions: the Spanish melon cultivar 'Piel de Sapo' (PS) and the Korean cultivar 'Songwhan Charmi' (SC) accession PI 161375. Gonzalo et al. (2005), using a double haploid line (DHL) population from the same cross, developed an expanded version of the melon map using only co-dominant transferable markers. The new composite map contained 327 markers, 97 of them SSRs.

A higher SSR marker density is desirable to expand the application of the melon genetic map to a larger group of breeding populations, however. genetic mapping is costly and time-consuming. A strategy to improve the efficiency of genetic mapping saturation ('bin mapping') by reducing the size of the mapping population was proposed by Vision et al. (2000). This strategy was later applied by Howad et al. (2005) to obtain a minimum set of plants to place new markers at a specific map position, and it was validated with a large set of SSR markers in the *Prunus* reference map. Briefly, the bin mapping approach consists of two steps. First, a high/medium density map is constructed using a mapping population of usual size (60–200 genotypes) and, second, new markers are added to the map with lower resolution by genotyping only a subset of selected, highly informative genotypes (the bin set). The optimal bin set of a given size has the maximum possible number of breakpoints evenly spaced throughout the genome, resulting in a high number of small bins of uniform size (Howad et al. 2005). This strategy has been recently used in strawberry (Sargent et al. 2008) and preliminarily studies have been carried out in melon (Moreno et al. 2008).

The main objectives of the current report were to test the bin mapping strategy for adding new markers to the Gonzalo et al. (2005) map and to further saturate it with newly developed EST-SSRs from the MELOGEN database and gSSRs from genomic libraries. We selected a bin set from the melon DHL mapping population (Gonzalo et al. 2005) based on number and distribution of recombination points through the genome to set up the bin mapping approach in melon and map the new SSRs. Additionally, the transferability of EST-SSRs to *Cucurbita* spp. species was evaluated in order to develop markers useful for these species.

Material and methods

Plant material and DNA extraction

Eight melon accessions representing several melon types (Table 1) were used to estimate the polymorphism of the newly developed SSR markers. This sample included "Piel de Sapo" (PS) and the "Songwhan Charmi" (SC), parents of the DHL mapping population ($N = 72$) used by Gonzalo et al. (2005), for the construction of a linkage map.

Thirty-one accessions covering a broad sample of variation were used to test the transferability of EST-SSR markers among *Cucurbita* spp. (Electronic Supplementary Material S1). The selection was based on results obtained in previous studies (Ferriol et al. 2003a, b, 2004a, b, 2007). Thirteen belonged to *C. pepo*, representing the eight edible-fruited cultivar groups of the subspecies *pepo* and subspecies *ovifera*, one accession representative of the special Bubango landrace from the Canary Islands and one accession used for ornamental purposes. Eleven and seven accessions, respectively, representing the extant morphological diversity of *C. moschata* and *C. maxima* were also used. They included accessions from their area of origin, *C. moschata* for Central and South America and *C. maxima* subsp. *maxima* for South America, along with Spanish landraces and a wild accession belonging to subsp. *andreana* (the ancestor of cultivated *C. maxima*).

DNA from all genotypes was extracted using the modified CTAB method (Doyle and Doyle 1990). The DNA of the 31 accessions of the *Cucurbita* genus was extracted from a mixture of leaves of five individuals per accession in order to sample the genetic variability within heterogeneous accessions.

Table 1 Melon genotypes used in this research

Plant designation	Accession number	Seed source[a]	Country of origin	Cultivar group[b]
Flexuosus, snake cucumber	PI 435288	1	Iraq	Flexuosus[1,2]
2564	PI 124112	1	India	Momordica[1]
Momordica	PI 414723	2	India	Momordica[1]
Songwhan Charmi	PI 161375	2	Korea	Chinensis[1], Conomon[2]
C. trigonus	Ames24297	1	Pakistan	Wild melon
Vedrantais			France	Cantalupensis[1,2]
Piel de sapo		2	Spain	Inodorus[1,2]
KLM-1683	PI 536481	1	Maldivas	Unknown

Codes are the same as those used by Monforte et al. (2003). Seed source, country of origin and cultivar group are also indicated

[a] 1. NCRPIS (North Central Regional Plant Introduction Station, Ames, IA, USA). 2. Semillas Fitó (Barcelona, Spain)

[b] According to: 1. Pitrat et al. (2000); 2. Munger and Robinson (1991)

gSSRs

Simple-sequence repeat markers from small-insert genomic libraries were developed following the procedure described by Aranzana et al. (2003). Briefly, 500 ng of DNA were digested with *Eco*RI. The resulting fragments were ligated to the adapters prepared with the oligo-nucleotides 17-mer (CTCGTAGACTGCGTACC) and 18-mer (AATTGGTA CGCAGTCTAC) and amplified by PCR. The amplified fragments were hybridized to a biotinylated oligonucleotide with the (5′55555TCTCTCTCTCTCTCTC) sequence (5′ indicates biotin, 5 indicates dInosine). The hybrids were bound to streptavidin-coated paramagnetic particles (Promega Kit 'MagneSphere® Magnetic Separation Products', Madison, WI, USA) and the $(AG)_n$ sequences were washed, eluted and cloned. Clones containing SSR motifs were selected by hybridizing filters containing their DNA with the primer $(CT)_{10}$, end-labeled with [γ-^{32}P]-ATP. Positive clones were selected for DNA sequencing. Primer pairs were designed from the microsatellite flanking regions using the program PRIMER3 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA). Primers were designated with GCM—followed by a three digit number. A set of 23 gSSRs from Ritschel et al. (2004) were also tested (ESM S2).

EST-SSRs

SSR motifs were searched within 30,000 ESTs from the Spanish Melon Genomics Project (MELOGEN) (http://www.melogen.upv.es). EST sequences containing perfect repetitions of more than nine units were selected for primer design. SSRs located within sequences homologous to conserved protein domains (PFAM) were excluded in order to select putative sequences with higher polymorphism. One hundred and seventy-three primer pairs were designed using the PRIMER3 software to amplify fragments ranging from 80 to 300 bp. In addition, ten primer pairs were designed from ESTs of the International Cucurbit Genomics Initiative (ICuGI) (http://www.icugi.org/cgi-bin/ICuGI/EST/search.cgi) following the same procedure as above. EST-SSRs were designated with ECM followed by a two/three digit number. EST functional annotation was established by comparing EST sequences with Uniref90 database (http://www.ebi.ac.uk/uniref/) using BlastX (Altschul et al. 1997).

SSR genotyping

Temperature-gradient PCR was performed in a PTC-200 Thermocycler (MJ Research, Waltham, MA, USA) to obtain the optimum annealing temperature (T_{an}) for each primer pair using a sample of the PS genotype. PCR reactions were performed in a final volume of 15 µl with 1× Taq buffer [10 mM Tris–HCl, 50 mM KCl, 0.001% gelatine, (pH 8.3)], 1.5–3.5 mM $MgCl_2$, 166 µM dNTPs, 5 pmol of each primer, 2 U Taq DNA polymerase and 20 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. For visualization using LI-COR IR^2 sequencer (Li-Cor Inc, Lincoln, NE, USA), a 20-nucleotide sequence from the M13 cloning vector (CACGACGTTGTAAAACGACC) was attached to the 5-end of the forward primers. PCR were performed as above but 2 pmol of each primer and 0.66 pmol of IRD700- or IRD800-labelled oligonucleotide complementary to the 20-mer M13 sequence were added.

Amplified bands were visualized in standard polyacrylamide gels by silver nitrate staining (Caetano-Anollés and Gresshoff 1994) or using a LI-COR IR^2 sequencer; 5 µl of loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) were added to the PCR mix and samples were denatured at 96°C for 2 min. Electro-

phoresis was performed in denaturing conditions at 50°C in TBE (90 mM Tris–borate, 2 mM EDTA pH 8.0 and 7.5 M urea) buffer using 6% polyacrylamide gels (AA:BIS = 19:1).

For the 132 EST-SSRs tested to evaluate the transferability and utility in genetic diversity studies within the *Cucurbita* genus, the PCR reactions were performed in a final volume of 25 µl with 1× Taq buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM each primer, 0.6 U Taq DNA polymerase (Netzyme, Molecular Netline Bioproducts,. N.E.E.D., S.L., Valencia, Spain), and 25 ng genomic DNA. Different annealing temperatures, from 50 to 55°C, were tested. The cycling conditions were as follows: an initial cycle at 94°C for 3 min, followed by 30 cycles at 94°C, 30 s, T_{an} °C, 30 s, and 72°C, 30 s and a final cycle at 72°C for 5 min. Some loci required further adjustments to PCR conditions, and a wider range of conditions were then tested (T_{an} and $MgCl_2$ concentrations). Touch-down PCR was also used in some cases. In order to test the amplification conditions, two accessions per species were tested for each SSR loci. Those loci that amplified in each species were tested in the remaining accessions of the corresponding species. After amplification, fragments were resolved using polyacrylamide gel electrophoresis (12% acrylamide–bisacrylamide, TBE 1×) followed by silver nitrate staining.

Genetic variability analysis

Number of alleles, allele frequencies and the polymorphism information content (PIC) were calculated for each locus for the eight melon genotypes using the PowerMarker software (Liu and Muse 2005).

The influence of different factors (SSR origin, type and length of the repeat, number of repetitions and position of the repeat within the EST) in the PIC value was also investigated. A *t*-test was used to compare mean PIC of EST-SSRs and gSSRs, and di- and trinucleotide EST-SSRs. Mean PIC of trinucleotide EST-SSRs placed in ORF, 5′-UTR and 3′-UTR were compared by ANOVA. Correlation between number of repeat units and PIC was studied by linear regression analysis for dinucleotide and trinucleotide EST-SSRs. This analysis was performed both globally and separately for SSRs placed in the ORF 5′- or 3′-UTRs. All statistical analyses were performed with JMP 5.1.2 software (SAS Institute, Cary, NC, USA).

In the analysis of *Cucurbita* species, the number of alleles and the PIC for each marker, and Nei's genetic diversity (Nei 1973) for each species, were calculated with Popgene v.1.32 (Yeh et al. 1997).

Selection of the bin set and map construction

The marker data set from Gonzalo et al. (2005) was used to select a bin set subset with the following criteria: maximum number of recombination breakpoints, minimal number of plants, minimal number of joint genotypes that correspond to more than one bin (duplicate bins), smallest maximum bin length, and maximum number of bins (minimal average bin length). Fourteen was considered a desirable size as a set of 16 individuals (fourteen DHL genotypes and the two parents) fits well in standard 96-well PCR plates. A set of eight additional DHL were also selected in order to verify further the bin mapping strategy. The selection of genotypes was done by both visual inspection and MapPop software (Vision et al. 2000). For the first method, the number and position of recombination crossover sites (or breakpoints) was calculated for each genotype. To minimize the effect of possible genotyping errors, double recombination sites were not taken into account. Initially, the genotypes with a higher number of recombination breakpoints were selected and the map was divided in bins. Some bins covered a large genetic distance, giving regions with very low mapping resolution. In these cases, we looked for alternative DHLs until we found a combination that gave a more uniform distribution of bins. We also checked that the joint genotypes defined by the DHLs of the bin set were specific for each bin (no duplicate bins).

When using the MapPop software two parameter options were used, namely the SAMPLEMAX command, which chooses a sample with a minimum, expected maximum bin size and the SAMPLEEXP command, which chooses a sample with minimum expected average bin size. Bin length was calculated as the distance between the position of the last marker of a bin and the position of the last marker of the previous bin. Total number of bins, the average bin length and the maximum bin length obtained by the three methods were compared and a final set of fourteen DHLs that best followed the above criteria was selected. Eight additional DHLs were selected to confirm marker positioning with the 14-set.

The selected DHLs (14 plus 8) were characterized with all gSSRs and EST-SSRs polymorphic between the parents of the mapping population. SSRs were mapped by visually matching the joint genotype obtained with that of the bin set. Markers that fitted in regions with low marker saturation or did not correspond to any of the detected bins were selected to genotype the whole reference population of 72 DHLs and were added to the original map dataset (Gonzalo et al. 2005). Linkage analysis and map construction was performed 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 LOD > 2.0. The remaining markers were then located with the try command. Distances were calculated with the Kosambi function

(Kosambi 1944) and maps were drawn with MapChart, version 2.1 (Voorrips 2002). Linkage group nomenclature was according to Périn et al. (2002). This map was used as the framework to redefine the number, joint genotype and position of the bins. Bins were coded with the linkage group number of the bin location, followed by a three-digit number, corresponding to the distance of the last marker included in the bin to the origin of the linkage group.

Results

SSR characterization

Two hundred and twenty-five primer pairs flanking SSR motifs were designed, 183 primer pairs were designed from ESTs (EST-SSRs) and 42 from genomic clones. Amplification products were obtained for 144 (79%) EST-SSRs and 36 (86%) gSSRs. A total of 157 (88%) SSRs were polymorphic among the melon genotypes used in this study, with a similar proportion of polymorphic SSRs for EST-SSRs (87.5%) and gSSRs (89%). All primer pairs amplified a single reproducible polymorphic band, except ECM60 that amplified three polymorphic bands (ECM60a, ECM60b, ECM60c) giving a total of 159 new polymorphic SSR loci. (See ESM S3 or http://www.melogen.upv.es/genomica/melon/table_3.php).

PIC values for EST-SSRs ranged from 0.21 to 0.82 (mean, 0.58), and for gSSR, 0.30 to 0.79 (mean, 0.54), with the means not being significantly different ($P = 0.29$). Mean PIC values of dimeric (0.55) and trimeric (0.41) EST-SSRs were not significantly different ($P = 0.17$). Looking at the effect of SSR position within the EST on the PIC, the mean PIC values for SSRs in the 5′-UTR (0.57), in the 3′-UTR (0.59) and in the ORF (0.61) were not significantly different ($P = 0.59$).

The correlation between SSR length and PIC was positive and significant both for EST-SSRs ($r = 0.42$, $P < 0.001$) and gSSRs ($r = 0.43$, $P = 0.04$). Correlations were also investigated among di- and trimeric EST-SSRs separately, with only the former having a clear positive correlation between length and PIC ($r = 0.53$, $P < 0.001$) whereas the latter showed no significant correlation ($r = 0.17$; $P = 0.25$). The lack of correlation between SSR length and PIC for trimeric EST-SSRs was independent of position within the EST, whereas the significant correlation found for dimeric EST-SSRs was found in both 5′-UTR ($r = 0{,}56$, $P < 0.001$) and 3′-UTR ($r = 0.64$, $P = 0.0013$) sequences.

Transferability of *C. melo* EST-SSRs to *Cucurbita* spp.

Nineteen out of 132 *Cucumis* EST-SSR primer pairs (13.6%) amplified in at least one *Cucurbita* species, 12.1% in *C. maxima* and *C. moschata* and 10.6% in *C. pepo,* indicating a low conservation of the sequences flanking the SSR between these two related genera. Eleven of these amplified in the three species, five in two species and three only in one species (Table 2). However, 38.9, 33.3 and

Table 2 Estimates of genetic diversity in a sample of 31 *Cucurbita* spp. accessions using a set of melon EST-SSRs that amplified in at least one species

SSR locus	No. alleles/Nei's genetic diversity (1973)			No. alleles *Cucurbita* spp.	PIC
	C. pepo	*C. moschata*	*C. maxima*		
ECM61	7/0.80	3/0.65	2/0.34	10	0.86
ECM116	4/0.62	3/0.42	2/0.34	6	0.71
ECM139	4/0.58	3/0.56	3/0.61	9	0.85
ECM53	5/0.69	2/0.40	1	6	0.76
ECM156	2/0.50	1	2/0.25	5	0.75
ECM147	–	2/0.30	1	3	0.68
ECM213	–	2/0.40	3/0.57	4	0.71
ECM208	1	1	3/0.65	4	0.38
ECM130	1	2/0.30	1	3	0.63
ECM55	1	1	1	1	n.a
ECM79	1	–	1	1	n.a
ECM106	–	–	1	1	n.a
ECM174	1	1	1	1	n.a.
ECM178	1	1	1	1	n.a
ECM129	1	–	1	1	n.a
ECM195	1	1	1	1	n.a
ECM86	1	1	–	1	n.a
ECM168	–	1	–	1	n.a
ECM215	–	1	–	1	n.a

The number of alleles and Nei's genetic diversity for each locus within each species are given in the first three columns. The total number of alleles and polymorphism information content (PIC) across species are also given

– No amplification, *1* no polymorphism, *n.a.* PIC could not be calculated due to lack of polymorphism

31.3% of the amplified bands were polymorphic between the accessions of *C. moschata*, *C. maxima* and *C. pepo,* respectively. Thus, the percentage of melon EST-SSR useful for genetic diversity studies in the genus *Cucurbita* was very low, less than 5.3% in all cases. These polymorphic EST-SSRs contained mostly di-nucleotide repeats.

Selection of the bin set

An average of 8.9 recombination breakpoints (ranging from 3 to 14) was observed in the DHL genomes, representing less than one recombination per linkage group. To define the bin set by visual inspection, the 19 DHLs with 12 or more recombination breakpoints were initially selected to study how they divided the genome in bins. We found some bins covered large genetic distances (>50 cM) and three additional DHLs with breakpoints in these regions were selected. From these 22 DHLs, a final set of 14 (bin set composed of DHLs 14, 39, 45, 85, 90, 117, 127, 135, 136, 1,007, 1,051, 1,127, 1,131, 1,152) was selected, defining 103 bins with an average bin length of 11.0 cM and a maximum bin length of 43 cM. No duplicate bins were observed.

When using a saturated map, the genotypes of the bin set should differ only in one point between two contiguous bins because each bin should be defined by an unique recombination point. However, we frequently found that the genotypes of the bin set between two contiguous bins differed in two or more genotypic points (two or more DHLs have different alleles at the marker loci defining the bin), indicating two or more recombination points between bins. Theoretically, there should exist a bin-set genotypic combination that would fit in that intermediate position. We term this expected genotypic combination "putative bin" (ESM 4). This situation occurred because the original melon map was not completely saturated. It is expected that when saturating the map with new markers, new combined genotypes will be compatible with a "putative bin" that fits between two contiguous bins that differ in two or more genotypic points. Thus, we selected eight additional DHLs (52, 84, 128, 138, 1,109, 1,019, 1,123 and 1,128) to help resolve any conflicting data and provide a more solid evidence during the bin mapping process.

SAMPLEMAX and SAMPLEEXP commands implemented in the MapPop software were also used for the selection of the bin set. An average bin length of 16.43 or 14.56 cM, and a maximum bin length of 78 or 85 cM were obtained, respectively. Given that both average and maximum bin length were longer in these sets compared with the set selected by visual inspection, we chose the latter for defining the melon bin set.

Bin mapping

Eighty-one EST-SSRs (83 loci), 25 gSSRs from our genomic libraries (GCM- and 5A6U) and 14 gSSRs (CMBR-) from Ritschel et al. (2004), polymorphic between PS and SC, were analyzed with the bin set. We found four different situations: (i) 84 SSRs corresponded to one of the defined bins; (ii) 24 SSRs had a joint genotype that was not coincident with any bin but was compatible with a 'putative bin' between two contiguous bins; (iii) 11 markers could not be clearly assigned to any bin or putative bin and (iv) two SSRs (ECM56 and GCM246) defined putative duplicate bins in LG IV and IX. Analysis of one additional DHL (52) resolved their assignment to one single bin, placing ECM56 in a putative bin in LG IX and GCM246 in the same bin as TJ12a in LG IV.

To investigate the accuracy of the bin mapping approach, 47 SSR loci were selected to be assayed in the whole mapping population. These included 23 SSR loci placed in bins covering large genome distances (i), 11 markers assigned to 'putative' intermediate bins (ii), 11 unassigned markers (iii) and the two SSRs placed in duplicate bins (iv).

All markers except ECM127, including unassigned ones, could be mapped. The markers assigned to putative intermediate bins were placed in the expected positions, confirming the putative intermediate bins. A total of 221 loci were used to construct a new linkage map including the 175 markers, previously mapped by Gonzalo et al. (2005), and 46 new SSR loci (36 EST-SSRs, seven GCM-, two CMBR- and 5A6U loci). The map spanned 1244 cM, with an average of 104 cM per linkage group, ranging from 62 to 141 cM. Map density was 5.7 cM/marker, and the maximum gap was 27 cM between markers CMTCN14 and CMAGN33 on LG XII (Fig. 1). The 136 SSR loci included in this map represent a density of 9.0 cM/SSR, with SSR markers covering 85% of the total genome length (considering that a marker covers a 10 cM-interval at each side). For the rest of the bin-mapped markers, the eight additional DHLs (see above) were also genotyped. In all cases, the genotype of the additional DHLs was compatible with the bin map position estimated with the fourteen set.

The new linkage map was used to redefine the number, position and joint genotype of the bins. The final bin map (Fig. 2) consisted of 296 (including 212 SSRs) distributed in 122 bins, including 19 new bins. Eleven of these bins were tested and confirmed with the entire DHL population. The remaining new bins were also confirmed by genotyping the eight additional DHLs. The average bin length was 10.2 cM with a maximum bin length of 33 cM (bin I:137). Map density was 4.2 cM/marker (5.9 cM/SSR).

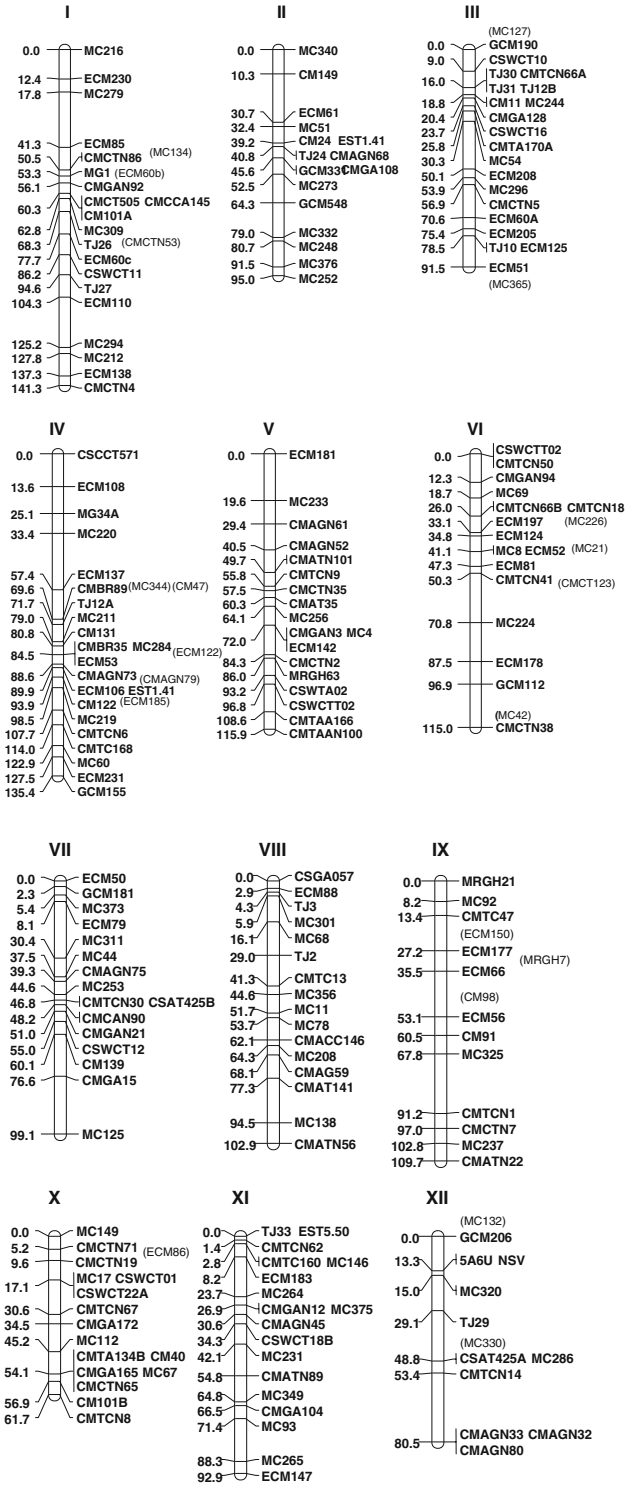


Fig. 1 Genetic linkage map of *Cucumis melo* based on the DHL population derived from the Piel de Sapo (PS) and Songwhan Charmi (SC) cross (Gonzalo et al. 2005). This map includes the markers previously reported by (Gonzalo et al. 2005) and 46 new SSRs (36 EST-SSRs, seven gSSRs developed in this work, and two gSSRs from Ritschel et al. 2004). *Markers* that could not be assigned at LOD > 2.0 are indicated in *brackets*

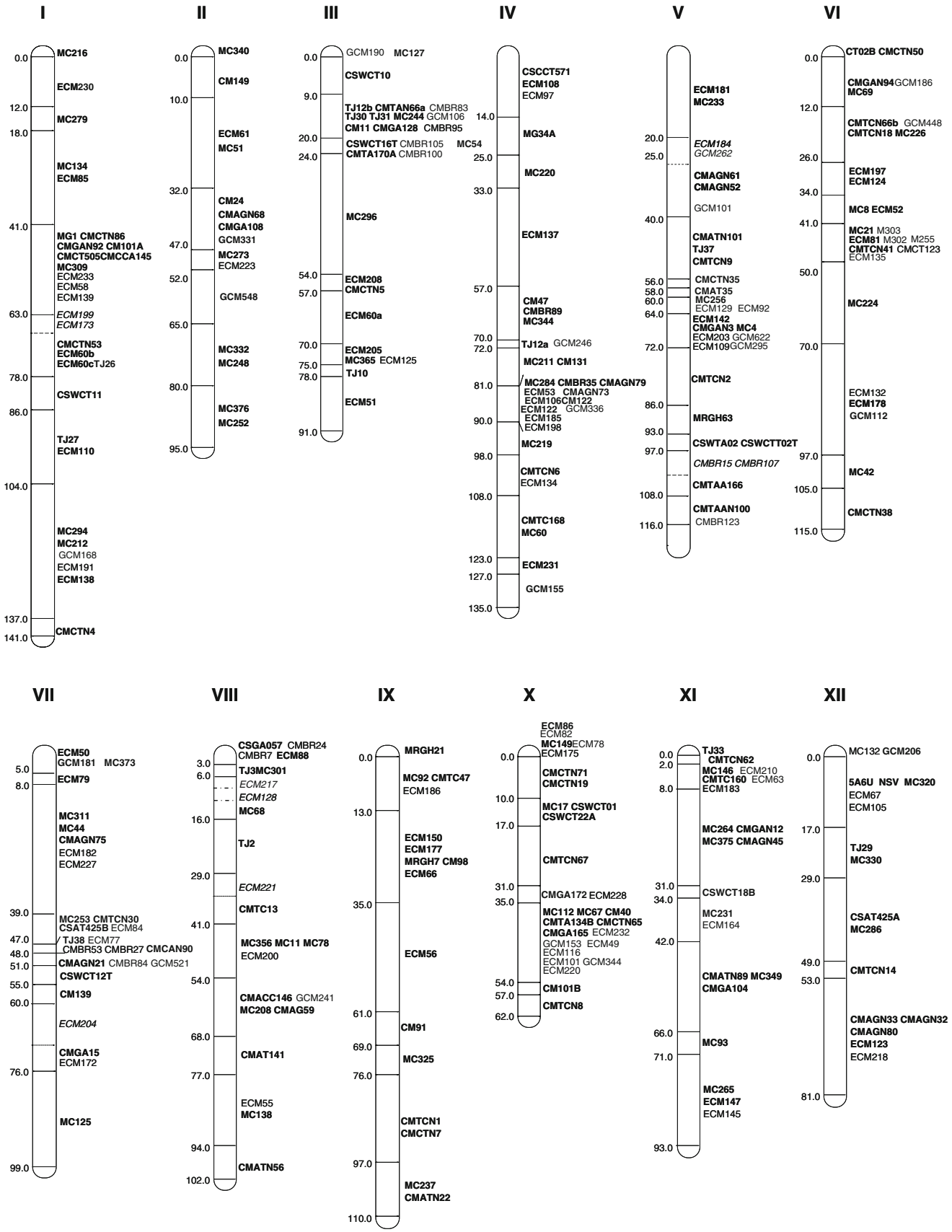
The number of markers per bin ranged from 1 to 13, with an average of 2.5 markers/bin. The number of SSRs per bin ranged from 1 to 10, with an average of 1.7 SSRs/bin. The bin with more SSRs was X:054 (ten SSRs in 19 cM), followed by bin IV:090 (nine SSRs in 9 cM) and bin III:020 (eight SSRs in 11 cM). These were also the bins where more markers were located (13, 11 and 10 respectively).

Discussion

Characterization of EST-SSRs

The EST-SSR primer pairs developed in the current work gave an amplification rate (79%) similar to that of gSSRs (83%). This is high compared with similar studies reported in other species (60–90% on average, reviewed by Varshney et al. 2005). The high quality of the sequence information available in MELOGEN and its accurate annotation may account for the success, together with careful optimization of PCR conditions.

EST-SSR polymorphism was very high and very similar to gSSRs both in terms of polymorphism rate (87 vs. 89%) and PIC (0.58 vs. 0.54). It has been reported in a wide spectrum of species, including rice (Cho et al. 2000), barley (Thiel et al. 2003, Chabane et al. 2005), wheat (Eujayl et al. 2002), alpine lady-fern (Woodhead et al. 2003), pine (Chagne et al. 2004) and spruce (Rungis et al. 2004), that gSSRs are more polymorphic than EST-SSRs. One possible explanation is that most of the accessions used for polymorphism investigation are quite distant to each other, so some SSR alleles may be allelic by state but not by descend, producing an underestimation of the SSR polymorphism. The underestimation could be more important for gSSRs, making that the polymorphism for gSSRs and EST-SSRs looks very similar. A higher range of melon genotypes, more closely related, should be investigated to address this hypothesis. On the other hand, the high proportion of polymorphic EST-SSRs could be a consequence of the stringent selection criteria applied to the ESTs for marker development. A minimum of nine and seven repetition units was set for dimeric and trimeric SSRs, respectively, based on observations of Thiel et al. (2003), who stated that above this threshold no further augmentation of the polymorphism level occurs in a set of SSRs derived from barley ESTs. SSRs within sequences homologous to known protein domains were also excluded as they are expected to be highly conserved among taxa. The EST-SSRs designed in the current study proved to be as efficient as the gSSRs, both in terms of amplification rate and polymorphism, making this set of EST-SSRs a valuable source of molecular markers in melon.


I
0.0 MC216
ECM230
12.0
MC279
18.0
MC134
ECM85
41.0
MG1 CMCTN86
CMGAN92 CM101A
CMCT505CMCCA145
MC309
ECM233
ECM58
ECM139
63.0
ECM199
ECM173
CMCTN53
ECM60b
ECM60cTJ26
78.0
CSWCT11
86.0
TJ27
ECM110
104.0
MC294
MC212
GCM168
ECM191
ECM138
137.0
141.0 CMCTN4
II
0.0 MC340
CM149
10.0
ECM61
MC51
32.0
CM24
CMAGN68
CMGA108
47.0 GCM331
MC273
52.0 ECM223
GCM548
65.0
MC332
MC248
80.0
MC376
MC252
95.0
III
0.0 GCM190 MC127
CSWCT10
9.0
TJ12b CMTAN66a CMBR83
TJ30 TJ31 MC244 GCM106
CM11 CMGA128 CMBR95
20.0 CSWCT16T CMBR105
24.0 CMTA170A CMBR100
MC296
54.0
ECM208
57.0 CMCTN5
ECM60a
70.0
ECM205
75.0 MC365 ECM125
78.0 TJ10
ECM51
91.0
IV
CSCCT571
ECM108
ECM97
14.0
MG34A
25.0
MC220
33.0
ECM137
57.0
CM47
CMBR89
MC344
70.0
72.0 TJ12a GCM246
MC211 CM131
81.0
MC284 CMBR35 CMAGN79
ECM53 CMAGN73
ECM106CM122
ECM122 GCM336
ECM185
90.0 ECM198
MC219
98.0
CMTCN6
ECM134
108.0
CMTC168
MC60
123.0
ECM231
127.0
GCM155
135.0
MC54
V
ECM181
MC233
20.0
ECM184
25.0 GCM262
CMAGN61
CMAGN52
GCM101
40.0
CMATN101
TJ37
CMTCN9
56.0 CMCTN35
58.0 CMAT35
60.0 MC256
ECM129 ECM92
64.0
ECM142
CMGAN3 MC4
ECM203 GCM622
72.0 ECM109GCM295
CMTCN2
86.0
MRGH63
93.0
CSWTA02 CSWCTT02T
97.0
CMBR15 CMBR107
CMTAA166
108.0
CMTAAN100
116.0 CMBR123
VI
0.0 CT02B CMCTN50
CMGAN94GCM186
MC69
12.0
CMTCN66b GCM448
CMTCN18 MC226
26.0
ECM197
ECM124
34.0
MC8 ECM52
41.0
MC21 M303
ECM81 M302 M255
CMTCN41 CMCT123
ECM135
50.0
MC224
70.0
ECM132
ECM178
GCM112
97.0
MC42
105.0
CMCTN38
115.0
VII
ECM50
GCM181 MC373
5.0
ECM79
8.0
MC311
MC44
CMAGN75
ECM182
ECM227
39.0
MC253 CMTCN30
CSAT425B ECM84
47.0 TJ38 ECM77
48.0 CMBR53 CMBR27 CMCAN90
51.0 CMAGN21 CMBR84 GCM521
55.0 CSWCT12T
60.0 CM139
ECM204
76.0
CMGA15
ECM172
MC125
99.0
VIII
CSGA057 CMBR24
CMBR7 ECM88
3.0
6.0 TJ3MC301
ECM217
ECM128
16.0 MC68
TJ2
29.0
ECM221
CMTC13
41.0
MC356 MC11 MC78
ECM200
54.0
CMACC146 GCM241
MC208 CMAG59
68.0
CMAT141
77.0
ECM55
MC138
94.0
CMATN56
102.0
IX
0.0 MRGH21
MC92 CMTC47
ECM186
13.0
ECM150
ECM177
MRGH7 CM98
ECM66
35.0
ECM56
61.0
CM91
69.0
MC325
76.0
CMTCN1
CMCTN7
97.0
MC237
CMATN22
110.0
X
ECM86
ECM82
MC149ECM78
0.0 ECM175
CMCTN71
CMCTN19
10.0
MC17 CSWCT01
CSWCT22A
17.0
CMTCN67
31.0
CMGA172 ECM228
35.0
MC112 MC67 CM40
CMTA134B CMCTN65
CMGA165 ECM232
GCM153 ECM49
ECM116
ECM101 GCM344
ECM220
54.0
CM101B
57.0 CMTCN8
62.0
XI
0.0 TJ33
CMTCN62
2.0
MC146 ECM210
CMTC160 ECM63
8.0 ECM183
MC264 CMGAN12
MC375 CMAGN45
31.0
34.0 CSWCT18B
MC231
ECM164
42.0
CMATN89 MC349
CMGA104
66.0
MC93
71.0
MC265
ECM147
ECM145
93.0
XII
0.0 MC132 GCM206
5A6U NSV MC320
ECM67
ECM105
17.0
TJ29
MC330
29.0
CSAT425A
MC286
49.0
CMTCN14
53.0
CMAGN33 CMAGN32
CMAGN80
ECM123
ECM218
81.0

◀ **Fig. 2** The bin map of *Cucumis melo* obtained by selectively genotyping fourteen selected DHLs. The *vertical bars* represent linkage groups, divided in bins defined by the joint genotype of the selected DHLs. *Markers in bold* are also included in the linkage map (Fig. 1) and were used for the estimation of bin size. Genetic distances are shown on the *left*, indicating the position of the last marker included in the bin according to the framework map. *Markers in italics* defined new bins, although the exact position and size of these bins could not be estimated because these markers were not analyzed with the whole population. The hypothetical position of the last marker of these bins is indicated by a *dashed horizontal line* within the linkage group bar without specification of the genetic distance

We also studied differences in the levels of polymorphism (PIC) depending on the position of the SSR motif within the EST (ORF, 5′- or 3′-UTR) or the type of repeat (di- vs. trinucleotide repeats). Trinucleotide SSRs usually have a lower level of polymorphism compared to dinucleotide SSRs, for example in rice (Cho et al. 2000), barley (Chabane et al. 2005), wheat (Eujayl et al. 2001, Gadaleta et al. 2006) and wild emmer wheat (Li et al. 2002). Different levels of polymorphism between trimeric SSRs belonging to the ORF, 5′- or 3′-UTRs has also been reported (Scott et al. 2000). We did not find any difference in polymorphism in the different regions of the EST. Interestingly, melon trinucleotide ESTs are mainly (AAG)-repeats while trinucleotide repeats in cereals are mainly composed of GC- rich motifs (up to 77% in barley, reviewed by Li et al. 2004). Cho et al. (2000) observed in rice that ATT and CTT (=AAG) motifs derived from random genomic clones were much more variable than GC-rich trinucleotides, proposing that trinucleotide SSRs are a heterogeneous group where each class of sequences with a particular SSR motif has its own potential for variability. Thus, the relatively low level of variability of trinucleotide EST-SSRs observed in cereals could be due to their high GC-content rather than the length of the repeat motif (trinucleotide) itself. The low GC-content observed in melon trinucleotide SSRs could explain their high variability.

The relationship between SSR length (number of repetition units) and degree of polymorphism of the SSRs has been extensively described in both gSSR and EST-SSRs (McMurray 1995; Brinkmann et al. 1998; Innan et al. 1997; Schug et al. 1998; Weber 1990; Goldstein and Clark 1995; Grist et al. 1993; Thomas and Scott 1993; Loridon et al. 1998; Thiel et al. 2003; Cho et al. 2000). During DNA replication, longer stretches of repetitions facilitate the slipped-strand mispairing producing deletion or addition of SSR units. We also found a correlation between repeat number and PIC in our set of dinucleotide EST-SSRs but no correlation was observed for trinucleotide EST-SSRs. One possible explanation is that the selected trinucleotide EST-SSRs had a high number of repeat units that may be near the upper limit to affect protein function or expression. Augmentation in the number of repeats could be limited by natural selection.

Transferability to *Cucurbita*

Recent research has shown that ESTs are a potentially rich source of SSRs for related taxa (Ellis and Burke 2007). For example, SSR amplification rates of around 35% have been reported from tomato (*Solanum* sp.) to pepper (*Capsicum* sp.) (Frary et al. 2005), and 40–80% in cereals (Gao et al. 2003; Gupta et al. 2003; Tang et al. 2006). Results reported between genera in the *Rosaceae* family are lower, about 10% on average (Decroocq et al. 2003). We found limited transferability between *C. melo* L. and *Cucurbita* spp., similar to that previously reported for genomic SSR by Ritschel et al. (2004) who found that less than 16 out of 67 melon gSSR generated PCR products in *C. moschata*, *C. maxima* or *C. pepo*. Similar results were previously reported by Katzir et al. (2000). The low transferability observed can be attributed to the low level of conservation in the flanking regions among these genera, consistent with the genetic distance between them. We would expect, for instance, better transferability of the melon EST-SSR to cucumber (*C. sativus* L.). In terms of the capacity for detecting polymorphism, the level of EST-SSRs transferability between the genera *Cucumis* and *Cucurbita* was extremely low (about 5%). In consequence, transfer of melon microsatellite markers (either EST-SSRs or gSSRs) is not an efficient procedure for marker development in *Cucurbita*. Recently, Gong et al. (2008) demonstrated that genomic SSRs developed from *C. pepo* and *C. moschata* had a high inter-species transferability within *Cucurbita* genus, although transferability to *Cucumis* genera has not been tested.

Application of the bin mapping strategy

The bin mapping strategy was applied for the first time by Howad et al. (2005) to saturate the reference map of *Prunus*. By genotyping only six plants, 264 microsatellite markers were added to the previous map. The genetic map of melon from Gonzalo et al. (2005) used to implement the bin mapping approach in this species was twice as long (1,223 vs. 519 cM) and much less saturated (7 vs. 1 cM/marker) than the *Prunus* map. A primary objective was to test if the bin mapping strategy could also be efficient in longer and less saturated maps.

Fourteen plants were chosen for the bin set, more than twofold the number of plants used in the *Prunus* bin mapping, although the number of recombinant gametes was comparable, as 14 DHLs provide information on 14 recombinant gametes, while six F_2 plants provide information on 12 recombinant gametes. As a consequence of the low saturation of the melon framework map, we found a larger number of intermediate putative bins compared with the

Prunus bin mapping. The bin mapping approach was tested by genotyping the entire DHL mapping population with 47 new SSRs: all these markers mapped where predicted by bin mapping, except ECM127, proving that bin mapping is an efficient and robust method that can be applied not only to highly saturated maps but also to medium-density ones. Thus, by generating only 1,936 genotypic data points, 109 out of 122 SSR loci tested (89%) could be placed correctly by bin mapping in the melon map without the need for further genotyping, compared with the 8,954 data points that would have been required to map these markers using the whole DHL population.

The definitive bin map includes 296 markers distributed in 122 bins (2.38 markers/bin) and a map density of 4.2 cM/marker, with 212 SSRs (5.8 cM/SSRs), some covering regions previously lacking them. Some examples are ECM230 placed in a gap of 68 cM in LG I, ECM108, ECM97 and ECM137 placed in a gap of 66 cM in LG IV, ECM132, ECM178 and GCM112 in a gap of 65 cM in LG VI or ECM186, ECM150, ECM177, ECM66 and ECM56 in a gap of 61 cM in LG IX. A single duplicated bin was obtained, as in *Prunus*, although this position can be resolved by genotyping only one extra DHL. This map could be used as a framework for future map saturation to build a consensus genetic map of this species by merging the existing genetics maps, as recently proposed in the International Cucurbit Genomics Initiative (http://www.icugi.org).

According to our results, the implementation of the bin mapping approach for efficient and accurate map saturation for medium/low resolution maps should combine a two-step strategy. Using the bin set to rapidly saturate the genome and then genotype a larger mapping population (classical approach) in those cases where the markers map in intermediate putative bins. As the map marker density increases, the number of putative bins decreases, making it less necessary to genotype markers with the whole population. The advantage of the bin mapping approach is that it allows the position of markers or candidate genes with a relatively low expenditure of time and money. Then markers located at positions of interest may be placed with more accuracy using larger populations.

Fifty-eight of the ESTs selected for SSR marker development showed homology to annotated protein sequences and could be assigned to one or more gene ontologies (Gonzalez-Ibeas et al. 2007). We can highlight ESTs involved in catabolism (14), signal transduction (12), development (6), transcription factors (4) and stress response (3). The study of these functional markers in germplasm collections may give insight into functional variability and its possible relationship with phenotypic variability. Furthermore, clustering of the melon EST-SSRs was observed, indicating the existence of gene-rich regions in the melon genome, e.g., bins I:063-I:078 (9 ESTs in 37 cM), IV:090 (5 ESTs in 9 cM), V:064-V:072 (5 ESTs in 12 cM) and XI:008 (4 ESTs in 8 cM). Combining mapping and expression analysis of these EST-SSRs will also help to define expression QTLs (eQTLs) in melon.

EST-SSRs can also provide anchor points for synteny studies. A certain degree of microsynteny between melon and the genomes of *Arabidopsis thaliana*, *Medicago truncatula* and *Populus trichocarpa* has been previously found (van Leeuwen et al. 2003; Deleu et al. 2007). Much higher degree of microsynteny and macrosynteny is expected with the closely related *Cucumis sativus* L., whose complete genome sequence will soon be released (Huang et al. 2008). Comparison of melon and cucumber genomes is particularly interesting because, even though they are closely related phylogenetically (Ghebretinsae et al. 2007), they differ dramatically in chromosome number (7 for cucumber and 12 for melon). The EST-SSRs mapped in the current report could be used as anchor points for comparing both genomes, giving insight into the evolution of genome structure.

In summary, we have developed an important set of mainly highly polymorphic EST-SSRs and gSSRs, defined a minimum set of DHL genotypes that allows rapid mapping of new markers (as the SSR markers recently developed by Fukino et al. 2007), and increased the melon genetic map density with these new SSR markers. These results provide a new set of high quality markers for various breeding applications, from varietal identification to marker-assisted selection. They also facilitate the construction of a highly dense melon map useful for the analysis of specific genome regions, the construction of physical maps and the whole genome sequence.

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References

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acid Res 25:3389–3402

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

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

Brinkmann B, Klintschar M, Neuhuber F, Huhne J, Rolf B (1998) Mutation rate in human microsatellites: influence of the struc-

ture and length of the tandem repeat. Am J Hum Genet 62:1408–1415

Brotman Y, Silberstein L, Kovalski I, Klingler J, Thompson G, Katzir N, Perl-Treves R (2000) Linkage groups of *Cucumis melo*, including resistance gene homologues and known genes. VII Eucarpia Metting on Cucurbit Genetics and Breeding. Acta Hortic 510:441–448

Caetano-Anollés G, Gresshoff PM (1994) staining nucleic acids with silver: an alternative to radioisotopic and fluorescent labeling. Promega Notes 45:13

Chabane K, Ablett GA, Cordeiro GM, Valkoun J, Henry RJ (2005) EST versus genomic derived microsatellite markers for genotyping wild and cultivated barley. Genet Resour Crop Evol 52:903–909

Chagne D, Chaumeil P, Ramboer A, Collada C, Guevara A, Cervera MT, Vendramin GG, Garcia V, Frigerio JM, Echt C, Richardson T, Plomion C (2004) Cross-species transferability and mapping of genomic and cDNA SSRs in pines. Theor Appl Genet 109:1204–1214

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

Cordeiro GM, Casu R, McIntyre CL, Manners JM, Henry RJ (2001) Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. Plant Sci 160:1115–1123

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

Decroocq V, Fave MG, Hagen L, Bordenave L, Decroocq S (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor Appl Genet 106:912–922

Deleu W, González V, Monfort A, Bendahmane A, Puigdomènech P, Arús P, Garcia-Mas J (2007) Structure of two melon regions reveals high microsynteny with sequenced plant species. Mol Genet Genomics 278:611–622

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

Ellis JR, Burke JM (2007) EST-SSRs as a resource for population genetic analyses. Heredity 99:125–132

Eujayl I, Sorrells ME, Baum M, Wolters P, Powell W (2001) Assessment of genotypic variation among cultivated durum wheat based on EST-SSRS and genomic SSRS. Euphytica 119:39–43

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

Eujayl I, Sledge MK, Wang L, May GD, Chekhovskiy K, Zwonitzer JC, Mian MAR (2004) *Medicago truncatula* EST-SSRs reveal cross-species genetic markers for *Medicago* spp. Theor Appl Genet 108:414–422

Ferriol M, Picó B, Nuez F (2003a) Genetic diversity of some accessions of *Cucurbita maxima* from Spain using RAPD and SRAP markers. Genet Resour Crop Evol 50:227–238

Ferriol M, Picó B, Nuez F (2003b) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. Theor Appl Genet 107:271–282

Ferriol M, Picó B, Nuez F (2004a) Morphological and molecular diversity of a collection of *Cucurbita maxima* landraces. J Am Soc Hort Sci 129:60–69

Ferriol M, Picó B, Fernández de Córdoba P, Nuez F (2004b) Molecular diversity of a germplasm collection of squash (Cucurbita moschata) with SRAP and AFLP markers. Crop Sci 44:653–664

Ferriol M, Picó B, Nuez F (2007) Genetic diversity of *Cucurbita* spp in the Canary islands: a bridge between America and Europe. In: Bullita S (ed) Plant genetic resources of geographical and other islands. Conservation evaluation and use for plant breeding. Proceedings of XVII Eucarpia Gen Res Sect Meeting. Castelsardo, Italy, pp 33–40

Frary A, Xu Y, Liu J, Mitchell S, Tedeschi E, Tanksley S (2005) Development of a set of PCR-based anchor markers encompassing the tomato genome and evaluation of their usefulness for genetics and breeding experiments. Theor Appl Genet 111:291–312

Fukino N, Sakata Y, Kunihisa M, Matsumoto S (2007) Characterisation of novel simple sequence repeat (SSR) markers for melon (*Cucumis melo* L.) and their use for genotype identification. J Hort Sci Biotech 82:330–334

Gadaleta A, Manzini G, Mulè G, Blanco A (2006) Characterization of dinucleotide and trinucleotide EST-derived microsatellites in the wheat genome. Euphytica 153:73–85

Gao LF, Tang JF, Li HW, Jia JZ (2003) Analysis of microsatellites in major crops assessed by computational and experimental approaches. Mol Breed 12:245–261

Ghebretinsae AG, Barber MT, Barber JC (2007) Relationships of cucumbers and melons unraveled: molecular phylogenetics of *Cucumis* and related genera (*Benincaseae*, *Cucurbitaceae*). Am J Bot 94:1256–1266

Goldstein DB, Clark AG (1995) Microsatellite variation in North American populations of *Drosophila melanogaster*. Nucleic Acids Res 23:3882–3886

Gong L, Stift G, Kofler R, Pachner M, Lelley T (2008) Microsatellites for the genus *Cucurbita* and an SSR-based genetic linkage map of *Cucurbita pepo* L. Theor Appl Genet (in press). doi:10.1007/s00122-008-0750-2

Gonzalez-Ibeas D, Blanca J, Roig C, González-To M, Picó B, Truniger V, Gómez P, Deleu W, Caño-Delgado A, Arús P, Nuez F, Garcia-Mas J, Puigdomènech P, Aranda MA (2007) MELOGEN: an EST database for melon functional genomics. BMC Genomics 8:306. doi:10.1186/1471-2164-8-306

Gonzalo MJ, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arús P, Monforte AJ (2005) Simple-sequence repeat markers used in merging linkage maps of melon (*Cucumis melo* L.). Theor Appl Genet 110:802–811

Grist SA, Figaira FA, Morley AA (1993) Dinucleotide repeat polymorphisms isolated by the polymerase chain reaction. BioTechniques 15:304–309

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003) Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol Genet Genom 270:315–323

Han ZG, Guo WZ, Song XL, Zhang TZ (2004) Genetic mapping of EST-derived microsatellites from the diploid *Gossypium arboreum* in allotetraploid cotton. Mol Genet Genom 272:308–327

Howad W, Yamamoto T, Dirlewanger E, Testolin R, Cosson P, Cipriani G, Monforte AJ, Georgi L, Abbott AG, Arús P (2005) Mapping with a few plants: using selective mapping for microsatellite saturation of the *Prunus* reference map. Genetics 171:1305–1309

Huang S, Du Y, Wang X, Gu X, Xie B, Zhang Z, Wang J, Li R, Li S, Ren Y, Wang J, Yang H, Jin W, Fei Z, Kilian A, Staub JE, van der Vossen E, Li G (2008) The Cucumber Genome Initiative-an international effort to unlock the genetic potential of an orphan crop using novel genomic technology. Plant Animal Genomes XVI Conference. htpp://www.intl-pag.org/16/abstracts/PAG16_W19_524.html

Innan H, Terauchi R, Miyashita NT (1997) Microsatellite polymorphism in natural populations of wild plant *Arabidopsis thaliana*. Genetics 146:1441–1452

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

Katzir N, Tadmor Y, Tzuri G, Leshzeshen E, Mozes-Daube N, Danin-Poleg Y, Paris HS (2000) Further ISSR and preliminary SSR analysis of relationships among accessions of *Cucurbita pepo*. Acta Hortic 510:433–439

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln SE, Newburg L (1987) Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural population. Genomics 1:174–181

Li YC, Korol AB, Fahima T, Nevo E (2004) Microsatellites within genes: structure, function, and evolution. Mol Biol Evol 21:991–1007

Li YC, Röder MS, Fahima T, Kirzhner V, Beiles A, Korol AB, Nevo E (2002) Genetic effects on SSR variation in a natural population of wild emmer wheat, *Triticum dicoccoides* in Yehudiyya microsite, Israel. Heredity 89:127–132

Liou PC, Chang YM, Hsu WS, Cheng YH, Chang HR, Hsiao CH (1998) Construction of a linkage map in *Cucumis melo* (L.) using random amplified polymorphic DNA markers. International symposium on biotechnology of tropical and subtropical species. Acta Hortic 461:123–132

Liu K, Muse SV (2005) Powermarker: integrated analysis enviroment for genetic marker data. Bioinformatics 21:2128–2129

Loridon K, Cournoyer B, Goubely C, Depeiges A, Picard G (1998) Length polymorphism and allele structure of trinucleotide microsatellites in natural accessions of *Arabidopsis thaliana.* Theor Appl Genet 97:591–604

McMurray CT (1995) Mechanisms of DNA expansion. Chromosoma 104:2–13

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

Moreno E, Obando J, Dos-Santos N, Fernández-Trujillo JP, Monforte AJ, Garcia-Mas J (2008) Candidate genes and QTLs for fruit ripening and softening in melon. Theor Appl Genet 116:589–602

Munger HM, Robinson RW (1991) Nomenclature of *Cucumis melo* L. Cucurbit Genet Coop Rep 14:43–44

Nei M (1973) Análisis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Oliver M, Garcia-Mas J, Cardus M, Pueyo N, López-Sese AL, Arroyo M, Gomez-Paniagua H, Arús P, de Vicente MC (2001) Construction of a reference Linkage map for melon. Genome 44:836–845

Panaud O, Chen X, McCouch SR (1995) Frequency of microsatellite sequences in rice (*Oryza sativa* L.). Genome 38:1170–1176

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

Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Forster J, Leon J (2000) Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars. Theor Appl Genet 101:652–660

Pitrat M, Hanelt P, Hammer K (2000) Some comments on intraspecific classification of cultivars of melon. Acta Hort 510:29–36

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. doi:10.1186/1471-2229-4-9

Rossetto M, McNally J, Henry RJ (2002) Evaluating the potential of SSR flanking regions for examining taxonomic relationships in the *Vitaceae*. Theor Appl Genet 104:61–66

Rungis D, Bérubé Y, Zhang J, Ralph S, Ritland CE, Ellis BE, Douglas C, Bohlmann J, Ritland K (2004) Robust simple sequence repeat markers for spruce (*Picea* spp.) from expressed sequence tags. Theor Appl Genet 109:1283–1294

Saha MC, Mian MA, Eujayl I, Zwonitzer JC, Wang L, May GD (2004) Tall fescue EST-SSR markers with transferability across several grass species. Theor Appl Genet 109:783–791

Sargent DJ, Cipriani G, Vilanova S, Gil-Ariza D, Arús P, Simpson DW, Tobutt KR, Monfort A (2008) The development of a bin mapping population and the selective mapping of 103 markers in the diploid *Fragaria* reference map. Genome 51:120–127

Schug M, Hutter C, Wetterstrand K, Gaudette M, Mackay T, Aquadro CF (1998) The mutation rates of di-, tri- and tetranucleotide repeats in *Drosophila melanogaster*. Mol Biol Evol 15:1751–1760

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

Tang JF, Gao LF, Cao YS, Jia JZ (2006) Homologous analysis of SSR-ESTs and transferability of wheat SSR-EST markers across barley, rice and maize. Euphytica 151:1–87

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

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor Appl Genet 86:985–990

Varshney RK, Graner A, Sorrells ME (2005) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23:48–55

Van Leeuwen H, Monfort A, Zhang HB, Puigdomenech P (2003) Identification and characterisation of a melon genomic region containing a resistance gene cluster from a constructed BAC library. Microcolinearity between *Cucumis melo* and *Arabidopsis thaliana*. Plant Mol Biol 51:703–718

Vision TJ, Brown DG, Shmoys DB, Durrett RT, Tanksley SD (2000) Selective mapping: a strategy for optimizing the construction of high-density linkage maps. Genetics 155:407–420

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

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

Weber JL (1990) Informativeness of human (dC-dA)n(dG-dT)n polymorphisms. Genomics 7:524–530

Woodhead M, Russell J, Squirrell J, Hollingsworth PM, Cardle L, Ramsay L, Gibby M, Powell W (2003) Development of EST-SSRs from the alpine lady-fern, *Athyrium distentifolium*. Mol Ecol Notes 3:287–290

Yeh FC, Yang R-C, Boyle TBJ, Ye Z-H, Mao JX (1997) POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada. Available at http://www.ualberta.ca/~fyeh/faq.htm