Linkage map of *Cucumis melo* including phenotypic traits and sequence-characterized genes

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Abstract: A new linkage map of *Cucumis melo*, derived from the F_2 progeny of a cross between PI 414723 and *C. melo* 'TopMark' is presented. The map spans a total of 1421 cM and includes 179 points consisting of random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeats (ISSRs), simple sequence repeats (SSRs), and restriction fragment length polymorphism (RFLP) markers. The map also includes an aphid resistance trait (*Vat*) and the sex type gene, *andromonoecious* (*a*), the two of which are important in resistance breeding and the control of hybrid seed production, as well as a seed-color gene, *Wt-2*. Most RFLPs represent sequence-characterized cDNA probes from *C. melo* and *Cucumis sativus*. These include resistance gene homologues and genes involved in various aspects of plant development and metabolism. A sub-set of our SSR and RFLP markers were also mapped, as part of this study, on additional mapping populations that were published for this species. This provides important reference points ("anchors"), enabling us to identify several linkage groups with respect to other melon maps.

Key words: Cucumis melo, melon, genetic map, molecular markers, resistance gene homologues.

Résumé : Une nouvelle carte génétique du *Cucumis melo*, dérivée d'une population F_2 issue du croisement entre PI 414723 et *C. melo* 'TopMark' est présentée. La carte totalise 1421 cM et inclut 179 marqueurs RAPD (« random amplified polymorphic DNA »), AFLP (« amplified fragment length polymorphism »), ISSR (« inter-simple sequence repeat »), SSR (« simple sequence repeat ») ou RFLP (« restriction fragment length polymorphism »). La carte comprend également un locus conférant la résistance à un puceron (*Vat*), ce qui en fait un locus important en amélioration variétale, et un locus déterminant le sexe, *andromonoecious* (*a*), lequel est important lors de la production de semence hybride. Finalement, la carte comprend également le locus *Wt-2*, lequel détermine la couleur des graines. La plupart des marqueurs RFLP font appel à des sondes d'ADNc de séquence connue qui proviennent du *C. melo* ou du *Cucumis sativus*. Ceux-ci comprennent des homologues de gènes de résistance et des gènes impliqués dans divers aspects du développement ou du métabolisme de la plante. Un sous-ensemble des marqueurs SSR et RFLP a aussi été employé sur d'autres populations de cartographie chez cette espèce. Cela a procuré des marqueurs d'ancrage, lesquels ont permis de déterminer la correspondance entre les groupes de liaison identifiés dans ce travail et ceux définis dans les travaux antérieurs.

Mots clés : Cucumis melo, carte génétique, marqueurs moléculaires, homologues de gènes de résistance.

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Introduction

Cucumis melo (melon) is an important vegetable crop worldwide, in both temperate and warm climates. Within the Cucurbitaceae, it belongs to the genus *Cucumis*, and to the sub-genus *melo*, which originated in Africa and has $2n = 24$ chromosomes. Modern breeding of hybrid varieties with improved disease resistances and fruit quality includes the use of molecular markers, which render the breeding process more efficient. Melon displays important intra-specific genetic variation in fruit traits (Stepansky et al. 1999) and has been the subject of detailed molecular and genetic studies on aspects of fruit maturation and sugar accumulation (e.g., Ayub et al. 1996; Flores et al. 2001; Perin et al. 2002*c*). To the molecular geneticist, melon offers the additional advantage of a very small genome (C = 0.45 pg), only three times that of *Arabidopsis* (Arumuganathan and Earle 1991).

Genetic maps are required for detailed studies of the genome, e.g., for dissecting quantitative traits, map-based cloning of agronomic traits and comparative genome studies among related species. A number of genetic maps were constructed for *Cucumis melo* in recent years (Baudracco Arnas and Pitrat 1996; Wang et al. 1997; Oliver et al. 2001; Perin et al. 2002*a*; Danin-Poleg et al. 2002). These maps differ in their degree of genome-saturation, in mapping parents' genotypes, and in the traits that segregate in each map. Also, various types of molecular markers were used in each map. RFLP (restriction fragment length polymorphism) and SSR (simple sequence repeats) provide codominant markers that increase mapping precision and that are transferable between mapping populations, and even among related species. AFLP (amplified fragment length polymorphism), ISSR (inter-simple sequence repeats), and RAPD (random amplified polymorphic DNA) are high-throughput, dominant fingerprinting markers that can fill in the gaps in a map and saturate regions of interest. This study provides a 179-point linkage map of melon with different types of markers based on a cross between an Indian landrace, PI 414723, and a cantaloupe-type cultivar, *C. melo* 'Top-Mark'. The map includes three phenotypic traits and about 40 sequenced RFLP markers, most of which are homologous to genes of known function. The linkage map and most (88%) of the 176 molecular markers placed on it have not been published previously, and constitute a significant addition to the present coverage of the melon genetic map. In addition, several anchor points were generated by mapping 30 of the RFLP and SSR markers on the map developed by Perin et al. (2002*a*), hereafter referred to as the INRA reference map. The anchor points allow cross-identification of several linkage groups in our map with respect to the INRA reference map, as well as to other maps that incorporated the same "anchors". The present map facilitates the sharing of molecular markers and linkage data, whose development required considerable resources, among cucurbit researchers. Moreover, the RFLP anchors represent an addition of ~20 unpublished new loci to the reference map of Perin et al. (2002*a*).

Materials and methods

Mapping population and scoring of phenotypic traits

The F_2 mapping population was derived from a cross between PI 414723 (McCreight et al. 1992) as the female parent, and *Cucumis melo* var. *cantalupensis* 'Topmark' (Zink and Gulber 1987) as the pollen parent. F_1 individuals were self-pollinated, and 113 F_2 individuals were grown in the greenhouse, sampled for DNA extraction, and self-fertilized. At anthesis, plants were scored twice for their sex type. The population segregated between andromonoecious plants, i.e., bearing staminate and perfect flowers, and monoecious plants, bearing staminate and pistillate flowers. The resulting F_3 seeds were inspected for seed-coat color. Families of F_3 plants were grown and scored for resistance to *Aphis gossypii* according to Klingler et al. (2001). Part of the molecular markers were also scored using two RIL populations constructed by Perin et al. (2002*a*) who used the two RIL populations to generate the INRA reference map. This allowed us to correlate our linkage data with the INRA reference map.

Source of RFLP probes

Most RFLP probes were cDNA clones. Part of the clones were from *C. sativus* (cucumber) cDNA libraries made from floral buds (Perl-Treves et al. 1998). These clones are designated by a code with the suffix "CS". As a second source, we used a cDNA library from melon hypocotyls of the breeding line AR5 in the λ-ZAPII vector (Stratagene, La Jolla, Calif.), enriched for large-insert clones. These were coded with the suffix "CM". Randomly isolated cDNA clones were excised from the different libraries by helper phage coinoculation according to manufacturer's instructions to yield plasmid clones in the Bluescript vector. Cucumber or melon cDNA clones that revealed strong similarity to functionally characterized proteins were named according to their sequence homology, while others that show poor similarity to known proteins, or strong similarity to poorly characterized ones, retained a code-number designation.

CM-PT7 is a genomic clone from a melon *Pst*I genomic library (Silberstein et al. 1999). Genomic fragments PTH3 and PTH2, with homology to Pto-kinase, were cloned by degenerate-primer PCR, using the forward primer 5′-GGIGCIGCIWRIGGIYT-3′ and the reverse primers 5′-ACICCRAAIGARTAIACRTC-3′ or 5′-ACICCRAARCTRTAITCACR-3′, kindly provided by R. Fluhr and Q. Pan of the Israeli Plant Genome Center, Rehovot. A longer PTH2 cDNA was then isolated from the melon AR5 hypocotyls cDNA library and used as probe. Cloning of NBS–LRR homologues was carried out by applying degenerate PCR primers to melon genomic DNA and by RT-PCR as described by Brotman et al. (2002).

RFLP analysis and sequencing of RFLP markers

For Southern blots, 3- to 5-μg DNA samples were restriction digested according to manufacturer's instructions (Roche Diagnostics, Basel, Switzerland, or MBI Fermentas, Vilnius, Lithuania), run on 0.8% w/v agarose gels, and blotted to charged nylon membranes (Genescreen Plus, Du Pont, Wilmington, Dela.) using the alkaline blotting method supplied by the manufacturer. Seven restriction enzymes were used: *Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Xba*I, *Mva*I, and *Bsu*15I. Hybridization was performed at 65 °C for the melon cDNA and genomic clones, and at 55 °C for the cucumber cDNA clones, in 6% mass fraction of polyethylene glycol (PEG),

5% mass fraction of SDS, 5× SSPE, and 50 μg denatured salmon DNA/mL. Probes were labeled by the random hexamer method (Roche protocol). Automated sequencing was carried out using an ABI Prism 310 Genetic Analyzer and the BigDye Terminator Kit (Perkin-Elmer Corp., Foster City, Calif.). DNA sequences were analyzed for homology to sequences in the Owl, Swissprot, and GeneBank databases using the BLAST method (Altschul et al. 1990).

For comparative mapping with the INRA map, samples of parental DNA of three genotypes, namely *C. melo* 'Vedrantais', PI 414723, and PI 161375, which served as mapping parents of the two RIL populations by Perin et al. (2002*a*), were digested with four different enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I), blotted, and hybridized with the RFLP probes. In cases of polymorphism, about 60 individuals from the appropriate RIL population were scored, allowing us to place the marker in a specific interval on the INRA reference map.

RAPD, ISSR, SSR, and AFLP markers

RAPD–PCRs were performed using random decamer sets (Operon Technologies, Alameda, Calif., sets OPA, B, D, and R; and University of British Columbia, Vancouver, sets No. 1, 2, 4, and 5), according to Williams et al. (1993). Each reaction included 30 ng genomic DNA, 0.2 μmol/L primer, 0.5 U *Taq* DNA polymearse, 0.1 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 10 mmol/L Tris–HCl (pH = 9), 50 mmol/L KCl, 0.1% volume fraction of Triton X-100, and 0.2 mg/mL bovine serum albumin (BSA) in a 25-μL volume. Amplification included 40 cycles of 1 min at 94 °C, 90 s at 36 °C, 2 min at 72 °C. Inter SSR analysis was performed according to Gupta et al. (1994) and Stepansky et al. (1999), using the ISSR primer set No. 9 of the University of British Columbia, Vancouver. The 25-μL reaction volume included 30 ng genomic DNA, 1 μmol/L primer, 0.5 U *Taq* DNA polymearse, 0.2 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 10 mmol/L Tris–HCl (pH = 8.4), 50 mmol/L KCl, 0.1% Triton X-100, and 0.2 mg BSA/mL. Amplification included 35 cycles of 1 min at 95 °C, 30 s at the annealing temperature (5°C below the approximate melting point temperature (T_m) of each primer), and 5 min at 72 °C. Markers generated using Operon primers were named starting with OP, followed by the Operon primer set letter (e.g., OPA for set A), followed by the number of the primer in the set and the approximate size of the fragment in base pairs. RAPD or ISSR primer bands generated using the University of British Columbia primer sets were designated by the suffix "UB", similarly followed by the primer's number and the fragment size. SSR markers were developed by Katzir and coworkers. Primer sequences, PCR, and electrophoresis conditions were according to Katzir et al. (1996), Danin-Poleg et al. (2001), and Danin-Poleg et al. (2002). AFLP analysis was carried out according to Vos et al. (1995). DNA was digested with *Eco*RI and *Mse*I and ligated to the appropriate adaptors using the AFLP Core Reagent Kit and the AFLP Starter Primer Kit (Life Technologies, Carlsbad, Calif.). Primers having three selective nucleotides were used to produce multi-banded patterns. Markers are named according to their 3-base selective sequences, the *Eco*RI site added nucleotides, followed by the *Mse*I site-specific nucleotides, followed by the approximate size in base pairs.

Scoring of molecular markers and computational analysis

All molecular markers were scored three times, with at least two persons independently evaluating each data point. Segregation of the markers among the F_2 progeny was analyzed by the χ^2 test. Badly skewed markers, having $p < 0.01$ according to a 3:1 inheritance model for dominant markers (RAPD, ISSR, and AFLP), or 1:2:1 for codominant markers (for SSR and RFLP), were eliminated from the data set. The RFLP and most of the RAPD markers were scored on all of the 113 mapping individuals, while for the SSR, AFLP, and part of the RAPD we adopted a sequential sampling strategy (Motro and Soller 1993): markers were first scored on a subset of 50–60 individuals and more individuals were added only when required for linkage determination, or for better resolution of marker order. The MAPMAKER software (Lander et al. 1987) was used to generate a linkage map. Markers were assigned to linkage groups using the "group" command with a threshold of LOD = 3, θ = 0.3. A LOD threshold value of 4 was used to construct a framework for each linkage group, using the "two point : three point" command, and markers having LOD values of at least 3 were progressively added to the group using the commands "try" and "ripple".

For comparative mapping with the INRA reference map, RFLP markers were scored on approximately 60 individuals from either one or both RIL populations (Perin et al. 2002*a*), depending on availability of polymorphism between the two pairs of RIL mapping parents (Vedrantais and PI 414723, or Vedrantais and PI 161375). The RILs were examined for a 1:1 segregation mode between two homozygous classes, typical of RIL population structure, heterozygous samples (about 5% of the plants) were deleted, and linkage analysis was performed using MAPMAKER as detailed above.

Results and discussion

Development of a linkage map based on a cross between PI 414723 and 'TopMark'

An F_2 mapping population was developed using a cross between an Indian accession, *C. melo* var. *momordica* (PI 414723) and *C. melo* var. *cantalupensis* 'TopMark'. The two parents differed in a large number of traits, three of which showing simple, monogenic inheritance, were scored and mapped. According to Neuhausen (1992), Silberstein et al. (1999), and Stepanski et al. (1999), these two parental genotypes are also well diverged at the molecular level.

Aphid resistance was scored using 64 F_3 families derived by self-fertilization of F_2 plants, and resistance exhibited a monogenic dominant mode of inheritance (Klingler et al. 2001). Resistance derived from PI 414723 or PI 161375 (the *Vat* locus) is an important agricultural trait, since it protects the plants from direct damage caused by the widespread pest, *Aphis gossypii*, and indirectly restricts the spreading of severe viral pathogens such as *zucchini yellow mosaic virus* (ZYMV), transmitted by the aphid. Breeding for aphid resistance by phenotypic selection, i.e., controlled infestation of breeding progenies, is slow and tedious. Molecular markers for this trait will facilitate breeding and possibly the cloning of the respective gene.

Fig. 1. Linkage groups of *Cucumis melo*. Values at the left of intervals represent map distances in centiMorgans (Haldane function). Types of markers: RFLPs are indicated in bold uppercase letters; SSR markers are underlined. The suffixes CS and CM indicate if the probe is of cucumber or melon origin, respectively. The same suffix in SSR markers indicates the origin of the sequence upon which the primers were based. RAPD and ISSR are preceded by the suffix UB or OP, for primers purchased from University of British Columbia laboratories or Operon Technologies, respectively. The suffix is followed by the primer identification code and the approximate size of the RAPD band in bp. AFLP markers are recognized by the three selective nucleotide sequence of the *Eco*RI adaptor primer, followed by those of the *Mse*I adaptor primer, and the approximate band size in base pairs. Markers that were placed on the RILs map by Perin et al. (2002*a*) are indicated by arrows, and their assignment to linkage groups on the latter map is indicated.

The sex determining gene *a*, which specifies a monoecious plant phenotype with stamen-less pistillate flowers, as opposed to hermaphrodite flowers in the homozygous recessive state, segregated 48 monoecious : 21 andromonoecious among the F_2 individuals scored, compatible with a 3:1 inheritance as reported in numerous studies since Rosa (1928). This trait is of considerable importance during hybrid seed production, eliminating the need for hand emasculation of pistillate flowers before pollination. The trait is often introgressed by breeders into their maternal breeding lines to replace the andromonoecious habit typical of older melon varieties. Markers for the trait would therefore assist breeders, and its molecular characterization may shed light on the control of flower sex in cucurbits. Seed coat color of the F_3 seeds extracted from F_2 fruits segregated at 56 orange : 27 white, which is rather skewed, but still compatible with a 3:1 model of inheritance ($p = 0.11$); previous studies showed single-gene inheritance of the same trait and designated the gene *Wt-2* (Hagiwara and Kamimura 1936; Perin et al. 2002*a*). Other morphological traits such as leaf shape and hairiness, fruit shape, color, netting, and dehiscence were scored in our population, but could not be analyzed at the F_2 generation because of the more continuous distribution of phenotypes and apparent environmental influence, requiring replicated scoring in more appropriate populations.

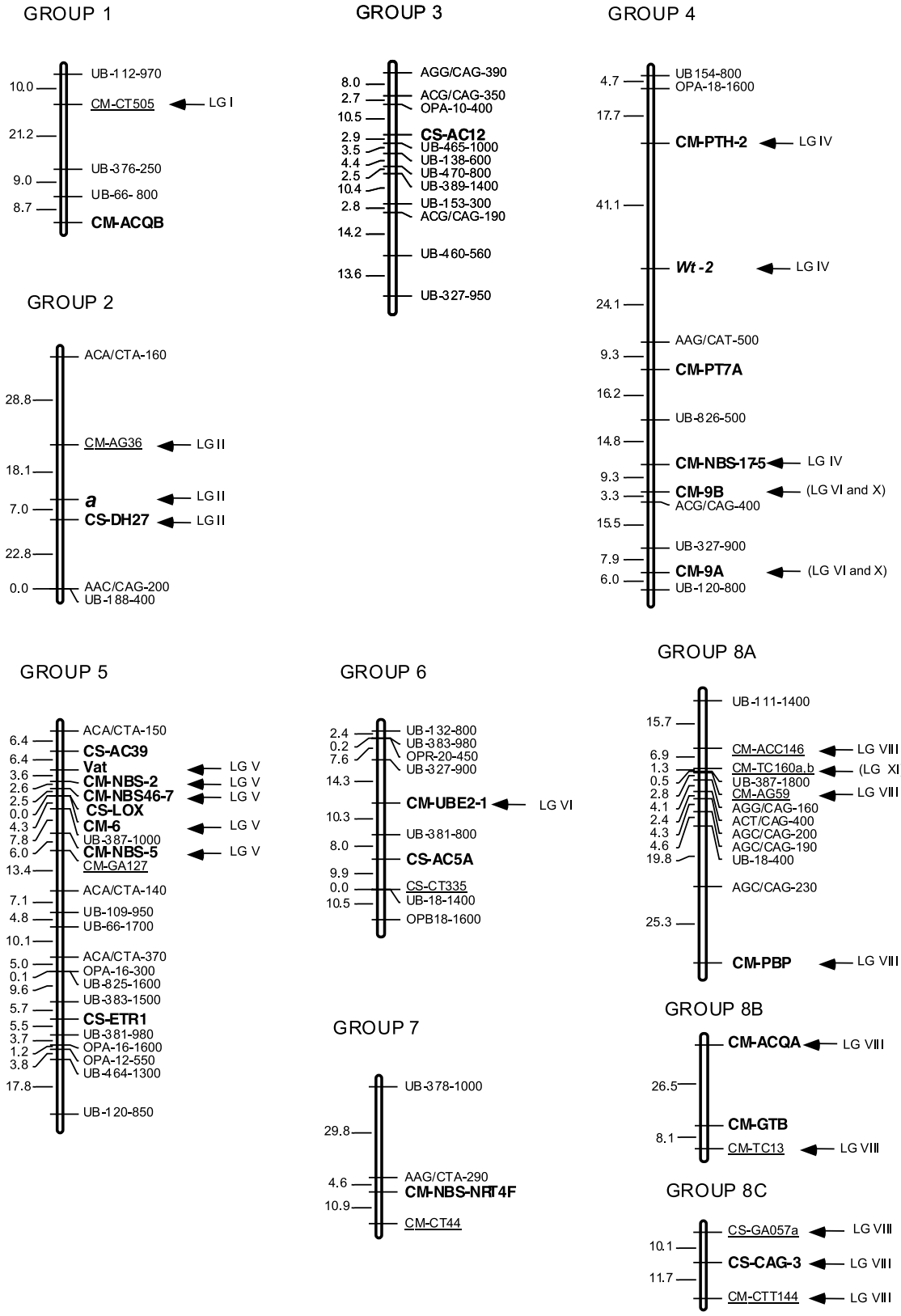
A total of 176 molecular markers were placed on the map, including RFLP, RAPD, AFLP, ISSR, and SSR markers (Fig. 1). Table 1 reports the distribution of the 179 markers and phenotypic trait loci among the 24 linkage groups identified. These include 11 larger groups, with 6 to 23 points, and several smaller groups with 2 to 5 markers. Groups 8A, 8B, and 8C could be identified as being part of the same group, using comparative data from another population (see below). Adding more markers may help to unite the smaller groups and merge the linkage groups into the expected $n = 12$. However, linkage group merging would require the use of other mapping populations, since the seeds and DNA of the founding F_2 plants have been nearly exhausted. The total length of the linkage groups in the present map is 1420.6 cM, with an average distance of 7.9 cM between points. These values are rather similar to the other published melon maps: Baudracco-Arnas and Pitrat (1996), 110-point map, 1390 cM; Wang et al. (1997) with 204 points spanning 1942 cM; Oliver et al. (2001), 412 points and 1197 cM; Perin et al. (2002), 668 points and 1654 cM. The map by Danin-Poleg et al. (2002) is less extensive, with 74 points and a total length of 610 cM.

RFLP and SSR markers

The 41 RFLP and 16 SSR linked markers can be regarded as the skeleton of the map, because of the robustness of these two techniques. Both techniques involve co-dominant markers that can provide suitable anchor points to align different maps. In addition, long stretches of hybridizing sequence in RFLP probes often result in distinct signals across species boundaries, enabling comparative mapping between related species. In the present study, cucumber cDNA clones, in addition to melon clones, were successfully hybridized with melon genomic blots to provide RFLP markers.

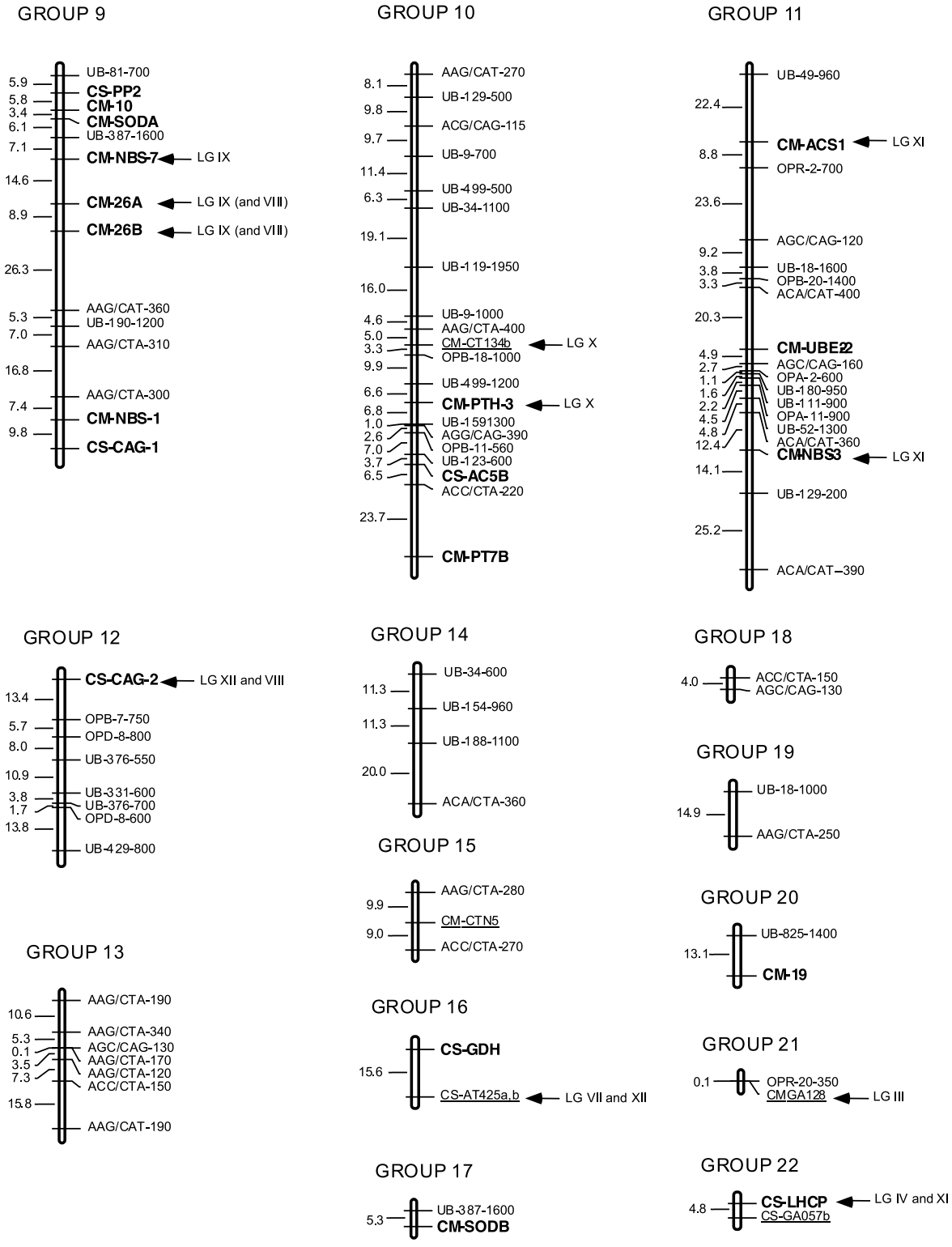
An additional advantage of RFLP markers is the sequence information that can be obtained, providing important clues on functional genes that may reside in a given locus. The map includes 41 RFLP loci, detected using 35 different probes, 6 of which detected 2 RFLP loci each. We thus sequenced 32 of these probes (three probes did not yield good quality sequences), either fully or in part. As a result, most RFLP markers on the map represent homologues of functionally characterized genes. The genomic target sequence that hybridizes to a cDNA probe may represent an expressed gene and, in the case of co-segregation with a phenotypic trait, it may provide a candidate gene for that trait. Alternatively, hybridizing bands may also consist of pseudogenes. Sequence-characterized RFLP probes are listed in Table 2, with reference to their GenBank accession numbers. Some of the clones represent proteins of unknown function, and only some descriptive data about their expression pattern, e.g., "cold regulated" or "floral bud expression". Clones that are homologous to well-characterized genes include photosynthesis genes (a light harvesting-complex protein) and housekeeping enzymes such as lipoxygenase, superoxide dismutase, and ubiquitin-conjugating enzymes. "Structural" proteins may include aquaporin and phloem lectin PP2; ethylene-related genes include an ACC synthase and an ethylene receptor. Other developmental regulators include the three *Agamous* homologues that encode MADS box transcription factors active in flower differentiation, and a protein that has an AP2 domain, typical of a family of plant-specific transcription factors.

A particularly interesting group of clones consists of 10 resistance gene homologues. They belong to two gene families. The protein members of the NBS–LRR super-family have a nucleotide binding site and a leucine rich repeat. The latter domain is believed to confer, in most cases, the specificity of the plant–pathogen interaction (Bent 1996; Ellis et al. 2000). The second family represented in this study is the Pto-receptor kinases (Hardie 1999). *Pto*, a gene conferring *Pseudomonas* resistance in tomato, was the first R gene to be cloned (Martin et al. 1993). It encodes an intracellular serine (threonine) protein kinase, and the physical interaction of PTO with the bacterial gene product, AVRPTO, provided the first direct demonstration of a "gene-for-gene"



molecular interaction (Scofield et al. 1996; Tang et al. 1996). Using sets of degenerate primers to the conserved motifs in proteins belonging to these two families of resistance genes, we have cloned melon genomic fragments, as well as cDNAs showing homology to both *Pto* and NBS domains. Cloning details and linkage data regarding a few of the NBS–LRR homologous sequences were reported in our previous papers (Klingler et al. 2001; Brotman et al. 2002), whereas the Pto-like clones were not reported previously. Out of the eight NBS-related sequences placed on the pres-

Fig. 1 (*concluded*).



ent map, three mapped to linkage group 5, close to the *Vat* locus specifying resistance to the aphid pest, *Aphis gossypii*. None cosegregates with the actual resistance trait, but they appear to form a cluster that contains the *Vat* gene, multiple R gene homologues, as well as an additional disease resistance gene for powdery mildew, *Pm-w* (Perin et al. 2002*a*). A fourth NBS clone, NBS-3, maps to linkage group 11, and is in tight linkage to the *Fusarium* race 1 and race 0 resistance locus, *Fom-2*, on the INRA reference map (see below). These data show that cloning and mapping R gene homologues is an efficient strategy to target molecular markers to the vicinity of R genes in melon, as has already been shown

Table 1. Linkage groups of *Cucumis melo*.

Linkage group	RFLP	RAPD	ISSR	SSR	AFLP	Traits	Total loci	Length (cM)
1	1	3	—	1	—	—	5	48.9
2	1	1	—	1	2	*a*	6	76.7
3	1	8	—	—	3	—	12	75.5
4	5	4	1	—	2	*Wt-2*	13	169.9
5	7	10	1	1	3	*Vat*	23	127.4
6	2	7	—	1	—	—	10	63.2
7	1	1	—	1	1	—	4	45.3
8A	1	3	—	3	5	—	12	87.7
8B	2	—	—	1	—	—	3	34.6
8C	1	—	—	2	—	—	3	21.8
9	8	3	—	—	3	—	14	124.4
10	3	11	—	1	5	—	20	161.1
11	3	10	—	—	5	—	18	164.9
12	1	7	—	—	—	—	8	57.3
13	—	—	—	—	7	—	7	42.6
14	—	3	—	—	1	—	4	42.6
15	—	—	—	1	2	—	3	18.9
16	1	—	—	1	—	—	2	15.6
17	1	1	—	—	—	—	2	5.3
18	—	—	—	—	2	—	2	4.0
19	—	1	—	—	1	—	2	14.9
20	1	—	1	—	—	—	2	13.1
21	—	1	—	1	—	—	2	0.1
22	1	—	—	1	—	—	2	4.8
Sum	41	74	3	16	42	3	179	1420.6

Note: Distribution of molecular markers and phenotypic traits among the different linkage groups. Group lengths are given in map units (cM).

for several other species (e.g., Leister et al. 1996; Kanazin et al. 1996). On the other hand, the two PTO homologues that we mapped in this study, PTH2 and PTH3, were not linked to disease resistance loci that are present either on this map or on the INRA reference map (Fig. 1).

The 16 SSR markers placed on this map were developed from melon or cucumber genomic sequences surrounding microsatellite repeat elements (Katzir et al. 1996; Danin-Poleg et al. 2000). Such elements exhibit frequent polymorphism in the number of repeats between melon accessions, and can be scored as codominant markers, whereas the specificity in the flanking sequence allows for reliable comparative mapping among different populations and related species (Danin-Poleg et al. 2000). Like sequence-characterized RFLP, SSR can provide useful sequence-tags that can help in connecting genetic maps with physical maps made of large-insert clone contigs.

RAPD, ISSR, and AFLP markers

RAPD, ISSR, and AFLP generate multi-banded patterns; these PCR-based techniques differ in the specific protocols employed to generate such patterns. The presence versus absence of a band is usually screened as a dominant marker trait, although in some cases, alternative alleles can be recognized and a pair of bands may then be scored as a codominant gene, as demonstrated by Perin et al. (2002*a*). In our case, we did not encounter such band pairs that we could designate as being allelic with high confidence. AFLP, RAPD, and ISSR markers are considered less useful for long-range genome analysis, because it is sometimes difficult to re-map them in different mapping populations. While their primary purpose would be to generate initial maps, saturate gaps, and link skeletal markers to each other, our experience indicates that RAPD and AFLP bands of interest can be excised, sequenced, and converted to robust, single-locus CAPs or dCAPS markers. Moreover, we find that part of the RAPD, ISSR, and AFLP patterns can be directly scored in different populations (e.g., Perin et al. 2002*a*).

Table 3 enlists the rates of polymorphism uncovered between the two mapping parents using four major marker types, as well as the final number of markers included in the map out of a larger number that was tentatively scored in the F_2 progeny. The specific rates of polymorphism and the efficiency of their conversion to mapped markers may vary according to the genotypes compared and the technical difficulties in genotyping the progeny. Nevertheless, our comparison of different marker types in a single study indicates that 60% of the RFLP probes and a similar proportion of RAPD primers uncovered at least one polymorphism between the two parents. SSR markers revealed a higher rate of polymorphism. As for AFLP, because of the high number of bands per primer, all primers tested were polymorphic. Rate of polymorphism calculated on a per-band basis was also higher with AFLP (37%), compared with RAPD (21%). We could typically score, in this population, one or two RAPD bands per polymorphic reaction, with many of the better primers yielding two scorable markers. With AFLP, we could score an average of 4.4 bands per polymorphic primer-pair, but the cost of the technique in time and materials was much higher. Table 3 also shows that only a propor-

Table 2. Sequence characterization of RFLP probes.

	Sequence descriptors					
Probe name	Species[a]	Type[b]	Sequence available[c]	GenBank accession No.	Sequence reference	Sequence homology
NBS-1	CM	g	F	AF354504	Brotman et al. 2002	NBS–LRR resistance genes
NBS-2	CM	g	F	AF354505	Brotman et al. 2002	NBS–LRR resistance genes
NBS-3	CM	g	F	AF354506	Brotman et al. 2002	NBS–LRR resistance genes
NBS-5	CM	g	F	AF354507	Brotman et al. 2002	NBS–LRR resistance genes
NBS-6 (syn.NBS17-5)	CM	g	F	AF354509	Brotman et al. 2002	NBS–LRR resistance genes
NBS-7	CM	g	F	AF354508	Brotman et al. 2002	NBS–LRR resistance genes
NBS-46-7	CM	g	F	AF354516	Brotman et al. 2002	NBS–LRR resistance genes
NRT-4F	CM	g	EST	BQ294501	This study	Limited similarity to NBS–LRR genes
PTH-2	CM	c	F	AF354500	This study	Pto serine (threonine) protein kinase R gene
PTH-3	CM	g	F	AF354501	This study	Pto serine (threonine) protein kinase R gene
ACS-1	CM	c	F	D30805	Miki et al. 1995	ACC synthase, GenBank accession No. D30805
ETR-1	CS	g	F	AB026498	Yamasaki et al. 2000	ETR1 ethylene receptor
CAG-1	CS	c	F	AF022377	Perl-Treves et al. 1998	Agamous transcription factor
CAG-2	CS	c	F	AF022378	Perl-Treves et al. 1998	Agamous transcription factor
CAG-3	CS	c	F	AF022379	Perl-Treves et al. 1998	Agamous transcription factor
PP2	CS	c	F	AF517155	Clark and Thompson, ms submitted	Cucumber phloem lectin
DH-27	CS	c	F	AF104397	Kahana et al., unpublished	Proline-rich floral bud protein
UBE2-1	CM	c	EST	BQ294492	This study	Ubiquitin-conjugating enzyme, E2
UBE2-2	CM	c	EST	BQ294493	This study	Ubiquitin conjugating enzyme, E2
CM-6	CM	c	EST	BQ294494	This study	Similar to src2, a cold-regulated soybean protein, GenBank accession No. AB00130
CM-9	CM	c	EST	BQ294495	This study	Unidentified protein
LHCP	CS	c	EST	BQ294490	This study	Chlorophyll *a*/*b* binding protein
SOD	CM	c	EST	BQ294498	This study	Cytosolic Cu, Zn superoxide dismutase
ACQ	CM	c	EST	BQ294499	This study	Aquaporin
PBP	CM	c	EST	BQ294500	This study	Similar to sunflower pollen basic protein, GenBank accession No. T13992
GTB	CM	c	EST	BQ294496	This study	GTP-binding protein, e.g., T07059
AC-39	CS	c	EST	BQ294487	This study	Unknown protein
LOX	CS	c	EST	BQ294486	This study	Lipoxygenase, e.g., P38417
AC12	CS	c	EST	BQ294489	This study	Similar to RNA-binding protein, GenBank accession No. AC018748_1
AC5	CS	c	EST	BQ294488	This study	Similar to GTPase activating protein, NC_003076
GDH	CS	c	EST	BQ294491	This study	Cytosolic glyceraldehyde 3-phosphate dehydrogenase, e.g., P26517
CM19	CM	c	EST	BQ294497	This study	AP2-domain containing protein, e.g., AF071893

[a]Source of clones. CS, *Cucumis sativus*; CM, *Cucumis melo*.
[b]Molecule type. c, cDNA; g, genomic fragment amplified by PCR.
[c]F, clone fully sequenced; EST, partial sequence.

Table 3. Rates of polymorphism and mapping efficiency using four molecular marker systems.

	Markers assayed			Loci scored[b]	
	Total	Non- polymorphic	Polymorphic[a]	Total scored	Mapped
RFLP probes	101	40	61 (60%)	59	41 (69%)
SSR primer- pairs	28	7	21 (75%)	24	16 (67%)
RAPD primers	80	32	48 (60%)		
RAPD bands	688	541	147 (21%)	120	74 (62%)
AFLP bands	283	178	105 (37%)	56	42 (75%)

[a]Number and percentage of polymorphic markers represent. RFLP probes that detected polymorphism between the two mapping parents using seven restriction enzymes, SSR primer pairs, RAPD primers and RAPD fragments, and AFLP fragments.

[b]Number and percentage of polymorphic markers that were successfully mapped in the F_2 progeny out of those that were tentatively scored. Rates are calculated per individual RAPD or AFLP bands, and RFLP or SSR loci (in some cases, we scored two loci per RFLP or SSR marker).

tion of the polymorphism detected between the parents could be successfully mapped: some bands could not be scored reliably in the F_2 progeny; others had unacceptable deviation from Mendelian segregation or did not link to the rest of the map. As for ISSR markers, these were generated using primers that correspond to the microsatellite core-repeat, with one or two selective nucleotides added at the 3′ end. ISSR primers often produced polymorphic PCR patterns on agarose gels; however, only a small proportion segregated properly and was scorable in the F_2 population. Danin-Poleg et al. (2002) reported similar problems with ISSR markers, whereas Perin et al. (2002*a*) reported their successful application.

Comparative mapping of selected RFLP and SSR markers on other populations

In the last few years, several research groups have developed their own mapping populations and, as a result, a few different maps for *Cucumis melo* have been recently published. Table 4 reviews the main features of these maps.

Many advanced genetic studies, like targeting a particular gene for map-based cloning, would require high resolution at specific map-regions. Other applications such as marker-assisted breeding would also benefit from increased availability of mapped markers. Identifying the linkage groups in a given map in terms of the other published maps renders marker exchange between maps possible, and alleviates the cost of producing new markers for each project. We therefore set out to correlate several of our linkage groups with those of Perin et al. (2002*a*), who have recently published the most saturated map for this species. Such data allow cross identification also with the linkage groups of Oliver et al. (2001), whose detailed map can also serve as a melon reference map; the latter includes numerous RFLP and SSR markers in common with the map of Perin et al. (2002*a*).

Eleven out of 15 SSR primer-pairs used in the present study had been successfully mapped and linked to the INRA map by Danin-Poleg et al. (2000). The linkage data are summarized in Table 5. They provide us with a set of "anchor points" to connect the map reported in the present study to that of Perin and co-workers. For example, SSR marker CM-CT505 that maps to our linkage group 1 (this study), was mapped by Danin-Poleg et al. (2000) to group G of Baudracco-Arnas and Pitrat (1996), between markers CM101_2 and CM CCA145. Group G corresponds to group I in the new INRA reference map by Perin et al. (2002*a*). The same SSR anchors were also applied by Danin-Poleg and co-workers to the mapping population of Oliver et al. (2001), and also to a third map, developed by Danin-Poleg et al. (2002), hence their importance in allowing cross-reference between all these different populations.

Eighteen of the RFLP probes mapped in this study were successfully used as additional anchor points between our map and the INRA map. For this purpose, about 60 individuals from the appropriate RIL population (Perin et al. 2002*a*) were scored, allowing us to place the marker in a specific interval on the reference map (Table 5). These markers were not published by Perin et al. (2002*a*) and therefore provide 22 novel data points to the INRA reference map as well. The comparative mapping data can also be viewed in Fig. 1, where SSR and RFLP markers used as anchors are marked by arrows, indicating the linkage groups to which they mapped in the INRA reference map.

The number of anchor points mapped in this study is not sufficient for a complete identification of all linkage groups in our map. In several instances, a desired marker could not be mapped onto the INRA map owing to lack of polymorphism. In a few other cases, ambiguity in group-assignment arises owing to hybridization of an RFLP probe to multiple bands (i.e., a gene family is detected). In spite of these limitations, the data in Table 5 provide sufficient identification of seven linkage groups, i.e., when more than a single anchor per group identifies the same linkage group in the INRA map. In a few other cases (groups 1, 6, 12, 16, 21, 22), data from a single common marker is presently available. We named our linkage groups, wherever possible, according to their likely identification in the INRA map.

Thus, group 1 is tentatively identified as group I in the INRA map by a single SSR marker, CM-CT505. For group 2, three anchor points provide its identification with INRA group II: these are the *andromonoecious* trait, RFLP marker CS-DH27, which is at present the closest marker that has been published for this trait (7 cM) and an SSR marker, CM-AG36. The latter marker exhibits tight linkage to the *Zym* locus, conferring resistance to the economically destructive potyvirus *zucchini yellow mosaic virus* (Danin-Poleg et al. 2002; Perin et al. 2002*a*). Group 4 is identified as INRA group IV by virtue of three RFLP probes: CM-9, which hybridizes to two unlinked loci on the INRA map, of which one is on LG IV, and the two resistance gene homologues, PTH-2 and NBS-17-5. In addition, the morphological trait of seed coat color, *Wt-2*, maps to this group on both maps.

The correspondence of our linkage group 5 with INRA group V relies on five anchor points: the *Vat* locus, which

Table 4. Main features of the published genetic maps of *Cucumis melo*.

Reference	Institute	Mapping population	No. of points	Map length (cM)	Main marker types	Phenotypic traits
Baudracco-Arnas and Pitrat (1996)	INRA, France	F_2 of 'Vedrantais' × PI 161375	110	1390	RFLP, RAPD	R genes: *Fom-1*, *Fom-2*, *Vat*, *nsv*; Morphological: *p*
Wang et al. (1997)	Clemson University, S.C.	BC_1 of (MR1 × 'Ananas Yokneam') × 'Ananas Yokneam'	204	1942	AFLP	None
Oliver et al. (2001)	IRTA, Spain	F_2 of PI 161375 × 'Pinonet Piel de Sapo'	412	1197	RFLP, AFLP, RAPD, SSR	Morphological: *p*
Perin et al. (2002*a*, 2002*b*, 2002*c*)	INRA, France	RIL of 'Vedrantais' × PI 161375 RIL of 'Vedrantais' × PI 414723	668	1654	AFLP, ISSR, RFLP	R genes: *Fom-1*, *Fom-2*, *Vat*, *nsv*, *Pm-x*, *Pm-w*, *Prv*, *Zym*; Morphological: 14 seed, seedling, flower and fruit traits; QTL: fruit shape and ripening traits
Danin-Poleg et al. (2002)	ARO, Israel	F_2 of PI 414723 × 'Dulce'	74	610	SSR, RAPD	R-genes: Zym, *Fom-1*; Other: *a*, flesh acidity
Silberstein et al. (this study)	Bar Ilan University, Israel	F_2 of PI 414723 × 'Topmark'	179	1420	RAPD, RFLP, AFLP, SSR	R-gene: *Vat*; Morphological: *a*, *Wt-2*

was scored on both populations, and several markers that are linked to it: three resistance gene homologues and another RFLP, CM-6. Group 6 can be tentatively assigned to INRA group VI owing to a single anchor point, UBE2-1.

Eight of the anchor points that map to INRA linkage group VIII are localized, on our map, on groups 8A, 8B, and 8C. The numerous SSR and RFLP anchors in this region prove that these three groups, which remained unlinked owing to the unsaturation of our map, are in fact part of a single large group, and their likely orientation with respect to each other is depicted in Fig. 1. The only marker that does not support this alignment is SSR CM-160a, b, but we note that this SSR reaction yielded, in our population, an atypical pattern with multiple bands that were difficult to relate to the products obtained in the other populations.

Two RFLP probes are common to our group 9 and INRA's group IX, and two other probes identify groups 10 and X, providing unambiguous identification of these two groups. In group 11, we have mapped a single anchor point, the resistance gene homologue NBS-3, to INRA group XI. This marker is tightly linked (0.7 cM) to the *Fusarium* races 0 and 1 resistance locus, *Fom-2*. Tightly linked markers for this locus have been published and used for marker-assisted breeding (Zheng et al. 1999; Wang et al. 2000; Zheng and Wolff 2000), but our RFLP marker indicates that *Fom-2* is probably part of an NBS–LRR gene cluster, and therefore the gene itself may be a member of the same family. Another RFLP, CM-ACS1, supports the identification of group 11 with group XI: the same RFLP clone for melon ACC synthase, (GenBank accession No. D30805; Miki et al. 1995) was mapped by Oliver et al. (2001) to their linkage group G5, which was shown, using two common SSR markers, to correspond to group XI of Perin et al. (2002*a*) and Danin-Poleg et al. (2000).

The single locus of CS-CAG-2 on our map may correspond to either one of two CS-CAG-2 loci that segregate in the INRA mapping population, (mapping at group VIII and group XII), so a reliable identification of our group 12 is not possible.

In addition to the reference to the INRA map, our SSR mapping data provide additional comparative information that was previously unavailable. SSR marker CM-GA127 could not be mapped onto the INRA map by Danin-Poleg and co-workers owing to lack of polymorphism, therefore group IX of the linkage map by Danin-Poleg et al. (2002), where CM-GA127 resided, could not be identified in terms of another map. Our data provide the missing identification and suggest that group IX of Danin-Poleg et al. (2002) probably corresponds to our group 5 and to group V of the INRA reference map. Along the same lines, CM CT44 was not mapped on the INRA maps, but was mapped by Danin-Poleg et al. (2002) on linkage group X. We can therefore suggest that our group 7 corresponds to group X of Danin-Poleg (2000).

Another SSR marker, CS-CT335 (on our group 6), was not placed on any other melon map, but was mapped by Danin-Poleg et al. (2000) on the cucumber map of Kennard et al (1994), and assigned to linkage group I. A few more SSR markers appearing on our map were placed on the cucumber map by the same authors: CM-CT44 (our group 7) maps to cucumber group D, CM-CTT144 and CS-GA057

Table 5. Mapping of selected SSR and RFLP markers on the melon reference map developed at INRA (Perin et al. 2002*a*).

	Marker name	Linkage group, present study	Linkage group, INRA map[a]	Flanking markers[b]
SSR markers	CM-CT505	1	I	CM101_2 : CM-CCA145
	CM-AG36	2	II	*Zym* : distal end[c]
	CM-GA128	21	III	CM82 : C19_1.3
	CM-ACC146	8A	VIII	CMAG59 : C04_0.8
	CM-AG59	8A	VIII	CM-ACC146 : C02_0.6
	CM-CTT144	8B	VIII	AE19 : distal end
	CS-GAO57[d]	8B and 22	VIII	CM173A : CM-CTT144
	CM-TC13	8C	VIII	CM173A : CM173B
	CM-CT134b	10	X	CM13 : B11_0.6
	CM-TC160b	8A	XI	G12_0.3 : E07_1.3
	CS-AT425a, b	16	a, XII; b, VII	CM39a : U_3000[c]; E07 : F14_1.6
RFLP markers	CS-DH27	2	II	*a* : E42/M31_21a
	CM-NBS-17-5 (= NBS 6)	4	IV	E33/M40_8a : N_950
	CM-PTH-2	4	IV	E32/M56_9 : H36/M37_18
	CM-NBS-2	5	V	E43/M44_9 : E46/M40_8
	CM-NBS-5	5	V	E43/M44_9 : E46/M40_8
	CM-NBS-46-7	5	V	E43/M44_9 : E46/M40_8
	CM-6	5	V	E43/M44_9 : E46/M40_8
	CM-UBE2-1	6	VI	T_680 : O_1250
	CM-PBP	8A	VIII	E39/M42_1 : E40/M34_9
	CM-ACQ[d]	a: 8Bb and 1	VIII	E38/M43_17 : H40/M37_11
	CS-CAG-3	8B	VIII	CM-CTT144 : distal end
	CM-NBS-7	9	IX	H36/M45_61 : E32/M56_55
	CM-26[e]	9	IX	E39/M42_9 : D_1100
			VIII	E39/M42_1 : E40/M34_9
	CM-PTH 3	10	X	E43/M44_6 : H33/M43_24
	CM-9[e]	4	VI	E43/M44_30 : O_1250
			X	AI1400 : O_330
	CM-NBS-3	11	XI	CMTC160 : *Fom-2*
	CS-LHCP[e]	22	XI	E40/M34_11 : *S-2*
			IV	E38/M43_24 : CM47
	CS-CAG-2[e]	12	XII	AM_1050 : distal end
			VIII	E42/M31_86 : E42/M31_52

[a]Linkage group designations on the INRA map are according to Perin et al. (2002*a*).
[b]Unless noted otherwise, SSR flanking markers are from Fig. 3 and Fig. 4 in Danin-Poleg et al. (2000), who mapped the SSR markers on the F_2 map by Baudracco-Arnas and Pitrat (1996). Linkage group correspondence between the 1996 and 2002 INRA maps are according to Perin et al. (2002*a*). RFLP-flanking markers are according to Fig. 3, Perin et al. (2002*a*).
[c]SSR-flanking markers from Fig. 3, Perin et al. (2002*a*).
[d]Two CS-GAO57 and two CM-ACQ loci segregated in the present study, only one of each in the INRA map.
[e]A single locus for CS-CAG-2, CM-9, and CS-LHCP segregated in the present study, two loci per probe in the INRA reference map. Two CM-26 bands were linked to the same group in the present study, whereas two bands mapped to different groups in the INRA map.

(our group 8) to cucumber group B, and CS-425a,b (on our group 16) maps to cucumber group D. Mapping a larger set of common markers on the two species maps should be possible, and would allow us to trace the evolutionary history of the two *Cucumis* genomes, including the intriguing difference in chromosome number (n = 7 in *C. sativus*, n = 12 in *C. melo*).

In conclusion, our map (Fig. 1) covers diverse linkage data and provides many new, useful markers, such as sequence-tagged RFLP homologous to genes of diverse functions. A few markers were located in the proximity of important traits, namely the *Vat* locus that specifies aphid resistance and the *andromonoecious* gene controlling sex type. Parallel regions between different melon maps were identified to facilitate the future use of genomic tools in this important horticultural crop.

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. 1990. Basic local alignment search tool. J. Mol. Biol. **215**: 403–410.

Arumuganathan, K., and Earle, E.D. 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. **9**: 208–218.

Ayub, R., Guis, M., Ben Amor, M., Gillot, L., Roustan, J.P., Latche, A., Bouzayen, M., and Pech, J.C. 1996. Expression of ACC oxidase antisense gene inhibits ripening of cantaloupe melon fruits. Nat. Biotechnol. **14**: 862–866.

Baudracco-Arnas, S., and 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.

Bent, A.F. 1996. Function meets structure in the study of plant disease resistance genes. Plant Cell, **8**: 1757–1771.

Brotman, Y., Silberstein, L., Kovalski, I., Perin, C., Dogimont, C., Pitrat, M., Klingler, J., Thompson, G.A., and Perl-Treves, R. 2002. Resistance gene homologues in melon are linked to genetic loci conferring disease and pest resistance Theor. Appl. Genet. **104**: 1055–1063.

Danin-Poleg, Y., Reis, N., Baudracco-Arnas, S., Pitrat, M., Staub, J.E., Oliver, M., Arus, P., deVicente, C.M., and Katzir, N. 2000. Simple sequence repeats in *Cucumis* mapping and map merging. Genome, **43**: 963–974.

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

Danin-Poleg, Y., Tadmor, Y., Tzuri, G., Reis, N., Hirschberg, J., and 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.

Ellis, J., Dodds, P., and Pryor, T. 2000. Structure, function and evolution of plant disease resistance genes. Curr. Opin. Plant Biol. **3**: 278–284.

Flores, F., Ben Amor, M., Jones, B., Pech, J.C., Bouzayen, M., Latche, A., and Romojaro, F. 2001. The use of ethylene-suppressed lines to assess differential sensitivity to ethylene of the various ripening pathways in Cantaloupe melons. Physiol. Plant. **113**: 128–133.

Gupta, M., Chyi, Y.-S., Romero-Severson, J., and Owen, J.L. 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theor. Appl. Genet. **89**: 998–1006.

Hagiwara, T., and Kamimura, K. 1936. Cross-breeding experiments in *Cucumis melo*. Tokyo Horticultural School Publication, Tokyo, Japan.

Hardie, D.G. 1999. Plant protein serine threonine kinases: Classification and functions. Annu. Rev. Plant Physiol. Plant Mol. Biol. **50**: 97–131.

Kanazin, V., Mareck, L.F., and Shoemaker, R.C. 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. **93**: 11 746 – 11 750.

Katzir, N., Danin-Poleg, Y., Tzuri, G., Karchi, Z., Lavi, U., and Cregan, P.B. 1996. Length polymorphism and homologies of microsatellites in several cucurbitaceae species. Theor. Appl. Genet. **93**: 1282–1290.

Kennard, W.C., Poetter, K., Dijkhuizen, A., Meglic, V., Staub, J.E., and Havey, M.J. 1994. Linkages among RFLP, RAPD, isozyme, disease resistance, and morphological markers in narrow and wide crosses of cucumber. Theor. Appl. Genet. **89**: 42–48.

Klingler, J., Kovalski, I., Silberstein, L., Thompson, G.A., and Perl-Treves, R. 2001. Mapping of cotton-melon aphid resistance in melon. J. Am. Soc. Hortic. Sci. **126**: 56–63.

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

Leister, D., Ballvora, A., Salamini, F., and Gebhardt, C. 1996. A PCR based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat. Genet. **14**: 421–429.

Martin, G.B., Brommonschenkel, S., Chunwongse, J., Frary, A.,Ganal, M.W., Spivey, R., Wu, T., Earle, E.D., and Tanksley, S.D. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science (Washington, D.C.), **262**: 1432–1436.

McCreight, J.D., Bohn, G.W., and Kinshaba, A.N. 1992. "Pedigree" P.I. 414723 melon. Cucurbit Genet. Coop. Rep. **15**: 51–52.

Miki, T., Yamamoto, M., Nakagawa, H., Ogura, N., Mori, H., Imaseki, H., and Sato, T. 1995. Nucleotide sequence of a cDNA for 1-aminocyclopropane-1-carboxylate synthase from melon fruits. Plant Physiol. **107**: 297–298.

Motro, U., and Soller, M. 1993. Sequential sampling in determining linkage between marker loci and quantitative trait loci. Theor. Appl. Genet. **85**: 658–664.

Neuhausen, S.L. 1992. Evaluation of restriction fragment length polymorphism in *Cucumis melo*. Theor. Appl. Genet. **83**: 379–384.

Oliver, M., Garcia-Mas, J., Cardus, M., Pueyo, N., Lopez-Sese, A., Arroyo, M., Gomez-Paniagua, H., Arus, P., and de Vincente, M.C. 2001. Construction of a reference linkage map for melon. Genome, **44**: 836–845.

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

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

Perin, C., Gomez-Jimenez, M., Hagen, L., Dogimont, C., Pech, J.C., Latche, A., Pitrat, M., and Lelievre, J.M. 2002*c*. Molecular and genetic characterization of a non-climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol. **129**: 300–309.

Perl-Treves, R., Kahana, A., Rosenman, N., Xiang, Y., and Silberstein, L. 1998. Expression of multiple AGAMOUS-like genes in male and female flowers of cucumber (*Cucumis sativus* L.). Plant Cell Physiol. **39**: 701–710.

Rosa, J.T. 1928. The inheritance of flower types in *Cucumis* and *Citrullus*. Hilgardia, **3**: 233–250.

Scofield, S.R., Tobias, C.M., Rathjen, J.P., Chang, J.H., Lavelle, D.T., et al. 1996. Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. Science (Washington, D.C.), **274**: 2063–2065.

Silberstein, L., Kovalski, I., Huang, R., Anagnostou, K., Kyle, M.M., and Perl-Treves, R. 1999. Molecular variation in *Cucumis melo* as revealed by RFLP and RAPD markers. Sci. Hortic. **79**: 101–111.

Stepansky, A., Kovalski, I., and Perl-Treves, R. 1999. Intraspecific classification of *Cucumis melo* based on morphological and molecular evaluation. Plant Syst. Evol. **217**: 313–333.

Tang, X., Frederick, R.D., Zhou, J., Halterman, D.A., Jia, Y., and Martin, G.B. 1996. Initiation of plant disease resistance by physical interaction of *avrPto* and *Pto* kinase. Science (Washington, D.C.), **274**: 2060–2063.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Vandelee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP — a new technique for DNA-fingerprinting. Nucleic Acids Res. **23**: 4407–4414.

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

Wang, Y.H., Thomas, C.E., and Dean, R.A. 2000. Genetic mapping of a fusarium wilt resistance gene (*Fom-2*) in melon (*Cucumis melo* L.). Mol. Breed. **6**: 379–389.

Williams, J.G.K., Hanafey, M.K., Rafalsky, J.A., and Tingey, S.V. 1993. Genetic analysis using random amplified polymorphic DNA markers. Meth. Enzymol. **218**: 704–741.

Yamasaki, S., Fujii, N., and Takahashi, H. 2000. The ethylene-regulated expression of *CS-ETR2* and *CS-ERS* genes in cucumber plants and their possible involvement with sex expression in flowers. Plant Cell Physiol. **41**: 608–616.

Zheng, X.Y., and Wolff, D.W. 2000. Randomly amplified polymorphic DNA markers linked to fusarium wilt resistance in diverse melons. Hortscience, **35**: 716–721.

Zheng, X.Y., Wolff, D.W., Baudracco-Arnas, S., and Pitrat, M. 1999. Development and utility of cleaved amplified polymorphic sequences (CAPS) and restriction fragment length polymorphisms (RFLPs) linked to the *Fom-2* fusarium wilt resistance gene in melon (*Cucumis melo* L.). Theor. Appl. Genet. **99**: 453–463.

Zink, F.W., and Gulber, W.D. 1987. U.C. PMR 45 and U.C. Top Mark fusarium wilt-resistant (fom-3) muskmelon breeding lines. HortScience, **22**: 172.