Theor Appl Genet (1997) 95:791–798

Y.-H. Wang · C. E. Thomas · R. A. Dean

A genetic map of melon (*Cucumis melo* L.) based on amplified fragment length polymorphism (AFLP) markers

Received: 12 March 1997 / Accepted: 20 May 1997

Abstract Genetic maps facilitate the study of genome structure and evolution, and the identification of monogenic traits or Mendelian components of quantitative traits. We evaluated 228 RAPD, microsatellite and AFLP markers for linkage analysis in melon (*Cucumis melo* L.) varieties MR-1 (resistant to *Fusarium* wilt, powdery and downy mildews) and Ananas Yokneum (AY; susceptible to these diseases) and constructed a detailed genetic map. The mapping population consisted of 66 backcross progenies derived from AY × (MR-1 × AY). Despite a relatively low level of polymorphism in the species, AFLP markers were found to be more efficient in mapping the melon genome than RAPD or microsatellite markers. The map contains 197 AFLPs, six RAPDs and one microsatellite marker assigned to 14 major and six minor linkage groups, and covers 1942 cM with the average distance between adjacent markers of approximately 10 cM. The maximum distance allowed between markers is 27.5 cM. About 11% of the intervals (20 out of 173) are over 20 cM (but less than 27.5 cM). The map has immediate utility for identifying markers linked to disease resistance genes that are suitable for marker-assisted breeding. The use of microsatellite markers for integration with other maps is also discussed.

Key words Melon · *Cucumis melo* L. · Genetic mapping · AFLP · RAPD · Microsatellite · DNA markers

Communicated by M. A. Saghai Maroof

Y.-H. Wang · R. A. Dean (✉)
Department of Plant Pathology and Physiology, 120 Long Hall, Clemson University, Clemson, SC 29634-0377, USA
Fax: +1-864-656-0274
e-mail: rdean@clemson.edu

C. E. Thomas
US Vegetable Laboratory, USDA-ARS, 2875 Savannah Highway, Charleston, SC 29414, USA

Introduction

Melon (*Cucumis melo* L.) is a valuable cash crop grown throughout the world. It is a member of the genus *Cucumis*, in the family Cucurbitaceae. *C. melo* includes a diverse group of annual, trailing-vine plants. Despite diversity in horticultural traits such as fruit shapes [there are seven different horticultural groups of melons (McCreight et al. 1993)] polymorphism at the DNA level among the groups is relatively low (Shattuck-Eidens et al. 1990; Neuhausen 1992). The plant is diploid with a base chromosome number of 12 (Dane 1991) and a genome size of $4.5–5.0 \times 10^8$ bp (Arumuganathan and Earle 1991). About 30% of the genome consists of repeated DNA sequences (Bendich and Anderson 1974).

Genetic maps with a relatively high-density of markers are useful for a number of purposes. For example, they serve to locate, or tag, gene(s) of interest to facilitate marker-assisted breeding and map-based cloning; they also provide useful clues to understand the biological basis of complex traits and phenomena (Lee 1995). Genetic studies of melon have identified over 90 genes (Pitrat 1994). However, before the advent of molecular markers, linkage mapping was limited to known genes or phenotypic markers. Pitrat (1991) analysed 28 phenotypic markers in an F_2 population and found that 23 of the markers fell into eight linkage groups. Development of DNA markers has greatly facilitated mapping. Baudracco-Arnas and Pitrat (1994) developed a mapping strategy for melon using RAPD and RFLP markers that resulted in assigning 77 DNA markers to 12 linkage groups with 2–12 markers in each group. The map was extended to 14 linkage groups covering 1390 cM with 102 markers, including five phenotypical markers (Baudracco-Arnas and Pitrat 1996). Twenty three percent of the map distance contained nine gaps larger than 30 cM. Microsatellite markers have also been developed for melon (Katzir et al. 1996).

Recently, amplified fragment length polymorphism (AFLP™) was developed (Vos et al. 1995) as a new DNA marker system combining the features of RFLP and PCR. It involves three steps: (1) digestion of genomic DNA with restriction enzymes and subsequent ligation of adapters; (2) amplification of the restriction fragments; and (3) denaturing polyacrylamide-gel analysis of the amplified fragments. It is an efficient technique to generate large numbers of markers for genetic mapping compared with other DNA marker systems (van Eck et al. 1995; Mackill et al. 1996; Keim et al. 1997). In addition to the construction of genetic maps, the AFLP technique has been used efficiently to identify specific genes (Thomas et al. 1995; Cervera et al. 1996) or genes differentially expressed (Bachem et al. 1996), to enrich markers in certain regions of the genome (Ballvora et al. 1995), and to determine genetic diversity (Maughan et al. 1996).

In the present paper we describe the construction of a melon genetic map based primarily on AFLP markers using a backcross population. The AFLP marker system proved to be more efficient in mapping the melon genome than RAPD or microsatellite markers. The linkage map reported in this paper using AFLP markers not only provides a framework to better understand the genome structure of melon, but also constitutes a useful resource for a genetic improvement of the crop.

Materials and methods

Plant materials

MR-1 is a melon breeding line developed by the USDA (Thomas 1986) which is resistant to downy and powdery mildews, *Fusarium* wilt (except *F. oxysporum* f. sp. *melonis* race 1, 2), and Alternaria leaf blight (Thomas 1986; Zink and Thomas 1990; McCreight et al. 1993). Ananas Yokneum (AY), a cultivar from Israel, is susceptible to downy and powdery mildews and *Fusarium* wilt. Crosses and backcrosses between MR-1 and AY were generated at the USDA, ARS, USA, Vegetable Laboratory in Charleston, S.C. A population of 66 progenies from a single fruit derived from AY × (MR-1 × AY) was grown in growth chambers (Wechter et al. 1995) for DNA isolation.

DNA isolation

Leaves of 4–6-week-old plants were collected. Nuclear DNA was isolated according to Weising et al. (1995) and Wing et al. (1995). Two to four grams of fresh leaves were ground in liquid nitrogen and transferred to a 200-ml beaker containing 40 ml of ice-cold 1 × homogenization buffer (HB) (10 mM Tris-HCl, 80 mM KCl, 10 mM EDTA, 1 mM spermidine, 500 mM sucrose and 0.15% β-mercaptoethanol) and 0.5% Triton X-100. The homogenates were stirred on ice for 10 min and filtered first through one layer and then again through two layers of Miracloth (Calbiochem, La Jolla, Calif.). The slurry was clarified by centrifugation at 1800 g at 4°C for 20 min. Pellets were washed with cold wash buffer (same as 1 × HB except for the addition of 0.5% Triton X-100) 2–3 times and re-suspended in 1 × TE (10 mM Tris, 1 mM EDTA, pH 7.5). One-tenth volume of lysis solution (0.2 M EDTA, 10% sarkosyl and 2% proteinase K) were added before being incubated at 65°C for 10 min, followed by 2-h incubation at 37°C. Samples were then centrifuged for 15 min at 5000 g and DNA was precipitated by the addition of 0.1 vol of 3 M sodium acetate and 2 vol of ethanol. DNA pellets were washed once with 70% ethanol and re-suspended in 100 µl of TE or H_2O. DNA quantity and quality were checked by a fluorometer and by electrophoresis through a 1% agarose gel.

RAPD analysis

Five to twenty nanograms of DNA were used for PCR in a 20-µl reaction containing 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 1 mM primer (primer # 1-200 and # 500-600 from University of British Columbia, Canada, or Kits A-B, D-H, J-K, M, O-T, W-X, AA, AH, AW from Operon Technologies, Alameda, Calif.), 0.2 U of *Tfl* DNA polymerase (Epicenter, Madison, Wis.) and 0.8-µl of 20 × reaction buffer (supplied by manufacturer). The reaction was overlaid with one drop of mineral oil. Amplification was performed in a Perkin Elmer DNA Thermal Cycler (Perkin Elmer, Foster City, Calif.) with one cycle of 94°C for 5 min and 45 cycles of 94°C for 1 min, 44°C for 1 min and 72°C for 2 min, followed by a final extension for 5 min at 72°C. PCR products were analysed on a 1.5% agarose gel for 4.5 h at 5 V/cm in 1 × TAE buffer (40 mM Tris, 20 mM glacial acetic acid, 1 mM EDTA). Gels were stained with ethidium bromide for 20 min and de-stained for10 min. Polymorphic bands were scored directly from the gel to minimize any scoring error and only stable bands were scored as potential markers. Each gel was photographed using Polaroid 667 film.

Microsatellite marker analysis

Six pairs of microsatellite primers (CMTC13, CMCT58, CMAG59, CMGA127, CMGA128 and CSLHCPA: each including sense and antisense primers) were synthesized as described by Katzir et al. (1996). The following PCR conditions were used: 1 cycle of 94°C for 4 min; 15 cycles of 94°C for 1 min, 65°C for 1 min with a decrease of 1°C every cycle, and 72°C for 2 min; 30 cycles of 94°C for 1 min, 51°C for 1 min and 72°C for 2 min. The temperature was held at 4°C after cycling. Products were run in 4% agarose gels at 5 V/cm in 1 × TBE buffer (90 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.0) for 4 h. Gels were stained and photographed as described above.

AFLP analysis

All reagents required for AFLP analysis were obtained from Life Technologies (Geithersburg, Md.) as kits except for the following: *Taq* polymerase was from Perkin Elmer, γ-^{32}P and γ-^{33}P [ATP] were from Amersham (Arlington Heights, Ill.) and additional primers were synthesized by Integrated DNA Technologies (Coralville, Iowa). Manufacturer's procedures were followed with some minor modifications. PCR products were separated by electrophoresis on 6% polyacrylamide gels (19 : 1 acrylamide : N,N′ methylene-bis-acrylamide; 8.3 M urea; 1 × TBE buffer) with 0.4-mm spacers. Sharkstooth or conventional combs were used. Electrophoresis was performed using 1 × TBE buffer on a BioMax STS-45I standard thermoplate sequencer gel-electrophoresis unit (Kodak, Conn.). Gels were pre-run for 20 min before 2–4-µl samples were loaded into each well. Gels were run at 70 W for 3 h, vacuum dried, and exposed to BioMax MR film (Kodak, Conn.) overnight.

Scoring of data

Bands generated by RAPD and AFLP analyses were scored as dominant markers. Only those bands present in MR-1, but absent in

AY and segregating in the backcross population in a 1:1 ratio, were scored. Microsatellite markers, although co-dominant, were also scored as dominant markers; i.e., if both alleles were present in the progenies, the marker was scored as present; if only the AY allele was present, the marker was scored as absent. F_1 plants were not typed because their genotypes can be inferred from parents and backcross progenies. For linkage analysis, markers present in a progeny were scored as "H" and absent as "A". Unreliable, ambiguous bands were scored as missing ("−"). Because the mapping population consisted of backcross progenies, AFLP or RAPD band presence implies a heterozygote and absence a homozygote. All scored markers were re-scored independently. The re-scored data were used for linkage analysis.

Marker nomenclature

RAPD markers were named using the primer number of the manufacturer followed by the number of polymorphic bands identified by the same primer in descending molecular-weight order. AFLP markers were named using the selective nucleotides in the *Eco*RI primer/the selective nucleotides in the *Mse*I primer followed by numbers in descending molecular-weight order. Primer pairs are presented the same way in the text. Microsatellite markers were named SSR (simple sequence repeats) followed by the primer number.

Linkage analysis

Data were analysed using Mapmaker Version 2.0 (Courtesy of S. Tingey, Du Pont, Del.). Markers were first grouped using a minimum LOD score of 4.0 and a maximum recombination value (θ) of 0.24. For each linkage group, markers were ordered by using a minimum LOD score of 6.0 and a maximum θ of 0.24 using the First-Order command. The maximum distance allowed in each linkage group was 27.5 cM, which corresponded to a recombination ratio of 0.25 by the Kosambi map function. The ordered marker sequences were confirmed using the Ripple command. Markers ordered with low confidence were placed again using the Try command. Linkage maps were generated with the Map command using the Kosambi map function. Chi-square tests were performed to check whether individual markers segregated randomly.

Results

RAPD and microsatellite analyses

Five hundred and fifty one primers were screened against the parents, MR-1 and AY. The random 10-mers amplified 5–16 PCR products per PCR reaction. About 17% (97) of the primers amplified fragments present in MR-1 and absent in AY. Initially, the map was planned to be constructed with RAPD markers. We switched to AFLP after 12 RAPD markers were analysed because we had problems scoring some of the marker bands, even directly from the gel. Some of the polymorphic bands were inconsistent between different PCR reactions. Therefore, only six RAPD markers were used in final map construction, including primer #596 which amplified a 1.6-kb fragment linked to *Fom-2* (named 596-1 in linkage group III in Fig. 1) (Wetcher et al. 1995). Baudracco-Arnas and Pitrat (1996) also concluded that RAPD analysis was not the best solution for melon map construction.

Among the six pairs of microsatellite primers reported by Katzir et al. (1996), only one (SSR127 in our map) was polymorphic between the parents used in our study. This marker has a core sequence of $(GA)_{13}A(GA)_2$ (Katzir et al. 1996). The other five pairs amplified fragments in each parent of the same size. Lack of polymorphism was also confirmed by running the same PCR products on 5% non-denaturing polyacrylamide gels. Therefore, only one microsatellite marker was mapped.

AFLP analysis

Thirteen *Eco*RI primers (eight with +3 and five with +2 selective nucleotides) and ten *Mse*I (eight with +3 and two with +2 selective nucleotides) primers were used. A total of 109 primer combinations (Table 1) were

Table 1 AFLP primer combinations tested and number of mappable markers in each pair used for map construction. Numbers in parentheses are number of markers scored in MR-1. Top row are *Mse*I primers with 3 or 2 selective nucleotides shown and left column are *Eco*RI primers with 3 or 2 selective nucleotides. ND – primer pairs not used

Primer	M-CAA	M-CAC	M-CAG	M-CAT	M-CTA	M-CTC	M-CTG	M-CTT	M-CC	M-CG	Average	Total
E-AAC	5 (8)	3 (3)	6 (6)	2 (2)	3 (4)	1 (3)	4 (5)	1 (4)	5 (7)	5 (7)	3.5 (4.9)	35 (49)
E-AAG	2 (7)	(1)	(2)	3 (3)	5 (5)	6 (7)	(2)	1 (3)	(3)	ND	3.4 (3.6)	17 (33)
E-ACA	(1)	(3)	1 (4)	7 (7)	(3)	(6)	(2)	(0)	7 (7)	6 (6)	5.2 (4.3)	21 (39)
E-ACT	(2)	(1)	2 (6)	4 (5)	(2)	(2)	(1)	(0)	8 (8)	ND	4.6 (3.4)	14 (27)
E-ACC	(4)	3 (4)	ND	1 (3)	(3)	(3)	2 (5)	(2)	ND	1 (1)	1.7 (3.1)	7 (25)
E-ACG	(1)	(2)	5 (6)	(2)	4 (6)	(2)	(5)	2 (4)	(1)	ND	3.6 (3.2)	11 (29)
E-AGC	5 (5)	1 (2)	4 (4)	(3)	1 (2)	5 (5)	(1)	(3)	ND	ND	3.2 (3.1)	16 (25)
E-AGG	(4)	3 (4)	(0)	(0)	2 (6)	2 (4)	3 (5)	(1)	2 (2)	ND	2.4 (3.7)	12 (26)
E-TA	1 (3)	(6)	(2)	3 (7)	5 (7)	(3)	9 (10)	6 (6)	ND	ND	4.8 (5.5)	24 (44)
E-TG	2 (7)	3 (4)	(1)	4 (6)	4 (7)	(3)	5 (5)	(3)	ND	ND	3.6 (4.5)	18 (36)
E-TT	(1)	ND	6 (7)	(4)	ND	4 (6)	ND	7 (7)	ND	ND	5.6 (5.0)	17 (25)
E-AT	(5)	(3)	6 (9)	3 (5)	(2)	(4)	4 (5)	(5)	ND	ND	4.3 (4.7)	13 (38)
E-TC	(4)	5 (5)	(2)	4 (5)	(2)	7 (10)	(3)	(2)	ND	ND	3.2 (4.1)	16 (33)
Average	3.0 (4.0)	3.0 (3.1)	4.2 (4.1)	3.4 (4.0)	3.4 (4.4)	4.1 (4.5)	4.5 (4.1)	3.4 (3.1)	5.5 (4.7)	4.0 (4.7)		
Total	15 (22)	18 (38)	30 (49)	31 (52)	24 (49)	25 (58)	27 (49)	17 (40)	22 (28)	12 (14)		221 (429)

tested against the parents, resulting in 7214 amplified bands with an average of 65 bands per primer pair. Four primer pairs, i.e., AGG/CAG, AGG/CAT, ACA/CTT and ACT/CTT, failed to detect polymorphism between the parents. Parental screening with the primer pairs was repeated once to reproduce the polymorphic bands using DNA samples from different isolations. From the 109 primer pairs, 429 (6%) of the total bands present in MR-1 and absent in AY were reproducibly scored in the parents. Fifty seven primer pairs were used for mapping, which included 306 markers scored in the parents. Only 221 markers (72%) could be confidently scored and mapped in the backcross progenies (Table 2). The remainder were either not well amplified, did not segregate in the population, or else were too faint to score although the parental bands were strong and easy to score. The average number of mapped markers for +3/+3 (*Eco*RI + 3/*Mse*I +3) primer pairs was 3.1, compared with 5.5 for +3/+2 or 4.9 for +2/+3 primer pairs (Table 2). From Table 2 it is unclear why +3/+2 primer pairs identified fewer total number of bands than +3/+3 since theoretically the former should generate more bands. On average, *Mse*I + CTT produced the least number of mappable markers while *Eco*RI + AAC produced the most markers, both total and mappable (Table 1).

Table 2 Number of polymorphic markers used for mapping and detected by a combination of primers with two or three selective nucleotides

*Eco*RI/*Mse*I primer pair	No. of primer pairs scored	Total no. of bands (average)	Total no. of polymorphic bands (average)
3/3	32	2059 (64)	99 (3.1)
3/2	6	341 (57)	33 (5.5)
2/3	18	1277 (71)	89 (4.9)
Total	57	3677	221

Map construction

Out of total 228 markers analysed, 204 were placed in 20 groups using a minimum LOD score of 4.0 (corresponding to a 99.5% confidence level) and a maximum recombination value of 0.24. One hundred and eighty eight markers were assigned to 14 major groups and 16 to six minor groups. The 14 major groups ranged from 41.5 to 317 cM in length and each group carried 5–28 markers with an average of 13 markers (Table 3). The six minor groups contained a total of 16 markers with 2–4 markers in each group ranging from 19 to 38.8 cM in length. Twenty four markers (all AFLPs) were not linked to any group. Five of these (21%) showed skewed segregation, one towards AY and four towards MR-1; ten were the products of four specific primer pairs, ACT/CAT (1-4), AGC/CTC (3-4), TT/CTT (5 and 7) and AT/CTG (2-3). Lowering the LOD score to 3.0 placed 2 of 24 unassigned markers into linkage groups, and also resulted in the combination of two of the major linkage groups, I and XI.

Markers in each group were arranged into a specific order using minimum LOD scores of 6.0 and a maximum recombination value (θ) of 0.24. The orders for each linkage group are presented in Fig. 1. Markers that could not be placed confidently after grouping are

Table 3 Marker distribution among the linkage groups of melon (*C. melo* L.)

Linkage group	Marker number	Length (cM)	Average distance (cM)	Number of skewed markers[a]	Number of markers not placed[b]
I	28	317.0	13.2	4 (14)	3
II	21	207.0	12.9	5 (24)	4
III	17	175.5	11.0	2 (12)	0
IV	22	164.2	7.8	0 (0)	0
V	12	150.8	15.1	4 (33)	1
VI	15	138.5	10.6	2 (13)	1
VII	15	127.6	9.1	0 (0)	0
VIII	10	111.0	13.8	0 (0)	1
IX	12	115.6	10.5	3 (25)	0
X	8	86.5	12.3	2 (25)	0
XI	8	59.3	8.5	3 (38)	0
XII	8	51.4	8.6	0 (0)	1
XIII	7	63.7	10.6	0 (0)	0
XIV	5	41.5	10.4	0 (0)	0
Others	16	132.7	NA	3 (19)	0
Total	204	1942.0	11.0	28	11

[a] Based on a *P* value of 0.05. In parentheses are the percentage of skewed markers in each group
[b] Number of markers that cannot be confidently placed on the map were included in the total number of markers in each linkage group. Averages between adjacent markers were obtained from ordered markers

indicated in the legend to Fig. 1. The map contains 197 AFLPs, six RAPDs and one microsatellite marker. The total length of the map is 1942 cM. The average distance between ordered adjacent markers is 11 cM with 11% of the intervals greater than 20 cM (Table 3). Linkage groups X and XI were initially grouped together based on a minimum LOD score of 4.0 and maximum θ of 0.24. However, they were broken into two linkage groups because the maximum genetic distance allowed between adjacent markers in the same group is 27.5 cM (see Discussion; Fig. 1).

Marker segregation

A total of 33 (14%) of 228 markers, all of them AFLPs, showed segregation patterns skewed away from a 1 : 1 ratio at $P = 0.05$. Twenty six of them displayed overpresence (skewed towards MR-1) and seven underpresence of markers (skewed towards AY). Among all data points (total number of progenies multiplied by total number of markers), 7703 (52%) represent presence vs 7115 (48%) absence of marker bands, indicating a slight, but significant ($\chi^2 = 23.76$, $P \leq 0.001$), overpresence of MR-1-specific markers in the backcross progenies. Twenty eight of the thirty three skewed markers were assigned to eight major and three minor linkage groups (Table 3). The skewed markers were not clustered in their respective linkage groups. However, in linkage group II all four skewed markers could not be ordered, whereas in other linkage groups some of the skewed markers tended to be mapped towards the ends. This is true in linkage groups I, III, V, VI, X, and XI. In linkage group IX, three markers (AAG/CTC6/, ACT/CC8 and AAG/CTA3) were skewed towards AY and represented the only skewed markers in that group.

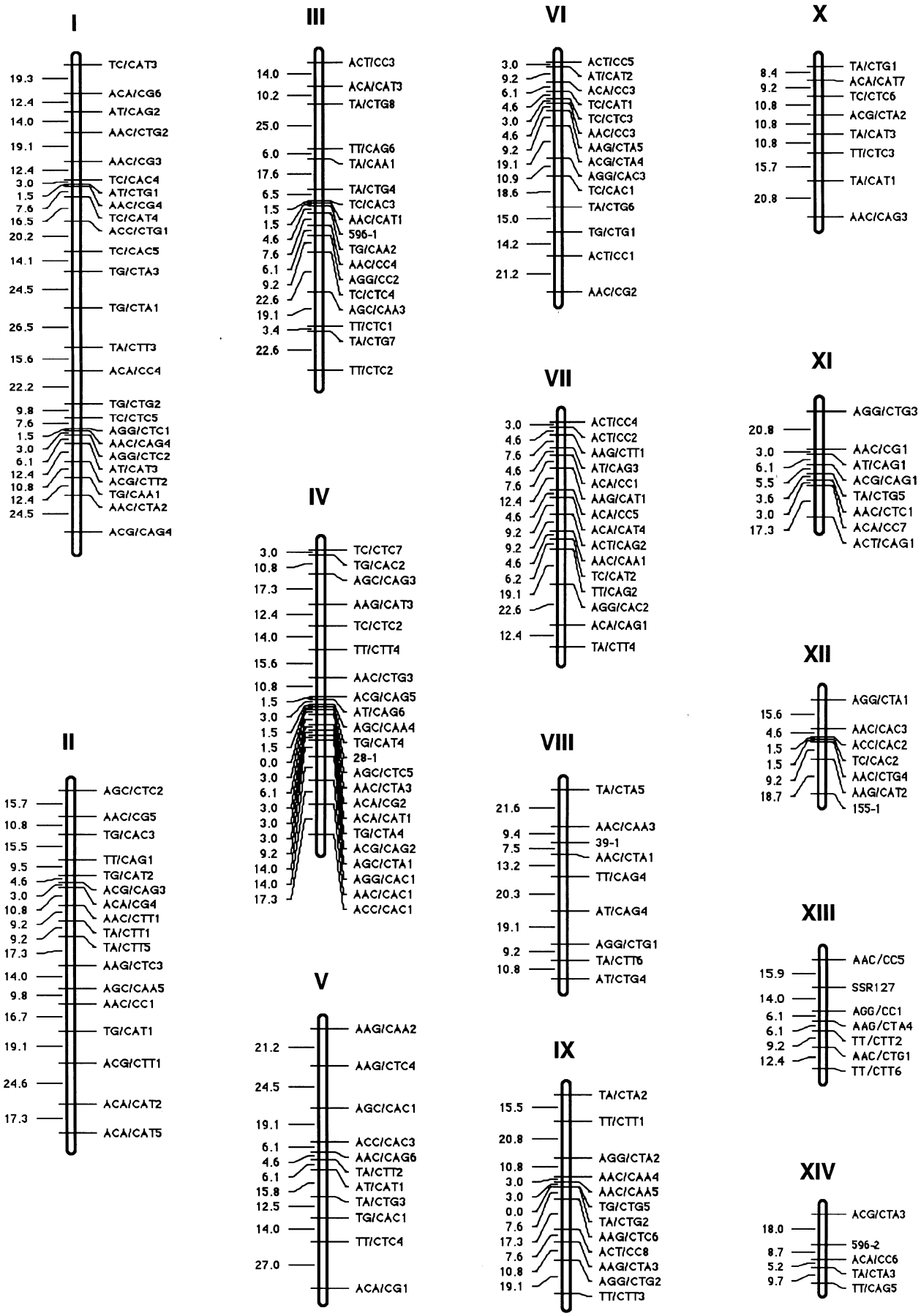
Discussion

Genetic variation within *C. melo*

Polymorphism in melon at the DNA level has been reported to be low. Shattuck-Eidens et al. (1990) surveyed a 1572-bp genomic region in eight melon varieties from diverse geographic areas and found only two single-base changes, both C-T transitions, among the melons. Furthermore, few polymorphisms could be detected by RFLP analysis of these lines. Neuhausen (1992) found that the majority (59%) of unique-sequence probes resulted in only two hybridization patterns among 44 geographically diverse melon lines and that polymorphisms were due to infrequent base changes. A low level of polymorphism was also confirmed by Baudracco-Arnas and Pitrat (1996) using RFLP analysis. Microsatellite markers have also been used to evaluate polymorphism in melons. Katzir et al. (1996) tested seven microsatellite markers (SSRs, simple sequence repeats) in eight melon varieties and found that five detected length polymorphisms among the varieties (3–5 alleles per SSR), a polymorphism level similar to that of cucumber which also has a low level of polymorphism (Staub et al. 1996). Using RAPD markers, Gracía-Rodríuez et al. (1996) found that variability among European melon lines was relatively low compared to lentils, which also exhibit low variability (Sharma et al. 1996). In creating our map, 551 RAPD random 10-mer primers were screened and 97 (17%) were found to amplify bands in MR-1 but were absent in AY. Baudracco-Arnas and Pitrat (1996) also reported a similar degree of polymorphism (18.3%) between the parental lines Vedrantais and Songwhan Charmi using RAPD markers. Because genome variations among different melon varieties has been reported to result from infrequent base changes, it was suggested that use of four-base restriction enzymes may aid the detection of polymorphism in such a species (Kennard et al. 1994). We have used AFLP markers, which combine the use of both four- and six-base restriction enzymes, to detect polymorphism in melon and found an average of 3.9 polymorphic markers per primer pair in the parents used in this study. This appears low compared to an average of nine (Keim et al. 1997) or 18 (Maughan et al. 1996) per primer pair with AFLP in soybean. It would be necessary to try other four-base cutters in combination with different six-base cutters to see if a higher level of polymorphism can be detected. Regardless of this, the polymorphic bands observed in the present study were sufficient to construct a linkage map of melon with moderate density and in a short period of time. In this study we only used *Eco*RI selective nucleotides of A-- or T- or A- and *Mse*I selective nucleotides of C-- or C-. Use of *Eco*RI and *Mse*I enzymes with different combinations of selective nucleotides would in principle provide enough markers for high-density mapping.

Mapping populations

Many plant genetic maps have been generated using either F_2 or backcross populations based either on RFLP or RAPD markers. The first molecular map of melon (Baudracco-Arnas and Pitrat 1996), consisting of 102 loci, was constructed using F_2 populations. For dominant marker systems, such as RAPD and AFLP, backcross populations are more suitable compared to F_2 populations although only one (rather than two) recombinant gamete is sampled in each plant (Reiter et al. 1992). We also noted that population size affects the precision in estimating the recombination ratio (r), which can be improved with larger populations. However, for phase-known double backcrosses, as used in this study, a population of 66 progenies is able to estimate a maximum r of 0.24–0.25 (Ott 1991). Such


I
19.3 TC/CAT3
12.4 ACA/CG6
14.0 AT/CAG2
19.1 AAC/CTG2
12.4 AAC/CG3
3.0 TC/CAC4
1.5 AT/CTG1
7.6 AAC/CG4
16.5 TC/CAT4
20.2 ACC/CTG1
14.1 TC/CAC5
24.5 TG/CTA3
26.5 TG/CTA1
15.6 TA/CTT3
22.2 ACA/CC4
9.8 TG/CTG2
7.6 TC/CTC5
1.5 AGG/CTC1
3.0 AAC/CAG4
6.1 AGG/CTC2
12.4 AT/CAT3
10.8 ACG/CTT2
12.4 TG/CAA1
24.5 AAC/CTA2
ACG/CAG4
II
15.7 AGC/CTC2
10.8 AAC/CG5
15.5 TG/CAC3
9.5 TT/CAG1
4.6 TG/CAT2
3.0 ACG/CAG3
10.8 ACA/CG4
9.2 AAC/CTT1
9.2 TA/CTT1
17.3 TA/CTT5
14.0 AAG/CTC3
9.8 AGC/CAA5
16.7 AAC/CC1
19.1 TG/CAT1
24.6 ACG/CTT1
17.3 ACA/CAT2
ACA/CAT5
III
14.0 ACT/CC3
10.2 ACA/CAT3
25.0 TA/CTG8
6.0 TT/CAG6
17.6 TA/CAA1
6.5 TA/CTG4
1.5 TC/CAC3
1.5 AAC/CAT1
4.6 596-1
7.6 TG/CAA2
6.1 AAC/CC4
9.2 AGG/CC2
22.6 TC/CTC4
19.1 AGC/CAA3
3.4 TT/CTC1
22.6 TA/CTG7
TT/CTC2
IV
3.0 TC/CTC7
10.8 TG/CAC2
17.3 AGC/CAG3
12.4 AAG/CAT3
14.0 TC/CTC2
15.6 TT/CTT4
10.8 AAC/CTG3
1.5 ACG/CAG5
3.0 AT/CAG6
1.5 AGC/CAA4
1.5 TG/CAT4
0.0 28-1
3.0 AGC/CTC5
6.1 AAC/CTA3
3.0 ACA/CG2
3.0 ACA/CAT1
3.0 TG/CTA4
9.2 ACG/CAG2
14.0 AGC/CTA1
14.0 AGG/CAC1
17.3 AAC/CAC1
ACC/CAC1
V
21.2 AAG/CAA2
AAG/CTC4
24.5 AGC/CAC1
19.1 ACC/CAC3
6.1 AAC/CAG6
4.6 TA/CTT2
6.1 AT/CAT1
15.8 TA/CTG3
12.5 TG/CAC1
14.0 TT/CTC4
27.0 ACA/CG1
VI
3.0 ACT/CC5
9.2 AT/CAT2
6.1 ACA/CC3
4.6 TC/CAT1
3.0 TC/CTC3
4.6 AAC/CC3
9.2 AAG/CTA5
19.1 ACG/CTA4
10.9 AGG/CAC3
18.6 TC/CAC1
15.0 TA/CTG6
14.2 TG/CTG1
21.2 ACT/CC1
AAC/CG2
VII
3.0 ACT/CC4
4.6 ACT/CC2
7.6 AAG/CTT1
4.6 AT/CAG3
7.6 ACA/CC1
12.4 AAG/CAT1
4.6 ACA/CC5
9.2 ACA/CAT4
9.2 ACT/CAG2
4.6 AAC/CAA1
6.2 TC/CAT2
19.1 TT/CAG2
22.6 AGG/CAC2
12.4 ACA/CAG1
TA/CTT4
VIII
21.6 TA/CTA5
9.4 AAC/CAA3
7.5 39-1
13.2 AAC/CTA1
20.3 TT/CAG4
19.1 AT/CAG4
9.2 AGG/CTG1
10.8 TA/CTT6
AT/CTG4
IX
15.5 TA/CTA2
20.8 TT/CTT1
10.8 AGG/CTA2
3.0 AAC/CAA4
3.0 AAC/CAA5
0.0 TG/CTG5
7.6 TA/CTG2
17.3 AAG/CTC6
7.6 ACT/CC8
10.8 AAG/CTA3
19.1 AGG/CTG2
TT/CTT3
X
8.4 TA/CTG1
9.2 ACA/CAT7
10.8 TC/CTC6
10.8 ACG/CTA2
10.8 TA/CAT3
15.7 TT/CTC3
20.8 TA/CAT1
AAC/CAG3
XI
20.8 AGG/CTG3
3.0 AAC/CG1
6.1 AT/CAG1
5.5 ACG/CAG1
3.6 TA/CTG5
3.0 AAC/CTC1
17.3 ACA/CC7
ACT/CAG1
XII
15.6 AGG/CTA1
4.6 AAC/CAC3
1.5 ACC/CAC2
1.5 TC/CAC2
9.2 AAC/CTG4
18.7 AAG/CAT2
155-1
XIII
15.9 AAC/CC5
SSR127
14.0 AGG/CC1
6.1 AAG/CTA4
6.1 TT/CTT2
9.2 AAC/CTG1
12.4 TT/CTT6
XIV
18.0 ACG/CTA3
596-2
8.7 ACA/CC6
5.2 TA/CTA3
9.7 TT/CAG5

a population size would not be able to detect linkage at >27.5 cM with as much confidence.

Skewed markers

The percentage of skewed markers was similar to that previously reported in melon (12%; Baudracco-Arnas and Pitrat 1996) and higher than those of other plants (Kesseli et al. 1994), but lower than for cucumber (Kennard et al. 1994). In Baudracco-Arnas and Pitrat's (1996) study in melon, skewed markers did not show systemic bias towards either parent. The reason for markers being skewed towards MR-1 in our study is not clear, but may be due to a number of factors. MR-1 is a breeding line known for its resistance to multiple diseases, although poor for desirable horticultural traits. Even in the absence of apparent pathogen pressure, simply the presence of genes conferring multiple disease resistance may offer some advantages to its overall fitness (Thomas 1986). AY, on the other hand, is an old cultivar and may be less fit than highly selected, more recent, cultivars such as MR-1.

Map construction

Melon contains 12 chromosomes; however, the 204 MR-1-specific markers fell into 14 major and six minor linkage groups. This may be a consequence of the fairly small population size used in this study; perhaps key recombinants were not identified and thus certain gaps remain to be filled. It has been suggested that increasing population size, and not the number of markers, would most likely reduce the number of linkage groups (Kesseli et al. 1994; Keim et al. 1997). Because the map covers 1942 cM with over 200 markers, there is at least one marker for every 10 cM in the map on average. Eleven markers could not be ordered after grouping. This may affect the resolution of the map overall, but high-resolution mapping of regions around genes of interest can be readily accomplished. In the long run the order of assigned markers will be resolved when more progenies are used and more markers are developed. Furthermore, the AFLP markers can be converted to either PCR primers or RFLP probes to facilitate integration with other linkage maps (Baudracco-Arnas and Pitrat 1996). In an attempt to anchor our linkage map, we included six microsatellite markers previously identified as polymorphic in other melon lines. Microsatellite markers have the advantage of being easy to utilize and are reproducible among different labs. Seven microsatellite markers have been incorporated by Katzir's group into Baudracco-Arnas and Pitrat's (1996) map (Katzir et al. 1997). The microsatellites contained $(TC)_n$, $(CT)_n$, $(AG)_n$, $(GA)_n$ and $(AT)_n$ motifs with *n* ranging from 8 to 20 (Katzir et al. 1996). Unfortunately, only one microsatellite detected a length polymorphism between the parents and was mapped into linkage group XIII. Additional microsatellite markers are currently being developed.

Map application

The immediate utility of the map will be to locate markers linked to disease-resistance genes on the map since MR-1 possesses genes conferring resistance to several races of Fusarium wilt, downy and powdery mildews, which are mostly controlled by monogenes (McCreight et al. 1993). Using F_2 progenies of the same cross we previously identified *Fom-2* that confers resistance to races 0 and 1 of *F. oxysporum* f. sp. *melonis*, which is linked to RAPD marker 596-1 (Wetcher et al. 1995). Markers for other resistance genes can be readily identified in a similar manner. Markers linked to resistance genes will then be automatically placed on the map. Therefore, our map is particularly useful to study the disease resistance-gene organization in the genome of melons, an advantage that may not be immediately available for other maps.

Acknowledgements This is technical contribution No. 4325, of the South Carolina Agricultural Experimental Station, Clemson, SC 29634. We thank Dr. David Heckel and Dr. Halina Shorupska for providing some of the RAPD primers and AFLP starter reagents, and for insightful discussions throughout this work; Pat Wechter for help in quantitating DNA concentrations; Barbara Blackmon for assistance through the study; Dr. Eric Fang for reviewing the manuscript. This work is supported by USDA-ARS, Clemson University and the Wade Stackhouse Graduate Fellowship to Y.-H. Wang.

Fig. 1 A genetic map of melon (*C. melo* L.). The 14 major linkage groups are arbitrarily labeled by *Roman letters* and shown here. On the left of the *vertical double lines* are map distances in cM as calculated by the Kosambi function and on the right are DNA markers. Markers assigned to linkage groups, but not ordered, are: I – ACA/CG3, AAG/CAA1, AAG/CTC5; II – TG/CTG3, AT/CAG5, ACA/CC2, TA/CTG9; V – ACT/CC6; VI – TA/CAT2; VIII – AAC/CAG2; XII – ACC/CTG2. The six minor groups not shown are: ACC/CAT1-AAC/CAT2-AGC/CAG1-AGC/CAA1; ACT/CC7-AAC/CAG5-AAG/CTC1; AAC/CAG1-153-1-AAC/CAC2; AAC/CAA2-TA/CTA1; ACC/CG1-TA/CTA4; and AAG/CTA2-AGC/CTC1. See Table 3 for a summary of the map

References

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:211–215

Bachem CWB, van der Hoeven RS, de Bruijn SM, Vreugdenhil D, Zabeau M, Visser RGF (1996) Visualization of differential gene expression using a novel method of RNA fingerprinting based on AFLP: analysis of gene expression during potato tuber development. Plant J 9:745–753

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

Baudracco-Arnas S, Pitrat M (1994) Molecular polymorphism between two *Cucumis melo* lines and linkage groups. In: Lester GE, Dunlap JR (eds) Proceedings: Cucurbitaceae '94 – evaluation and enhancement of cucurbit germplasm. Gateway Printing 1995, Edinburg, Texas, USA, pp 197–200

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

Bendich AJ, Anderson RS (1974) Novel properties of satellite DNA from muskmelon. Proc Natl Acad Sci USA 71 : 1511–1515

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

Dane F (1991) Cytogenetics of the genus *Cucumis*. In: Tsuchiya T, Gupta PK (eds) Chromosome engineering in plants: genetics, breeding, evolution, part B. Elsevier, Amsterdam, pp 201–214

García-Rodríuez E, Alvarez I, Lozano R (1996) Random amplified polymorphic DNAs (RAPDs) as markers to determining genetic relationships among *Cucumis melo* L. genotypes. Cucurbitaceae '96/EUCARPIA VI, Malaga, Spain, May 1996

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

Katzir N, Danin-Poleg Y, Reis N, Pitrat M (1997) Simple sequence repeats for germplasm analysis and mapping in melon (*Cucumis melo* L.). P55 (Poster), Plant and Animal Genome V, January 12–16 1997, San Diego, California, USA

Keim P, Schupp JM, Travis SE, Clayton K, Webb DM (1997) A high-density soybean genetic map based upon AFLP markers. Crop Sci 37 (in press)

Kennard WC, Poetter K, Dijkhuizen A, Meglic V, Staub JE, Havey MJ (1994) Linkage among RFLP, RAPD, isozyme, disease-resistance, and morphological markers in narrow and wide crosses of cucumber. Theor Appl Genet 89 : 42–48

Kesseli RV, Paran I, Michelmore RW (1994) Analysis of a detailed genetic linkage map of *Lactuca sativa* (lettuce) constructed from RFLP and RAPD markers. Genetics 136 : 1435–1446

Lee M (1995) DNA markers and plant breeding programs. Adv Agron 55 : 265–344

Mackill DJ, Zhang Z, Redona ED, Colowit PM (1996) Level of polymorphism and genetic mapping of AFLP markers in rice. Genome 39 : 969–977

Maughan PJ, Saghai Maroof MA, Buss GR, Huestis GM (1996) Amplified fragment length polymorphism (AFLP) in soybean: species diversity, inheritance, and near-isogenic line analysis. Theor Appl Genet 93 : 392–401

McCreight JD, Nerson H, Grumet R (1993) Melon *Cucumis melo* L. In: Kalloo G, Berch BO (eds) Genetic improvement of vegetable crops. Pergamon Press, Oxford, pp 267–294

Neuhausen SL (1992) Evaluation of restriction fragment length polymorphism in *Cucumis melo*. Theor Appl Genet 83 : 379–384

Ott J (1991) Analysis of human genetic linkage. 2nd edn. The Johns Hopkins University Press, Baltimore, MD, USA

Pitrat M (1991) Linkage groups in *Cucumis melo* L. J Hered 82 : 406–411

Pitrat M (1994) Gene list for *Cucumis melo* L. Cucurbit Genet Coop Rep 17 : 135–148

Reiter RS, Williams JGK, Feldman KA, Rafalski A, Tingey SV, Scolnik PA (1992) Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc Natl Acad Sci USA 89 : 1477–1481

Sharma SK, Knox MR, Ellis THN (1996) AFLP analysis of the diversity and phylogeny of *Lens* and its comparison with RAPD analysis. Theor Appl Genet 93 : 751–758

Shattuck-Eidens DM, Bell RN, Neuhausen SL, Helentjaris T (1990) DNA sequence variation within maize and melon: observations from polymerase chain reaction amplification and direct sequencing. Genetics 126 : 207–217

Staub J, Serquen F, Bacher J (1996) Genetic map construction and map merging in cucumber (*Cucumis sativus* L.). Cucurbitaceae '96/EUCARPIA VI, Malaga, Spain, May 1996

Thomas CE (1986) Downy and powdery mildew-resistant muskmelon breeding line MR-1. HortScience 21 : 329

Thomas CM, Vos P, Zabeau M, Jones DA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato *Cf-9* gene for resistance to *Cladosporium fulvum*. Plant J 8 : 785–794

van Eck HJ, van der Voort JR, Draaistra J, van Zandvoort P, van Enckevort E, Segers B, Peleman J, Jacobsen E, Helder J, Bakker J (1995) The inheritance and chromosomal localization of AFLP markers in non-inbred potato offspring. Mol Breed 1 : 397–410

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

Wechter WP, Whitehead MP, Thomas CE, Dean RA (1995) Identification of a randomly amplified polymorphic DNA marker linked to the *Fom-2 Fusarium*-wilt resistance gene in muskmelon MR-1. Phytopathology 85 : 1245–1249

Weising K, Nybom H, Wolff K, Meyer W (1995) DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, pp 57–58

Wing RA, Zhang HB, Woo SS (1995) BAC workshop manual. Texas A and M University, College Station, Texas, USA

Zink FW, Thomas CE (1990) Genetics of resistance to *Fusarium oxysporum* f. sp. *melonis* races 0, 1, and 2 in muskmelon line MR-1. Phytopathology 80 : 1230–1232