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A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers

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Abstract One hundred and ten markers were analysed for linkage in 218 F_2 plants derived from two divergent cultivars ('Védrantais' and 'Songwhan Charmi') of *Cucumis melo* (L.). Thirty-four RFLPs, 64 RAPDs, one isozyme, four disease resistance markers and one morphological marker were used to construct a genetic map spanning 14 linkage groups covering 1390 cM of the melon genome. RAPD and RFLP markers detected similar polymorphism levels. RFLPs were largely due to base substitutions rather than insertion/deletions. Twelve percent of markers showed distorted segregation. Phenotypic markers consisted of two resistance genes against *Fusarium* wilt (*Fom-1* and *Fom-2*), one gene (*nsv*) controlling the resistance to melon necrotic spot virus, one gene (*Vat*) conferring resistance to *Aphis gossypii*, and a recessive gene for carpel numbers (3 vs 5 carpels: *p*).

Key words *Cucumis melo* · RFLP · RAPD · Molecular markers · Genetic map

Introduction

Melon (*Cucumis melo* L.) is a cross-pollinated diploid ($2x = 2n = 24$) species of African origin. Breeding for disease resistance is an important objective of melon improvement programs. In the field, *Fusarium* wilt, powdery and downy mildew, and several viruses, including cucumber mosaic virus, cause severe damage in France (Quiot et al. 1979). Resistance to *Fusarium oxysporum* f. sp. *melonis* races 0 and 1 (*Fom*-2) and races 0 and 2 (*Fom-1*), melon necrotic spot virus (*nsv*), and aphid infection (*Vat*) are controlled by single genes, whereas resistance to cucumber mosaic virus is controlled by several genes. Molecular markers linked with these genes would speed up breeding programs. To-date, more than 90 genes in melon have been described (Pitrat 1994). The genetic map consists of eight linkage groups with 23 disease resistance, flower biology, and vegetative characters (Pitrat 1991). It is not possible to assign these groups to chromosomes because neither trisomic nor monosomic plants have been described. Molecular markers have not yet been used for constructing a genetic map in melon. However, some phylogenic studies with isozymes or RFLPs have been conducted on several cultivars or accessions of *C. melo* (Esquinas 1981; Dane 1983; Perl-Treves et al. 1985; Staub et al. 1987; Neuhausen 1992). Isozyme variability has been studied in 125 melon accessions with six enzyme systems which revealed 11 loci (Esquinas 1981). Another study analysed the intraspecific and interspecific variability in accessions of different *Cucumis* species with nine enzyme systems (Staub et al. 1987). Perl-Treves et al. (1985) showed 24% polymorphism between six melon varieties with 29 enzyme systems. More recently, Neuhausen (1992) used RFLP markers to study phylogenetic relationships among 44 melon accessions and showed a high polymorphism level (90%). To construct a map of melon, we used RFLP, RAPD, isozyme, disease resistance and morphological markers. The population used in this study segregated for a number of quantitative traits of economic importance, such as cucumber mosaic virus resistance, papaya ring spot virus resistance, fruit shape, fruit quality, and other morphological traits.

Materials and methods

Genetic material

The map was generated from the analysis of an F_2 population derived from an intraspecific cross between two inbred lines: 'Védrantais' (VD) a Cantaloup Charentais type (from Vilmorin, France) and PI 161375 or 'Songwhan Charmi' (SC) (from Korea). VD possesses good horticultural characteristics and one gene for resistance to races 0 and 2 of *F. oxysporum* f sp *melonis* (*Fom-1*) (Risser 1973). SC possesses

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several genes for disease resistance: *Fom-2*, controlling resistance to races 0 and 1 of *F. oxysporum melonis* (Risser 1973); *nvs*, controlling resistance to melon necrotic spot virus (MNSV) (Coudriet et al. 1981); and *Vat*, conditioning resistance to *Aphis gossypii* (Pitrat and Lecoq 1980). SC also possesses the recessive gene *p* controlling the number of carpels, five vs three, in VD (Rosa 1928). Two-hundred-and-eighteen F_2 plants were grown in the greenhouse and self-pollinated to obtain F_3 families. A bulk of 100 individuals from each F_3 family was used as a source of DNA. The analyses of monogenic resistances and morphological markers were scored on F_3 families (20 individuals) to determine the genotype of the F_2 parental plant.

Disease resistance tests

F. oxysporum f sp melonis resistance

Fusarium resistance was evaluated as described by Risser and Mas (1965) by dipping the roots of 20 plantlets of each F_3 family in a conidial suspension before transplanting to sand. Two weeks after inoculation susceptible plants died, whereas resistant ones remained green.

Melon necrotic spot virus resistance

Melon seeds (20 per F_3 family) were sown in a flat and grown under greenhouse conditions. Plants to be tested were used 15–20 days after sowing, when the first leaf was expanding. Inoculum was prepared by grinding 1 g of young leaves of VD with necrotic lesions in 4 ml of 0.03 M Na_2HPO_4 solution containing 0.2% sodium diethyldithiocarbamate. Prior to inoculation, 0.4 g of carborundum and 0.5 g of activated charcoal were added to the sap extract (Marrou 1967). Necrotic lesions appeared 5–7 days after inoculation. Melon necrotic spot virus induced necrotic lesions on susceptible plants, whereas resistant plants (allele *nsv* in SC) exhibited no symptoms.

A. gossypii infection resistance

A. gossypii resistance was tested as described by Pitrat and Lecoq (1980) on 20 plants per F_3 family. Aphids remain under the leaves of susceptible plants and produce larvae, whereas aphids leave the resistant plants and produce very few larvae.

Placenta number

The number of carpels was determined on five flowers of ten plants from each F_3 family. The '5-carpels' character (vs '3 carpels') is controlled by one recessive gene (allele *p* in SC) (Rosa 1928).

Molecular and isozyme markers

Isozyme detection

Crude extraction was performed on ice by grinding 50 mg of young leaf with 100 µl of the extraction buffer (0.065 M Tris-Glutathione, pH = 7.6 and 0.5 M sucrose). Horizontal starch-gel electrophoresis was performed according to a modified procedure following Wendel and Weeden (1990). Gels were stained for 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44) with 50 ml of staining-buffer (0.1 M Tris-HCl, pH = 7.5, 10 mg 6-phosphogluconic acid, 7.5 mg $NADP^+$, 10 mg Tetrazolium thiazolyl blue, 2 mg phenazine methosulphate, 100 mg $MgCl_2$) at 37 °C for 2 h. The analysis was performed on a bulk of ten F_3 plants for each F_2 genotype.

DNA extraction and RFLP analysis

The extraction of genomic DNA has been previously described (Baudracco-Arnas 1995). The melon cDNA library and *pMEL1* gene (ACC oxidase) used as RFLP probes were provided by J. C. Pech of Laboratoire Ethylène et Mécanismes Moléculaires de la Maturation of ENSA Toulouse (France), and templates were isolated by the method described in Balagué et al. (1993). The procedures for the digestion by endonuclease restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I), agarose-gel electrophoresis, blotting DNA onto nylon membranes, and Southern hybridization have been described by Lefebvre et al. (1993). All autoradiograms were scored blindly at least three times to limit errors in scoring.

RAPD analysis

The protocol for RAPD markers was adapted from that of Williams et al. (1990). The DNA used was the same as that prepared for RFLP procedures. Primers were purchased from Operon Technologies, Inc., Alameda, Calif., USA. Reactions were performed in a 20-µl solution containing 1 × buffer (50 mM KCl, 10 mM Tris-HCl, pH = 8.8, 0.1% Triton X-100), 2.5 mM $MgCl_2$, 130 µM of each nucleotide, 0.2 µM primer, 0.8 units *Taq*DNA polymerase (Promega) and 20–30 ng of DNA. The reactions were conducted in sealed 200-µl tubes covered with plastic film and incubated for one cycle of 3 min at 92 °C, 45 cycles of 1 min at 92 °C, 1 min at 40 °C and 2 min at 72 °C, and 1 cycle of 10 min at 72 °C in a Techne PHC-3 thermocycler. After amplification 4 µl of blue dye (0.25% bromophenol blue, 30% glycerol and 0.125 M Na_2EDTA) were added to each tube. The samples were loaded into a 2% agarose gel in TAE buffer, pH = 8 (40 mM Tris-HCl, 20 mM acetic acid and 1 mM Na_2EDTA). In order to confirm polymorphic markers, the amplifications were repeated at least three times, and only the qualitative polymorphisms were considered. All gels were scored blindly at least three times to limit errors in scoring.

Data analysis

After scoring the segregation of molecular markers and monogenic characters, each locus was tested for goodness of fit expected 3:1 or 1:2:1 ratios by a chi-square test. The order of markers was analysed with Mapmaker version 3.0 (Lander and Botstein 1989). Markers were initially associated with two-point comparisons of the *group* command (threshold LOD 5.0, threshold recombination value 0.3). Markers were then placed in sequence via three-point analyses of the *order* command. Scoring errors were detected by checking for double cross-overs. Finally, candidate orders were confirmed with multipoint analyses of the *compare* and *ripple* commands observing a window of five contiguous loci. Markers that could not be confidently located were placed via the *try* command. Recombination frequencies were transformed to Kosambi distances (Kosambi 1944).

Results

Inheritance of disease resistance genes and morphological characters

The segregation data for the phenotypic characters observed in the F_3 progenies are listed in Table 1. *Vat*, *nsv*, *Fom-2* and *p* fit the expected 1:2:1 ratio for monogenic control. For *Fom-1* a significant deviation from 1:2:1 ratio was observed, with an excess of heterozygous genotypes.

Isozyme analysis

Two zones of 6-phosphogluconate dehydrogenase activity were observed. Two isozymes were detected, the most-anodal one (6PGD-1) was monomorphic while

Table 1 Segregation and chi-square goodness-of-fit analysis disease resistances, pentamerous fruit, and isoenzyme measured in F_3 families

Markers	Observed numbers	χ^2 Value (1:2:1 segregation)	Probability
Disease resistance			
Vat	44:117:51	2.74	0.253
nsv	48:112:58	1.08	0.582
Fom-1	36:125:55	8.69	0.013*
Fom-2	50:117:51	1.18	0.553
Morphological			
p	41:116:57	3.9	0.142
Isoenzyme			
6Pgd-2	54:106:56	0.11	0.946

* Indicates a significant ($P < 0.05$) deviation from the expected segregation

the lowest migrating isozyme (6PGD-2) was polymorphic (Esquinas 1981). The *6Pgd-2* locus segregated in the expected 1:2:1 ratio (Table 1).

RAPD analysis

The screening of 383 primers revealed 279 polymorphisms. Out of 1995 amplified products (average of 5.2 per primer), 366 were polymorphic between VD and SC (18.3% polymorphism). Thirty eight primers were used to reveal 68 markers. Twelve allowed the amplification of a fragment specific from VD and another from SC, the F_1 containing both. These fragments segregated independently and showed no significant deviation from a 1:2:1 ratio. For the 56 remaining dominant RAPDs, nearly equivalent numbers were recessive (i.e. null) for each parent (25 for VD, 31 for SC). Five markers showed a significant deviation ($P < 0.05$) from the expected 3:1 ratio (Table 2). All showed an over-abundance of the absent class. Six other markers presented a highly significant deviation ($P < 0.01$) from the 3:1 ratio (Table 2); four showed an over-abundance of the absent class and two exhibited over-abundance of the present class (one in favor of VD and one in favour of SC). The majority of these biased markers were on linkage groups D and G. On linkage group D, the three biased markers were bulked on the same region. Linkage group G, with three biased markers, was constituted of dominant markers only, and so was difficult to order.

RFLP analysis

DNA from the two parental lines and from the F_1 (VD × SC) was individually digested with each of six restriction endonucleases and then hybridized with a maximum of 131 probes. DNA digested with *Dra*I, *Bam*HI and *Xba*I displayed significantly less polymorphism than DNA digested with *Eco*RI and *Eco*RV (Table 3). Among the restriction fragment patterns of 724 probe-enzyme combinations (6 enzymes and 131 probes; with some missing data), 150 probe-enzyme combinations (with 73 probes) detected polymorphism

Table 2 Markers displaying deviation from Mendelian ratios (1:2:1 or 3:1). SC/SC, VD/VD and SC/VD represent respectively the number of F_2 individuals scored homozygous for the 'Songwhan Charmi' allele, homozygous for the 'Védrantais' allele, or heterozygous. Differences in the totals of the two or three classes between markers are the result of missing data. Italic markers are co-dominant markers

Group	Locus	SC/SC	SC/VD	VD/VD	VD/VD or SC/VD	SC/SC or SC/VD	χ^2 3:1	χ^2 1:2:1
5	*Fom-1*	36	125	55				8.69*
A	C19-1.0	66			144		4.63*	
B	C07-0.6	68			145		5.45*	
C	*AE10*	66	95	41				6.90*
D	B11-0.8			66		126	9.00**	
D	Q10-1.6			55		119	4.05*	
D	G19-0.9			66		144	4.62*	
F	F14-1.6	65			140		4.92*	
G	C04-0.7	80			126		21.03**	
G	C11-0.4			29		184	17.72**	
G	C18-0.9	75			124		17.09**	
J	*CM93-1*	41	99	30				4.89*
Unlinked	C11-0.6	16			189		32.32**	
Unlinked	F13-0.4	73			134		14.03**	

* $P < 0.05$, ** $P < 0.01$

Table 3 Polymorphism level displayed by the six restriction endonucleases used for DNA digestion in the two inbred lines 'Vedrantais' and 'Songwhan Charmi'

Restriction endonuclease	Polymorphism level (%)	Number of tested probes
*Bam*HI	13.9	108
*Dra*I	10.6	113
*Eco*RI	29.5	129
*Eco*RV	32.3	127
*Hin*dIII	22.6	124
*Xba*I	14.6	123

between the two parental lines (20.6%). About 63% of the probes detected RFLPs with at least one enzyme.

Two restriction enzymes, *Eco*RI and *Eco*RV, were used for the segregation analysis on the F_2 progeny because they produced the highest polymorphism level. The 32 probes (revealing 36 loci) that gave the most clearly readable patterns were studied. Only one RFLP marker (*CM93-1*) deviated significantly from the expected 1:2:1 ratio ($P < 0.05$) (Table 2). Twenty five were hybridized on DNA digested with *Eco*RI and seven were hybridized on DNA digested with *Eco*RV. For two probes, the patterns observed in the two parents differed for only one band (dominant marker). For 27 probes the pattern differed for two bands, one specific for VD and the other specific for SC, the F_1 having both. In such cases, the two bands were alleles of a single locus. The probe *CM173* gave a different segregation pattern with DNA digested by *Eco*RI (*CM173-a*) and with *Eco*RV (*CM173-b*). For the remaining two probes, the patterns of the two parental lines differed for several bands. One probe gave two loci (*CM93-1*) and *CM93-2*) and the other one gave three loci (*CM39-1*, *CM39-2* and *CM39-3*).

RFLPs may be generated either by base substitution or insertion/deletion events. In the latter case, RFLPs should be generated by any enzyme whose restriction sites encompass the insertion/deletion, and the probability of different enzymes detecting polymorphism with a given probe would not be independent. The probabilities of obtaining RFLPs with one probe and two different restriction enzymes are given in Table 4 where the 15 possible enzyme pairs are considered. For ten pairs independence was accepted, which suggested that the polymorphism should result more often from individual nucleotide substitutions than from insertion/deletion events. This result was confirmed by the fact that the majority of probes were polymorphic with only one or two restriction enzymes (Fig. 1).

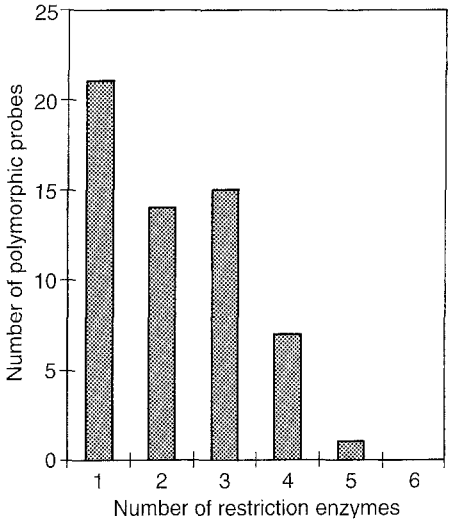

Fig. 1 Number of polymorphic cDNA probes detected with 1–6 restriction enzymes

Table 4 Independence between enzymes to reveal polymorphism. Non-bracketted numbers are for observed class sizes (number of probes revealing polymorphism, or its absence, with these enzymes). The bracketted numbers represent the theoretical class size under the hypothesis of independence

Pair of enzymes		Polymorphism with				χ^{2a}
1	2	1 and 2	1	2	Neither 1 or 2	
*Eco*RI	*Eco*RV	15 (10.9)	20 (24.1)	22 (26.1)	61 (56.9)	3.06
*Eco*RI	*Xba*I	9 (5.3)	27 (30.7)	9 (12.7)	77 (73.3)	4.26
*Eco*RI	*Hin*dIII	17 (8.5)	20 (28.5)	11 (19.5)	74 (65.5)	15.88*
*Eco*RI	*Bam*HI	10 (4.5)	22 (27.5)	5 (10.5)	69 (63.5)	11.03*
*Eco*RI	*Dra*I	3 (3.5)	30 (29.5)	9 (8.5)	70 (70.5)	0.13
*Eco*RV	*Xba*I	11 (5.9)	28 (33.1)	7 (12.1)	79 (67.9)	7.73*
*Eco*RV	*Hin*dIII	16 (9.4)	24 (30.6)	12 (18.6)	67 (60.4)	9.03*
*Eco*RV	*Bam*HI	10 (4.9)	24 (29.1)	5 (10.1)	65 (59.9)	9.19*
*Eco*RV	*Dra*I	7 (3.9)	28 (31.1)	5 (8.1)	69 (65.9)	4.25
*Xba*I	*Hin*dIII	6 (4.1)	13 (14.9)	19 (20.9)	79 (77.1)	1.41
*Xba*I	*Bam*HI	5 (2.1)	10 (12.9)	10 (12.9)	87 (79.1)	5.40
*Xba*I	*Dra*I	3 (1.8)	13 (14.2)	9 (10.2)	84 (82.8)	1.15
*Hin*dIII	*Bam*HI	6 (3.5)	18 (20.5)	9 (11.5)	71 (68.5)	2.82
*Hin*dIII	*Dra*I	2 (2.4)	22 (21.6)	9 (8.6)	78 (78.4)	0.09
*Bam*HI	*Dra*I	4 (1.7)	11 (13.3)	8 (10.3)	80 (77.7)	3.84

[a] Calculated chi-square values

* $P < 1\%$ with P = probability of independence

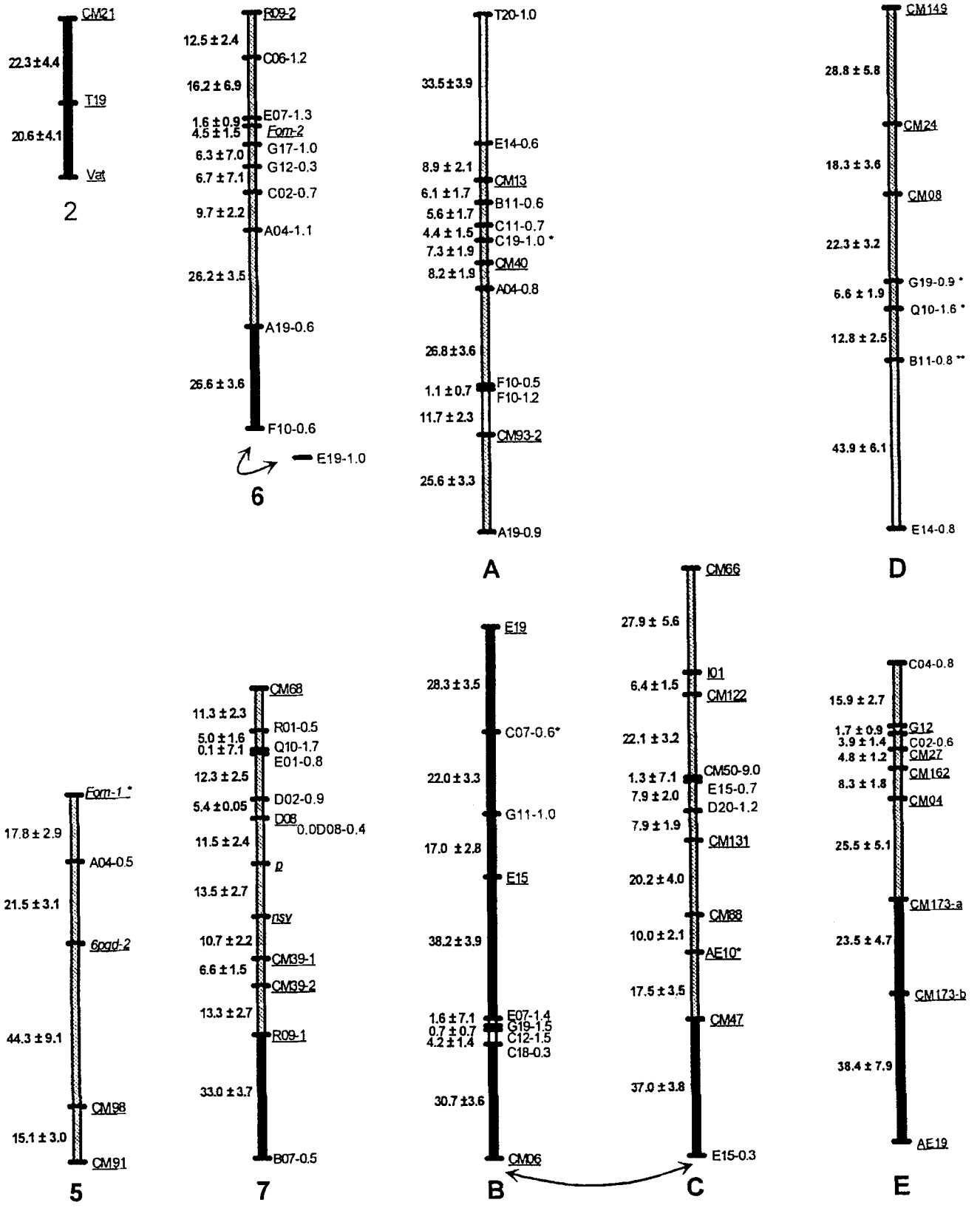

Fig. 2 Partial linkage of melon. The 14 linkage groups are schematically represented by *vertical double lines*. The linkage group letters were arbitrarily assigned. Symbols for loci are shown on the right of the lines. *Numbers* on the left correspond to the map distance ± standard error between markers in cM (Kosambi function). RFLP markers are prefixed with 'CM' for random melon cDNA clones (the restriction enzymes revealing polymorphism for each probe are available from the authors). *6Pgd-2* is an isozyme locus corresponding to second region of the 6-phoshogluconate dehydrogenase. Nomenclature for RAPD loci indicates the Operon primer kit designation (a capital letter and a number). The relative molecular weights in kb are indicated on the dominant markers (RAPD bands or RFLP bands). The *underlined markers* are co-dominants markers. Loci with a distorted segregation ratio are denoted by *, $P < 0.05$; **, $P < 0.01$. The unlinked markers are C11-0.6**, E07-0.7, F13-0.4**, G12-0.4, *pMEL1* (ACC oxidase), and *CM142*

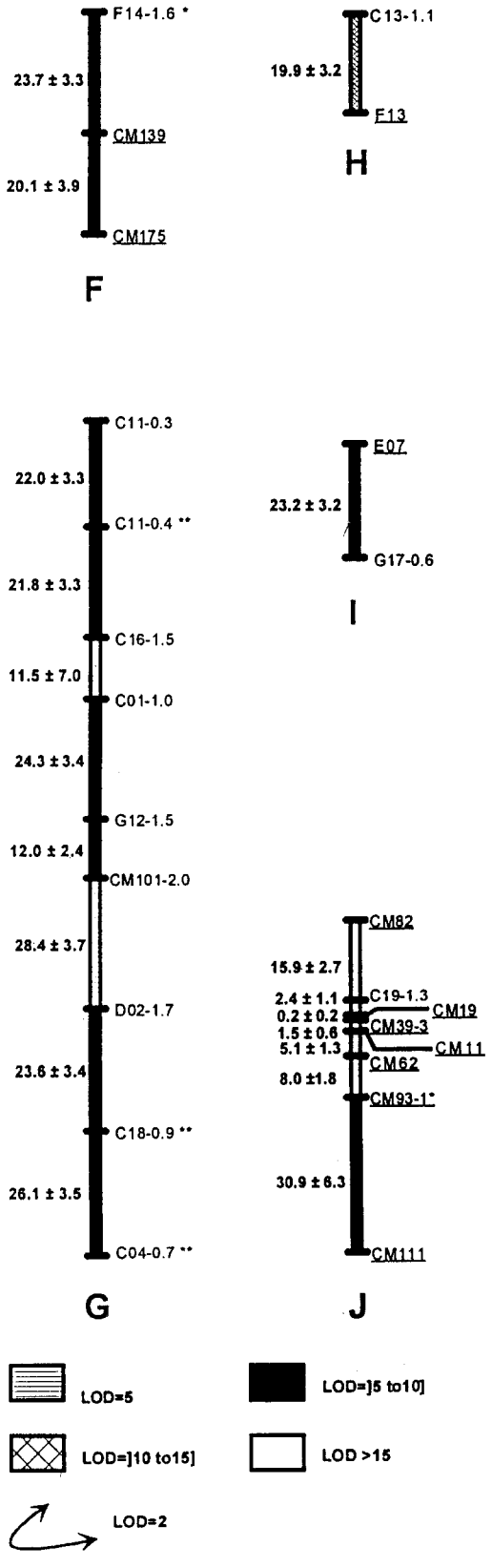

Fig. 2. (Continued)

Linkage analysis

Segregation data were recorded for a total of 110 markers: 36 RFLPs, 68 RAPDs, one isoenzyme, four disease resistance genes and one morphological character. Fifty eight out of the one hundred and ten markers were dominant markers. Two-point linkage analysis of the segregating 110 loci using Mapmaker resulted in 14 linkage groups containing 102 loci while seven markers remained unassigned to any linkage group (Fig. 2) with LOD ≥ 3 to LOD ≥ 5 and $r \leq 0.3$. The number of linkage groups (14) is higher than the chromosome number (12) indicating an unsaturated map. These groups covered 1390 cM (sum of the individual distances in cM at LOD ≥ 5). The number of mapped loci for each linkage group varies between two and twelve, with genetic lengths ranging from 19.9 to 169.7 cM. The average interval between two loci was 17.7 cM with a maximum of 44.3 cM between two loci on linkage group 5. Only 39% of the intervals between markers were smaller than 10 cM. When using a LOD score of 2.0, two of the 14 linkage groups (B and C) formed a longer group and one RAPD marker (E19-1.0) is linked to group 6. The number of linkage groups is the same with a LOD of 3.0, 4.0 or 5.0. Some probes detected two linked loci (*CM39-1* and *CM93-2* in group 7, *CM173-a* and *CM173-b* in group E), suggesting duplication. For the two probes *CM39* and *CM93*, loci were found respectively on linkage groups J (*CM39-3*) and 7 (*CM39-1*, *CM39-2*), and J (*CM93-1*) and A(*CM93-2*), suggesting translocations. Only one gene of known function coding for ACC oxidase (*pMEL1*) showed polymorphism between VD and SC with *Eco*RV. Unfortunately, this gene remained unlinked.

Discussion

Polymorphism analysis

'Vedrantais' and 'Songwhan Charmi' were chosen primarily because they are very different for disease resistances. They also differ for horticultural traits such as fruit flesh color, fruit shape, seed size and soluble solids content.

RAPD and RFLP markers revealed a similar polymorphism between the two parental genotypes. With these genotypes use of the RAPD method does not seem to be the solution for an increased number of markers.

When six restriction enzymes were used on melon, only 5 enzyme pair out of 15 showed dependence, suggesting that the polymorphism resulted from individual nucleotide substitution rather than insertion/deletion/duplication. Neuhausen (1992) and Shattuck-Eidens et al. (1990) also reported that point mutations are the basis of RFLPs in melon.

Even though many RAPD bands were polymorphic, it is worth noting that for each RAPD profile only a few bands were polymorphic. RAPDs with skewed segregation showed no systematic bias toward one parent. Heun and Helentjaris (1993) observed the same result and ascribed skewed segregation ratios to the low reproducibility of certain RAPDs, primarily those with weakly amplifying fragments. RAPDs were evenly distributed

throughout the melon genome. Several authors showed that RAPDs were predominantly of a repetitive nature (Paran and Michelmore 1993; Giese et al. 1994), and therefore may detect polymorphism in regions not accessible to RFLP analysis. However, RAPDs were generally no more clustered than RFLPs indicating that both types of markers are equally applicable for melon genome analysis.

Progeny analysis

Of 110 markers analysed in the F_2 progeny, 88% fit the expected 1:2:1 or 3:1 Mendelian ratios. A small proportion of markers (12%) deviated significantly from the expected ratio. The majority of biased markers are RAPDs (12 of 14 markers). Three biased markers were on linkage group D and three others on linkage group G. These biased markers led to some difficulty in the ordering of these two groups. Nine out of the eleven biased dominant RAPDs showed an over-abundance of the absent class, as in cucumber (Kennard et al. 1994).

Three of the thirty one probes (*CM39*, *CM93*, and *CM173*) showed several segregating bands, suggesting the existence of duplicated sequences. These sequences were scattered throughout the genome (Fig. 2). Duplicated sequences have also been reported for other crops, such as lettuce (Landry et al. 1987), pea (Ellis et al. 1992) and bean (Adam-Blondon et al. 1994). Here they were correlated with chromosomal translocations and inversions (Ellis et al. 1992) or with sequence transpositions (Mc-Couch et al. 1988; Shattuck-Eidens et al. 1990; Lark et al. 1993).

Linkage map

A linkage map of melon, based on genes that control disease resistances and flower biology or vegetative characters, has already been published (Pitrat 1991). This linkage map contained 23 genes on eight linkage groups. The map of melon described in the present paper includes a morphological marker, disease resistance genes, and isozyme and DNA markers. At least one molecular marker was found near genes controlling disease resistances (*Fom-1*, *Fom-2*, *Vat* and *nsv*). Two of these genes are in terminal positions (*Vat* in group 2 and *Fom-1* in group 5). The four disease resistance genes allowed this map to be compared with the linkage groups already published. For each resistance gene, linked RAPDs or RFLPs were found; T19 is 20.6 ± 4.1 cM from *Vat* on group 2; A04-0.5 is 17.8 ± 2.9 cM from *Fom-1* on group 5; *Fom-2* is flanked by two RAPD markers, G17-1.0 (4.5 ± 1.5 cM) and E07-1.3 (1.6 ± 0.9 cM), on group 6; and *nsv* between one morphological gene *p* (13.5 ± 2.7 cM) and *CM39-1* (10.7 ± 2.2 cM) on group 7. *Fom-2* which was tightly linked with one RAPD, less than 2 cM, may be a good candidate for the detection of SCARs (Paran and Michelmore 1993).

In group G, dominant markers were difficult to order. The use of a large Lod score (10.0) permitted the construction of the group by three sets of three dominant markers, but the order between sets is not definite. For each pair of loci, the standard error of the recombination fraction was calculated (Allard 1956). In eight cases, two markers were in repulsion phase. In each case the standard error is larger than the distance, making the order of the markers tentative. For other locus pairs, the linkage of markers corresponded to the three other possibilities (two co-dominants, one co-dominant and one dominant, or two dominants in coupling phase), and the standard errors were lower than the distances (Fig. 2).

The population size seems relatively large for constructing a map (218 individuals). It was necessary to use such a population size to decrease the standard error existing between the dominant markers in repulsion (Allard 1956). For example, with a population size of 50 individuals and two dominant markers linked in repulsion phase ($r = 0.1$), the standard error was 0.14. This decreased to 0.07 with a population of 200 individuals. Moreover, for QTL analysis, a large population size is important for analysis precision.

Melon genome-length estimate

The map obtained from the analysis using a Lod score greater than 5.0, and a maximum recombination frequency of 0.3, was composed of 14 linkage groups and seven independent markers. Since the number of linkage groups is higher than the number of chromosomes in *C. melo* ($2n = 2x = 24$), and there are seven unassigned loci, it can be concluded that the genome is not fully covered. An estimate of the genetic length of the genome is useful for evaluating the overall relationship between physical and genetic distance, as measured by the number of megabases (Mb) of DNA per Morgan, and for evaluating the percentage of the genome covered by a linkage map. Assuming that linkage groups and unlinked markers are flanked by chromosome segments whose size is equal to the highest recombination value detected in the progeny (44.3 cM), the total genetic length was estimated as being 2276 cM (maximum overlap) and 3250 cM (if all chromosome segments did not overlap each other). Another method proposed by Kennard et al. (1994) used meiotic analysis. Meiotic analysis (Ramachandran and Seshadri 1986) revealed an average of 1.97 chiasmata per chromosome pair, corresponding to 2364 cM (1.97 chiasmata per chromosome, 12 chromosomes and 100 cM per chiasma) in melon. These different values indicate that the melon map covers 43–61% of the total genome. Since the haploid nuclear DNA content of melon was estimated to be 454–502 Mb (Arumuganatham and Earle 1991), one centimorgan equals approximately 480–680 kb.

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