Theor Appl Genet (2002) 104:1017–1034
DOI 10.1007/s00122-002-0864-x

C. Périn · L.S. Hagen · V. De Conto · N. Katzir
Y. Danin-Poleg · V. Portnoy · S. Baudracco-Arnas
J. Chadoeuf · C. Dogimont · M. Pitrat

A reference map of *Cucumis melo* based on two recombinant inbred line populations

Received: 2 February 2001 / Accepted: 25 October 2001 / Published online: 27 March 2002

Abstract A composite genetic melon map was generated based on two recombinant inbred line (RI) populations. By analyzing the segregation of 346 AFLPs, 113 IMAs and phenotypic characters on a RI population of 163 individuals derived from the cross Védrantais × PI 161375, a first map was constructed. About 20% of the molecular markers were skewed, and the residual heterozygosity was estimated at 4.43% which was not significantly different from the theoretical value of 4.2%. The genome distribution of molecular markers among the 12 linkage groups was not different from a random distribution with the exception of linkage group XII which was found significantly less populated. The genome distributions of IMAs and AFLPs were complementary. AFLPs were found mainly in the middle of each linkage group and sometimes clustered, whereas IMAs were found mainly at the end. A total of 318 molecular markers, mainly AFLP and IMA markers, were mapped on 63 RIs of the second population, Védrantais × PI 414723. Comparison of the maps enables one to conclude that AFLPs and IMAs of like molecular size, amplified with the same primer combination, correspond to the same genetic locus. Both maps were joined through 116 common markers comprising 106 comigrating AFLPs/IMAs, plus five SSRs and five phenotypic markers. The integrated melon map contained 668 loci issuing from the segregation of 1,093 molecular markers in the two RI populations. The composite map spanned 1,654 cM on 12 linkage groups which is the haploid number of chromosomes in melon. Thirty two known-function probes, i.e. known-function genes (9) and morphological traits (23), were included in this map. In addition, the composite map was anchored to previously published maps through SSRs, RFLPs and phenotypic characters.

Keywords Melon · *Cucumis melo* · AFLP · IMA · Genetic mapping · RIL

Communicated by C. Möllers

C. Périn · L.S. Hagen · C. Dogimont · M. Pitrat (✉)
INRA, Station de Génétique et d'Amélioration des fruits et légumes, BP 94, 84143 Montfavet Cedex, France
e-mail: Michel.Pitrat@avignon.inra.fr
Tel.: +33 (0)4-32-72-27-17, Fax: +33 (0)4-32-72-27-02

V. De Conto
SEMINIS Research, Mas de Rouzel, chemin des Canaux, 30900 Nîmes, France

N. Katzir · Y. Danin-Poleg · V. Portnoy
ARO, Newe Ya'ar Research Center, Ramat Yishay 30095, Israël

S. Baudracco-Arnas
ASL, Site Agroparc, B.P. 1202, 84911 Avignon Cedex 9, France

J. Chadoeuf
INRA, Unité de Biométrie, Domaine St Paul, Site Agroparc, 84914 Avignon Cedex 9, France

Introduction

Cucumis melo L. is a diploid species (2n = 2*x* = 24) of African origin with a high phenotypic variation probably due to a large diversification center from the Mediterranean sea to Eastern Asia and several independent domestication events. Selection for different types of fruit, sweet or not sweet for instance (Stepansky et al. 1999), could also explain the high range of variation observed earlier by Naudin (1859). Melon has a small-sized genome (0.45 pg) (Arumanagathan and Earle 1991), and can be used for genetic and molecular studies due to its short generation time, high polymorphism, and possible genetic transformation through *Agrobacterium tumefaciens* (Guis et al. 1998). More than 109 genes have been described in the current gene list for *C. melo* (Pitrat 1998), and classical genetic linkage maps with a few morphological and disease-resistance genes (Pitrat 1991), or isozyme markers (Staub et al. 1998) exist. In addition, several partial maps with molecular markers have been published: on 244 F_2 individuals with 110 RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) markers (Baudracco-Arnas and Pitrat 1996), on 66 BC_1 individuals with 196 AFLP (amplified fragment length polymor-

phism) markers (Wang et al. 1997), on 77 F_2 plants with 125 RAPD markers (Liou et al. 1998), on 64 F_2 individuals with 107 markers (RAPDs, RFLPs, Inter Microsatellite Amplification = IMA and Simple Sequence Repeats = SSRs) (Brotman et al. 2000), on 93 F_2 individuals with 411 markers (RFLPs, AFLPs, RAPDs, SSRs and isozymes) (Oliver et al. 2001).

Immortalized populations like RI provide an easy way to exchange genetic material (i.e. seeds or DNA) between several laboratories and to add an infinite number of molecular markers. An immortalized population can be scored for many characters such as resistance to several diseases. A replicated progeny is also a powerful tool to study complex physiological processes or to map quantitative trait loci (QTLs) (Causse et al. 1995; Goldman et al. 1995; Nandi et al. 1997). Furthermore, in a RI population, both dominant and codominant markers can be mapped with the same precision (for a review see Burr and Burr 1991). The probability to separate tightly linked genes is higher in an RI population than in doubled-haploid lines (Burr and Burr 1991).

Combining the use of several RI populations through the development of a composite map presents some advantages in comparison with a one-population mapping strategy (Burr and Burr 1991; Causse et al. 1996; Sewell et al. 1999). Monomorphic molecular markers in one cross could be polymorphic in a second one and allow a better genome coverage (Causse et al. 1996). Hence, robustness and genome coverage would be better in a multi-population mapping strategy and give a more representative map of the species. According to Beavis and Grant (1991), Burr and Burr (1991) or Helentjaris et al. (1986), such a map should be derived from several crosses, especially to compare QTL and major genes identified in several genetic backgrounds.

Species of secondary economic importance like *C. melo* require alternative molecular marker technologies than RFLPs, mostly used in high-density map construction of economically important species like rice, maize, tomato, or wheat. PCR-based markers, such as RAPD (Williams et al. 1990), AFLP (Vos et al. 1995) and IMA (Zietkiewicz et al. 1994), are easy to handle and can generate more than a thousand markers in a few months. RAPDs and AFLPs were successfully used for the quick development of genetic maps in several species like *Arabidopsis* with RAPD markers (Reiter et al. 1992), barley (Qi et al. 1998), and rice (Maheswaran et al. 1997; Nandi et al. 1997), loblolly pine (Remington et al. 1999), potato (Rouppe van der Voort et al. 1998b), eucalyptus (Marques et al. 1998) and melon (Wang et al. 1997) with AFLP markers. IMA enables amplification of genomic DNA between adjacent inversely oriented simple-sequence repeats as revealed on an agarose or acrylamide gel (Zietkiewicz et al. 1994). The use of this technique on plants has been only scarcely reported. However, it was used for studying diversity in rice (Blair et al. 1999), maize (Kantety et al. 1995) and melon (Stepansky et al. 1999), mapping disease resistance genes in chickpea (Ratnaparkhe et al. 1998a, b) and mapping a fertility restorer gene in rice (Akagi et al. 1996). The usefulness of IMA in the construction of dense maps was never really demonstrated, even if some of them were successfully mapped in Douglas-fir (Tsumura et al. 1996). IMA allows one to test the polymorphism and the distribution of microsatellite sequences and does not require cloning, sequencing and primer designing. Hence, the number of bands produced by a primer combination is a function of microsatellite abundance in the genome. Moreover, IMA could help to select motifs for the future development of microsatellite markers.

Whether a map constructed with PCR high-volume marker technologies can be used to relate several independent maps remains unclear, even if recent results tend to be optimistic for AFLP markers, for instance in rice (Nandi et al. 1997), potato (Rouppe van der Voort et al. 1998b), barley (Qi et al. 1998) and tomato (Haanstra et al. 1999). In this paper, we present the development, based on two RI populations, of a dense genetic map which could be used as a reference map for *C. melo*.

Materials and methods

Plant material

Two RI populations were developed between three melon lines: Védrantais, a French line with the Charentais type (Vilmorin release) as a common parent, PI 161375, a Korean line, and PI 414723, an Indian line. The population (Védrantais × PI 161375) (named Ved161 in this paper) with 163 $F_6/F_7/F_8$ RI lines and the population (Védrantais × PI 414723) (named Ved414 in this paper) with 63 $F_6/F_7/F_8$ were derived by single-seed-descent from individual plants of the F_2 progeny after five to seven selfings with no conscious selection in any generation. In the Ved161 population, the following number of RI lines were used: 163 for AFLPs, 120 for SSRs and RFLPs, 93 for IMAs and RAPDs, between 120 and 163 for phenotypic traits. In the Ved414 population, all 63 RI lines were tested for AFLPs, SSRs, RFLPs, IMAs, and phenotypic traits.

DNA extraction

DNA was extracted from fresh young leaf tissue following the CTAB procedure described in Lefebvre et al. (1993).

AFLP analysis

The AFLP analysis was performed as described by Vos et al. (1995) with minor modifications. All chemical reagents were purchased from Life Technology. Adapters and primers were obtained from Keygene (Netherlands). The adapter and primer sequences are summarized in Table 1. A pre-amplification was performed with one selective nucleotide for each primer; this nucleotide was a A for *Eco*RI and *Hin*dIII, and could be a A (abbr. *Mse*I-A) or a C (abbr. *Mse*I-C) for *Mse*I. The second PCR amplification was performed with primers having three selective nucleotides (Table 1). The *Eco*RI or *Hin*dIII specific amplification primer was end-labeled with ^{33}P-γATP using T4 polynucleotide kinase (Pharmacia). AFLP products were denatured for 3 min at 93 °C and cooled on ice. For each sample between 1.5 to 3 µl were loaded on a 4.5% denaturing polyacrylamide gel and electrophoreses were carried out in 1 × TBE at 130 W for 2 h 15 min. The lower buffer was 1 × TBE supplemented with 0.5 M Na Acetate. The gel was removed using a Whatman paper, dried for 1 h 30 min at 75 °C and

Table 1 Sequences of adapters and primers combinations for AFLPs

Adapters	
*Eco*RI adapters: 5′-CTCGTAGACTGCGTACC-3′	3′-CTGACGCATGGTTAA-5′
*Hin*dIII adapters: 5′-CTCGTAGACTGCGTACC-3′	3′-CATCTGACGCATGGTCGA-5′
*Mse*I adapters: 5′-GACGATGAGTCCTGAG-3′	3′-TACTCAGGACTCAT-5′
Primers	
*Eco*RI + 1 primer = E01 = 5′-GACTGCGTACCAATTCA	
*Eco*RI + 3 primers	
E32 = 5′-GACTGCGTACCAATTCAAC	E33 = 5′-GACTGCGTACCAATTCAAG
E35 = 5′-GACTGCGTACCAATTCACA	E38 = 5′-GACTGCGTACCAATTCACT
E39 = 5′-GACTGCGTACCAATTCAGA	E40 = 5′-GACTGCGTACCAATTCAGC
E42 = 5′-GACTGCGTACCAATTCAGT	E43 = 5′-GACTGCGTACCAATTCATA
E46 = 5′-GACTGCGTACCAATTCATT	
*Hin*dIII + 1 primer = H01 = 5′-GACTGCGTACCAGCTTA	
*Hin*dIII + 3 primers	
H33 = 5′-GACTGCGTACCAGCTTAAG	H36 = 5′-GACTGCGTACCAGCTTACC
H40 = 5′-GACTGCGTACCAGCTTAGC	
*Mse*I + 1 A as selective nucleotide primer = M01 = 5′-GATGAGTCCTGAGTAAA	
*Mse*I + 3 primers (A)	
M31 = 5′-GATGAGTCCTGAGTAAAAA	M34 = 5′-GATGAGTCCTGAGTAAAAT
M35 = 5′-GATGAGTCCTGAGTAAACA	M37 = 5′-GATGAGTCCTGAGTAAACG
M40 = 5′-GATGAGTCCTGAGTAAAGC	M41 = 5′-GATGAGTCCTGAGTAAAGG
M42 = 5′-GATGAGTCCTGAGTAAAGT	M43 = 5′-GATGAGTCCTGAGTAAATA
M44 = 5′-GATGAGTCCTGAGTAAATC	M45 = 5′-GATGAGTCCTGAGTAAATG
*Mse*I + 1 C as selective nucleotide primer = M02 = 5′-GATGAGTCCTGAGTAAC	
*Mse*I + 3 primers (C)	
M48 = 5′-GATGAGTCCTGAGTAACAC	M51 = 5′-GATGAGTCCTGAGTAACCA
M56 = 5′-GATGAGTCCTGAGTAACGC	

Table 2 IMA codes and primers sequences used for mapping

Name	code[a]	Tm[b]	Primer sequence (5′–3′)[c]	Name code[a]	Tm[b]	Primer sequence (5′–3′)[c]
A	–	55	$HVH(TGT)_5$	X –	47	$(AG)_8R$
B	UBC 807	55	$(AG)_8T$	Y –	50	$CCG(CA)_6$
C	UBC 845	55	$(CT)_8RG$	Z –	50	$(CA)_6R$
D	UBC 823	55	$(TC)_8C$	AA –	50	$VHV(GT)_6$
E	UBC 891	55	$HVH(TG)_7$	AB –	50	$HVH(CA)_6$
F	UBC 880	55	$GGA(GAG)_2AG(GA)_2$	AC –	50	$(AG)_8CA$
G	–	55	$(AG)_8VC$	AD –	50	$(AGC)_4AY$
H	UBC 842	55	$(GA)_8YG$	AE –	50	$(AG)_8CT$
I	UBC 887	55	$DVD(TC)_7$	AF –	50	$VHV(CT)_6$
J	UBC 840	55	$(GA)_8YT$	AG –	50	$CCG(CT)_6$
K	UBC 836	55	$(AG)_8YA$	AH –	44	$(GATA)_4N$
L	UBC 844	55	$(CT)_8RC$	AI –	50	$CCT(GA)_6$
M	UBC 835	55	$(AG)_8YC$	AJ –	50	$(AG)_8Y$
N	UBC 888	55	$BDB(CA)_7$	AK –	50	$(GGGT)_4$
O	UBC 841	55	$(GA)_8YC$	AL –	50	$(GGAA)_4$
P	UBC 884	55	$HBH(AG)_7$	AM –	50	$RY(CT)_6$
Q	UBC 895	55	AGAGTTGGTAGCTCTTGATC	AN –	50	$CCC(AG)_6$
R	–	55	$BDT(CA)_7$	AO –	44	$(AGC)_4GR$
S	–	55	$BDB(CAC)_5$	AP –	44	$(GCT)_4Y$
T	UBC 868	55	$(GAA)_6$	AQ –	44	$(GGGC)_4$
U	UBC 824	55	$(TC)_8G$	AR –	44	$(ATC)_6$
V	UBC 809	55	$(AG)_8G$	AS –	44	$(CCT)_6$
W	UBC 810	55	$(GA)_8T$	AT –	44	$(AG)_8CC$

[a] Codes are according to the University of British Columbia (UBC) or were synthesized (–)
[b] Tm = annealing temperature (°C)
[c] N = (A, G, C, T); R = (A, G); Y = (C, T); B = (C, G, T); D = (A, G, T); H = (A, C, T); V = (A, C, G)
[c] Tm = annealing temperature (°C)

exposed to X-Omat X-ray film for 4 to 6 days. Each polymorphic AFLP fragment was identified by the primer combination (for instance E46/M35, as indicated in Table 1) followed by a number indicating the relative molecular weight of the band. Bands were coded by decreasing molecular weight, i.e. that number 1 is the less migrating.

IMA analysis

Primers for IMA were purchased from British Columbia University, Life Technology or were newly synthesized (Table 2). All products were purchased from Life Technology. Amplifications were performed in 2 mM of $MgCl_2$, 0.4 mM of each dNTP,

Table 3 Known function genes mapped on the composite melon map

Gene symbol	Type[a]	Product/phenotype[b]	Linkage group	Population[c]	Reference
a	Pheno	Andromonoecious	II	2	Rosa 1928
Al-3	Pheno	Abscission layer-3	VIII	1	Périn et al. 1999
Al-4	Pheno	Abscission layer-4	IX	1	Périn et al. 1999
Bi	Pheno	Bitter seedling	Unlinked	2	Lee and Janick 1978
CMAT35	SSR	ACC oxidase 1	V	1	Lasserre et al. 1996
CmACO2	RFLP	ACC oxidase 2	VIII	1	Lasserre et al. 1996
CmACS1	SSR	ACC synthase 1	XI	1	Yamamoto et al. 1995
CmACS5	RFLP	ACC synthase 5	III	1	Périn et al. 2002
CmGT2	RFLP	Transcription factor	II	1	J.C. Pech, pers. comm
CmERS1	RFLP	Ethylene receptor	I	2	Périn et al. 2002
CmXET6	RFLP	Xylotransferase	V	1	Rose et al. 1998
Ec	Pheno	Empty cavity	III	2	Périn et al. 1999
ech	Pheno	Exaggerated curvature of the hook	I	1	Périn et al. 2002
Fom-1	Pheno	Fusarium races 0 and 2 resistance	IX	1	Risser 1973
Fom-2	Pheno	Fusarium races 0 and 1 resistance	XI	1/2	Risser 1973
gf	Pheno	Green flesh color	IX	1	Hughes 1948
h	Pheno	Halo cotyledon	II	3	Nugent and Hoffman 1974
me-2	Pheno	Mealy flesh	Unlinked	2	Ganesan 1988
CSAT425a	SSR	Malate dehydrogenase	XII	2	Katzir et al. 1996
mt-2	Pheno	Spots on the rind	II	1/2	Périn et al. 1999
nsv	Pheno	Melon necrotic spot virus resistance	XII	1	Coudriet et al. 1981
p	Pheno	Pentamerous (five carpels)	XII	2	Rosa 1928
6-Pgd-2	Iso	6-phosphogluconate dehydrogenase-2	IX	3	Baudracco-Arnas and Pitrat 1996
pin	Pheno	Pine seed shape	III	1	This work
Pm-x	Pheno	Powdery mildew x resistance	II	3	Pitrat 1991
Pm-w	Morph	Powdery mildew w resistance	V	3	Pitrat 1991
Prv	Pheno	Papaya ringspot virus resistance	IX	2	Webb 1979
s-2	Pheno	Sutures on the rind	XI	1/2	Bains and Kang 1963
spk	Pheno	Speckled fruit epidermis	VII	1	This work
Vat	Pheno	Virus aphid transmission resistance	V	1/2	Pitrat and Lecoq 1980
Wt-2	Pheno	White testa (seed)	IV	2	Hagiwara and Kamimura 1936
Zym	Pheno	Zucchini yellow mosaic virus resistance	II	2	Pitrat and Lecoq 1984

[a] Pheno = phenotypic characters, RFLP = restriction fragment length polymorphism, SSR = simple sequence repeat, Iso = Isozyme
[b] Phenotype of the character for the morphological characters, function based on sequence homology for the probes
[c] Gene segregating in the RI populations: 1 = Védrantais × PI 161375 or 2 = Védrantais × PI 414723, 3 = based on linkages with two common markers on this map

0.5 μM of primer, 1 U of Platinum Taq and 20 ng of melon total DNA in a 20-μl final volume. The PCR cycles were as follows: initial denaturation at 94 °C for 3 min, followed by 45 cycles of 30 s at 94 °C, 45 s at 55 °C (or 50 °C, 44 °C, 47 °C according to primers, see Table 2), 2 min at 72 °C, and a final extension at 72 °C for 5 min. PCR products were electrophoresed on 2% agarose gels and detected by staining with ethidium bromide. IMA markers were named with a one or two letter(s) code followed by the approximate molecular weight of the band in bp.

RAPD analysis

PCR reactions and revelation of RAPD markers were previously described (Baudracco-Arnas and Pitrat 1996). Thirteen RAPD primers (16 markers) previously mapped in the F_2 population were used in this study. RAPD markers purchased from Operon technologies were named OP followed by the code name and the approximate molecular weight of the band in bp.

SSR analysis

SSR primers, sequences and PCR conditions were previously described (Katzir et al. 1996; Danin-Poleg et al. 2000).

RFLP analysis

DNA (5–8 μg) was digested with three restriction enzymes (*Eco*RI, *Eco*RV and *Hin*dIII) and used to make blots. Southern-blotting and hybridization procedures were performed as described by Baudracco-Arnas and Pitrat (1996). Some of the RFLP are known-function genes and are described in Table 3. RFLP probes from a ripe fruit cDNA library (CM13, CM24, CM27, CM39a, CM39b, CM98, CM101, CM122, CM173) were previously described (Balagué et al. 1993; Baudracco-Arnas and Pitrat 1996).

Disease resistance tests

At least 20 plants of each RI line were inoculated and two independent tests were performed for each pathogen. One recessive gene controls the resistance to melon necrotic spot carmovirus (MNSV) in PI 161375. MNSV induces necrotic lesions on susceptible plants, whereas resistant plants (allele *nsv*) exhibit no symptoms (Coudriet et al. 1981). Resistance to zucchini yellow mosaic potyvirus (ZYMV) and to papaya ringspot potyvirus (PRSV) are conferred by two dominant independent genes in PI 414723 (alleles *Zym* and Prv^2) (Webb 1979; Pitrat and Lecoq 1983, 1984). Both viruses induce yellow mosaic on susceptible plants. On the resistant plants, sporadic necrotic lesions appear on the inoculated cotyledons without systemic symptoms upon inoculation with ZYMV and with a systemic necrotic reaction with PRSV. Strains used were the poorly aphid-transmissible strains E15-PAT and

R1A, both of pathotype 0 of ZYMV (Lecoq and Pitrat 1984) and the strain E2 of PRSV. These viruses were mechanically inoculated (Marrou 1967). Resistance to *Fusarium oxysporum* f. sp. *melonis* races 0 and 1, and races 0 and 2, is controlled by two dominant independent genes, *Fom-2* and *Fom-1*, respectively (*Fom-1* carried by Védrantais and *Fom-2* carried by PI 161375 and PI 414723) (Risser 1973; Risser et al. 1976). Fusarium resistance was tested as described by Risser and Mas (1965) by dipping the roots of plantlets in a suspension of 10^6 conidia per ml, before transplanting them in soil. Two weeks after inoculation, susceptible plants died whereas resistant ones remained green. Isolates 'Fom26' of race 1 and 'Spain' of race 2 were used. One dominant gene confers resistance to *Aphis gossypii* and to virus transmission by *A. gossypii* in PI 161375 and in PI 414723 (allele *Vat*) (Pitrat and Lecoq 1980). We used the test of non-acceptance described by Pitrat and Lecoq (1982) to characterized the behavior of each line in both RI populations. On susceptible plants aphids remain under the leaves and produce larvae, whereas on resistant plants they leave the plants and there are very few larvae.

Morphological characters

White seed testa color (vs yellow) is controlled by one single dominant gene (symbol *Wt-2*) (Hagiwara and Kamimura 1936) which is present in PI 414723. PI 161375 has seeds with a pine-seed shape (known as piñonet in spanish) whereas Védrantais has flat seeds. This character segregates as a single recessive gene. We propose the name *pine-seed shape* and the symbol *pin*. Seedling bitterness (due to the presence of cucurbitacins) is controlled by one dominant gene (symbol *Bi*) (Lee and Janick 1978). Védrantais and PI 161375 seedlings are bitter, PI 414723 ones are non-bitter. Bitterness has been evaluated by tasting at least ten seedlings per RI line. The triple response of dark-grown seedlings in the presence of ethylene gave a very strong hook curvature (360°) of hypocotyls in PI 161375. Védrantais and PI 414723 present a moderate (180°) curvature. This trait is controlled by one recessive gene named *exaggerated curvature of the hook* (symbol *ech*) (Périn et al. 2002). The *andromonoecious* gene (symbol *a*) controls the presence (vs absence) of stamens in female flowers that are hermaphroditic (vs female) (Rosa 1928; Poole and Grimball 1939). Védrantais and PI 161375 are both andromonoecious, PI 414723 is monoecious. The five-placentas character, controlled by the *pentamerous* gene (symbol *p*), is present in PI 161375, whereas Védrantais and PI 414723 have three placentas (Rosa 1928; Baudracco-Arnas and Pitrat 1996). The presence of *sutures* (symbol *s-2*) on the fruit skin is under the control of one recessive gene, present in Védrantais, but neither in PI 161375 nor PI 414723 (Bains and Kang 1963; Périn et al. 1999). The presence of dark spots (about 1 cm in diameter) on the fruit epidermis (vs no spots) is controlled by one recessive gene (*mottled rind* pattern, symbol *mt-2*) (Périn et al. 1999). The *mt-2* allele is present in PI 161375 and PI 414723, but not in Védrantais. The speckled epidermis of the fruit is controlled by one recessive gene. We propose the name *speckled fruit epidermis* (symbol *spk*), present in PI 161375 and PI 414723 but not in Védrantais. This character has been analyzed only in the Ved414 population. Abscission layer formation on the peduncle of the fruit at maturity is controlled by two independent dominant genes (*Abscission layer-3* and *Abscission layer-4*, symbols *Al-3* and *Al-4*) (Takada et al. 1975; Périn et al. 1999; Périn et al. 2002). *Al-3* and *Al-4* are present in Védrantais and PI 414723, but not in PI 161375. The green flesh color of mature fruit is controlled by one recessive gene (symbol *gf*) present in PI 161375 (Hughes 1948; Périn et al. 1999); Védrantais has an orange flesh color. An empty cavity at maturity with placental separation is controlled by one dominant gene (symbol *Ec*) present in PI 414723 but not in Védrantais (Périn et al. 1999). Mealy flesh texture is controlled by one recessive gene (symbol *me-2*) (Ganesan 1988; Périn et al. 1999); the *me-2* is present in PI 414723 but not in Védrantais.

Linkage map construction

After visual scoring by two independent persons, a χ^2_{1ddl} goodness of fit value for 1:1 segregation was calculated for each maker. All allelic or highly skewed ($p < 0.001$) markers were discarded for map elaboration to eliminate spurious linkages. The maps were constructed using MAPMAKER version 3.0 (Lander et al. 1987). All pairs of linked markers were first identified using the "group" command with a LOD score of 3.0 and a recombination fraction of 0.3. Ordering of markers was done by alternatively using the commands "group" and "order". Candidate orders were confirmed with the "ripple" and "compare" commands. Markers that could not be confidently located were placed via the "try" command. Map units (cM) were calculated using the Kosambi function (Kosambi 1944).

Map merging

Map merging was made with a common file of both populations using MAPMAKER v3.0. Markers segregating in only one population were coded as missing data in the other population. All markers belonging to the same linkage group, and which could be ordered with the command "order" at a LOD score better than 2.0, define a framework map. The order of this framework map was checked using the "ripple" command. Other markers were placed with the "try" command at their most probable position on this framework. In order to avoid map length inflation, only the framework map was used to compute genetic distances.

Marker distribution

The repartition of the AFLP and IMA markers on the melon genome was studied on the Ved161 population, which has a higher number of RI lines and of markers than the Ved414 population.

Across linkage groups

The ability of these markers to uniformly cover the melon genome was investigated by confronting the expected and observed marker distribution across linkage groups using χ^2 (0.05). The genome distribution of molecular markers among linkage groups was analyzed using a Poisson's law distribution (with μ = average density of the molecular marker types analyzed). We calculated the theoretical number of molecular markers for a 100-cM equivalent length for each linkage group and each marker type, and we tested it against Poisson's law for a significant threshold of 0.05.

Within linkage groups

Poisson's law was also used to test the distribution of molecular markers within the linkage groups. Each linkage group was divided in 10-cM intervals and the number of molecular markers of each type was evaluated. The minimum and the maximum number of markers in a single interval according to a Poisson distribution (μ = average density of each molecular marker type) were determined for a threshold of 0.05.

Results

Map of the Ved161 population

AFLP and IMA polymorphism

The polymorphism between the two parental lines, Védrantais and PI 161375, was assayed for 23 AFLP

Table 4 Polymorphism, codominant and skewed markers in the RI population 'Védrantais' × 'PI 161375'

Molecular marker		No. of primer combinations	Total (and per primer) number of visible bands	Number (and percentage) of polymorphic bands		Number of codominant bands	Number (and percentage) of skewed markers
Type	Primer			Total	Per primer		
AFLP	*Hin*dIII/*Mse*I-A	6	345 (57.5)	124 (36%)	20.7	20	21 (17%)
AFLP	*Eco*RI/*Mse*I-A	10	814 (81.4)	262 (32%)	26.2	36	55 (21%)
AFLP	*Eco*RI/*Mse*I-C	7	446 (63.7)	78 (18%)	11.1	9	15 (19%)
IMA	Di-nucleotide	31	381 (12.3)	89 (23%)	2.6	–	
IMA	Tri- or tetra-nucleotide	14	144 (10.3)	33 (23%)	2.4	–	

primer combinations (Table 1) and 46 IMA primers (Table 2). Each AFLP primer combination produced an average of about 70 bands, while the IMA primers produced an average of 11.3 bands; 28.9% of the AFLP and 23.2% of the IMA bands were polymorphic between parental lines. Significant differences were found between AFLP primer-enzyme combinations (Table 4). *Hin*dIII/*Mse*I-A were the most polymorphic (35.9%) followed by *Eco*RI/*Mse*I-A (32.2%). *Eco*RI/*Mse*I-C showed a significantly lower polymorphism rate (17.5% and $p < 0.01$ *t*-test) than the *Hin*dIII/*Mse*I-A combinations. In terms of the number of markers per combination (Table 4), the *Eco*RI/*Mse*I-A combinations generated the highest number of polymorphic bands with an average of 26.2 polymorphic bands per primer combination. No difference was observed between di- and tri/tetra-nucleotide repeat primers for IMA.

Marker segregation

The frequency of the Védrantais and PI 161375 alleles at all the loci studied was 51.8% and 48.2% respectively, fitting a 1:1 ratio ($p = 0.37$); 19.8% of the molecular markers were found distorted at $p < 0.05$, which is higher than previously found in the F_2 from which the RI population was directly derived (Baudracco-Arnas and Pitrat 1996). Skewed ratios of 14.7%, 22.4%, 22.4% and 23.25% were found respectively for *Eco*RI-*Mse*I with a C, *Eco*RI-*Mse*I with an A, *Hin*dIII-*Mse*I (A) and IMA. The most-skewed markers (5.1% of the total) were discarded for the map construction.

Codominant AFLP markers

AFLP markers were considered as allelic markers when:

(1) They were amplified with the same primer combination in both parental lines.
(2) A small electrophoretic mobility difference, 1 to 10 bp, was found between both bands.
(3) The two AFLP bands segregated complementarily.
(4) No double absence was found.

According to these criteria, a RI line which had the two AFLP bands of a codominant marker was declared heterozygous at that locus. For instance, E46/M40_10 and E46/M40_11 were two alleles of a codominant marker with differences in electrophoretic mobility of 3 to 5 bp (Fig. 1).

A total of 16.2% (Table 4) of the AFLP markers were codominant. For most of them, parental bands showed only a one to five base-pair difference on the acrylamide gel, suggesting that most polymorphisms between the two parents were due to small insertions/deletions. Codominant AFLP markers revealed residual heterozygosity of the RI lines. Among the entire population, 4.43% RI lines were heterozygous at a codominant marker locus on average, which is not significantly different ($P = 0.305$) from the theoretical value 4.20%.

Map construction

Among the 615 bands scored, 150 were discarded for map construction, mainly allelic markers, highly skewed markers or minor bands with instability between gels. In a first round, 465 markers were grouped at a LOD > 6.0 in 12 major and seven minor linkage groups. All the minor groups were sequentially added to the major groups' extremities at a minimum LOD score value of 3.3. Twelve linkage groups were finally recovered, corresponding to the haploid number of chromosomes in *C. melo*. Two markers, an AFLP and an IMA, were unlinked at a LOD score >3.0 despite a third scoring, which did not reveal specific errors. Altogether, 460 molecular markers were successfully mapped on 12 linkage groups. The map covered 1,411 cM with a mean distance between markers of 3.24 cM.

Distribution of molecular markers on the linkage groups

Thirteen molecular markers were mapped on the least-populated linkage group (LG XII) and 59 on the most-populated one (LG IV) (Table 5). The linkage group LG IV was the longest covering 170 cM, and LG X was the smallest spanning only 76 cM; this represents more than a two-fold variation of the linkage groups' genetic lengths. The genetic length and the number of molecular markers in each linkage group were significantly correlated ($\rho = 0.7$ $P < 0.01$). However, the group LG XII was

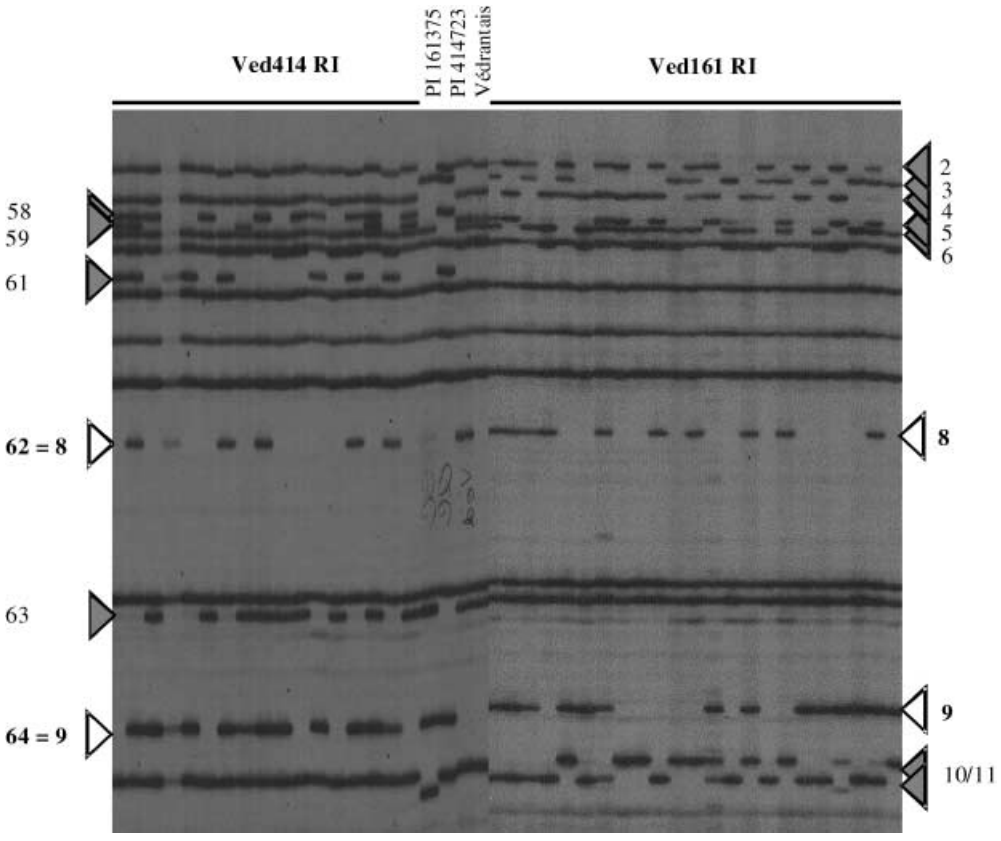

Fig. 1 Locus specificity of AFLP markers. Two partial views of AFLP gels from the RI populations Ved161 and Ved414 obtained with the same primer combination E46/M40 were aligned side by side. AFLP markers which segregated only in the Ved161 or Ved414 population are indicated by a *full arrow* at the right or the left of the figure respectively. Comigrating AFLP markers are indicated with an *empty arrow* on both sides of the figure. E46/M40_8 is a comigrating AFLP with a band amplified in the common parent Védrantais, whereas E46/M40_9 is a comigrating AFLP marker amplified in the two unrelated parents. E46/M40_10 and E46/M40_11 is a codominant marker with a electrophoretic mobility shift of 2 bp

Table 5 Distribution of molecular marker on linkage groups of the Ved161 population

Linkage group	Map length (cM)	Mean distance (cM)	Markers			AFLP				IMA
			Number	Framework[a]	LOD < 2.0[b]	Total	*Eco*RI/*Mse*I-C	*Hin*dIII/*Mse*I-A	*Eco*RI/*Mse*I-A	
I	103	2.51	41	30	11	39	6	7	26***	2
II	98	2.88	34	19	15	28	3	6	19	6
III	154	3.76	41	32	9	32	5	10	17	9
IV	170	2.88	59	43	16	43	10	6	27	16
V	97	2.55	38	22	16	31	10*	9	12	7
VI	100	2.56	39	26	13	28	5	5	18	11
VII	154	4.16	37	26	11	26	7	6	13	11
VIII	156	3.39	46	29	17	31	3	15	13	15
IX	111	3.47	32[c]	20	12	20	3	6	11	11
X	76	2.71	28	16	12	23	3	6	14	5
XI	104	2.21	47	27	20	33	6	12	15	14
XII	88	6.77	13***	10	3	9	2	1	6	4
Unlinked		–	5	–	5	3	–	–	–	2
Total	1,411	3.24	460	300	160	346	63	89	191	113

*, **, *** = significantly different from a Poisson law repartition at respectively $P < 0.05$, $P < 0.01$, $P < 0.001$

[a] Markers ordered with a LOD score higher than 2.0

[b] Markers placed with a LOD score lower than 2.0

[c] 31 molecular markers and one phenotypical gene (*Fom-1*)

found significantly less densely populated than expected with a random distribution ($P < 0.001$). Considering each type of molecular marker, an excess ($P < 0.05$) of *Eco*RI/*Mse*I-C on the linkage group LG V and a highly significant ($P < 0.001$) excess of *Eco*RI/*Mse*I-A markers on LG I were observed.

Distribution of map units within linkage groups

If the markers seemed to be uniformly distributed between linkage groups, except for LG XII, the situation was different within linkage groups. Regions with more frequent markers than expected were found on all link-

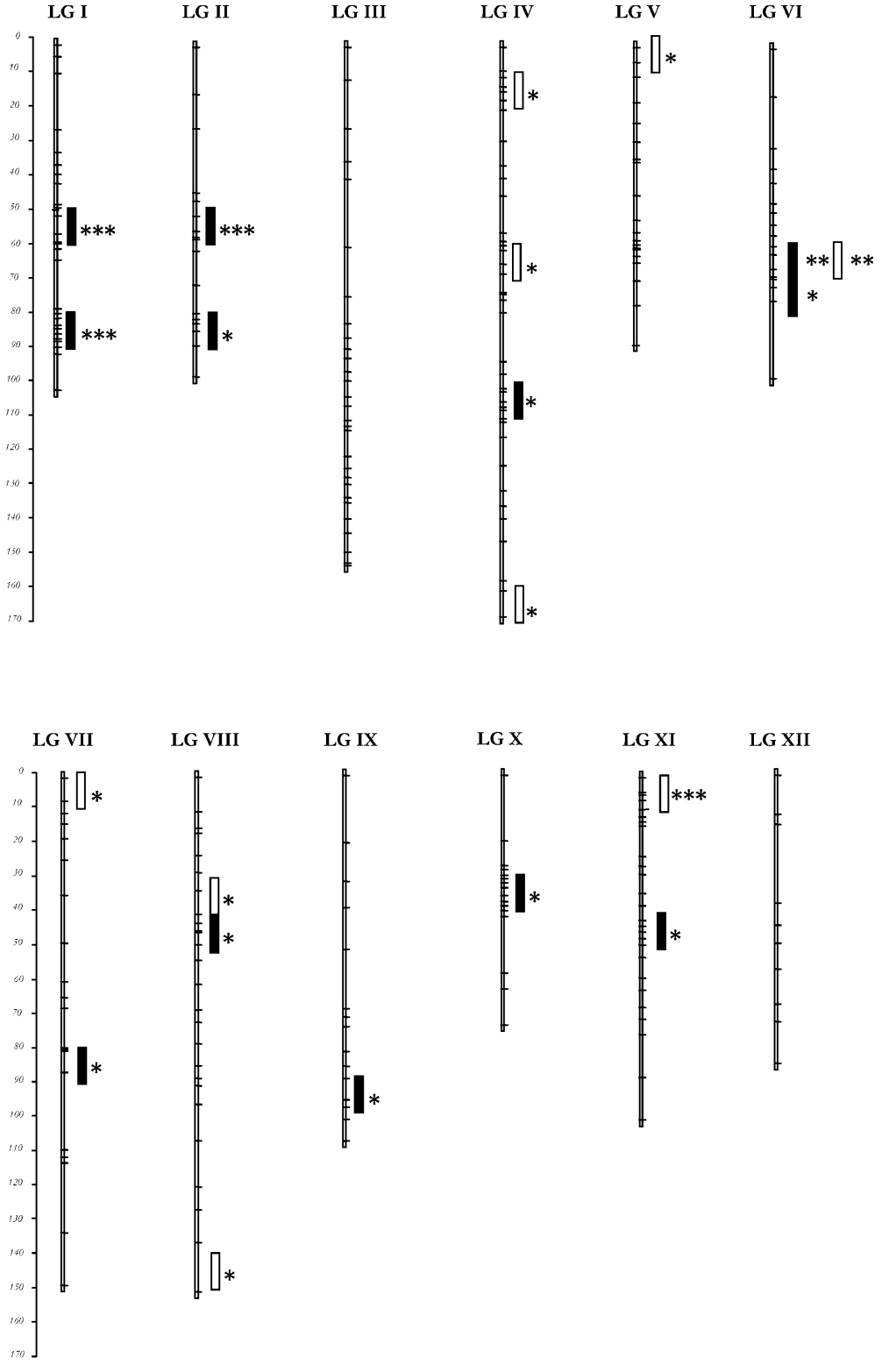

Fig. 2 Cluster position on 10-cM intervals for each linkage group of the Ved161 population. *, **, *** = significantly different from a Poisson law repartition at respectively $P < 0.05$, $P < 0.01$, $P < 0.001$. IMA cluster are drawn with *open boxes* whereas AFLP cluster are with *dark boxes*

age groups, except on LG III and on LG XII (Fig. 2) probably because fewer markers were mapped on them. A notable concentration of markers was detected near the middle of the linkage groups I, II, IV, VI, VII, VIII, X and XI at $P < 0.05$ or $P < 0.01$. Together, these clusters comprised 123 molecular markers covering a total genetic distance of 140 cM. So, 26.7% of the markers were mapped on 9.4% of the total genetic distance. A mean distance of 1.13 cM between two markers was recovered inside clusters and 3.9 cM outside, which represents a 3-fold variation in the mean density of markers.

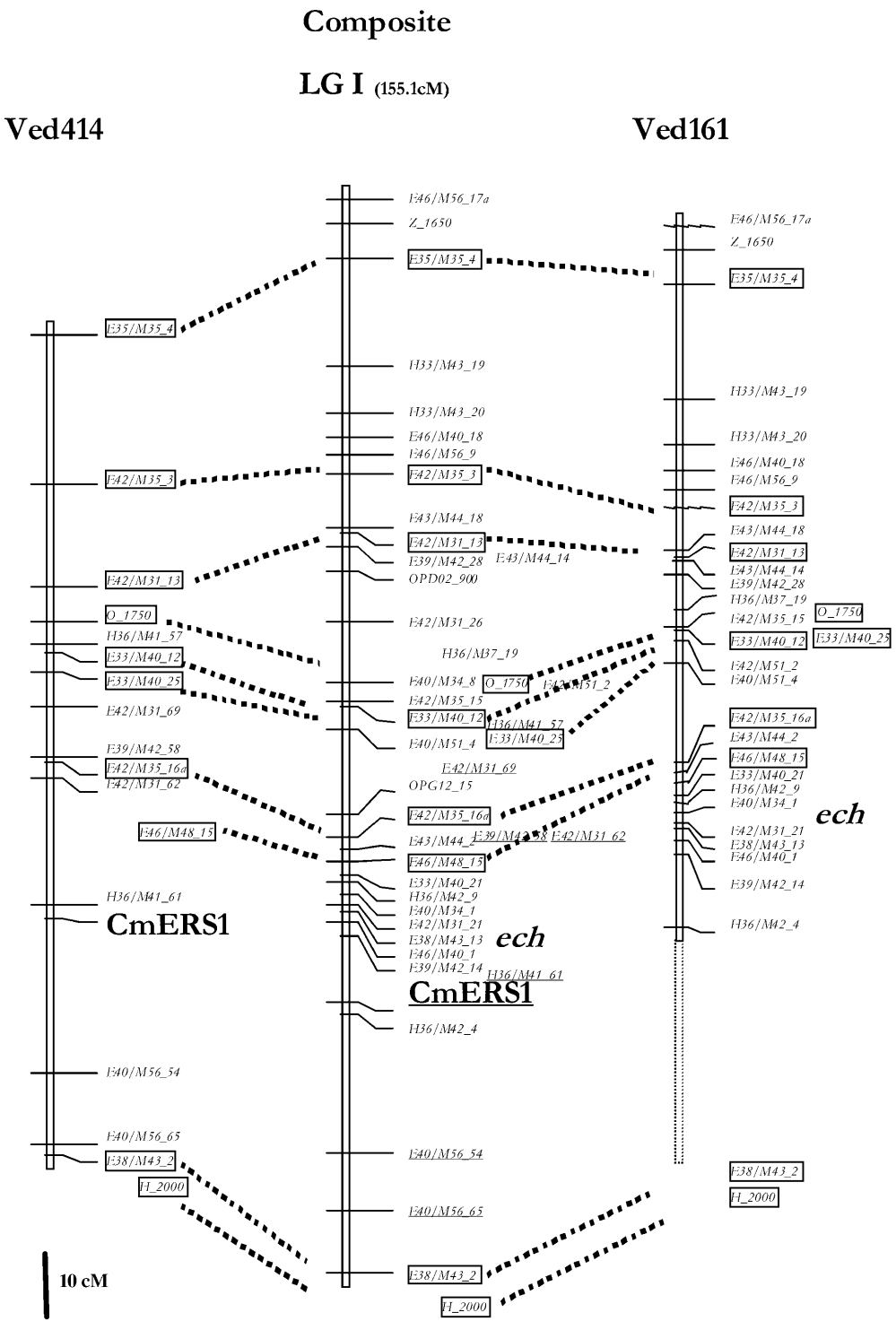

Fig. 3 Alignment of the Ved161, Ved414 and the composite map for linkage group I. Comigrating markers which segregated in both populations are in *boxes* and are related with *dashed lines* to build the composite map

Distribution of the different marker types within linkage groups

AFLP and IMA showed different distributions. According to Poisson's law, a higher number of AFLP markers were found in clusters; 30.3% of *Eco*RI/*Mse*I-A and 36.6% of *Hin*dIII/*Mse*I-A markers mapped in clustered genomic intervals, while only 14.2% of *Eco*RI/*Mse*I-C markers were found inside clusters. On the other hand, IMA markers were less clustered with only 8.8% of them included in AFLP-clustered genomic intervals, which is not significantly different from a random distribution (9.4% of markers expected). IMA markers were often found at linkage-group extremities and helped to extend genome coverage. IMA clustered markers were located in different regions than AFLP clusters, except for LG VI where a common cluster

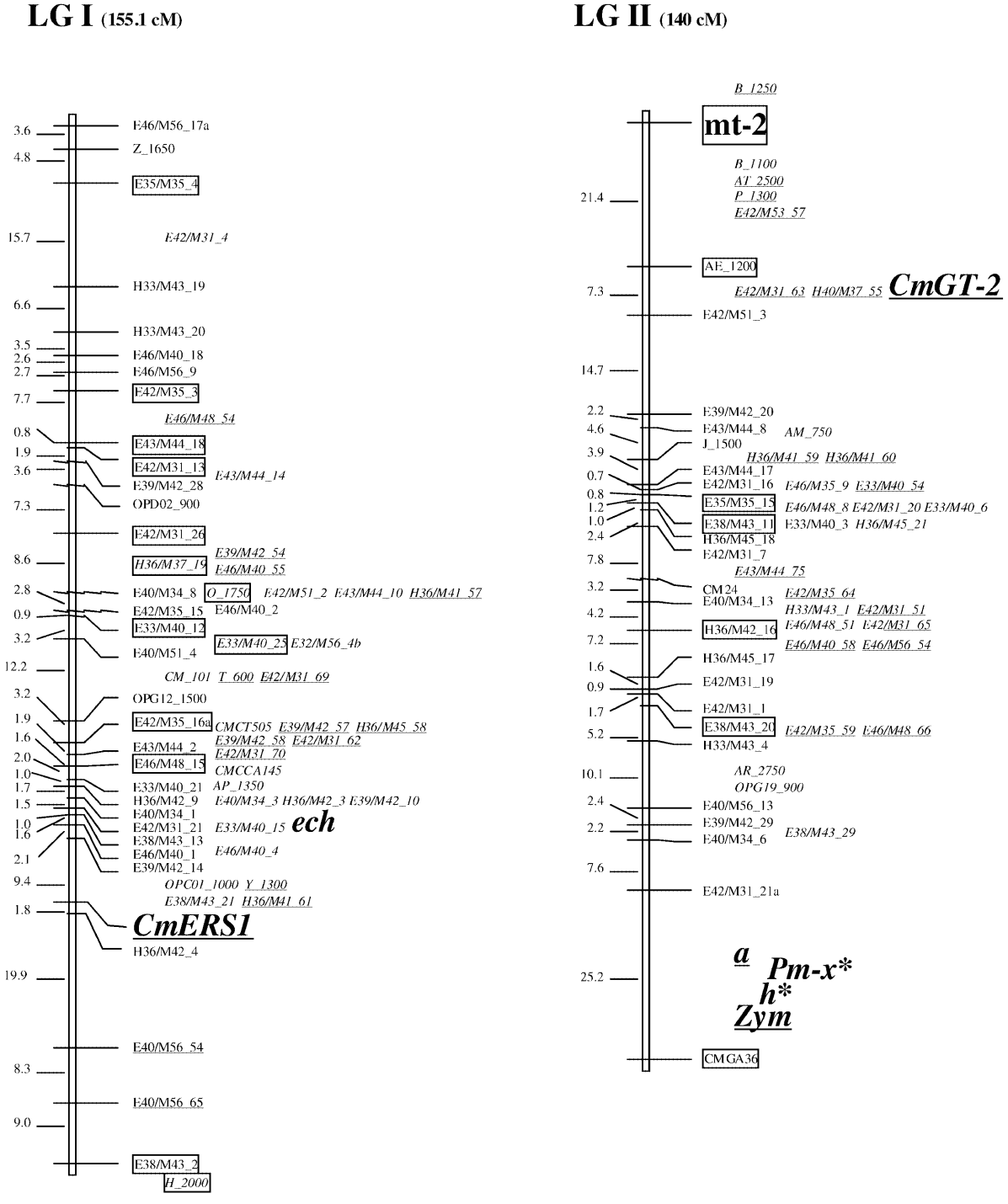

Fig. 4 Composite map of *C. melo*. Molecular markers are on the right side of each linkage group whereas genetic distance are on the left (cM Kosambi). Markers in *thick* are located with a confidence order better than 2.0 and give the framework map. Other markers are placed in the most probable position inside framework intervals and are in *italic*. Markers in *bold type* are know function genes, *underlined markers* segregate only in the Ved414 population whereas markers which segregated in both population are in *boxes*

was found (Fig. 2). Six out of the nine IMA clusters were found at linkage-group extremities. Intervals of 0–10 cM on linkage group LG VII and interval 140–150 cM on linkage group LG VIII contained three or more IMA markers. Closely related IMA primers sometimes gave products of similar size which were tightly linked. These were: AF = $CCG(CT)_6$, AG = $VHV(CT)_6$ and AM = $RY(CT)_6$ which, respectively, amplified markers AF_800, AG_800, AM_800 on LG VIII and also markers AF_1250, AG_1250, AM_1250 on LG XI; V = $(AG)_8G$ and X = $(AG)_8R$ which amplified markers V_490 and X_490 on

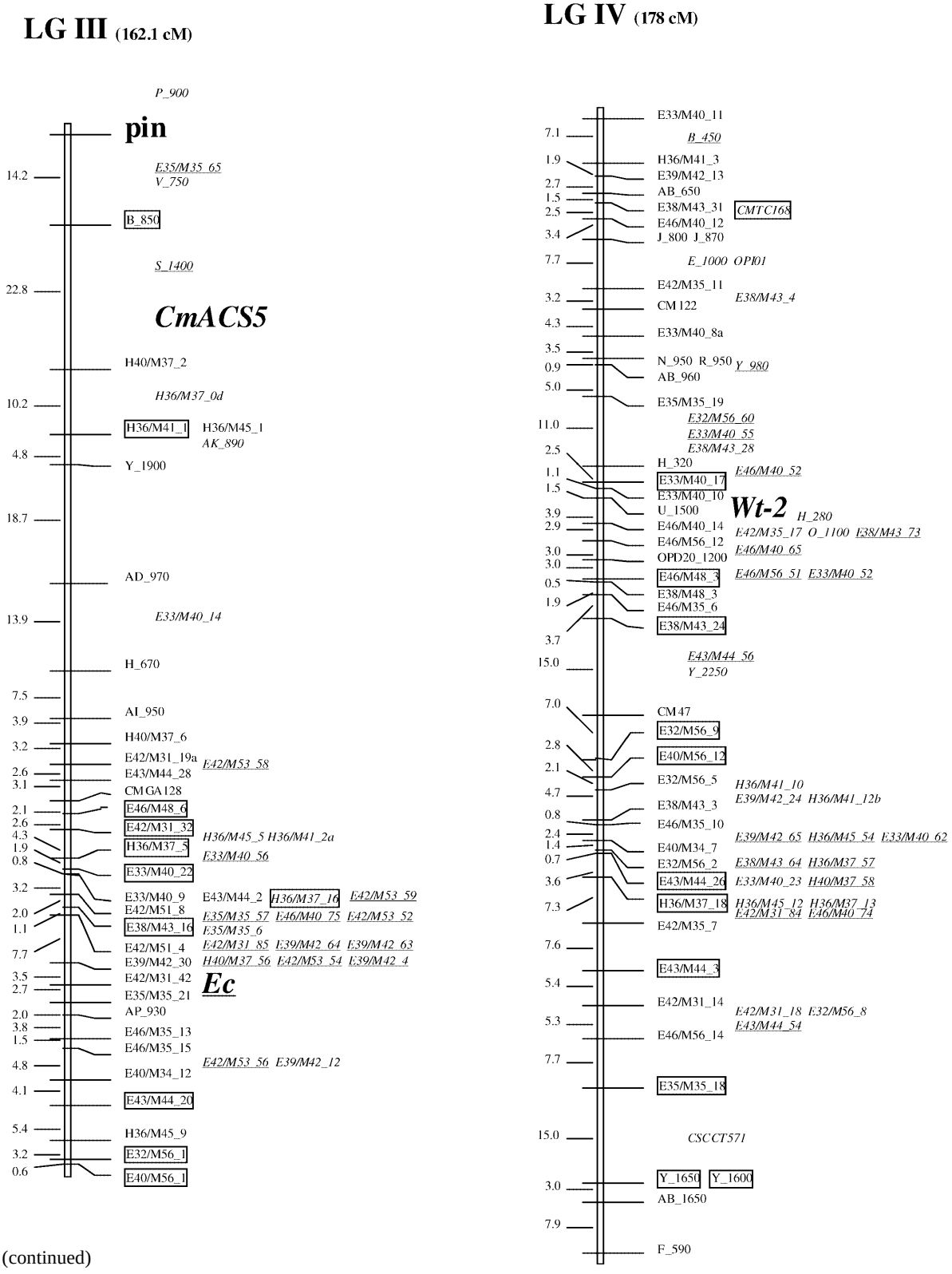

Fig. 4 (continued)

LGVII; N = BDB$(CA)_7$ and R = BDT$(CA)_7$ which amplified markers N_950 and R_950 on LG IV. These may represent amplification of the same DNA fragment most likely due to the degenerate nature of the IMA primers or tightly linked markers amplified in SSR-rich regions.

Map construction on the Ved414 population

An average of 21.7% polymorphic markers was found for AFLPs and IMAs between PI 414723 and Védrantais. The frequency of the Védrantais and PI 414723 al-

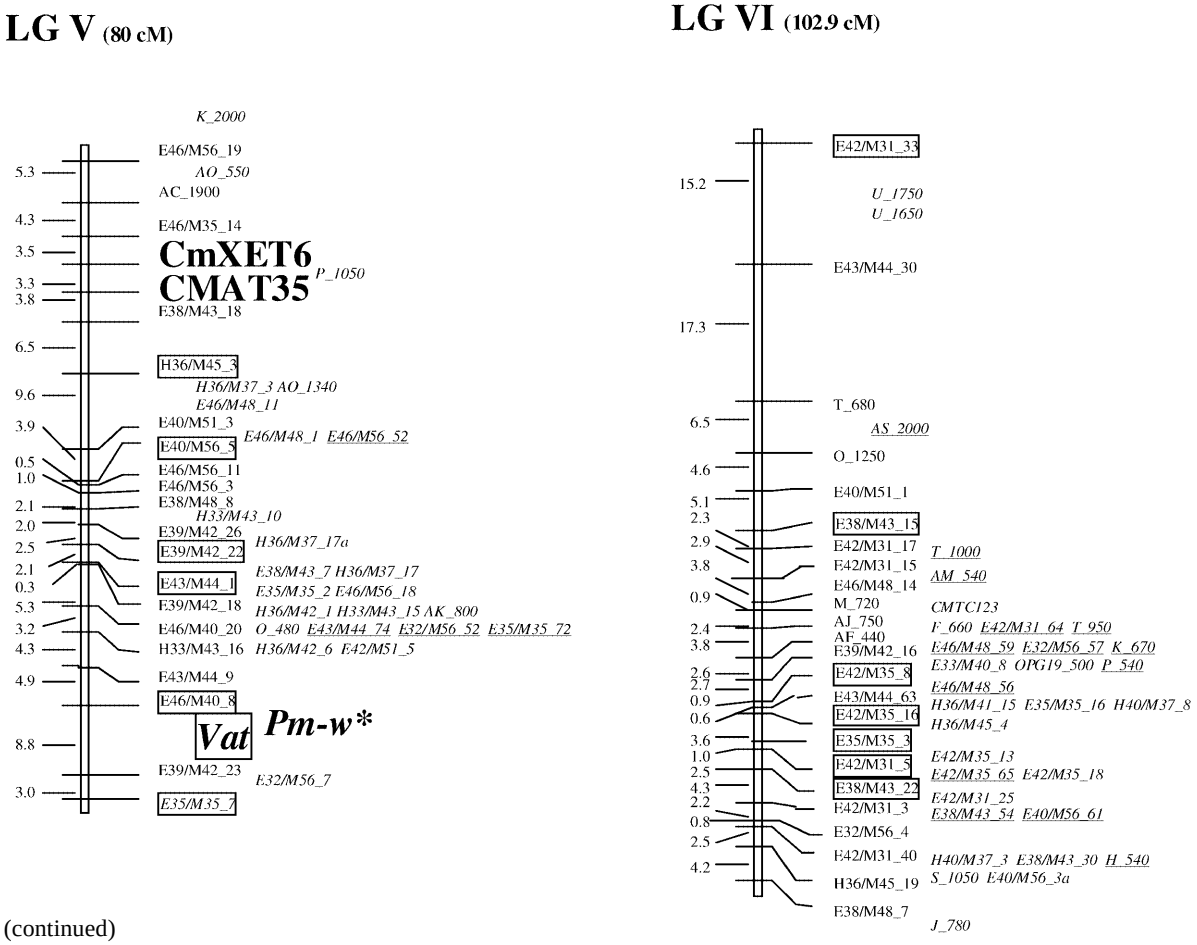

Fig. 4 (continued)

leles was 52% and 48% respectively. Sixteen of the markers were skewed at $P < 0.05$ and all the highly skewed markers ($P < 0.001$) were discarded for map construction. Codominant AFLPs, based on visual scoring of two bands on an acrylamide gel separated by a few base pairs, were often found (20.2%). We used these markers and other codominant markers (namely SSRs and RFLPs) to estimate the heterozygosity level of the Ved414 population; 4.12% of heterozygous RI loci was found, not significantly different from the theoretical value. A total of 318 molecular markers (including 233 AFLPs, 65 IMAs, 5 SSRs, 2 RFLPs, and 13 phenotypic) were used to build a 1,180-cM length map. Nineteen linkage groups and 12 unlinked markers were recovered at a LOD score higher than 3.0, indicating that the map was unsaturated according to the haploid number of melon chromosomes (n = 12).

Map merging

Locus specificity of AFLP and IMA markers

Since the two populations have Védrantais as a common parent, it was expected that the Védrantais-specific DNA fragment should map at the same genetic position. Comigrating bands of like molecular weight amplified on the two unrelated parents could correspond alternatively to the same allele shared by both parents or to two different loci. If molecular markers from AFLP or IMA:

(1) amplified with the same primer combination,
(2) gave a band with the same mobility in agarose or acrylamide gels (i.e. side by side), and
(3) mapped to the same locus on the two maps,

then we assumed that the molecular markers corresponded to the same locus in the two populations. We gave a common name from the Ved161 population, and pooled data from the two populations. A total of 66 comigrating markers from the common parental line (Védrantais), and 40 ones amplified on the two unrelated lines, were recovered. Similar profiles were obtained even when gel production or amplification conditions varied to some extent. For instance, the AFLP gels from Ved161 and Ved414 generated with the E46/M40 primer combination could easily be aligned side by side, and comigrating markers be identified by visual inspection; the markers E46/M40_62 and E46/M40_64 in the Ved414 population corresponded respectively to E46/M40_8 and E46/M40_9 in the Ved161 population (Fig. 1).

Finally, 88 AFLP and 18 IMA comigrating loci, along with five SSR and five phenotypic anchor markers, were

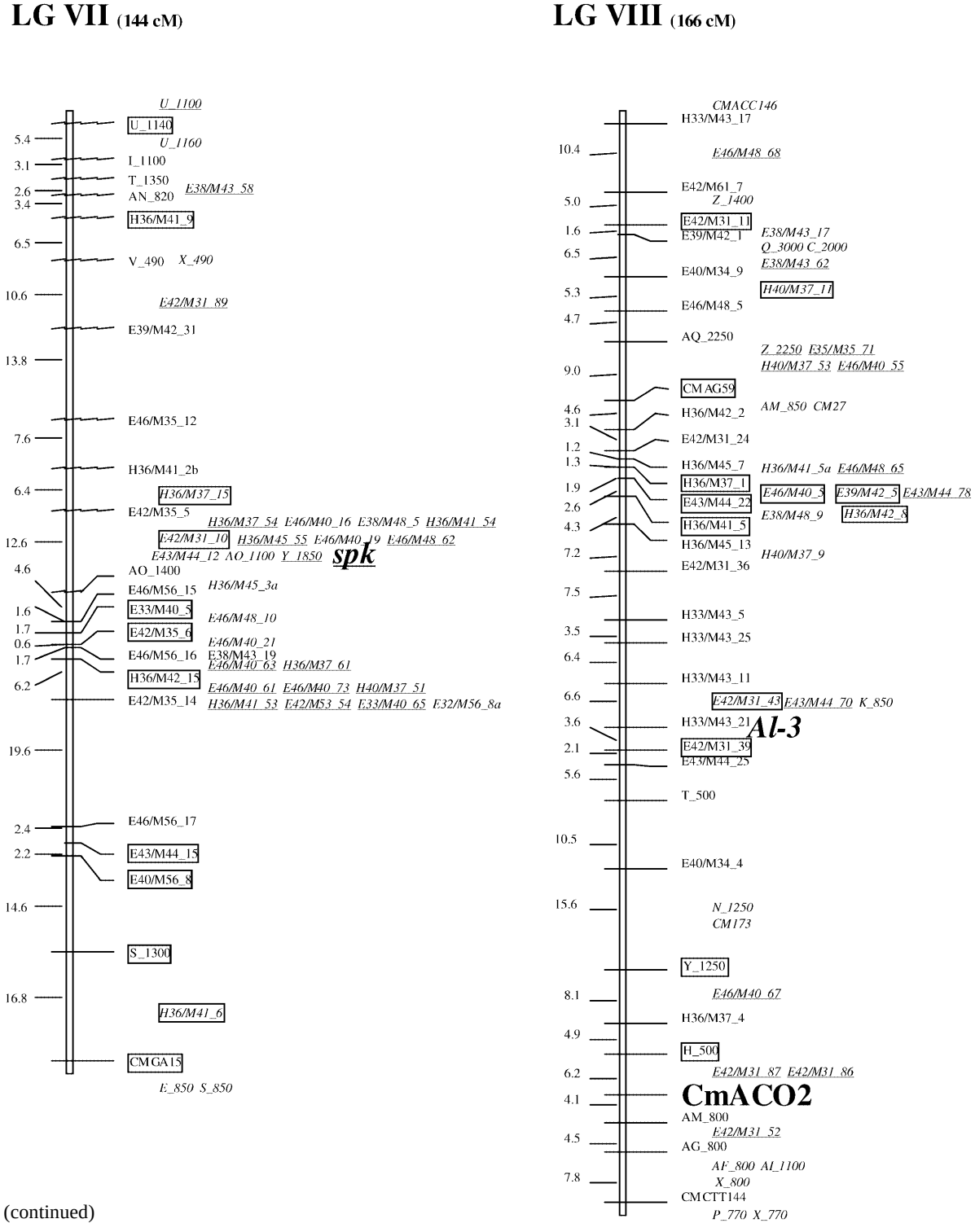

Fig. 4 (continued)

used to align the Ved161 and Ved414 linkage groups. Alignment analysis revealed a similar order between both maps; for example, on LG I (Fig. 3). In several cases, slight local order differences were found, presumably due to the lower resolution of the Ved414 map which is based on only 63 individuals.

Composite map construction

All the linkage groups of both populations were successfully aligned side by side using the 116 common markers. Hence, we related all of the 19 linkage groups of the Ved414 map to the 12 linkage groups of Ved161. A 668-point linkage composite map was built from the poly-

Table 6 Correspondance of the linkage groups of the composite map with other published maps

Composite map	Pitrat (1991)	Baudracco-Arnas and Pitrat (1996)[a]	Wang et al. (1997)[b]	Brotman et al. (2000)[c]	Danin-Poleg et al. (2000)[d]	Oliver et al. (2001)[e]
I	–	G	–	3 + 12	VIII	6
II	4	D	–	3	–	8
III	–	J	–	–	V	2
IV	–	C	–	10	–	10
V	2	2 + K	–	6	–	4
VI	–	B	–	–	–	12
VII	–	F	–	9	VI	3
VIII	–	E	–	8 + 13	I	1
IX	5	5	–	11	II	7
X	–	A	–	4 + 7	VII	9
XI	6	6	III	1	III	5
XII	7	7	–	2	–	11

Maps with molecular markers developed on the following populations
[a] F_2 Védrantais × PI 161375
[b] BC_1 Ananas Yokneum × (MR-1 × Ananas Yokneum)
[c] F_2 Top mark × PI 414723
[d] F_2 PI 414723 × Dulce
[e] F_2 Piel de sapo × PI 161375

morphic (AFLP, IMA, RAPD, SSR, RFLP, phenotypic) markers of the two segregating populations. Comigrating markers helped to identify remaining gaps in both maps and to increase map coverage in both populations. For instance, the Ved414 map helped to add the unlinked E38/M43_2 and H_2000 markers from the Ved161 population on LG I and extended the Ved161 map by more than 35 cM (Fig. 3). The map spanned 1,654 cM on 12 linkage groups which correspond to the haploid number of chromosomes in melon (Fig. 4). Ten markers remained unlinked, eight from Ved414 and two from Ved161, at a LOD score higher than 3.0. Two of the unlinked markers coming from the Ved414 population were the phenotypic markers *Bi* (seedling bitterness) and *me-2* (mealy flesh).

The high overall polymorphism between the three parental lines allowed us to map 21 phenotypic genes of which eight were disease/pest resistance genes (*Fom-1*, *Fom-2*, *Pm-x*, *Pm-w*, *Prv*, *Vat*, *Zym*) and eight were fruit quality genes (*Al-3*, *Al-4*, *Ec*, *gf*, *mt-2*, *p*, *s-2*, *spk*). We also mapped eight known-function genes by RFLP or SSR (Table 3). The 30 known-function genes (phenotypic, RFLPs, SSRs or isoenzyme markers), were found well-distributed among the linkage groups except for LG VI and LG X where none were located (Table 3 and Fig. 3). Some of the disease resistance genes were found tightly linked, for instance *Prv* and *Fom-1* on LG XI.

Correspondence of this composite map with the other published maps

Twenty nine markers were common (16 RAPDs, 7 RFLPs, 5 phenotypic) between the Ved161 population and the F_2 map (Baudracco-Arnas and Pitrat 1996). They were used to assign all but two minor RAPD linkage groups, I and J, of the F_2map to the Ved161 map (Table 6). The composite map contained 23 phenotypic markers, some of which had been used to build a classical genetic map (Pitrat 1991). Five linkage groups of this genetic map were also assigned to the composite map and to the F_2 map (Baudracco-Arnas and Pitrat 1996) (Table 6). Moreover, *Zym* and *a* were linked to LG II and correspond to LG 4 of the genetic map (Table 6) which had not been assigned before. In order to simplify the comparison of map positions among several maps, we propose the roman nomenclature I to XII to assign all new molecular markers or genes on the *C. melo* genome (Table 4). The linkage groups have not been physically assigned to chromosomes which are not easily distinguished. For the map developed by Wang et al. (1997) on the BC_1 Ananas Yokneum × (MR-1 × Ananas Yokneum), the only correspondance which can be made is between their group III where the marker 596-1 linked to the gene *Fom-2* is located and our group XI where this gene is located. Partial maps on the F2 Top mark x PI 414723 (Brotman et al. 2000) and on the F_2 PI 414723 x Dulce (Danin-Poleg et al. 2000) and recently a more complete map on the F_2 Piel de sapo x PI 161375 (Oliver et al. 2001) have been published. Correspondances were better due to several microsatellites which were located on these different maps, and RFLP for the last one (Table 6).

Discussion and conclusion

We have constructed a saturated map of melon based on a RI population with 163 lines derived from the cross between a Charentais line, Védrantais, and a Korean accession, PI 161375. This population is of high quality based on marker segregation; the ratio of skewed markers is moderate (19%), compared for instance with 75% of skewed markers for a RI population derived from an interspecific tomato cross (Paran et al. 1995). Moreover, the residual heterozygosity of our population is close to the theoretical value, unlike in other species such as rice (Cho et al. 1998) or tomato (Paran et al. 1995) which reported values several times higher.

A second unsaturated map was developed on a RI population derived from the cross between Védrantais and PI 414723, an Indian accession, to assay AFLP and IMA locus specificity. AFLP and IMA robustness was confirmed through visual inspection which demonstrated high reproducibility for most of the primer combinations used for both populations (Fig. 1). High reproducibility of AFLPs was already described in several populations (Alonso-Blanco et al. 1998; Rouppe van der Voort et al. 1998b) and the high reliability of IMA markers could be due to the high annealing temperature used. Only one PCR is needed for IMA as for RAPD and may replace them in the future. RAPD markers were extensively used in the past for plant map construction but are known to be weakly repeatable and very sensitive to scoring errors (Jones et al. 1997).

AFLPs were quite well-distributed on the Ved161 map and covered the whole genome. Although some clusters were found, 70% of AFLP markers mapped outside clustered regions which is considerably higher than for tomato or pepper where less than 5% of the AFLP markers, with restriction enzymes *Eco*RI-*Mse*I and *Hin*dIII-*Mse*I, mapped outside putative centromeric regions (Haanstra et al. 1999; Saliba-Colombani et al. 2000). AFLPs were also well distributed in rice (Maheswaran et al. 1997; Nandi et al. 1997; Cho et al. 1998) and *A. thaliana* maps (Alonso-Blanco et al. 1998), even if a variable clustering rate was found. Differences in the rate of repeated sequences between plant genomes could explain the striking difference in AFLP map distribution. Also, within a species, clustering rate depends on the molecular-marker type. In barley, AFLPs were more clustered around centromeres than RFLPs (Qi et al. 1998). On the tomato map, *Eco*RI-*Mse*I AFLPs were almost all linked to the presumed centromere, unlike *Pst*I-*Mse*I AFLPs which were more dispersed (Haanstra et al. 1999) but had lower polymorphism. A similar result was found in soybean (Young et al. 1999). By using a selective nucleotide A or C for *Eco*RI-*Mse*I AFLPs we found that *Eco*RI-*Mse*I-A were more polymorphic than *Eco*RI-*Mse*I-C, probably because plant genomes are AT-rich, and *Eco*RI-*Mse*I-A were mapped more often inside clusters than *Eco*RI-*Mse*I-C which were more randomly distributed.

In contrast to AFLPs, IMAs were mapped preferentially to distal positions of linkage groups and were under-represented in putative centromeric regions. The distal IMA distribution could be explained if the IMA markers had many scoring errors. Markers with scoring errors may give a high LOD score for a linkage group but are placed at the extremities rather than inside the linkage group to minimize the order variation of correctly scored markers. We did not find more scoring errors for IMAs, and they mapped with a high LOD score at the ends of the linkage group. The distribution of SSRs is quite variable in plants, albeit plant SSR maps are usually not very dense. SSRs were found well dispersed (Chen et al. 1997), or sometimes associated with centromeric regions in wheat (Bryan et al. 1997), sugar beet (Schmidt and Heslop-Harrison 1996) or tomato (Areshchenkova and Ganal 1999; Ganal et al. 1992). GATA and GACA repeats were associated with centromeres in tomato (Arens et al. 1995) but seemed well distributed in sugar beet (Schmidt and Heslop-Harrison 1996). In a fish species, rainbow trout, a variable number of tandem repeats (VNTRs) (Young et al. 1998) were mapped together on a haploid population with a similar repartition of IMAs as in our population. Moreover, AFLPs seemed to be concentrated in or near middle of the linkage groups (Young et al. 1998).

The development of a new map using a second population, Ved414, demonstrated that AFLP and IMA PCR-based markers are transportable between crosses and to be locus specific. The use of a common parental line, Védrantais, eased the identification of common markers between both RI populations, and our results demonstrate the usefulness of a common parent to generate allelic bridges. We expected that the Védrantais-specific markers would correspond to the same loci. Using the same set of primer combinations in both populations, we recovered 106 comigrating markers of which 62% were Védrantais specific and 38% were from the two unrelated parents. All of these comigrating markers were assigned to the same position with a good colinearity in both maps (Fig. 3). Thus, comigrating AFLPs and IMAs seemed to correspond to the same loci even if they came from two unrelated parents and could be used as anchors to compare or merge several maps of *C. melo*. While only sequence analysis of the putative alleles amplified in both unrelated parents would be conclusive as to the true allelism of comigrating markers, the locus specificity of AFLP markers has been demonstrated in many species (Waugh et al. 1997; Alonso-Blanco et al. 1998; Qi et al. 1998; Rouppe van der Voort et al. 1998b; Haanstra et al. 1999; Vuylsteke et al. 1999). Our results for IMA markers suggest strongly that they also may be locus-specific and that this merits further confirmation.

Comparison of maps produced in different laboratories has been done in the past using anchor loci such as the RFLP/SSR/phenotypic loci mapped in our composite map. However, the correspondance between maps made using mainly PCR-based high-throughput markers (e.g. the AFLP melon map of Wang et al. 1997) requires a data base where gel profiles could be accessible on a web site to allow the identification of possible comigrating bands. The potato AFLP data base is exemplary (Rouppe van der Voort et al. 1998a). As a first step, we related the linkage groups of this composite map to those already published on melon with our anchor loci.

The two previous partial melon maps, (Baudracco-Arnas and Pitrat 1996; Wang et al. 1997), estimated the total genetic map length to be between 2,276 and 3,250 cM, which is higher than the 1,654 cM reported here. However, these were only estimates extrapolated from the partial maps. Also, the marker type, number of loci, population type and population size can affect the outcome of a mapping project. The first map of *C. melo* was built with an F_2 population of 218 individuals with a

majority of RAPD markers (64 out of 99 markers). RAPDs are known to be weakly repeatable and very sensitive to scoring errors (Jones et al. 1997). Mapping precision of dominant markers in an F_2 population is low especially when they are in repulsion (Allard 1956). The second map (Wang et al. 1997) was built with a small population, 66 BC_1, and thus a lower mapping precision. Small mapping populations are known to be sensitive to scoring errors which cause map expansion (Maheswaran et al. 1997; Powell et al. 1997). In general, linkage-group lengths vary between 90 and 200 cM in most other plant genetic maps. For 12 chromosomes, this gives a gross estimate of total map length between 1,080 cM and 2,400 cM. The 1,654-cM map reported here is within this range.

PI 414723 and PI 161375 are two multi-disease resistant lines whereas Védrantais is a line of good agronomic value belonging to the French Charentais type. Overall polymorphism between Védrantais vs PI 161375 and Védrantais vs PI 414723 was around 25%. This high polymorphism rate helped to build a good map with a limited number of primer combinations and in a short time. For instance, the E42/M31 primer combination generated 43 markers in Ved161 and 36 markers Ved414, 14 being in common. This high polymorphism has also facilitated the mapping of the phenotypic traits and candidate genes reported here: 20 phenotypic genes (Table 3, Fig. 4) of which six were disease/pest resistance genes (*Fom-1*, *Fom-2*, *nsv*, *Prv*, *Vat*, *Zym*) and eight were fruit quality genes (*Al-3*, *Al-4*, *Ec*, *gf*, *mt-2*, *p*, *s-2*, *spk*), with six miscellaneous genes involved in flower morphology/sex type (*p*, *a*), seed morphology (*Wt-2*, *pin*) and seedling traits (*ech*). Moreover, based on linkage with at least two common markers on our map, two disease genes (*Pm-x*, *Pm-w*), one seedling morphology gene (*h*) and an isozym were also positioned. Most of the disease resistance genes mapped were involved in resistance to an economical pathogen of melon, for example to viruses (*nsv*, *Zym*, *Prv*), fungi (*Fom-1*, *Fom-2*) or aphids (*Vat*). We found some of these genes clustered, like *Prv* and *Fom-1* on LG IX, *Vat* and *Pm-w* on LG V, *Zym* and *Pm-x* on LG II, a feature already described in a lot of other plant species (Pan et al. 2000).

In conclusion, the composite map presented here is a significant contribution to the study of the melon genome. More than 668 loci cover all, or nearly all, of the *C. melo* genome. Thirty two phenotypic genes, most of them being genes of economic and/or fundamental interest, were located. These two populations constitute a permanent reference mapping resource useful for basic and applied investigations like positional cloning. Ved161 and Ved414 provide useful genetic material to identify loci involved in developmental and physiological traits, like fruit shape or fruit ripening (Périn et al. 2001, 2002), or polygenic disease resistance to cucumber mosaic virus (Dogimont et al. 2000).

Acknowledgements This work was supported in part by Conseil Régional Provence-Alpes-Côte d'Azur (France). All experiments were conducted according to the current laws in France and in Israel.

References

Akagi H, Yokozeki Y, Inagaki A, Nakamura A, Fujimura T (1996) A co-dominant DNA marker closely linked to the rice nuclear restorer gene, *Rf-I*, identified with inter-SSR fingerprinting. Genome 39:1205–1209

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Alonso-Blanco C, Peeters AJM, Koornneef M, Lister C, Dean C, Bosch NVD, Pot J, Kuiper MTR (1998) Development of an AFLP based linkage map of Ler, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a Ler/Cvi recombinant inbred line population. Plant J 14:259–271

Arumanagathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–209

Arens P, Odinot P, Heusden AWV, Lindhout P, Vosman B (1995) GATA and GACA repeats are not evenly distributed throughout the tomato genome. Genome 38:84–90

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

Bains MS, Kang US (1963) Inheritance of some flower and fruit characters in muskmelon. Indian J Genet Plant Breed 23:101–106

Balagué C, Watson C, Turner A, Rouge P, Picton S, Pech JC, Grierson D (1993) Isolation of a ripening and wound induced cDNA from *Cucumis melo* L. with homology to the ethylene forming enzyme. Eur J Biochem 212:27–34

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

Beavis WD, Grant D (1991) A linkage map based on information from four F_2 populations of maize (*Zea mays* L.). Theor Appl Genet 82:636–644

Blair MW, Panaud O, McCouch SR (1999) Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theor Appl Genet 98:780–792

Brotman Y, Silberstein L, Kovalski I, Klinger J, Thompson G, Katzir N, Perl-Treves R (2000) Linkage groups of *Cucumis melo*, including resistance gene homologues and known genes. Acta Hort 510:441–448

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

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Causse M, Rocher JP, Henry AM, Charcosset A, Prioul JL, de Vienne D (1995) Genetic dissection of the relationship between carbon metabolism and early growth in maize, with emphasis on key-enzyme loci. Mol Breed 1:259–272

Causse M, Santoni S, Damerval C, Maurice A, Charcosset A, Deatrick J, Vienne Dd (1996) A composite map of expressed sequences in maize. Genome 39:418–432

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95:553–567

Cho YG, McCouch SR, Kuiper M, Kang M-R, Pot J, Groenen JTM, Eun MY (1998) Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa* L.). Theor Appl Genet 97:370–380

Coudriet DL, Kishaba AN, Bohn GW (1981) Inheritance of resistance to muskmelon necrotic spot virus in a melon aphid resistant breeding line of muskmelon. J Am Soc Hort Sci 106:789–791

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

Dogimont C, Lecomte L, Périn C, Thabuis A, Lecoq H, Pitrat M (2000) Identification of QTLs contributing to resistance to dif-

ferent strains of cucumber mosaic cucumovirus in melon. Acta Hort 510:391–398
Ganal MW, Broun P, Tanksley SD (1992) Genetic mapping of tandemly repeated telomeric DNA sequences in tomato (*Lycopersicon esculentum*). Genomics 14:444–448
Ganesan J (1988) Genetic studies on certain characters of economic importance in muskmelon (*Cucumis melo* L.). Annamalai University, India
Goldman IL, Paran I, Zamir D (1995) Quantitative trait locus analysis of a recombinant inbred line population derived from a *Lycopersicon esculentum* ×*Lycopersicon cheesmanii* cross. Theor Appl Genet 90:925–932
Guis M, Roustan JP, Dogimont C, Pitrat M, Pech JC (1998) Melon biotechnology. Biotech Genet Engin Rev 15:289–311
Haanstra JPW, Wye C, Verbakel H, Meijer-Dekens F, Berg PVd, Odinot P, Heusden AWv, Tanksley S, Lindhout P, Peleman J (1999) An integrated high-density RFLP-AFLP map of tomato based on two *Lycopersicon esculentum* ×*L. pennelli* F_2 populations. Theor Appl Genet 99:254–271
Hagiwara T, Kamimura K (1936) Cross-breeding experiments in *Cucumis melo*. Tokyo Horticultural School Publication
Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769
Hughes MB (1948) The inheritance of two characters of *Cucumis melo* and their interrelationship. Proc Am Soc Hort Sci 52:399–402
Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F, Wiel CVd, Bredemeijer G, Vosman B, Matthes M, Daly A, Brettschneider R, Bettini P, Buiatti M, Maestri E, Malcevschi A, Marmiroli N, Aert R, Volckaert G, Rueda J, Linacero R, Vazquez A, Karp A (1997) Reproductibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol Breed 3:381–390
Kantety RV, Zheng X, Bennetzen J, Zehr BE (1995) Assessement of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. Mol Breed 1:365–373
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
Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175
Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181
Lasserre E, Bouquin T, Hernandez JA, Bull J, Pech JC, Balagué C (1996) Structure and expression of three genes encoding ACC oxidase homologs from melon (*Cucumis melo* L.). Mol Gen Genet 251:81–90
Lee CW, Janick J (1978) Inheritance of seedling bitterness in *Cucumis melo*. HortScience 13:193–194
Lefebvre V, Palloix A, Rives M (1993) Nuclear RFLP between pepper cultivars (*Capsicum annuum* L.). Euphytica 71:189–199
Liou PC, Chang YM, Hsu WS, Cheng YH, Chang HR, Hsiao CH (1998) Construction of a linkage map in *Cucumis melo* L. using random amplified polymorphic DNA markers. Acta Hort 461:123–131
Maheswaran M, Subudhi PK, Nandi S, Xù JC, Parco A, Yang DC, Huang N (1997) Polymorphism, distribution, and segregation of AFLP markers in a doubled haploid rice population. Theor Appl Genet 94:39–45
Marques CM, Araujo JA, Ferreira JG, Whetten R, O'Malley DM, Liu B-H, Sederoff R (1998) AFLP Genetic maps of *Eucalyptus globulus* and *E. tereticornis*. Theor Appl Genet 96:727–737
Marrou J (1967) Amélioration des méthodes de transmission mécanique des virus par absorption des inhibiteurs d'infection sur le charbon végétal. C R Acad Agric France 53:972–981
Nandi S, Subudhi PK, Senadhira D, Manigbas NL, Sen-Mandi S, Huang N (1997) Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol Gen Genet 255:1–8
Naudin C (1859) Essais d'une monographie des espèces et des variétés du genre *Cucumis*. Ann Sci Nat 11:5–87
Nugent PE, Hoffman JC (1974) Inheritance of the halo cotyledon mutant in muskmelon. J Hered 65:315–316
Oliver JL, Garcia-Mas J, Cardús M, Pueyo N, López-Sesé AI, Arroyo M, Gómez-Paniagua H, Arús P, de Vicente CM (2001) Construction of a reference linkage map of melon. Genome 44: 836–845
Pan QL, Liu YS, Budai-Hadrian O, Sela M, Carmel-Goren L, Zamir D, Fluhr R (2000) Comparative genetics of nucleotide binding site-leucine rich repeat resistance gene homologues in the genomes of two dicotyledons: tomato and *Arabidopsis*. Genetics 155:309–322
Paran I, Goldman I, Tanksley SD, Zamir D (1995) Recombinant inbred lines for genetic mapping in tomato. Theor Appl Genet 90:542–548
Périn C, Dogimont C, Giovinazzo N, Besombes D, Guitton L, Hagen L, Pitrat M (1999) Genetic control and linkages of some fruit characters in melon. Cucurb Genet Coop Rep 22:16–18
Périn C, Gomez-Jimenez MC, Hagen L, Dogimont C, Pech JC, Latché A, Pitrat M, Lelièvre JM (2002) Molecular and genetic characterisation of a non-climacteric phenotype in melon reveals two loci conferring altered ethylene response in fruit. Plant Physiol (in press)
Périn C, Hagen LS, Giovinazzo N, Besombes D, Dogimont C, Pitrat M (2001) Genetic control of fruit shape acts prior to anthesis in melon (Cucumis melo L.). Mol Genet Genom (in press)
Pitrat M (1991) Linkage groups in *Cucumis melo* L. J Hered 82:406–411
Pitrat M (1998) 1998 gene list for melon. Cucurb Genet Coop Rep 21:69–81
Pitrat M, Lecoq H (1980) Inheritance of resistance to cucumber mosaic virus transmission by *Aphis gossypii* in *Cucumis melo*. Phytopathology 70:958–961
Pitrat M, Lecoq H (1982) Relations génétiques entre les résistances par non acceptation et par antibiose du melon à*Aphis gossypii*. Recherche de liaisons avec d'autres gènes. Agronomie 2:503–508
Pitrat M, Lecoq H (1983) Two alleles for Watermelon Mosaic Virus 1 resistance in melon. Cucurb Genet Coop Rep 6:52–53
Pitrat M, Lecoq H (1984) Inheritance of Zucchini Yellow Mosaic Virus resistance in *Cucumis melo* L. Euphytica 33:57–61
Poole CF, Grimball PC (1939) Inheritance of new sex forms in *Cucumis melo* L. J Hered 30:21–25
Powell W, Thomas WTB, Baird E, Lawrence P, Booth A, Harrower B, McNicol JW, Waugh R (1997) Analysis of quantitative traits in barley by the use of amplified fragment length polymorphisms. Heredity 79:48–59
Qi X, Stam P, Lindhout P (1998) Use of locus-specific AFLP markers to construct a high-density molecular map in barley. Theor Appl Genet 96:376–384
Ratnaparkhe MB, Santra DK, Tullu A, Muehlbauer FJ (1998a) Inheritance of inter-simple-sequence-repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. Theor Appl Genet 96:348–353
Ratnaparkhe MB, Tekeoglu M, Muehlbauer FJ (1998b) Inter-simple-sequence-repeat (ISSR) polymorphisms are useful for finding markers associated with disease resistance gene clusters. Theor Appl Genet 97:515–519
Reiter RS, Williams JGK, Feldmann KA, Rafalski JA (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
Remington DL, Whetten RW, Liu BH, O'Malley DM (1999) Construction of an AFLP genetic map with nearly complete coverage in *Pinus taeda*. Theor Appl Genet 98:1279–1292

Risser G (1973) Étude de l'héréditéde la résistance du melon (*Cucumis melo*) aux races 1 et 2 de *Fusarium oxysporum* f.sp. *melonis*. Ann Amélior Plantes 23:259–263

Risser G, Mas P (1965) Mise en évidence de plusieurs races de *Fusarium oxysporum* f.sp. *melonis*. Ann Amélior Plantes 15:405–408

Risser G, Banihashemi Z, Davis DW (1976) A proposed nomenclature of *Fusarium oxysporum* f.sp. *melonis* races and resistance genes in *Cucumis melo*. Phytopathology 66:1105–1106

Rosa JT (1928) The inheritance of flower types in *Cucumis* and *Citrullus*. Hilgardia 3:233–250

Rose JK, Hadfield KA, Labavitch JM, Bennett AB (1998) Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol 117:345–361

Rouppe van der Voort JNAM, van Eck HJ, Draaistra J, van Zandvoort PM, Jacobsen E, Bakker J (1998a) An online catalogue of AFLP markers covering the potato genome. Mol Breed 4:73–77

Rouppe van der Voort JNAM, Eck HJV, Folkertsma RT, Zandvoort PV, Hutten RCB, Draaistra J, Gommers FJ, Jacobsen E, Helder J, Bakker J (1998b) Locus specificity of AFLP markers used to align genetic maps from different potato genotypes. Mol Gen Genet 255:438–447

Saliba-Colombani V, Causse M, Gervais L, Philouze J (2000) Efficiency of RFLP, RAPD, AFLP markers for the construction of an intraspecific map of the tomato genome. Genome 43:29–40

Schmidt T, Heslop-Harrison JS (1996) The physical and genomic organization of microsatellites in sugar beet. Proc Natl Acad Sci USA 6:8761–8765

Sewell MM, Sherman BK, Neale DB (1999) A consensus map for Loblolly pine (*Pinus taeda* L.). I. Construction and integration of individual linkage maps from two outbred three-generation pedigrees. Genetics 151:321–330

Staub JE, Meglic V, McCreight JD (1998) Inheritance and linkage relationships of melon (*Cucumis melo* L.) isozymes. J Am Soc Hort Sci 123:264–272

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

Takada K, Kanazawa K, Takatuka K (1975) Studies on the breeding of melon for resistance to powdery mildew. II. Inheritance of resistance to powdery mildew and correlation of resistance to other characters. Bull Veg Ornem Crops R Stn 2:11–31

Tsumura Y, Ohba K, Strauss SH (1996) Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theor Appl Genet 92:40–45

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

Vuylsteke M, Mank R, Antonise R, Bastiaans E, Senior ML, Stuber CW, Melchinger AE, Lubberstedt T, Xia XC, Stam P, Zabeau M, Kuiper M (1999) Two high-density AFLP linkage maps of *Zea mays* L.: analysis of distribution of AFLP markers. Theor Appl Genet 99:921–935

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

Waugh R, Bonar N, Baird E, Thomas B, Graner A, Hayes P, Powell W (1997) Homology of AFLP products in three mapping populations of barley. Mol Gen Genet 255:311–321

Webb RE (1979) Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* L. HortScience 14:265–266

Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Yamamoto M, Miki T, Ishiki Y, Fujinami K, Yanagisawa Y, Nakagawa H, Ogura N, Hirabayashi T, Sato T (1995) The synthesis of ethylene in melon fruit during the early stage of ripening. Plant Cell Physiol 36:591–596

Young WP, Wheeler PA, Coryell VH, Keim P, Thorgaard GH (1998) A detailed linkage map of rainbow trout produced using doubled haploids. Genetics 148:839–850

Young WP, Schupp JM, Keim P (1999) DNA methylation and AFLP marker distribution in the soybean genome. Theor Appl Genet 99:785–790

Zietkiewicz E, Rafalsky A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–183