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ORIGINAL PAPER

A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes

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Abstract A genetic map of melon enriched for fruit traits was constructed, using a recombinant inbred (RI) population developed from a cross between representatives of the two subspecies of *Cucumis melo* L.: PI 414723 (subspecies *agrestis*) and 'Dulce' (subspecies *melo*). Phenotyping of 99 RI lines was conducted over three seasons in two locations in Israel and the US. The map includes 668 DNA markers (386 SSRs, 76 SNPs, six INDELs and 200 AFLPs), of which 160 were newly developed from fruit ESTs. These ESTs include candidate genes encoding for enzymes of sugar and carotenoid metabolic pathways that were cloned from melon cDNA or identified through mining of the International Cucurbit Genomics Initiative database (http://www.icugi.org/). The map covers 1,222 cM with an average of 2.672 cM between markers. In addition, a skeleton physical map was initiated and 29 melon BACs harboring fruit ESTs were localized to the 12 linkage groups of the map. Altogether, 44 fruit QTLs were identified: 25 confirming QTLs described using other populations and 19 newly described QTLs. The map includes QTLs for fruit sugar content, particularly sucrose, the major sugar affecting sweetness in melon fruit. Six QTLs interacting in an additive manner account for nearly all the difference in sugar content between the two genotypes. Three QTLs for fruit flesh color and carotenoid content were identified. Interestingly, no clear colocalization of QTLs for either sugar or carotenoid content was observed with over 40 genes encoding for enzymes involved in their metabolism. The RI

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population described here provides a useful resource for further genomics and metabolomics studies in melon, as well as useful markers for breeding for fruit quality.

Introduction

Cucumis melo L. (melon) is a highly diversified species that is widely cultivated throughout the world. Melon fruits are highly variable in shape, size, rind form, firmness, color and flavor. Fruits of wild and cultivated genotypes accumulate various levels of soluble sugars, organic acids, pigments and aroma volatiles (Burger et al. 2006, 2009; Lester et al. 2001; Morales et al. 2004; Perin et al. 2002b; Portnoy et al. 2008), affecting fruit quality through complex networks of metabolic pathways that are active during fruit ripening.

Genetic maps are powerful tools for linking genes and traits. Several melon genetic maps have been developed (Baudracco-Arnas and Pitrat 1996; Danin-Poleg et al. 2002; Monforte et al. 2004; Perin et al. 2002b; Wang et al. 1997). Most of the markers comprising these maps were based on the random polymorphisms in the genome, including SSRs, RFLPs and AFLPs, and were not developed from sequences encoding for functional genes. A bin map has been recently developed that includes 126 SSR markers derived from melon expressed sequence tags (ESTs) (Fernandez-Silva et al. 2008) and 200 EST-based SNP markers (Deleu et al. 2009) resulting largely from non-fruit libraries. Quantitative trait loci (QTLs) for fruit traits, such as total soluble solids (TSS), fruit size and shape, climacteric ripening, netting, color and various metabolites, including β-carotene, sugars and organic acids have recently been identified in some melon maps (Cuevas et al. 2008, 2009; Eduardo et al. 2007; Monforte et al. 2004; Moreno et al. 2008; Obando-Ulloa et al. 2009; Paris et al. 2008; Park et al. 2009; Sinclair et al. 2006; Zalapa et al. 2007). A limited number of genes related to fruit development have also been mapped, particularly genes involved in ethylene biosynthesis and regulation (Moreno et al. 2008; Perin et al. 2002a, b) and carotenoid metabolism (Cuevas et al. 2008, 2009).

A more saturated melon map, including in particular candidate genes encoding for metabolic components of fruit quality, is necessary to discover possible links between genotype and phenotype and also to identify efficient markers for use in marker-assisted breeding. The International Cucurbit Genomics Initiative (ICuGI) (http://www.icugi.org/) currently includes approximately 35,000 ESTs, nearly one-third of which were derived from fruit libraries. This powerful database, formerly the melon EST Database (http://melon.bti.cornell.edu/), is an excellent source for candidate gene mining to map metabolic QTLs (Portnoy et al. 2008).

One of the metabolic pathways of particular interest in melon is that of sugar metabolism, since the sugar content is the primary determinant of fruit quality (Rosa 1928; Yamaguchi et al. 1977). Sucrose is the major sugar that accumulates during melon fruit ripening (Burger et al. 2002; Lingle and Dunlap 1987; Schaffer et al. 1996) and previous studies have clearly indicated that sucrose accumulation in melon is developmentally controlled by metabolism occurring in the fruit sink (Burger and Schaffer 2007; Hubbard et al. 1989; Schaffer et al. 1996).

The enzymes involved in sugar metabolism have been well studied (Burger and Schaffer 2007; Carmi et al. 2003; Dai et al. 2006; Gao et al. 1999; Gao and Schaffer 1999; Lingle and Dunlap 1987; Yu et al. 2008). Each of these enzymes' genes, especially the paralogous genes that compose the enzyme family and are expressed in melon fruit tissue, are obvious candidates for mapping together with the phenotypic traits of sugar content. To date, only one such gene encoding for a sucrose synthase has been mapped in melon (Deleu et al. 2009). However, the sugar-metabolism pathway is complex, with over 40 genes coding for it in melon (Dai et al. in preparation). In this study, we made a concerted effort to map an exhaustive compilation of these recently described melon genes representing the complete gene families coding for the enzymes of sugar metabolism.

We describe an enriched melon map based on a unique, publicly available, recombinant inbred (RI) population developed from a cross between representatives of the two subspecies of melon according to Pitrat et al. (2000): PI 414723 (*Cucumis melo* subsp. *agrestis* var. *momordica*) and 'Dulce' (*C. melo* subsp. *melo* var. *reticulatus*). These genotypes differ in numerous fruit quality traits, including flesh characters, such as sweetness, acidity and color, external rind characters, size, shape and firmness. This RI population is, therefore, an excellent tool for the mapping of fruit quality traits. We enriched the melon genetic map with ~700 markers, over two-thirds of which are codominant SSR, SNP and INDEL markers, and nearly 200 that are derived from fruit ESTs. Over 30 genes encoding for the pathway of sugar metabolism were mapped, together with QTLs for TSS, sucrose and glucose accumulation. The RI population enabled the identification of six QTLs for sugar content, each of which contributes to sucrose levels in an additive manner. Genes of the carotenoid pathway and QTLs of fruit color were also mapped. Finally, 29 bacterial artificial chromosomes (BACs) carrying fruit quality related genes distributed across all linkage groups were mapped, as a first step toward the generation of a physical map of the melon genome.

Materials and methods

Plant material

A recombinant inbred population (designated 414 × Dul) was developed from a cross between PI 414723-S_5 (*Cucumis melo* var. *momordica*), an Indian cooking melon line, and 'Dulce' (*C. melo* var. *reticulatus*), an American muskmelon (Danin-Poleg et al. 2002). The PI 414723 fruits were oval with a smooth rind, not firm, with pale-orange-colored acidic flesh that was not sweet. The 'Dulce' fruits were nearly round, firm with a heavily netted rind, and had dark orange-colored flesh that was sweet and lacked acidity. F_2 individuals ($n = 112$) originating from two F_1 plants were propagated by the single-seed descent method to create the population, consisting of 99 RI lines comprising a mix of F_6, F_7 and F_8 generations.

Evaluation of fruit traits

The two parents and their RI lines were grown in the open field in Newe Ya'ar, Israel, during the summers of 2006 and 2007, and in the open field in Davis, California, during the summer of 2008. Each line, in a complete randomized design, was represented by 12 and 10 plants in 2006 and 2007, respectively. In 2008, the experiment was designed in two random blocks of 16 plants each. The two parents were each grown in three replications of 10–12 plants, randomly distributed in the field. A single fruit per plant was harvested when the abscission layer developed.

The following fruit traits were evaluated and the phenotypes are illustrated in Fig. 1: (1) two rind netting traits: percentage of fruit covered by netting (NTC), scored between 1 (no cover, as in PI 414723) and 9 (100% cover, as in 'Dulce') and net density (NTD), scored between 1 (signifying very low density) and 9 (high density); (2) stripes (STR): rind stripes were scored between 1 (absence of stripes) and 9 (most conspicuous stripes). Stripe evaluation was performed on both young and mature fruits; (3) rind sutures (SUT) were scored in the mature fruit on a scale of 1 (no sutures, as in PI 414723) to 9 (100% sutures, as in 'Dulce'); (4) fruit firmness (FF) of the whole fruit, was measured using a penetrometer (Fruit Pressure Tester, McCormick, FT-327, Facchini, Italy) applied to the mid-portion of the intact fruit, with the results expressed in kilograms; (5) fruit weight (FW), length (FL) and width (FWI) were measured and shape (FSH) was calculated as the ratio between length and width; (6) TSSs and (7) pH of the flesh were measured by refractometer (Atago PAL-1, Atago, Japan) and pH meter (PH-03(II), ZD Instrument Corp., China), respectively, in juice squeezed from the fruit; (8) flesh color (FLC) was evaluated visually on a scale of 1 (white) to 9 (dark orange).

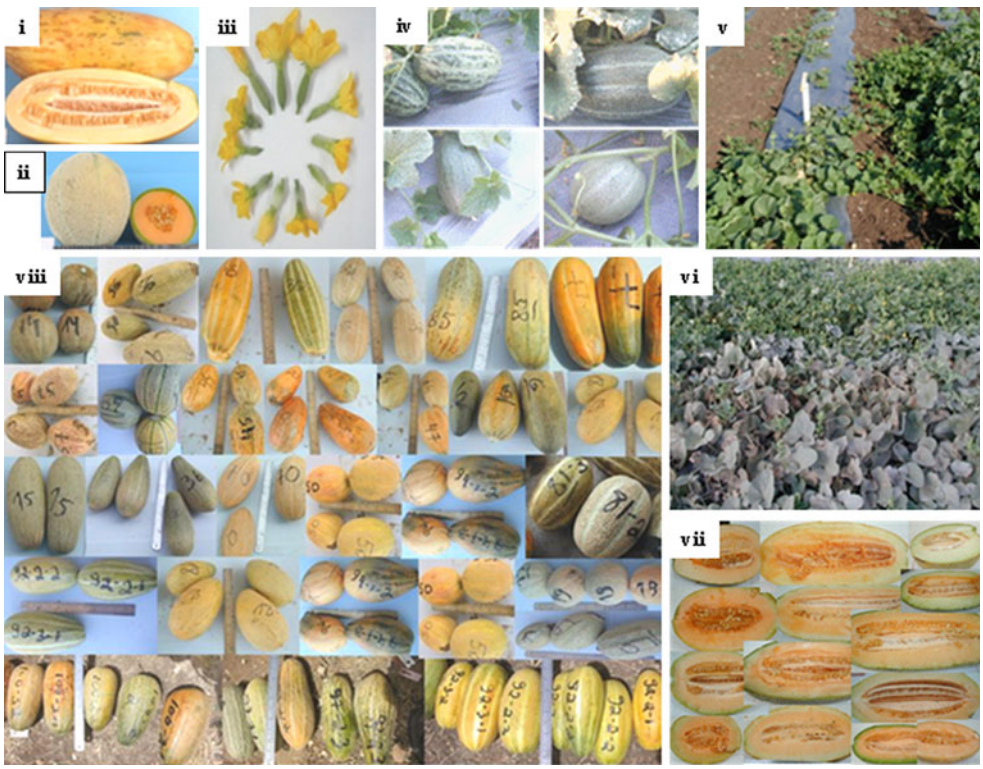


Fig. 1 Polymorphism of various characters in the 414 × Dul RI population and the parental lines. *i* PI 414723 mature fruit, *ii* 'Dulce' mature fruit, *iii* ovary size and shape of RI lines, *iv* young fruit rind: green color intensity, stripes and sutures in two RI lines, *v* growth habit, *vi* powdery mildew resistance, *vii* flesh color of ripe fruit of selected RI lines, *viii* mature fruit of selected RI lines: *each square* includes fruits from multiple plants of single RI lines, indicating high polymorphism between RI lines and homogeneity within them

Sugar analyses

Harvested fruits were brought to the laboratory and sampled as follows. Approximately, 1 g fresh weight of mesocarp tissue, taken from the center-equatorial portion of the fruit, was placed in 80% EtOH and stored at −20°C until extraction. The soluble sugars (glucose, fructose and sucrose) were extracted in hot 80% alcohol. Following further sample preparation, analysis was performed by HPLC using Aminex® Fast Carbohydrate Column (100 × 7.8 mm, BioRad Laboratories, Hercules, CA) as in Petreikov et al. (2006).

Carotenoid analysis

Mid-fruit section slices from three fruits were cut into small cubes and frozen in liquid nitrogen. Frozen samples were ground to a fine powder with an IKA-11 mill (IKA, Germany) in the presence of liquid nitrogen and approximately 0.5 g, accurately weighed, was extracted in 8 ml of hexane:acetone:ethanol (50:25:25, v/v), followed by 5 min saponification in 80% (w/v in methanol) KOH (Sigma). The saponified material was extracted twice with hexane. A Cary50Bio spectrophotometer (Varian, Palo Alto, CA) was utilized to measure carotenoid content at A_{450}. Quantification was based on a standard calibration of β-carotene authentic standard extracted under similar conditions. For the HPLC analysis, the hexane extractions were evaporated in a Savant SpeedVac apparatus (Savant, Holbrook, NY) and resuspended in 400 µl of acetonitrile:methanol:dichloromethane (45:5:50, v/v), passed through a 0.45-µm nylon filter (Intersep, Wokingham, UK) and kept at room temperature in the dark for no more than 24 h before analysis. A Waters HPLC equipped with Waters PDA detector 996, C18 Nova-Pak column ($250_{mm} \times 4.6_{mm}$ i.d., 60 Å, 4 µm), and a Nova-Pak Sentry Guard cartridge (Waters, Milford, MA) was utilized to fractionate carotenoids as described in Tadmor et al. (2005). Carotenoids were identified by comparison of retention time, UV–Vis spectra and co-injection spiking of authentic standards (Sigma) and were quantified utilizing standard curves of authentic standards, extracted under the same conditions, with MILLENIUM software (Waters). Carotenoid content was determined as µg/g frozen weight.

DNA preparation

DNA was extracted from young leaf tissue from ten plants of each of the parental lines and ten plants of each of the RI lines according to the preparation procedure described by Fulton et al. (1995).

Scoring for ZYMV resistance

Zucchini yellow mosaic virus (ZYMV) resistance was evaluated as reported previously (Danin-Poleg et al. 2002) with minor modifications. A non-aphid-transmissible ZYMV isolate (ZYMV-NAT) was used for inoculation. The isolate was propagated in *Cucurbita pepo* L. 'Ma'yan' (Hazera Genetics Ltd., Berurim, Israel). Plants were grown in a chamber under controlled conditions of 29/20°C day/night. Seedlings (n = 30–90) from each RI and from the parental lines were inoculated twice with ZYMV-NAT inoculum: 10 days after sowing and 2 days later. Plants were visually scored as resistant or susceptible 21 days after the first inoculation. Plant symptoms were evaluated relative to the response of the parental lines in each trial. Plants were classified as resistant if they were symptomless or had systemic chlorotic spots as in PI 414723. In some trials, the resistant plants had very severe symptoms indicative of a hypersensitive response. Plants were classified as susceptible if they had mosaic symptoms or leaf distortion and/or stunting, as in the susceptible parent 'Dulce'.

Statistical analysis

Means, standard deviations, trait distribution, pairwise correlation, ANOVA and Welch ANOVA analyses were conducted with the JMP program, v. 7.0 (SAS Institute Inc., Cary, NC).

Genotype × environment interaction was determined by ANOVA for each fruit trait. In addition, pairwise correlation coefficients were calculated between growing seasons according to Yan and Rajcan (2003).

Marker analysis and map construction

SSR markers

SSRs were identified within the ICuGI database (http://www.icugi.org/) and by searching sequenced genomic and cDNA clones for repetitive sequences using SPUTNIK (http://espressosoftware.com/sputnik/index.html) and ad hoc PERL scripts. Altogether, 386 SSRs were mapped: 113 were newly developed; 143 were previously published (Supplemental Table S1) and 130 markers were kindly contributed by Syngenta Seeds S.A.S. (designated Sys markers). Primer sequences for the newly developed SSRs are provided in Table 1 and Supplemental Table S2.

SSR analysis, primer selection and primer synthesis were conducted as previously described (Danin-Poleg et al. 2001; Katzir et al. 1996) with minor modifications. The PCR mixture for SSR amplification contained: 30-ng plant genomic DNA, 1.1 mM Mg^{2+}, 8–10 pmol primers, 0.2 mM

Table 1 List of polymorphisms and linkage group associations of sugar- and carotenoid-metabolism genes of melon

Gene/enzyme name	Code	ICuGI accession no.	Primers	Polymorphism	Detection method	Linkage group
Sugar-metabolism genes						
Acid α-galactosidase 1	*CmAAG1*	MU9934	F-ACGTTGGATGTGCAAAGGTGTGTGACAAAG R-ACGTTGGATGATTTGGGACACATTTTTTC EXT-GTGTGACAAAGTTTGAAATG	GAAATG[T/A]AAGTTC	Sequenom	LG10
Acid α-galactosidase 2	*CmAAG2*	MU9937	F-ACGTTGGATGCTCAACTCGTGGGTTGAGTG R-ACGTTGGATGCAATGGAGCCTTCGTATATG EXT-GTGGGTTGAGTGTATAATC	AGCACA[A/T]GATTAT	Sequenom	LG10
Alkaline α-galactosidase 1	*CmNAG1*	MU10936	F-CTGATCAGATCGGTAGTCGC R-CTGTAAAATCAATCAAACACACC	INDEL (deletion of 9 bp in PI 414723)	Genetic analyzer 3130*xl*	LG7
Alkaline α-galactosidase 2	*CmNAG2*	MU8249	F-ACGTTGGATGAACCCTCTTGGTAGGATTTC R-ACGTTGGATGCTTCGCCAGCTATTATATAG EXT-GCAACGACAAATGGCAAGTGATTG	TGATTG[A/G]AATTTA	Sequenom	LG8
Galactokinase 1	*CmGK*	MU7673	F-ACGTTGGATGGTTCCTCCTTCAACAGTTCC R-ACGTTGGATGTTCTGGGCTTATGAACCTTC EXT-AGACAGAGCAATAAGAAAATAC	AAATAC[C/T]GATAAC	Sequenom	LG6
UDPglu/Gal Ppase	*CmUGGP*	MU8498	F-ACGTTGGATGCTTGTGAAAATGGAAGCAGAG R-ACGTTGGATGGCTAGAGTGATGGCAATTAG EXT-TGGAAGCAGAGTTGGAC	TTGGAC[A/G]CTACAT	Sequenom	LG3
Galactose-1-P uridyltransferase	*CmUT*	MU6607	F-ACGTTGGATGAAAATCCTAACGCACCCCAC R-ACGTTGGATGGGGTTTTGAGACGATTTTCC EXT-CGTGTTCGACGAAATGAA	AATGAA[A/G]ATGACA	Sequenom	LG6
UDPglucose pyrophosphorylase	*CmUGP*	MU4025	F-ACGTTGGATGGCCGTTTGACAGGTTGTTAC R-ACGTTGGATGTTTCGCCAGTTTTTTCCCAC EXT-GTTGTTACATATCCGTGG	CCGTGG[G/A]GGCCCA	Sequenom	LG6
UDPglu epimerase 1	*CmUGE1*	MU4888	F-ACGTTGGATGCATTAGGATGTACTCAGGGC R-ACGTTGGATGTGCTCAGTTTTTGAGTGGTG EXT-CAAAAAATAACTGCATCCTATC	CCTATC[G/A]AAGTAG	Sequenom	LG9
UDPglu epimerase 2	*CmUGE2*	MU7372	F-ACGTTGGATGCAGTTCCTATGAACTTGTTTG R -ACGTTGGATGCCAGCAAAATGAATAACAGC EXT-TTGATTCTGTTTTGAGTTGAT	GTTGAT[G/T]TTTGTG	Sequenom	LG9
UDPglu epimerase 3	*CmUGE3*	MU10578	F-ACGTTGGATGCTTAACATGCCTTCTGCCTG R-ACGTTGGATGCTCGGCATCTTTCTTACCAA EXT-TGCCTGAAATTTAGACATC	TCGATT[G/C]GATGTC	Sequenom	LG7
Phosphoglucomutase (cytosolic)	*CmPGMc*	MU7548, MU17272	F-ACGTTGGATGGAAAATCGTCTGATAAGTT R-ACGTTGGATGCAACACACAAGATAAAATAC EXT-AATCGTCTGATAAGTTTAATTGC	ACTATT[A/G]GCAATT	Sequenom	LG9
Sucrose-phosphate synthase 1	*CmSPS1*	MU9726, MU9939, MU4702	F-ACGTTGGATGTTCACGAAAATCAGTTGCCG R-ACGTTGGATGTTTCTCCTCCTCAATGCTGG EXT-GGGAGCTGATCAAAATTCTAATGTT	AATGTT[G/A]GCAAGT	Sequenom	LG2

Table 1 continued

Gene/enzyme name	Code	ICuGI accession no.	Primers	Polymorphism	Detection method	Linkage group
Sucrose synthase 1	*CmSUS1*	MU5261	F-CCTGCAACATCATGAACTG R-CTCTCTCTAGGCCAATCTCC	TATTAA[G/A]CTTTAC	dCAP—*Hin*dIII	LG2
Sucrose synthase 2	*CmSUS2*	MU10266, MU10761	F-ACGTTGGATGTGTGGATTTCCTGGATGTGG R-ACGTTGGATGGTCACAAGGATTTTCAATGGC EXT-TCGACCACGATCGGGAC	GAAAAC[C/T]GTCCCG	Sequenom	LG9
Sucrose synthase 3	*CmSUS3*	MU11554	F-CCAGACTGAGGGAACTAGC R-CGTCTCTCCCCCAAGTTG	CGAGAC[A/T]CGTCGA	dCAP—*Taq*1	LG7
Hexokinase 1	*CmHK1*	MU8995, MU9178	F-AACAAGTAATGAGATGACAAAC R-CCCTTTATTTCAATCGTTAG	INDEL (deletion of 11 bp in Dulce)	Agarose gel	LG2
Hexokinase 2	*CmHK2*	FJ231523[d]	F-ACGTTGGATGCCTTCAAATGATGACGAAGC R-ACGTTGGATGCTAGGGAAGAATTGCATGAG EXT-TGTGCATTTTGCGATTT	CGATTT[T/G]TAAAGT	Sequenom	LG5
Fructokinase 1[a]	*CmFK1*	MU7625	F-CATGTTACTTCAGGAATCTG R-GACTGATAGAACCATAATGG	ATCCA[T/C]ACAAA	SNP by Sequence	LG6
Sucrose transporter 1	*CmSUT1*	MU4744	F-ACGTTGGATGATCTTTTTGACCCTACAGCC R-ACGTTGGATGTCTGTGCTGCTAGCATTGTC EXT-CCCTACAGCCTACTGAT	ACTGAT[A/G]TTAAAT	Sequenom	LG2
Sucrose transporter 2	*CmSUT2*	MU7073	F-ACGTTGGATGCACAGTTTTTCCTCTTCGCC R-ACGTTGGATGGCAGCGATAGTACAACTGAG EXT-TCTAAGCCCAATAGTTTGA	GTTTGA[C/T]TATCTT	Sequenom	LG9
Sucrose transporter 4[a]	*CmSUT4*	MU9727	F-CAAGAATAT CTAAGTCAACGAACA T R-ATGTGTATGTGTACCGAGCATTGAATC	SSR (AT)24	Genetic analyzer 3130*xl*	LG3
Neutral invertase 1	*CmNIN1*	MU9938, MU18322	F-ACGTTGGATGTATTCTGCCTTACGCTGCTC R-ACGTTGGATGGAAGGAGAGTGCACTAAGTC EXT-GCTGCTCCAGAGAGATGCTGAT	ATTAAC[A/C]ATCAGC	Sequenom	LG6
Neutral invertase 2	*CmNIN2*	MU5047	F-ACGTTGGATGGTTGCTTGCCTGGTTTTTCG R-ACGTTGGATGTAGAAACAACGGCTTGAGGG EXT-TCCATCGTTGTATTCCTCTTTTT	CTTTTT[A/C]TTTTT	Sequenom	LG10
Acid invertase 1	*CmAIN1*	FJ231519[d]	F-ACGTTGGATGTGACCCCGGACGCAATATCT R-ACGTTGGATGCCAACGTGAATTTTGTTGCC EXT-ACTGGGACCTTAAGAAC	GACTTT[C/T]GTTCTT	Sequenom	LG9
Acid invertase 2	*CmAIN2*	MU9591	F-ACGTTGGATGTCCCGCAACTTCACTACAAC R-ACGTTGGATGTTCGGGAGATGAGGTGTTAG EXT-TCCTTCTCGTTTCTTTA	GAGCAA[A/G]TAAAGA	Sequenom	LG9
Hexose transporter	*CmHTR1*	MU7247	F-ACGTTGGATGTTTCCGGCATCAACGTCATC R-ACGTTGGATGTTAAGGACGCGTCGGATTTG EXT-CATCATGTTCTACGCTCCTGT	TCCTGT[G/A]TTGTTC	Sequenom	LG8

Table 1 continued

Gene/enzyme name	Code	ICuGI accession no.	Primers	Polymorphism	Detection method	Linkage group
Hexose transporter	*CmHTR2*	MU11210, MU11325	F-ACGTTGGATGCCGCCTACAGAAAAACTGTG R-ACGTTGGATGCAAGCATACTCGCTAGCAAC EXT-CTGTGTTAGCTAGAATTTGATAA	CTGTTTA[T/C]TTATCA	Sequenom	LG3
Sugar isomerase	*CmSUI*	MU10474	F-ACGTTGGATGCAACCAGAGAATGGTTCGTC R-ACGTTGGATGGATTCCTCATCGTCCGCCAT EXT-CGCCGATGTGATTTGCT	GGAACG[A/T]AGCAAA	Sequenom	LG6
Sucrose transporter	*CmSUT6*	MU10398	F-ACGTTGGATGCCCTCTATCAATGCCATTGC R-ACGTTGGATGCGGTCAACGATTTTGTTGTC EXT-GCTTGATGAATAAACCACAC	CCACAC[C/G]CCTTGT	Sequenom	LG11
Phosphofructokinase	*CmPFK1*	MU3712	F-GCTGGGGCTAATGCAATAC R-CCCAGAAGCATCGGTAGC	CTGCTGT[T/C]GAAGAA	dCAP—*Taq*1	LG7
Carotenoid-metabolism genes						
Phytoene desaturase[b]	*CmPDS*	MU9070	F-AAACCTACTGCGACCAAC R-TGCAACAAAAAAATAGTTAG	INDEL (deletion of 30 bp in PI 414723)	Agarose gel	LG7
Phytoene synthase[b]	*CmPSY1*	MU10942	F-ACGTTGGATGATGACGAAAGAAACCGACCC R-ACGTTGGATGAGGGCAGAAAATGTGAAAGC EXT-CCCAAACCGACCCTTTTCCACTT	CCACTT[A/G]CCCCTC	Sequenom	LG9
Phytoene synthase 3[a]	*CmPSY3*	MU16943	F-TTGATGAGCTTTACCTTTATTGC R-AAGGCTATGGGAAGTGATAACAG	TTGAA[G/T]ATAAG	dCAP—*Mbo*II	LG7
Phytoene synthase 4	*CmPSY4*	MU14339	F-AAACCTACTGCGACCAAC R-TGCAACAAAAAAATAGTTAG	INDEL (deletion of 80 bp in PI 414723)	Agarose gel	LG5
Carotenoid isomerase[c]	*CmCRITSO*	MU4592	F-ACGTTGGATGTGCTATCAGCGCCTTCTTTG R-ACGTTGGATGTCCTGTTTTTCACACTCCAC EXT-AGCGCCTTCTTTGCTTCGTA	GAAGA[G/A]TACGAA	Sequenom	LG4
Lycopene β-cyclase[b]	*CmBCYC*	MU9517	F-ACGTTGGATGTTGGTTTCAGGAACAAGCTC R-ACGTTGGATGAGGGTCGTCTGAAATTGAGG EXT-AACAAGCTCCAAAAGAGCACTA	GCACTA[T/C]TTCTCAC	Sequenom	LG8
ζ-Carotene desaturase[b]	*CmZDS*	MU4107, MU13961	F-ACGTTGGATGTGTGGCAACATTGTTGTCTC R-ACGTTGGATGAATCCTCCAATCTTGAAGAC EXT-GTCTCTTCTTTGCCTCT	CCTCT[A/G]GAGCTA	Sequenom	LG2
Geranylgeranyl hydrogenase	*CmGGPR*	MU7586	F-TGAGATTGCTTCAACTATGTG R-GGAAGTGTTTTGGACGTTG	INDEL (deletion of 11 bp in Dulce)	Agarose gel	LG2
Geranylgeranyl pyrophosphate synthase	*CmGGPS*	MU9935	F-ACGTTGGATGAGAAACAGAGGATTGGATGG R-ACGTTGGATGGGAACGTACTGAAGAACCC EXT-TGGATGACGTTGCCAAC	GCCAAC[C/A]CCCATT	Sequenom	Un linked group

Table 1 continued

Gene/enzyme name	Code	ICuGI accession no.	Primers	Polymorphism	Detection method	Linkage group
β-Carotene hydroxylase[b]	*CmCRTR*	MU9511	F-ACGTTGGATGTCTCAGATGACAAAAACAG R-ACGTTGGATGTCTGGCACTCTTCCTTATGG EXT-AGTATATAGTGAAGAGGGATT	GGGATT[A/C]GAAACC	Sequenom	LG6
Carotenoid cleavage dioxygenase	*CmCCD*	MU5659	F-ACGTTGGATGCCGCCATATGTTACCTACAG R-ACGTTGGATGTCATGCATCATGACAGGAGC EXT-ACATGACCCAGTACCAATCAC	AATCAC[A/G]ATTCCA	Sequenom	LG1

Accession numbers are available at http://www.icugi.com

[a] Mapped on F2 population

[b] Mapped previously (Cuevas et al. 2008, 2009)

[c] Sequences mapped through BAC end sequencing

[d] Accession number from NCBI

dNTPs, 1× *Taq* buffer and 1 unit of *Taq* polymerase (Sigma-Aldrich, St Louis, MO) in a total volume of 25 µl. SSR amplification products were separated on either a 2.5% agarose gel or by fluorescence labeling using a 3130*xl* Genetic Analyzer (Applied Biosystems, Foster City, CA).

SNP markers

SNP mining of the ICuGI database (http://www.icugi.org/) was performed by two methods. (1) Selected EST sequences of various melon genotypes present in the ICuGI database were compared in silico. Putative SNPs were validated by amplification and re-sequencing of genomic DNA from PI 414723 and 'Dulce'. (2) For candidate genes of interest, where polymorphism was not detected within the database, genomic DNA from PI 414723 and 'Dulce' was amplified with primers designed according to the ICuGI database. Preliminary screening for polymorphism of the amplicons was performed by either the denaturing gradient gel electrophoresis (DGGE) technique of single-strand conformational polymorphism (SSCP) (BioRad Laboratories) or denaturing high-performance liquid chromatography (DHPLC), performed at the Galilee Technology Center, Migal (Qiryat-Shmona, Israel). Amplicons of the genomic DNA were sequenced by Macrogen Inc. (Seoul, South Korea sequences) and then aligned and screened for polymorphism using the DNAMAN program (version 4.2; Lynnon Corp. Vaudreuil-Dorion, QC, Canada).

Altogether 76 newly developed SNP markers and six INDEL markers were mapped (Supplemental Table S1). Primer sequences for the newly developed SNPs and INDELs are provided in Table 1 and Supplemental Table S3.

SNP genotyping was carried out by two methods: dCAP (dCAPS Finder 2.0) (Neff et al. 2002) or the Sequenom (San Diego, CA) MassARRAY Compact Analyzer, at the Cancer Research Center and Pediatric Hematology-Oncology, Sheba Medical Center, Tel Hashomer, Israel. Assays and primers were designed using the MassARRAY assay Design 2 software. Sequenom assay was performed as described by Zilberman et al. (2009). PCR amplifications were carried out in standard 384 well plates, in a 5 µl final volume containing 20 ng of template DNA, 0.1 U of *Taq* polymerase (HotStarTaq; Qiagen, Valencia, CA), 0.2 mM of each dNTP, 200 nmol of each primer, 1 mM $MgCl_2$ and 1× HotStar buffer. Specific primers were used for each gene. PCR thermal cycling was carried out in an ABI-9700 instrument under the following conditions: 15 min at 95°C, followed by four cycles of 20 s at 95°C, 30 s at 65°C and 60 s at 72°C, four cycles of 20 s at 95°C, 30 s at 58°C and 60 s at 72°C, and 38 cycles of 20 s at 95°C, 30 s at 53°C and 60 s at 72°C. To remove the non-incorporated dNTPs, PCR products were incubated with Shrimp alkaline

phosphatase (SAP) (0.3 U, in a total volume of 7 µl; 20 min at 37°C and 5 min at 85°C). The MassEXTEND (Sequenom) assay was then conducted in a final volume of 9 µl containing 1 µM extension primer, 0.2 µl termination mix (50 nM each of ddA, ddG, ddT and ddC) and 1.25 U ThermoSequenase (Sequenom) in 0.22× PCR buffer. The cycling conditions were: incubation for 2 min at 94°C followed by 99 cycles of 5 s at 94°C, 5 s at 52°C and 5 s at 72°C. Following this step, 3 µg MassEXTEND cleanup resin (Sequenom) and 16 µl double-distilled water were added to remove extraneous salts. A Samsung nanodispenser was used to apply 15 nl of the extension products from each well of the sample plate onto the SpectroChips (Sequenom). Mass spectra were recorded on a Bruker Biflex MALDI-TOF mass spectrometer (Bremen, Germany) operated in linear mode, and were finally analyzed by MassARRAY Typer software (Sequenom). Analyzer accuracy, as reported by the manufacturer, varies between 97 and 99%.

Three of the markers (*CmFK1*, *CmSUT4 and CmPSY3*) were mapped in the F_2 population and their suggested location on the RI map was based on anchor markers.

AFLP analysis

The AFLP procedure was performed as described previously (Vos et al. 1995; Vuylsteke et al. 2007). Briefly, total genomic DNA was extracted and double-digested with *Eco*RI and *Mse*I. Appropriate linkers were ligated to the digested genomic DNA and two PCR reactions with increasing selectivity were performed. In all, 99 RI lines from the 414 × Dul RI population were analyzed using 20 *Eco*RI–*Mse*I primer combinations. Gel images were electronically scanned and markers scored using proprietary technology developed at Keygene N.V. (Wageningen, The Netherlands). When possible, relative band intensities of individual AFLP markers were used to attribute homozygous and heterozygous scores to individual AFLP markers.

Sugar-metabolism genes

Genes belonging to the sucrose metabolism pathway were selected based on the study of Dai et al. (in preparation) and polymorphisms were identified based on the comparative sequencing of the parental lines. Information related to these genes is summarized in Table 1.

Mapping of selected BACs

To identify specific BACs that contain fruit genes of interest, specific probes were designed for hybridization with the melon BAC library (MR1, Clemson University, Clemson, SC). Standard hybridization procedures were used with a radioactively labeled probe on BAC library membranes (Luo et al. 2001). Positive BACs were rechecked by PCR using the primers that had been used to prepare the probes. Representative PCR products were further sequenced to validate the results.

Map construction and QTL analysis

Mapping was performed using JoinMap® 3.0 software (Van Ooijen and Voorrips 2001). Markers were grouped at a minimum LOD score of 4.0 and a recombination frequency value of 0.4. JoinMap® 3.0 software uses Kosambi mapping functions to translate recombination frequency into map distance. Linkage group (LG) designation was according to Perin et al. (2002b). This marking system was selected as the system for the merged melon map of the ICuGI that is currently in preparation (Monforte et al. personal communication), which includes the 414 × Dul map. Shared markers from other melon genetic maps (Danin-Poleg et al. 2002; Gonzalo et al. 2005; Moreno et al. 2008; Perin et al. 2002b) were used to unite the minor linkage groups into the 12 known melon linkage groups.

Using the MapQTL® 5 software (Van Ooijen 2004), QTLs and their significance were calculated using interval mapping (IM), multiple QTL model (MQM) and permutation analysis. The QTL threshold base in the permutation analysis (1,000 permutation at $p = 0.05$) ranged between 2.0 and 2.3 for the various traits. A QTL was determined as significant if its LOD score was higher than the calculated threshold. However, for the most part, a stricter threshold of 3.0 was used, except for several cases which are discussed further on.

Results

Development and characterization of a RI population

An RI population was developed from a cross between PI 414723-S_5 (*Cucumis melo* var. *momordica*) and cv. 'Dulce' (*C. melo* var. *reticulatus*). This highly polymorphic population included 99 lines comprised 84 F_8, 13 F_7 and 2 F_6 families. The population was polymorphic in many characteristics (Fig. 1), including growth rate, leaf structure, ovary size, pathogen resistances and fruit traits. It was also polymorphic with respect to markers, with 113 polymorphic SSRs (55%) out of the 203 newly developed markers that were tested here. AFLP polymorphism was estimated at 20–25%.

The population was genotyped using 668 molecular markers described below. The distribution of the two parental genotypes within the RI lines was almost equal: the average RI line was homozygous for the PI 414723 allele (A) at 43.3% of the loci and homozygous for the 'Dulce'

allele (B) at 44.4% of them. The average RI line was heterozygous for 5.3% of the loci, which is higher than the expected value of 1.6%. The bias was contributed mainly by 10% of the RI lines, with six RI lines heterozygous for more than 20% of the loci (ca. 140 markers).

Development of an enriched linkage map

The 668 DNA markers used to construct the map (Fig. 2) comprised 386 SSRs, 200 AFLPs, 76 SNPs and six INDELs (Supplemental Tables S1, S4). All the SNPs, INDELs and 113 of the SSRs were newly developed markers. In addition, three monogenic characters were mapped: monoecious trait (*a*) was mapped to cM 9 at LG2; ZYMV resistance (*zymv*) was mapped to cM 4 at LG2; and pH of fruit flesh (*pH*) was mapped to cM 95 at LG8, all of which have been previously mapped using the 414 × Dul F_2 population (Danin-Poleg et al. 2002) from which the RI population described here was developed.

To enrich the map with genes related to fruit quality, we also mined the public database of melon ESTs (ICuGI) and developed markers for 161 ESTs expressed during fruit development (73 SNPs, 82 SSRs and six INDELs). One-third (33%) of the polymorphic ESTs were for genes involved in the metabolism of fruit quality components such as sugars (31 genes), carotenoids (11 genes), volatiles (five genes) and ethylene synthesis and regulation (six genes). Altogether, 131 SSRs, as well as most of the SNPs and INDELs were developed from ESTs (Supplemental Table S1).

The 414 × Dul RI genetic map covers 1,222 cM and is divided into 12 major and 7 minor linkage groups, with an average of 2.67 cM between markers. The largest linkage group (LG6) covers 131 cM. Using shared markers from other melon genetic maps, we merged the minor linkage groups into the 12 known melon linkage groups (Fig. 2; Supplemental Table S5). To relate the recombination map to a physical map, 29 of the markers, which were spread over all linkage groups, were used to isolate melon BACs. These BACs were positioned on the map according to their markers (Fig. 2; Supplemental Table S6).

Variability in fruit phenotypes in the RI population

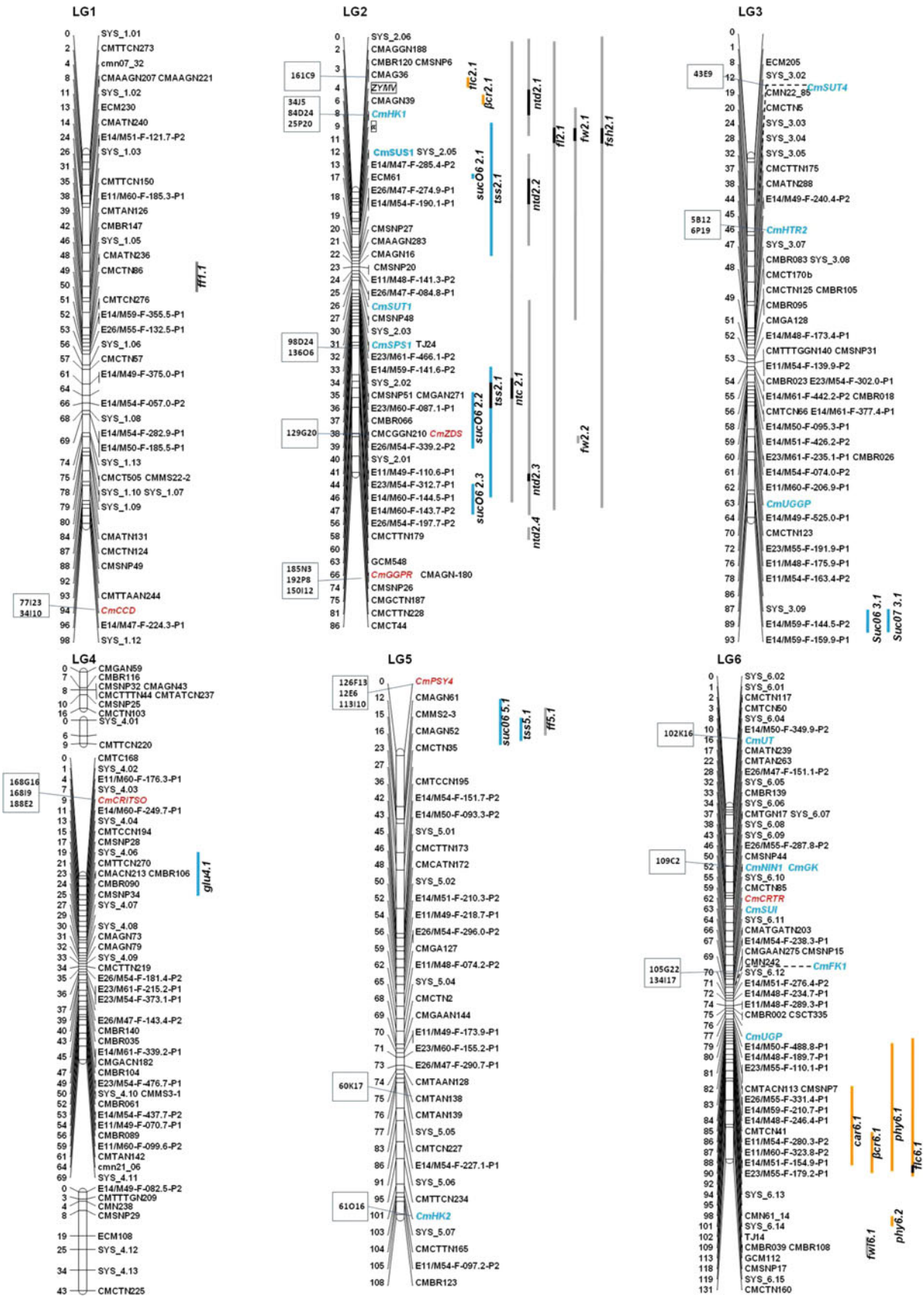
The RI population was used to characterize and map variation in fruit qualities such as shape, size and firmness. The RI lines, as well as the parental lines, were grown during three growing seasons at two different locations (Newe Ya'ar, Israel, during the summers of 2006 and 2007 and Davis, CA, USA during the summer of 2008), to enable accurate evaluation of quantitative traits. The trial effect was not significant according to ANOVA test ($P \geq 0.01$) for nine fruit traits: length, weight, width, stripes, sutures, net cover, net density, flesh color and pH, indicating low genotype × environment interaction. Growing season influence was significant according to ANOVA test ($P \leq 0.01$) for firmness, TSS and the individual sugar levels. Mean, minimum and maximum values of the fruit traits for the parents and the RI population are presented in Table 2. Correlation coefficients between the seasons, for the nine traits with no significant trial effect, were higher than 0.8 (Supplemental Table S7). Firmness, TSS and sugar levels showed somewhat lower correlation coefficients (<0.7), suggesting a higher environmental effect for them.

Significant variability was observed for all of the traits between families as indicated by Welch ANOVA test. The distribution mode of net density, fruit weight, fruit length, fruit width, flesh color and TSS among the RI population was normal according to the Shapiro–Wilk goodness-of-fit test. Transgressive segregation was detected for fruit size and flesh color: phenotypes with higher values than the parents for size were found in 24 families and phenotypes with lower values for flesh color in two families.

Identification of QTLs for fruit size, rind characters and fruit firmness

Trait evaluation was repeated for the three growing seasons. Although the ANOVA test showed significant differences between the growing seasons in the case of fruit firmness, QTL analyses of fruit size, rind characters and fruit firmness for each season were similar (Supplemental Table S8). Therefore, QTLs were calculated for means of traits over the three growing seasons to facilitate the construction of a clear reiterated map, with the exception of a

Fig. 2 Genetic map of the 414 × Dul RI population. Linkage group (LG) numbers are according to Perin et al. (2002b). Distances in centimorgans are marked on the *left side* and marker names are on the *right side* of each linkage group. Markers for sugar and carotenoid-metabolism genes are in *blue* and *orange*, respectively. Markers that were mapped in the F_2 population are connected by *dotted lines* according to suggested location based on anchor markers. Significant QTLs (by interval mapping analysis with LOD > 3 or by permutation test) are represented by lines and were calculated for means of fruit traits. *TSS* flesh color (*flc*), net cover (*ntc*), length (*fl*) and weight (*fw*) averages were calculated for three growing seasons. Shape (*fsh*), width (*fwi*), stripes (*str*), sutures (*sut*) and firmness (*ff*) averages were calculated for two growing seasons. QTLs that were calculated for specific growing season are: sucrose content (*suc06*) analyzed in 2006, sucrose content analyzed in 2007 (*suc07*), total carotenoids (*cr*) analyzed in 2006 by spectrophotometer, *β*-carotene (*βcr*), phytoene (*phyt*), α-carotene (*αcr*) and lutein (*lut*) analyzed in 2007 by HPLC, fruit weight in 2006 (*fw06*), net cover in 2008 (*ntc08*), rind stripes (*str06*) and sutures (*sut06*) in 2006. For QTLs with LOD > 5.0, LOD scores in the highest 10% are indicated by *dark black lines* within the broader QTL. *Rectangles* on the *left* of the linkage groups include BACs harboring the adjacent mapped markers. Three monogenic traits: ZYMV resistance (*zymv*), monoecious (α) and fruit acidity (*pH*) are framed. Sugar QTLs are indicated in *blue*, carotenoid QTLs are in *orange*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E14/M50-F-185.5-P1
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192P8
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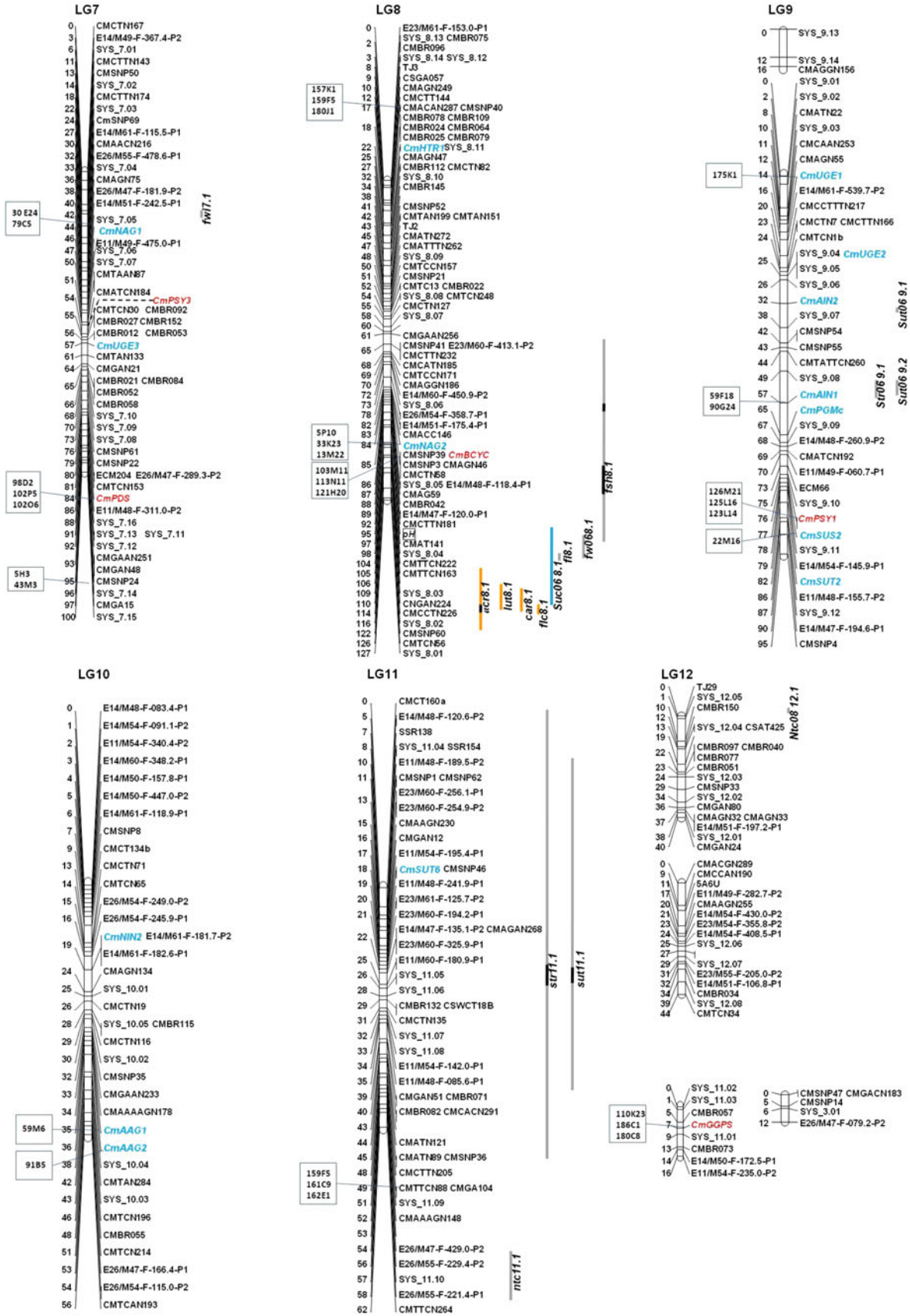

Fig. 2 continued

Table 2 Phenotypic data of the parental accessions and the RI population for nine traits

Trait	Years of trial	'Dulce', mean ± SD	PI 414723, mean ± SD	RI, mean ± SD	RI, max. ± SD	RI, min. ± SD
Net cover (1–9)	2006–2008	9.0 ± 0.0	1.3 ± 0.4	6.84 ± 2.5	9.0 ± .41	1.0 ± 0.0
Net density (1–9)	2006–2008	9.0 ± 0.0	1.3 ± 0.2	5.0 ± 1.9	9.0 ± 3.5	1.0 ± 0.0
Stripes (1–9)	2006–2007	8.8 ± 0.2	1.0 ± 0.1	4.8 ± 3.1	9.0 ± 4.0	1.0 ± 0.0
Sutures (1–9)	2006–2007	8.8 ± 0.2	1.1 ± 0.1	4.8 ± 2.8	9.0 ± 3.8	1.0 ± 0.0
Weight (kg)	2006–2008	1.0 ± 0.2	1.7 ± 0.1	1.4 ± 0.6	3.9 ± 1.0	0.4 ± 0.0
Length (cm)	2006–2008	12.9 ± 1.0	29.8 ± 0.6	20.4 ± 5.8	37.4 ± .4	8.8 ± 0.4
Width (cm)	2006–2007	12.2 ± 0.3	11.6 ± 0.5	11.8 ± 1.4	17.7 ± .3	8.8 ± 0.2
Flesh pH	2006–2008	6.2 ± 0.2	4.8 ± 0.2	5.4 ± 0.5	6.5 ± 0.9	4.0 ± 0.0
Firmness (kg)	2006	11.0 ± 1.8	4.0 ± 0.9	7.2 ± 2.3	11.9 ± 4.02	3.0 ± 0.3
	2007	11.7 ± 0.8	5.6 ± 1.3	9.4 ± 1.8	12.0 ± .1	4.9 ± 0

Means were averaged across years and locations (2006 and 2007 in Israel; 2008 in USA), except for firmness for which growing season influence was significant

few traits, as described below. Interval mapping analysis resulted in a total of 23 significant QTLs for nine traits: the external rind characters of net cover, net density, sutures and stripes, the fruit size characters of width, length shape and weight and fruit firmness. The number of QTLs ranged from 1 to 4 per trait (Fig. 2; Table 3). The most significant QTLs were for rind stripes (*str11.1*) with LOD > 15, fruit length *(fl2.1)* with LOD > 11 and rind netting coverage (*ntc2.1*) with LOD > 11.

Fruit size, as measured by weight, is influenced by its length and width. In the 414 × Dul RI population, both traits correlated equally with fruit size ($R^2 = 0.84$ for each with weight). However, while QTLs for length and weight co-segregated on LG2 with high LOD scores, there were no QTLs for width co-segregating with weight. Overlapping QTLs were observed on LG2: a QTL for length (*fl2.1*) with LOD = 11.8 and at the same position, a QTL for weight (*fw2.1*) with LOD = 8.6. Both of these traits had smaller additional QTLs on LG8, although the LG8 QTL for weight was only significant in the 2006 trial (Table 3; Supplemental Table S8). The QTLs for width mapped to different linkage groups, LG6 and LG7, with lower LOD values and no co-segregating QTLs for fruit size at these positions were detected. This suggested that fruit size in this population is determined predominantly by fruit length, which was itself correlated with fruit width. Two QTLs for fruit shape, calculated as the relation between fruit length and fruit width, were highly significant at LG2 and LG8 (Table 3), at the same positions as the QTLs for fruit length.

The major QTL for stripes (*str11.1*) in the RI population was localized to LG11 based on an evaluation of mature fruits. The relation between stripes and sutures in this population was quite close, because the correlation between them was high (0.87) and the QTLs for both traits, rind stripes (*str11.1*) with LOD > 15 and rind sutures (*sut11.1*) with LOD > 9, colocalized to a region on LG11. Verification of the phenotyping of each family for stripes was based on evaluations of young fruits, prior to the development of netting cover, to clearly distinguish between stripes and sutures.

Netting was estimated by two parameters: the proportion of the fruit that was covered by netting and the density of the net. These features were highly and positively correlated (0.8) and presented overlapping QTLs in LG2. There was one major QTL for net cover (*ntc2.1*) with LOD > 11 and three QTLs for net density (*ntd2.1*, LOD = 5; *ntd2.2*, LOD = 5; *ntd2.3*, LOD = 9), which were located along the net cover QTL. Despite the high correlation between the two traits, in four of the families, the fruit was almost entirely covered, but the net was very sparse. Additional minor QTLs for net cover were detected on LG11 (*ntc11.1*, LOD = 3.5) and LG12 (*ntc0812.1*, LOD = 3.5). The QTL on LG12 was identified only in the US trial in 2008.

Two significant QTLs were identified for fruit firmness, one at LG1 (*ff1.1*) and one at LG5 (*ff5.1*), with LOD of ~3.3 in both loci. The 'Dulce' alleles were associated with firm fruit at both QTLs, correlated with the parental phenotype.

IM analysis identified several QTLs that were extended along LG2: length, weight, netting and TSS. To further study these QTLs, MQM analysis was performed. The results showed a single QTL for weight and length at the same (LG2 cM 9–11) location; however, there was an additional QTL for length on this linkage group (cM 38). Interestingly, MQM analysis identified three QTLs for net cover on this linkage group (LG2 cM 33, cM 63–65 and cM 68–74), the major one (cM 33) overlapping the QTLs for net density and TSS. The other two had lower LOD scores and one of them (cM 68–74) had an opposite additive effect, in which

Table 3 Significant QTLs for fruit traits identified by interval mapping analysis

QTL name[a]	Trait	Linkage group	Position of max. LOD (cM)	Position (cM)[b]	Max. LOD[c]	Max. A[d]	Max. B[e]	Expl. (%)[f]	Additive[g]
tss2.1	TSS	LG2	17	9–2	3.37	7.04	7.91	14.50	−0.44
tss2.2	TSS	LG2	36	33–46	5.48	6.99	8.01	26.00	−0.58
tss5.1	TSS	LG5	36	15–17	3.10	6.99	7.85	14.30	−0.44
ntc11.1	Net cover	LG11	56	55–61	3.52	5.89	7.74	15.20	−0.93
ntc2.1	Net cover	LG2	36	1–53	11.57	6.04	8.61	43.50	−1.60
ntc08 12.1[h]	Net cover	LG12	12	11–12	3.54	6.11	8.14	16.10	−1.01
ntd2.1	Net density	LG2	7	0–11	4.99	4.45	6.00	23.10	−0.88
ntd2.2	Net density	LG2	18	14–22	5.07	4.49	5.94	21.50	−0.84
ntd2.3	Net density	LG2	45	26–53	8.79	4.46	6.21	40.80	−1.13
ntd2.4	Net density	LG2	59	59	3.23	4.21	5.58	14.40	−0.69
fl2.1	Length	LG2	10	0–51	11.80	23.88	18.18	44.60	3.89
fl8.1	Length	LG8	101	100–101	2.99	22.75	18.09	15.70	2.33
fsh8.1	Shape	LG8	76	61–96	4.48	1.99	1.63	22.40	0.2103
fsh2.1	Shape	LG2	10	0–47	9.98	2.02	1.61	39.60	0.2824
fw2.1	Weight	LG2	9	7–28	8.58	1.19	0.81	0.03	0.24
fw2.2	Weight	LG2	39	39	3.02	1.12	0.81	0.01	0.15
fw06 8.1[i]	Weight	LG8	101	95–104	4.14	1.78	1.21	21.10	0.31
fwi6.1	Width	LG6	104	102–111	3.64	11.31	12.43	17.60	−0.59
fwi7.1	Width	LG7	43	41–43	3.51	11.07	12.23	15.70	−0.60
str11.1	Stripes	LG11	27	2–45	15.96	3.66	7.27	61.70	−2.47
str06 9.1[i]	Stripes	LG9	54	51–56	3.38	5.82	3.14	19.80	1.41
sut11.1	Sutures	LG11	27	9–37	9.22	3.70	6.56	39.80	−1.83
sut06 9.1[i]	Sutures	LG9	38	37–38	3.05	5.81	3.58	13.60	1.12
sut06 9.2[i]	Sutures	LG9	53	51–55	3.29	5.87	3.31	19.80	1.33
ff1.1	Softness	LG1	49	49	3.38	7.68	9.12	14.70	−0.72
ff5.1	Softness	LG5	15	13–18	3.31	7.71	9.15	16.60	−0.77
flc2.1	Flesh color	LG2	7	7	3.03	4.42	5.65	18.40	−0.61
flc6.1	Flesh color	LG6	88	78–91	5.94	4.54	5.69	24.30	−0.67
flc 8.1[i,j]	Flesh color	LG8	111	110–112	2.48	5.30	6.49	12.00	−0.58
car6.1	Total carotenoids	LG6	90	85–90	3.76	15.59	22.41	16.80	−3.019
car8.1	Total carotenoids	LG8	110	107–113	4.24	15.26	22.34	18.80	−3.228
βcr6.1	*β*-carotene	LG6	88	82–89	4.00	9.44	12.86	17.70	−1.92
βcr2.1	*β*-carotene	LG2	7	5–7	2.97	9.34	12.86	17.50	−1.92
phy6.1	Phytoene	LG6	85	78–90	4.81	0.27	0.40	20.40	−0.07
phy6.2	Phytoene	LG6	101	98–100	3.59	0.27	0.40	15.80	−0.064
αcr8.1	α-carotene	LG8	110	103–120	11.18	0.05	0.16	44.60	−0.07
lut8.1	Lutein	LG8	110	106–113	4.10	0.02	0.08	18.20	−0.03

Fruit TSS, flesh color, netting, length and weight—averaged over 3 years

Fruit shape, width, stripes, sutures and softness—averaged over 2 years

Total carotenoids were analyzed in 2006 by spectrophotometer; *β*-carotene, phytoene, α-carotene and lutein were analyzed in 2007 by HPLC

[a] QTLs are defined by the trait abbreviation, linkage group number and QTL number

[b] Position of the QTL in centimorgans on the linkage group

[c] Maximum LOD score for each QTL

[d] Maximum score in trait values for PI414723 (A) alleles for each QTL

[e] Maximum score in trait values for 'Dulce' (B) alleles for each QTL

[f] Maximum percent of explanation for each QTL

[g] Additive effect is positive when PI414723 alleles increase the trait score and negative when 'Dulce' alleles increase the trait score

[h] Significant for 2006 season

[i] Significant for 2007 season

[j] Significant for 2008 season

the PI 414723 allele, and not the netted 'Dulce', contributed to the netting coverage (data not shown).

Identification of QTLs for fruit flesh color and carotenoids

Three QTLs for flesh color (*flc*) were detected over the three growing seasons by visual evaluation of the mature fruit. These QTLs were mapped to LG2, 6 and 8. In addition, carotenoid content was analyzed over two growing seasons using either the spectrophotometric method based on total carotenoid content or following chromatographic separation by HPLC. Because β-carotene was the predominant carotenoid (>90%) in both parental genotypes, the total carotenoid content was a reflection of the concentration of this pigment (Table 4). The HPLC separation quantified four carotenoid pigments: the predominant β-carotene, together with smaller amounts of α-carotene, phytoene and lutein. QTL analysis of the individual carotenoid components identified six QTLs for the four different carotenoids (Table 3).

The QTLs for flesh color colocalized with QTLs for carotenoid content. LG6 contained a major QTL for flesh color (*flc6.1*, LOD = 4.5), together with QTLs for total carotenoids (*car6.1,* LOD = 3.8), β-carotene (*βcr6.1,* LOD = 4.0) and two QTLs for phytoene (*phy6.1,* LOD = 4.8 and *phy6.2,* LOD = 3.6). LG8 contained a weaker QTL for flesh color (*flc8.1*) which was detected in both 2006 and 2007. This QTL was found to be significant by permutation analysis, with a LOD score of about 2.5. It colocalized with the QTL for total carotenoids (*car8.1*, LOD = 4.2), a weak QTL (LOD = 2) for β-carotene, together with QTLs for the minor carotenoids, α-carotene (*αcr8.1*) with LOD > 11 and lutein (*lut8.1*) with LOD ~4. In addition, a third and minor QTL on LG2 was identified in the 2007 population for flesh color, *flc2.1* (LOD = 3.0), together with a QTL for β-carotene (*βcr2.1*) with a lower LOD score of 2.9, which was significant by permutation analysis.

To test whether known carotenoid metabolism genes account for these QTLs, 11 genes of the carotenoid metabolism pathway were mapped; six of them for the first time. The genes were mapped to nine different linkage groups, but none mapped within the QTLs for fruit flesh color or carotenoids.

TSS and sugars

HPLC analysis of the flesh sugars in ripe fruit grown in Israel over two growing seasons was performed to estimate the distribution of sugar content and composition in the fruits of the population. The results were compared with TSS values of the same fruits. PI 414723 fruits had low TSS and low sucrose content relative to the 'Dulce' fruits (Table 4). Glucose and fructose contents were similar for both genotypes. The distribution of TSS among the population was normal according to Shapiro–Wilk test ($P \leq 0.2$). The distribution of sucrose content was lognormal according to KSL test ($P \leq 0.15$). None of the RI lines reached the

Table 4 Metabolic data of the parental accessions and the RI lines for TSS, sugars, flesh color and carotenoids in three seasons

Trait	Year of trial	'Dulce', mean ± SD	PI 414723, mean ± SD	RI, mean ± SD	RI, max. ± SD	RI, min. ± SD
TSS	2006	11.8 ± 1.4	5.4 ± 1.1	7.6 ± 1.8	10.8 ± 2.3	5.3 ± 0.6
	2007	13 ± 1.2	5.7 ± 0.8	8.3 ± 1.8	11.7 ± 2.5	5.7 ± 0.2
	2008	9.1 ± 2.1	4.7 ± 1.1	6.1 ± 1.7	8.9 ± 2.4	3.6 ± 0.1
Sucrose (mg/g)	2006	50.5 ± 18.2	4.9 ± 2.5	15.6 ± 10.5	41.2 ± 16.7	5.1 ± 2.1
	2007	53.2 ± 12.5	2.1 ± 0.7	10.6 ± 9.9	39.9 ± 17.3	1.8 ± 0.8
Glucose (mg/g)	2006	19.8 ± 5.9	16.5 ± 3.3	17.2 ± 5	25.5 ± 7.6	7.7 ± 1.4
	2007	14.2 ± 3.9	12.4 ± 1.91	15.4 ± 4.7	23.5 ± 8.2	8.9 ± 0.4
Fructose (mg/g)	2006	18.4 ± 4.4	17.5 ± 2.32	19.3 ± 4.5	27.4 ± 6.8	12.9 ± 0.8
	2007	16.7 ± 3.7	16.9 ± 1.70	17.4 ± 4.4	24.1 ± 7.9	11.8 ± 0.7
Flesh color (1–9)	2006	9.0 ± 0	2.4 ± 0.5	5.9 ± 1.7	8.5 ± 2.7	1.1 ± 0.3
	2007	9.0 ± 0.2	2.4 ± 0.5	5.3 ± 1.4	8.3 ± 2.3	1.0 ± 0
	2008	7.4 ± 1.5	1.9 ± 0.4	3.7 ± 1.1	6.6 ± 1.5	1 ± 0
Total carotenoids (μg/g frozen weight)	2006	49.99 ± 4.72	6.83 ± 2.75	18.43 ± 8.26	50.84 ± 11.08	0.78 ± 0.45
β-carotene (μg/g frozen weight)	2007	28.77 ± 4.64	2.59 ± 0.9	10.74 ± 4.32	22.02 ± 7.23	0.78 ± 0.07
Phytoene (μg/g frozen weight)	2007	0.17 ± 0.03	0.13 ± 0.05	0.32 ± 0.16	1.03 ± 0.4	0 ± 0
α-Carotene (μg/g frozen weight)	2007	0.19 ± 0.02	0 ± 0	0.09 ± 0.11	0.39 ± 0.18	0 ± 0
Lutein (μg/g frozen weight)	2007	0.01 ± 0.02	0.03 ± 0.06	0.04 ± 0.07	0.04 ± 0.25	0 ± 0

Table 5 Significant sugar and TSS QTLs identified by interval mapping and permutation analysis

QTL[a]	Trait	Year of trial	Linkage group	Position of max. LOD (cM)	Position (cM)[b]	Max. LOD[c]	Max. A[d]	Max. B[e]	Expl. (%)[f]	Additive[g]
suc2.1	Sucrose	2006	LG2	17	17	3.0	12.9	18.8	13.4	−3.0
suc2.2	Sucrose	2006	LG2	37	35–39	3.4	12.9	19.3	17.0	−3.4
suc2.3	Sucrose	2006	LG2	46	44–48	3.7	12.4	19.3	19.7	−3.5
suc3.1	Sucrose	2006	LG3	89	87–91	2.3	13.2	18.1	11.3	−2.6
suc3.1	Sucrose	2007	LG3	89	87–91	2.9	8.1	13.3	12.9	−2.7
suc5.1	Sucrose	2006	LG5	16	11–20	2.7	13.3	18.5	12.9	−2.8
suc8.1	Sucrose	2006	LG8	102	93–110	3.7	12.6	18.8	18.3	−3.4
tss2.1	TSS	Mean[h]	LG2	17	9–22	3.4	7.0	7.9	14.5	−0.4
tss2.2	TSS	Mean[h]	LG2	36	33–46	5.5	7.0	8.0	26.0	−0.6
tss5.1	TSS	Mean[h]	LG5	16	15–17	3.1	7.0	7.9	14.3	−0.4
glu4.1	Glucose	2006	LG4	23	19–25	3.6	18.7	16.1	15.8	1.3

[a] QTLs are defined by the trait abbreviation, linkage group number and QTL number

[b] Position of the QTL in centimorgans on the linkage group

[c] Maximum LOD score for each QTL

[d] Maximum score in trait values for PI414723 (A) alleles for each QTL

[e] Maximum score in trait values for 'Dulce' (B) alleles for each QTL

[f] Maximum percent of explanation for each QTL

[g] Additive effect is positive when PI414723 alleles increase the trait score and negative when 'Dulce' alleles increase the trait score

[h] Mean of TSS scores during three growing seasons

high TSS or sucrose levels of 'Dulce' (Table 4); however, a few families were characterized by high levels of sucrose approaching those of 'Dulce' parent.

Correlations of TSS and sucrose content were 0.7 for the 2006 growing season and 0.8 for the 2007 growing season (Supplemental Table S9). Correlation coefficients between TSS and either glucose or fructose content were low and insignificant according to pairwise analysis (data not shown). Therefore, particular attention was directed toward the analysis of QTLs for TSS and sucrose. In total, nine QTLs were found significant by IM and permutation analysis for sucrose and TSS and were distributed over four linkage groups (Fig. 2; Table 5). Of the nine QTLs, six were for sucrose levels and three for TSS. One additional QTL was identified for glucose content in the melon fruit at LG4, where the low sugar parent PI 414723 alleles contributed to higher glucose content, leading to transgressive segregation for this trait. No significant QTLs were detected for fructose content in this population. QTLs for sucrose and glucose content were calculated for each growing season individually since the environmental effect was significant for these traits. For TSS, however, QTLs were analyzed based on the average scores of the three growing seasons, since similar QTLs were identified when calculated for each growing season individually (Supplemental Table S8).

Three QTLs for sucrose accumulation were located on LG2 (*suc2.1*, LOD = 3.0, *suc2.2*, LOD = 3.4 and *suc2.3*, LOD = 3.7) and they overlapped the two TSS QTLs (*tss2.1*, LOD = 3.4 and *tss2.2*, LOD = 5.5) identified in this linkage group. QTL *suc2.1* overlapped QTL *tss2.1* at the proximal end of the linkage group, while the QTLs *suc2.2 and suc2.3* overlapped the additional QTL for TSS (*tss2.2*) at the distal end of LG2. An additional QTL for TSS at LG5 (*tss5.1*, LOD = 3.1) also overlapped a QTL for sucrose accumulation (*suc5.1*); however, this sucrose QTL was only significant by permutation analysis, with a lower LOD score (2.7).

An additional QTL for sucrose content was identified on LG8 (*suc8.1*, LOD = 3.7), near the locus controlling the pH of the fruit flesh. IM analysis did not identify a QTL for TSS in this linkage group. Nevertheless, MQM analysis for sucrose accumulation and TSS values from the 2006 growing season did show overlapping QTLs for TSS and sucrose, with LOD = 2.5, on LG8 (data not shown). One more QTL for sucrose accumulation was found to be significant by permutation analysis on LG3 for both growing seasons, with a lower LOD score (2.9), but analysis did not indicate a significant QTL for TSS in this region.

In all cases, 'Dulce' alleles (the sweet parent) contributed to higher sucrose content and elevated TSS values. Moreover, there was an additive contribution of the individual loci, such that with increasing number of 'Dulce' loci for the six QTLs, there was an incremental increase in sucrose content of the ripe fruit. Figure 3 shows this effect of increasing 'Dulce' alleles for the six QTLs. There appears to be a threshold effect in which sucrose levels do

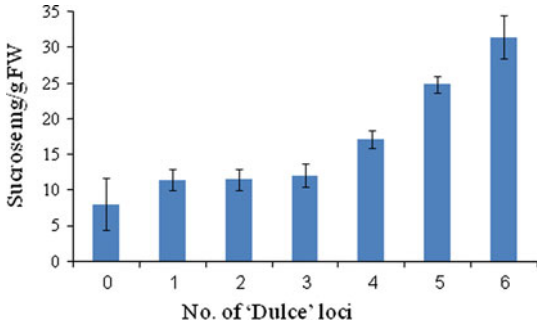

Fig. 3 Effect of number of sucrose QTLs with 'Dulce' genotype on sucrose content of the mature fruit. Data points represent means of sucrose content of the RIs harboring the corresponding number of homozygous 'Dulce' loci, from 0 to 6, and *bars* represent ±SE. Number of RI lines in each category ranged from 2 to 22 and are described in Supplementary Table S11

not increase significantly with less than three 'Dulce' loci, whereas above this threshold, the additive effect of 'Dulce' loci on sucrose content is observed. The effect of number of 'Dulce' loci appeared to be irrespective of which loci were involved: there were no differences between lines having similar numbers of 'Dulce' loci, regardless of which individual loci they were. Some of the genotypes represented permutations of the different sucrose QTLs which were underrepresented among the 99 RI lines and effects could not be accurately discerned between the low 'Dulce' genotypes. When all six sucrose QTLs were of the 'Dulce' genotype, the sucrose level was highest and closely approached the levels observed in the 'Dulce' parent.

In light of the clear identification of multiple QTLs for sucrose content, we tested the hypothesis that these QTLs encode genes involved in sugar metabolism and accumulation in the melon fruit. Polymorphisms between PI 414723 and 'Dulce' were identified for 31 sugar-metabolism genes (Table 1) which were mapped and checked for colocalization with the TSS and sucrose QTLs. For seven genes, no polymorphisms were identified. None of the 31 genes clearly mapped to QTLs for either TSS or sucrose content, although there were some weak colocalizations, such as for *CmSUS1* on LG2 (Fig. 2). The genes mapped to 9 of the 12 chromosomes without any indication of clustering.

Discussion

The 414 × Dul RI population

The 414 × Dul RI population is a novel, publicly available resource that has been proven to be highly homozygous and highly polymorphic, making it valuable for genetic studies of multiple quantitative traits. One indication of the quality of the RI population is the distribution of the alleles, which in this population is almost even. The heterozygosity in this population was found to be 5.3%, which is not significantly different from the heterozygosity level (4.43%) obtained in another melon RI population (Perin et al. 2002b). Mean heterozygosity of 90% of the population was 3.06%, twice the predicted value for this type of population (1.6%). The bias was contributed mainly by 10% of the RI lines, with six lines heterozygous for more than 20% of the loci. Similar results were obtained for two maize RI populations by Burr and Burr (1991), who suggested that unintentional selection can contribute to residual heterozygosity. Even higher residual heterozygosity, 16%, and skewed distribution of alleles have been reported in other RI populations (Paran et al. 1995).

It should also be noted that two regions, at two linkage groups, LG2 (0–37 cM) and LG8 (91–98 cM), were highly heterozygous in more than 10 families. In maize, excess residual heterozygosity was observed in pericentromeric regions in a nested association mapping population (McMullen et al. 2009). However in melon, the pericentromeric regions have not yet been defined, and the excess residual heterozygosity cannot therefore be associated with such regions.

The 414 × Dul linkage map

The 414 × Dul RI linkage map was created with almost 700 markers, 161 of which were newly developed fruit-EST markers, one-third of them from metabolic pathways of sucrose, carotenoids and volatiles. Almost 500 markers, including all of the fruit-EST markers, are codominant: SNPs, INDELs and SSRs. The resulting map spans over 1,222 cM with an average of 2.67 cM between markers, which is slightly more saturated than other detailed melon maps (e.g. Gonzalo et al. 2005; Perin et al. 2002b; Zalapa et al. 2007).

Previous melon maps were constructed using various population types (F_2, F_3, RI lines, DHL, NILs), marker types and nomenclatures, which does not enable easy comparisons between populations or maps. It was, therefore, suggested to use SSR markers as anchor points for syntenic analysis and map merging (Danin-Poleg et al. 2000, 2002; Gonzalo et al. 2005; Katzir et al. 1996; Monforte et al. 2004; Paris et al. 2008; Perin et al. 2002b). The map developed here is one of eight maps that are currently being merged as part of the ICuGI, and the nomenclature of the linkage groups is, therefore, according to Perin et al. (2002b). This nomenclature differs from the one used in the 414 × Dul F_2 map (Danin-Poleg et al. 2002), but good synteny was nevertheless found between the two maps based on the 33 common SSR markers as well as the common traits mapped in both 414 × Dul F_2 and 414 × Dul RI: pH, resistance to ZYMV (*zymv*), andromonoecious (*a*) and

stripes (*st*). The level of polymorphism detected by SSR markers (55%) was similar to the level detected by Danin-Poleg et al. (2000). The level of polymorphism detected by AFLPs (20–25%) was similar to the mean level described for the same primer combinations by Perin et al. (2002b). Among the mapped markers, 143 were from a set of markers used in previous maps (Cuevas et al. 2008, 2009; Danin-Poleg et al. 2002; Fernandez-Silva et al. 2008; Gonzalo et al. 2005; Monforte et al. 2004; Paris et al. 2008; Zalapa et al. 2007). In light of these common markers, a gap in LG12 on the 414 × Dul map could be merged based on Moreno et al. (2008) while gaps in six LGs (2, 4, 6, 8, 9 and 11) described by Cuevas et al. (2008) can be merged using the new information provided here. This set of anchor markers enabled comparison of our QTLs with previously published fruit QTLs (Cuevas et al. 2008, 2009; Eduardo et al. 2007; Monforte et al. 2004; Obando-Ulloa et al. 2009; Paris et al. 2008; Park et al. 2009; Perin et al. 2002c; Zalapa et al. 2007).

The 414 × Dul RI map was also enriched with 29 BAC clones anchored by markers that were scattered throughout the 12 linkage groups. To date, only a few BAC clones of melon have been mapped, to only four linkage groups, as part of map based cloning projects for resistance genes (Deleu et al. 2007; Joobeur et al. 2004; Luo et al. 2001; Morales et al. 2005; Park et al. 2004; van Leeuwen et al. 2003; Wang et al. 2002), and the *CmACS-7* gene (Boualem et al. 2008). This anchored set of BAC clones represents the first step in preparing a physical map of the melon genome.

QTL analysis of fruit traits

Fruit size and rind characters

Fruit size and shape have been studied in many plant species, including melon; for example, QTLs for these traits have been detected in pepper and tomato (Lippman et al. 2007; Zygier et al. 2005). In melon, QTLs for size and shape have been reported in various populations (Eduardo et al. 2007; Monforte et al. 2004; Perin et al. 2002c; Zalapa et al. 2007). The correlation between fruit size and shape can vary in different populations, since either the length or the width can be diverse and affect the size and weight. In the 414 × Dul RI population, the QTLs for length and weight colocalized on the same linkage groups (LG2 and LG8), similar to Perin et al. (2002c) and to a QTL for fruit weight in another population (Zalapa et al. 2007). Significant QTLs for width were detected on LG6 here as well as in two other populations (Eduardo et al. 2007; Monforte et al. 2004).

The immature fruit stripes trait was mapped, using the 414 × Dul F_2 population as a monogenic trait (Danin-Poleg et al. 2002), to the same linkage group as in the present study. Adjacent to the peak at LG11 cM 22, an SSR marker (CMAGAN268) that may be useful in marker-assisted breeding was mapped. It was shown here that both the stripe trait in the young ovary and the suture trait in the mature fruit colocalize and are presumably controlled by the same gene.

One major QTL for net cover was located on LG2; along this QTL were located three QTLs for net density. In another study, by Paris et al. (2008), a QTL for percentage of exocarp netting was identified at the same location, based on a common marker (TJ24). Two useful markers, CMSNP51 and CMGAN271 (LGS cM 34), were mapped within 1 cM from the peak of the netting QTL and may be helpful for breeding.

Firmness

The firmness QTLs detected here were mapped to LG1 and LG5. These positions harbor genes coding for ethylene synthesis and regulation, as well as a gene encoding for polygalacturonase, which have been mapped in other populations (Moreno et al. 2008; Perin et al. 2002b). The QTL for firmness on LG1 appears to colocalize with *CmERS1* and *CmPG4*, as can be predicted by a shared marker (CMCTN86) with the PI 161375 × 'Piel de Sapo' (SC × PS) melon genetic map (Moreno et al. 2008). The QTL on LG5 is close to *CmACO* based on a common marker (CMAGN61) with the map developed by Moreno et al. (2008). These genes are, therefore, strong candidates for controlling genetic variability of fruit firmness and shelf life.

Fruit flesh color

The parental lines of the RI population differed in the intensity of their orange flesh color, determined mainly by β-carotene content, as indicated by the high correlation between the two traits (Supplemental Table S10). In total, 11 QTLs were identified as related to intensity of the orange flesh color and to various components of carotenoid content (three for *flc*, two for *car*, two for *βcr*, two for *phy* and one each for *αcr* and *lut*). These were measured during two seasons by two different methods: a determination of crude total carotenoid content based on β-carotene as the predominant pigment, and a chromatographic separation of the major and three minor pigments. The 11 QTLs mapped to three major locations on LG2, LG6 and LG8. In all of these QTLs, RI lines that carried 'Dulce' alleles had higher contents than those carrying the PI 414723 alleles. On LG6, QTLs for both flesh color and β-carotene were mapped (*flc6.1* and *βcr6.1*). In an earlier report, orange flesh color was mapped to LG6 in another melon population (Monforte

et al. 2004), and recently, QTLs for β-carotene accumulation have also been mapped to the same position on LG6 in two different segregating populations (Cuevas et al. 2008, 2009). Interestingly, a QTL for phytoene, the first carotenoid in the biosynthetic pathway, has also been localized to the same position on LG6 (*phy6.1*). This suggests that the LG6 QTL may contain a gene that stimulates the carotenoid metabolic pathway in general, leading to the formation of β-carotene.

With regard to the QTLs on LG8 (*car8.1*, *flc8.1*, *αcr8.1* and *lut8.1*), a parallel QTL for β-carotene content was located in a population derived from a cross between white-fleshed and orange-fleshed genotypes (Cuevas et al. 2009) but not in populations derived from crosses between two orange-fleshed lines (Cuevas et al. 2008). Similarly, no QTL for flesh color was located on LG8 in a cross between green- and orange-fleshed genotypes (Perin et al. 2002b). Interestingly, a QTL for flesh color (*gf*) in the same region on LG8 was identified while analyzing segregants of a cross between white- and green-fleshed lines (Monforte et al. 2004). QTLs for the minor carotenoids, α-carotene and lutein were localized to a similar position on LG8. This could indicate that the QTL on LG8 is also a general enhancer of carotenoid biosynthesis.

There have been no previous reports of QTLs for flesh color or carotenoid content on LG2 (*flc2.1, bcr2.1*), which we mapped proximal to the monoecious (*a*) gene. Although Cuevas et al. (2008, 2009) reported QTLs for carotene levels on LG2, they were positioned significantly distal to monoecious (*a*) and the QTL reported here.

The common QTLs within the broad genetic variability of *C. melo* indicate their major roles in determining carotenoid accumulation. On the other hand, prior reports of fruit flesh color and carotenoid content in other populations (Cuevas et al. 2008, 2009; Monforte et al. 2004; Perin et al. 2002b) contain several QTLs which were not observed in the 414 × Dul RI population, e.g. a QTL on LG9 identified using crosses between green- or white- and orange-fleshed genotypes (Cuevas et al. 2009; Perin et al. 2002b). The LG9 QTL could be associated with the presence or absence of flesh carotenoids while the 414 × Dul RI population was established from a cross between two orange-fleshed parental lines: pale and intense, and thus does not segregate for such a trait. These additional QTLs present an intriguing opportunity for the accumulation of loci which may lead to increased carotenoid levels in melon fruit.

Seven genes from the carotenoid biosynthetic pathway have been mapped previously (Cuevas et al. 2008, 2009), five of which were re-mapped here to similar locations using different SNP markers. We also mapped six additional genes from this pathway. None of the 13 genes colocalized with the QTLs we identified and similarly, they did not colocalize with the QTLs described in the studies of Cuevas et al. (2008, 2009). Variation in total carotenoid content was also not found to be associated with structural carotenogenesis genes in tomato (Liu et al. 2003). However, genetic control of carotenoid composition, such as in high β-carotene (beta) or δ-carotene (delta) lines, is determined by structural genes of the carotenoid biosynthetic pathway (Liu et al. 2003; Ronen et al. 1999, 2000).

Unlike tomato or watermelon, for which there exists a natural variation in fruit carotenoid composition (Tadmor et al. 2005), in the case of melon, no natural genetic variability has yet been described for carotenoid pigment composition. All non-green melon cultivars, with flesh color ranging from cream to intense orange, vary only in their β-carotene content, and have only low levels of other carotenoids (Burger et al. 2006, 2009).

Sugar QTLs

Sugar concentration is the major factor influencing melon fruit quality (Yamaguchi et al. 1977) and sucrose is the main sugar accumulating during fruit ripening (Burger and Schaffer 2007; Rosa 1928; Schaffer et al. 1996). TSS is a common method of predicting fruit sugar content and was strongly correlated with sucrose accumulation in the present study. Similarly, our previous studies have consistently shown the overwhelming contribution of sucrose accumulation to both genetically determined and physiologically determined differences in total sugar content in melons (Burger and Schaffer 2007; Burger et al. 2000; Schaffer et al. 1996; Stepansky et al. 1999).

The results of this study point to strict additive and polygenic control of sucrose levels in the cross between 'Dulce' and P I414723. Six QTLs for sucrose, five of which colocalized with QTLs for TSS, were identified. The colocalization of these QTLs is a reflection of the determining role of sucrose in total sugar content of melon fruit. The loci are inherited independently and their effects are additive, with respect to both allelic dosage at each locus and additivity of cumulative loci. Each locus accounted for ~20% of the difference in parental sucrose or TSS levels, and the heterozygote for each locus was intermediate in contribution, when compared with the parental genotypes. The genotypic accumulation of 'Dulce' alleles for the six loci led to the highest sucrose and TSS levels in the RI population, nearly approaching those of 'Dulce' parent. The observation that the accumulation of all 12 'Dulce' alleles reached ~90% of the sucrose content of the 'Dulce' parent may indicate additional minor loci affecting sucrose accumulation, or merely the limiting biological variability of the small population of RI lines possessing all 12 'Dulce' alleles (only two lines out of 99). Further development of true breeding lines for each of the possible combinations of these six loci will further clarify their respective contributions to sucrose levels.

In a previous study based on segregating populations derived from a sweet *cantalupensis* melon ('Noy Yizre'el') and a *flexuosus* type non-sweet melon ('Faqqous'), we suggested the presence of a single major gene controlling sucrose levels (Burger et al. 2002). The results of the present study, based on the sweet *reticulatus* ('Dulce') and a non-sweet *momordica* type melon (PI 414723) do not support the presence of such a major locus in this population, and similarly, the segregation data for sugar levels in this population do not indicate the involvement of a single major gene (not shown). It is nevertheless possible that there is a major gene controlling sucrose levels in the *cantalupensis* × *flexuosus* population while in the *momordica* line, the allele for this major gene is identical to that in the sweet genotypes. In fact, the *momordica* line accumulates higher sucrose levels than the *flexuosus* lines (Burger and Schaffer 2007) and it is feasible that polygenic control by a few genes determines the sucrose levels in the *reticulatus* × *momordica*, 414 × Dul RI population. This exemplifies how using different genetic backgrounds can assist in identifying additional QTLs by eliminating the effect of major QTLs, thereby increasing the sensitivity.

QTLs for TSS/sucrose, similar to those detected here, have been described in previous studies comprising a broad spectrum of *C. melo* germplasm. One of the QTLs at LG2 (*tss2.1* and *suc2.1*) comprises markers, including that for monoecious (*a*), that have been shown to cosegregate with both TSS and sucrose in two different crosses, based on a low sugar accession from Zimbabwe, TGR1551 (*C. melo* subsp. *agrestis*) (Park et al. 2009; Sinclair et al. 2006). Similarly, QTLs for TSS on LG2 have been detected using additional populations based on crosses between the Korean accession PI 161375 and the *inodorus* cultivar 'Piel de Sapo' (PI × PS) (Monforte et al. 2004) and between a USDA breeding line and the American cantaloupe 'Top Mark' (Paris et al. 2008). The QTL at LG3 for sucrose accumulation (*suc3.1*) is at a similar location as QTLs for sucrose/TSS identified in PI × PS (Obando-Ulloa et al. 2009) and for TSS (Monforte et al. 2004). A QTL on LG5 has been described by both Monforte et al. (2004) and Paris et al. (2008) at a location similar to the QTLs identified here for TSS and sucrose (*suc5.1, tss5.1*). Finally, the QTLs for sucrose/TSS on LG8 (*suc8.1*) may be identical to the QTLs for sucrose/TSS identified by Obando-Ulloa et al. (2009) and QTLs for TSS identified in additional populations (Monforte et al. 2004; Paris et al. 2008).

One QTL for glucose was identified at LG4, on the same linkage group as in Obando-Ulloa et al. (2009). Additional QTLs for sucrose, glucose and fructose that were not identified here have also been described (Obando-Ulloa et al. 2009; Park et al. 2009).

The identification of well-defined QTLs for sucrose content enabled the performance of a colocalization test to determine whether any of the QTLs can be accounted for by enzymes of melon sugar metabolism. In this study, we focused on a comprehensive list of enzymes involved in sugar metabolism in melon, including all of the members of individual gene families (Table 1; Dai et al. in preparation). We identified polymorphisms for most of these genes and mapped 31 of them, including all the major genes which are significantly expressed in developing melon fruit (Dai et al. in preparation). The results of this colocalization mapping study point to a general absence of colocalization between genes coding for primary sugar metabolism and the QTLs for sucrose accumulation. Although previous studies have demonstrated that the activities of several of these enzymes, particularly acid invertase, sucrose-phosphate synthase and sucrose synthase, are correlated with sucrose accumulation in melon (Burger and Schaffer 2007; Lingle and Dunlap 1987; Yu et al. 2008), none of these genes mapped with QTLs for sucrose concentration. The only possible exception is the mapping of one of the three members of the sucrose synthase gene family in melon, *CmSUSY1,* with QTL *tss2.1*, with a LOD score of <3. *CmSUSY1* is expressed primarily in the young developing fruitlets and its expression declines strongly at the stage of sucrose accumulation (Dai et al. in preparation). Perhaps, genetic variation for the temporal decline in expression is responsible for extended sucrose cleavage and, hence, for genetic variation in sucrose content. Genetic variation for the decline in activity of acid invertase has been shown to be correlated with genetic differences in the developmental onset of sucrose accumulation (Burger and Schaffer 2007), but the *CmAIN2* gene did not colocalize with the sucrose QTLs. Further genetic dissection of this QTL will test the candidacy of the *CmSUSY1* gene for QTL *tss2.1*. In conclusion, these mapping results indicate that other genes determine sugar accumulation in the melon fruit, either structural genes that have not yet been mapped or regulatory genes that remain to be identified.

A similar general lack of colocalization of primary sugar-metabolism structural genes and QTLs for their related metabolites has also been reported in tomato (Bermudez et al. 2008; Causse et al. 2004). Similarly, in *Arabidopsis*, the major carbohydrate QTLs generally do not map with the expression QTLs of the structural genes, although QTLs for minor carbohydrates do colocalize with related structural genes (Keurentjes et al. 2008). However, there are some notable examples of colocalization of sugar content and carbohydrate metabolism genes, such as the colocalization of QTLs for TSS in tomato: *LIN5* (for apoplastic invertase, on chromosome 9-2-5; Fridman et al. 2004), *LS1* (for ADPgluPPase, on chromosome 1-4; Petreikov et al. 2009), and for sucrose accumulation: *sucr* (for soluble acid invertase activity, on chromosome 3–2; Chetelat et al. 1995).

The presence of the same QTLs in diverse segregating populations is likely indicative of major roles for these QTLs within the species. The strategy of fine-mapping and subsequent cloning and identification of these QTLs has been strengthened by the recent release of the cucumber genome sequence, which is highly microsyntenous with the melon genome (Huang et al. 2009), and future research will focus on the gene function of these important QTLs for fruit quality.

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References

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

Bermudez L, Urias U, Milstein D, Kamenetzky L, Asis R, Fernie AR, Van Sluys MA, Carrari F, Rossi M (2008) A candidate gene survey of quantitative trait loci affecting chemical composition in tomato fruit. J Exp Bot 59:2875–2890

Boualem A, Fergany M, Fernandez R, Troadec C, Martin A, Morin H, Sari MA, Collin F, Flowers JM, Pitrat M, Purugganan MD, Dogimont C, Bendahmane A (2008) A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. Science 321:836–838

Burger Y, Schaffer AA (2007) The contribution of sucrose metabolism enzymes to sucrose accumulation in *Cucumis melo*. J Am Soc Hortic Sci 132:704–712

Burger Y, Shen S, Petreikov M, Schaffer AA (2000) The contribution of sucrose to total sugar content in melon. In: Katzir N, Paris HS (eds) Cucurbitaceae 2000, 7th EUCARPIA meeting on cucurbit genetics and breeding. Acta Hortic, pp 479–485

Burger Y, Saar U, Katzir N, Paris HS, Yeselson Y, Levin I, Schaffer AA (2002) A single recessive gene for sucrose accumulation in *Cucumis melo* fruit. J Am Soc Hortic Sci 127:938–943

Burger Y, Sa'ar U, Paris HS, Lewinsohn E, Katzir N, Tadmor Y, Schaffer AA (2006) Genetic variability for valuable fruit quality traits in *Cucumis melo*. ISR J Plant Sci 54:233–242

Burger Y, Paris H, Cohen R, Katzir N, Tadmor Y, Lewinsohn E, Schaffer AA (2009) Genetic diversity of *Cucumis melo*. Hortic Rev 36:165–198

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

Carmi N, Zhang GF, Petreikov M, Gao ZF, Eyal Y, Granot D, Schaffer AA (2003) Cloning and functional expression of alkaline alpha-galactosidase from melon fruit: similarity to plant SIP proteins uncovers a novel family of plant glycosyl hydrolases. Plant J 33:97–106

Causse M, Duffe P, Gomez MC, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C (2004) A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. J Exp Bot 55:1671–1685

Chetelat RT, Deverna JW, Bennett AB (1995) Introgression into tomato (*Lycopersicon esculentum*) of the *L. chmielewskii* sucrose accumulator gene (*Sucr*) controlling fruit sugar composition. Theor Appl Genet 91:327–333

Cuevas HE, Staub JE, Simon PW, Zalapa JE, McCreight JD (2008) Mapping of genetic loci that regulate quantity of beta-carotene in fruit of US Western Shipping melon (*Cucumis melo* L.). Theor Appl Genet 117:1345–1359

Cuevas HE, Staub JE, Simon PW, Zalapa JE (2009) A consensus linkage map identifies genomic regions controlling fruit maturity and beta-carotene-associated flesh color in melon (*Cucumis melo* L.). Theor Appl Genet 119:741–756

Dai N, Petreikov M, Portnoy V, Katzir N, Pharr DM, Schaffer AA (2006) Cloning and expression analysis of a UDP-galactose/glucose pyrophosphorylase from melon fruit provides evidence for the major metabolic pathway of galactose metabolism in raffinose oligosaccharide metabolizing plants. Plant Physiol 142:294–304

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

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

Danin-Poleg Y, Tadmor Y, Tzuri G, Reis N, Hirschberg J, Katzir N (2002) Construction of a genetic map of melon with molecular markers and horticultural traits, and localization of genes associated with ZYMV resistance. Euphytica 125:373–384

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

Deleu W, Esteras C, Roig C, Gonzalez-To M, Fernandez-Silva I, Gonzalez-Ibeas D, Blanca J, Aranda MA, Arus P, Nuez F, Monforte AJ, Pico MB, Garcia-Mas J (2009) A set of EST–SNPs for map saturation and cultivar identification in melon. BMC Plant Biol 9:90

Eduardo I, Arus P, Monforte AJ, Obando J, Fernandez-Trujillo JP, Martinez JA, Alarcon AL, Alvarez JM, van der Knaap E (2007) Estimating the genetic architecture of fruit quality traits in melon using a genomic library of near isogenic lines. J Am Soc Hortic Sci 132:80–89

Fernandez-Silva I, Eduardo I, Blanca J, Esteras C, Pico B, Nuez F, Arus P, Garcia-Mas J, Monforte AJ (2008) Bin mapping of genomic and EST-derived SSRs in melon (*Cucumis melo* L.). Theor Appl Genet 118:139–150

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004) Zooming in on a quantitative trait for tomato yield using interspecific introgressions. Science 305:1786–1789

Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. Plant Mol Biol Rep 13:207–209

Gao ZF, Schaffer AA (1999) A novel alkaline alpha-galactosidase from melon fruit with a substrate preference for raffinose. Plant Physiol 119:979–987

Gao ZF, Petreikov M, Zamski E, Schaffer AA (1999) Carbohydrate metabolism during early fruit development of sweet melon (*Cucumis melo*). Physiol Plant 106:1–8

Gonzalo MJ, Oliver M, Garcia-Mas J, Monfort A, Dolcet-Sanjuan R, Katzir N, Arus P, Monforte A (2005) Simple-sequence repeat

markers used in merging linkage maps of melon (*Cucumis melo* L.). Theor Appl Genet 110:802–811

Huang S, Li R, Zhang Z, Li L, Gu X, Fan W, Lucas WJ, Wang X, Xie B, Ni P, Ren Y, Zhu H, Li J, Lin K, Jin W, Fei Z, Li G, Staub J, Kilian A, van der Vossen EA, Wu Y, Guo J, He J, Jia Z, Tian G, Lu Y, Ruan J, Qian W, Wang M, Huang Q, Li B, Xuan Z, Cao J, Asan Wu Z, Zhang J, Cai Q, Bai Y, Zhao B, Han Y, Li Y, Li X, Wang S, Shi Q, Liu S, Cho WK, Kim JY, Xu Y, Heller-Uszynska K, Miao H, Cheng Z, Zhang S, Wu J, Yang Y, Kang H, Li M, Liang H, Ren X, Shi Z, Wen M, Jian M, Yang H, Zhang G, Yang Z, Chen R, Ma L, Liu H, Zhou Y, Zhao J, Fang X, Fang L, Liu D, Zheng H, Zhang Y, Qin N, Li Z, Yang G, Yang S, Bolund L, Kristiansen K, Li S, Zhang X, Wang J, Sun R, Zhang B, Jiang S, Du Y (2009) The genome of the cucumber, *Cucumis sativus* L. Nat Genet 41:1275–1281

Hubbard NL, Huber SC, Pharr DM (1989) Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. Plant Physiol 91:1527–1534

Joobeur T, King JJ, Nolin SJ, Thomas CE, Dean RA (2004) The fusarium wilt resistance locus Fom-2 of melon contains a single resistance gene with complex features. Plant J 39:283–297

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

Keurentjes JJB, Sulpice R, Gibon Y, Steinhauser MC, Fu JY, Koornneef M, Stitt M, Vreugdenhil D (2008) Integrative analyses of genetic variation in enzyme activities of primary carbohydrate metabolism reveal distinct modes of regulation in *Arabidopsis thaliana*. Genome Biol 9:R129

Lester GE, Arias LS, Gomez-Lim M (2001) Muskmelon fruit soluble acid invertase and sucrose phosphate synthase activity and polypeptide profiles during growth and maturation. J Am Soc Hortic Sci 126:33–36

Lingle SE, Dunlap JR (1987) Sucrose metabolism in netted muskmelon fruit during development. Plant Physiol 84:386–389

Lippman ZB, Semel Y, Zamir D (2007) An integrated view of quantitative trait variation using tomato interspecific introgression lines. Curr Opin Genet Dev 17:545–552

Liu YS, Gur A, Ronen G, Causse M, Damidaux R, Buret M, Hirschberg J, Zamir D (2003) There is more to tomato fruit colour than candidate carotenoid genes. Plant Biotechnol J 1:195–207

Luo M, Wang YH, Frisch D, Joobeur T, Wing RA, Dean RA (2001) Melon bacterial artificial chromosome (BAC) library construction using improved methods and identification of clones linked to the locus conferring resistance to melon Fusarium wilt (*Fom-2*). Genome 44:154–162

McMullen MD, Kresovich S, Villeda HS, Bradbury P, Li HH, Sun Q, Flint-Garcia S, Thornsberry J, Acharya C, Bottoms C, Brown P, Browne C, Eller M, Guill K, Harjes C, Kroon D, Lepak N, Mitchell SE, Peterson B, Pressoir G, Romero S, Rosas MO, Salvo S, Yates H, Hanson M, Jones E, Smith S, Glaubitz JC, Goodman M, Ware D, Holland JB, Buckler ES (2009) Genetic properties of the maize nested association mapping population. Science 325:737–740

Monforte AJ, Oliver M, Gonzalo MJ, Alvarez JM, Dolcet-Sanjuan R, Arus P (2004) Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.). Theor Appl Genet 108:750–758

Morales M, Roig E, Monforte AJ, Arus P, Garcia-Mas J (2004) Single-nucleotide polymorphisms detected in expressed sequence tags of melon (*Cucumis melo* L.). Genome 47:352–360

Morales M, Orjeda G, Nieto C, van Leeuwen H, Monfort A, Charpentier M, Caboche M, Arus P, Puigdomenech P, Aranda MA, Dogimont C, Bendahmane A, Garcia-Mas J (2005) A physical map covering the nsv locus that confers resistance to Melon necrotic spot virus in melon (*Cucumis melo* L). Theor Appl Genet 111:914–922

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

Neff MM, Turk E, Kalishman M (2002) Web-based primer design for single nucleotide polymorphism analysis. Trends Genet 18:613–615

Obando-Ulloa JM, Eduardo I, Monforte AJ, Fernandez-Trujillo JP (2009) Identification of QTLs related to sugar and organic acid composition in melon using near-isogenic lines. Sci Hortic 121:425–433

Paran I, Goldman I, Tanksley SD, Zamir D (1995) Recombinant inbred lines for genetic mapping in tomato. Theor Appl Genet 90:542–548

Paris MK, Zalapa JE, McCreight JD, Staub JE (2008) Genetic dissection of fruit quality components in melon (*Cucumis melo* L.) using a RIL population derived from exotic × elite US Western Shipping germplasm. Mol Breed 22:405–419

Park Y, Katzir N, Brotman Y, King J, Bertrand F, Havey M (2004) Comparative mapping of ZYMV resistances in cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.). Theor Appl Genet 109:707–712

Park SO, Hwang HY, Crosby KM (2009) A genetic linkage map including loci for male sterility, sugars, and ascorbic acid in melon. J Am Soc Hortic Sci 134:67–76

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

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

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

Petreikov M, Shen S, Yeselson Y, Levin I, Bar M, Schaffer AA (2006) Temporally extended gene expression of the ADP-Glc pyrophosphorylase large subunit (AgpL1) leads to increased enzyme activity in developing tomato fruit. Planta 224:1465–1479

Petreikov M, Yeselson L, Shen S, Levin I, Schaffer AA, Efrati A, Bar M (2009) Carbohydrate balance and accumulation during development of near-isogenic tomato lines differing in the AGPase-L1 allele. J Am Soc Hortic Sci 134:134–140

Pitrat M, Hanelt P, Hammer K (2000) Some comments on infraspecific classification of cultivars of melon. In: Katzir N, Paris HS (eds) Cucurbitaceae 2000, 7th EUCARPIA meeting on cucurbit genetics and breeding. Acta Hortic, pp 29–36

Portnoy V, Benyamini Y, Bar E, Harel-Beja R, Gepstein S, Giovannoni JJ, Schaffer AA, Burger J, Tadmor Y, Lewinsohn E, Katzir N (2008) The molecular and biochemical basis for varietal variation in sesquiterpene content in melon (*Cucumis melo* L.) rinds. Plant Mol Biol 66:647–661

Ronen G, Cohen M, Zamir D, Hirschberg J (1999) Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. Plant J 17:341–351

Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of Beta and old-gold color mutations in tomato. Proc Natl Acad Sci USA 97:11102–11107

Rosa JT (1928) Change in composition during ripening and storage of melons. Hilgardia 3:421–443

Schaffer AA, Madore M, Pharr DM (1996) Cucurbits. In: Zamski E, Schaffer AA (eds) Photoassimilate distribution in plants and crops. Marcel Dekker, New York, pp 729–757

Sinclair JW, Park SO, Lester GE, Yoo KS, Crosby KM (2006) Identification and confirmation of RAPD markers and andromonoecious associated with quantitative trait loci for sugars in melon. J Am Soc Hortic Sci 131:360–371

Stepansky A, Kovalski I, Schaffer AA, Perl-Treves R (1999) Variation in sugar levels and invertase activity in mature fruit representing a broad spectrum of *Cucumis melo* genotypes. Genet Resour Crop Evol 46:53–62

Tadmor Y, King S, Levi A, Davis A, Meir A, Wasserman B, Hirschberg J, Lewinsohn E (2005) Comparative fruit colouration in watermelon and tomato. Food Res Int 38:837–841

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

Van Ooijen JW (2004) MapQTL® 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V, Wageningen

Van Ooijen JW, Voorrips RE (2001) JoinMap® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee 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, Peleman JD, van Eijk MJ (2007) AFLP technology for DNA fingerprinting. Nat Protoc 2:1387–1398

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

Wang YH, Choi WB, Thomas CE, Dean RA (2002) Cloning of disease-resistance homologues in end sequences of BAC clones linked to *Fom-2,* a gene conferring resistance to Fusarium wilt in melon (*Cucumis melo* L.). Genome 45:473–480

Yamaguchi M, Hughes DL, Yabumoto K, Jennings WG (1977) Quality of cantaloupe muskmelons—variability and attributes. Sci Hortic 6:59–70

Yan WK, Rajcan I (2003) Prediction of cultivar performance based on single- versus multiple-year tests in soybean. Crop Sci 43:549–555

Yu X, Wang X, Zhang W, Qian T, Tang G, Guo Y, Zheng C (2008) Antisense suppression of an acid invertase gene (MAI1) in muskmelon alters plant growth and fruit development. J Exp Bot 59:2969–2977

Zalapa JE, Staub JE, McCreight JD, Chung SM, Cuevas H (2007) Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. Theor Appl Genet 114:1185–1201

Zilberman DE, Safran M, Paz N, Amariglio N, Simon A, Fridman E, Kleinmann N, Ramon J, Rechavi G (2009) Does RNA editing play a role in the development of urinary bladder cancer? Urol Oncol. doi:10.1016/j.urolonc.2008.11.006

Zygier S, Chaim AB, Efrati A, Kaluzky G, Borovsky Y, Paran I (2005) QTLs mapping for fruit size and shape in chromosomes 2 and 4 in pepper and a comparison of the pepper QTL map with that of tomato. Theor Appl Genet 111:437–445