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ORIGINAL PAPER

Mapping of genetic loci that regulate quantity of beta-carotene in fruit of US Western Shipping melon (*Cucumis melo* L.)

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Abstract Melon (*Cucumis melo* L.) is highly nutritious vegetable species and an important source of β-carotene (Vitamin A), which is an important nutrient in the human diet. A previously developed set of 81 recombinant inbred lines (RIL) derived from Group Cantalupensis US Western Shipper market type germplasm was examined in two locations [Wisconsin (WI) and California (CA), USA] over 2 years to identify quantitative trait loci (QTL) associated with quantity of beta-carotene (QβC) in mature fruit. A moderately saturated 256-point RIL-based map [104 SSR, 7 CAPS, 4 SNP in putative carotenoid candidate genes, 140 dominant markers and one morphological trait (*a*) spanning 12 linkage groups (LG)] was used for QβC–QTL analysis. Eight QTL were detected in this evaluation that were distributed across four LG that explained a significant portion of the associated phenotypic variation for QβC ($R^2 = 8$ to 31.0%). Broad sense heritabilities for QβC obtained from RIL grown in WI. and CA were 0.56 and 0.68, respectively, and 0.62 over combined locations. The consistence of QβC in high/low RIL within location across years was confirmed in experiments conducted over 2 years. QTL map positions were not uniformly associated with putative carotenoid genes, although one QTL (*β-car6.1*) interval was located 10 cM from a β-carotene hydroxylase gene. These results suggest that accumulation of β-carotene in melon is under complex genetic control. This study provides the initial step for defining the genetic control of QβC in melon leading to the development of varieties with enhanced β-carotene content.

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Introduction

Carotenoids play essential functions in plants to include roles in phyto-hormone precursor action (Schwartz et al. 2003) and environmental adaptation through modulation of the photosynthetic apparatus (Demming-Adams and Adams 2002). In addition, some carotenoid pigments (e.g., α-carotene, β-carotene) are important for human health and nutrition (Mares-Perlman et al. 2002; Giovannucci 2002) resulting from their conversion to vitamin A by molecular cleavage and subsequent reduction.

The genes and enzymes involved in the biosynthesis of carotenoids pigments have received extensive research and review (Bartley and Scolnik 1995; Cunningham and Gantt 1998; Hirschberg 2001) to indicate that the carotenoid pathway is highly conserved in plants. The genes and cDNAs encoding nearly all the enzymes required for carotenoid biosynthesis in plants have been identified and sequenced, and their products have been characterized (Cunningham and Gantt 1998). Although the carotenoid biosynthetic pathway is known, the complex regulation and inheritance of carotenogenesis in plants remains poorly understood (Römer and Fraser 2005).

Melon (*Cucumis melo* L.; $2n = 2x = 24$) is an economically important, cross-pollinated vegetable species that is subdivided into seven cultivar groups (i.e., Flexuosus,

Conomon, Cantalupensis, Inodorus, Chito, Dudaim, and Momordica), where numerous market classes (e.g., Ogen, Galia, Charentais, and US Western Shipping) exist within many of these groups (Munger and Robinson 1991). Although fruit of these market classes are morphologically diverse, edible portions are rich in phytonutrients important for human health and nutrition (Mares-Perlman et al. 2002; Giovannucci 2002). Fruit of market classes having orange mesocarps, for instance, are a rich source of dietary carotenes (e.g., β-carotene; Gross 1987), where concentrations of β-carotene can range from 9 to 18 ppm in fresh tissue (Navazio 1994).

Genetic maps in melon have been constructed using a wide array of dominant (RAPD and AFLP) and codominant (RFLP, SSR, and SNP) markers employing F_2 and backcross populations (Baudracco-Arnas and Pitrat 1996; Danin-Poleg et al. 2002; Liou et al. 1998; Oliver et al. 2001; Silberstein et al. 2003; Wang et al. 1997). These maps, however, are unsaturated and define the position of only relatively few horticulturally important traits. More recently, genetic maps have been constructed using recombinant inbred lines (Perin et al. 2002a, b; Fukino et al. 2008; Zalapa et al. 2007b) and doubled haploid lines (DHL; Monforte et al. 2004) for the analysis of economically important qualitatively and quantitatively inherited traits. For instance, a 181-point (114 RAPD, 35 SSR, and 32 AFLP) genetic map constructed by Zalapa et al. (2007b) using RIL derived from Group Cantalupensis US Western Shipping germplasm spanned 1,032 cM with a mean marker interval of 5.7 cM on 15 linkage groups. Although this map was useful for identifying many yield and quality QTL (Zalapa et al. 2007b; Paris et al. 2008), it must, along with other currently constituted maps, be considered relatively unsaturated.

The inheritance and mapping of carotenoids in melon has not been documented, and enhanced, high carotene (>30 μg/g fresh wt.) germplasm is not publicly available. Recently, Monforte et al. (2004) evaluated the inheritance of orange mesocarp color using F_2 populations and double haploid lines, and hypothesized that three putative loci control the orange color expression in melon. This hypothesis, however, was not confirmed by comparative analysis of nearly isogenic lines differing in fruit color variation (Eduardo et al. 2007).

The recent dramatic increase in the number of published SSR markers for melon (>400; Chiba et al. 2003; Ritchel et al. 2004; Kong et al. 2007; Gonzalo et al. 2005; Fukino et al. 2007), and genomics resources [e.g., expressed sequence tag (EST) libraries; cucurbit genomics resource (http://cucurbit.bti.cornell.edu)] affords opportunities for map merging to increase map saturation and colinearity analyses. Given the need for increased saturation in melon maps and the economic importance of melon carotenes in human diets, a study was designed to: (1) increase the amount of SSR markers of the melon map published by Zalapa et al. (2007b); (2) use this revised map for identifying QTL associated with the quantity of β-carotene (QβC) in melon fruit; and (3) characterize and map putative carotenoid biosynthetic genes. This approach will allow the subsequent candidate gene analysis of QTL-associated sequences related to the quantity and expression of melon carotenoids.

Materials and methods

Plant material

A set of 81 recombinant inbred lines (F_7) was developed from a cross between US Department of Agriculture Group Cantalupensis breeding line 'USDA 846-1' (P_1; ~11 μg/g β-carotene) and western shipping type 'Top Mark' (P_2; ~13 μg/g β-carotene) (Zalapa 2005; Zalapa et al. 2007b), where both parents develop orange-fleshed (mesocarp) fruits. Although color pigmentation of mesocarp tissue between the parents is not visually different (Royal Horticultural Society 2005; 19B), the RIL population differ in mesocarp color pigmentation. These RIL have previously been employed for mapping of fruit yield- (Zalapa et al. 2007b) and quality-related components (Paris et al. 2008) in melon.

Experimental design

Experiments were conducted using these RIL in 2005 and 2006. In 2005, the two parental lines, 81 RIL, and two Cantalupensis commercial cultivars ('Sol Dorado', and 'Esteem' Syngenta Seeds, Gilroy, CA, USA) were evaluated at the University of California Desert Research and Extension Center in El Centro, CA, USA (DREC) and the University of Wisconsin Experimental Farm in Hancock, WI, USA (HES) during the spring and summer of 2005, respectively (Experiment 1). These locations differed dramatically in growing conditions, where average temperatures are typically 34°C in California (May–July) and 26°C in Wisconsin (May–August), and average relative humidity is 36 and 68% in California and Wisconsin, respectively.

The experimental design at both locations was a randomized complete block design (RCBD), consisting of three blocks with eight plants per plot. In Wisconsin, seeds were sown (Growing Mix No. 2; Conrad Fafard, Inc., Agawam, MA, USA) in a greenhouse (Madison, WI, USA), and then seedlings were transplanted to the field at the three-leaf stage every 0.35 m within rows on 2 m centers (72,600 plants/ha) into Planefield loamy sand

(Typic Udipasamment) soil at HES. In California, seeds were sown directly into Imperial silty clay Vertic Torrifluvents soil at the same row and plant spacing used in Wisconsin.

In order to confirm line performance and thus QTL placement, a subset of 20 RIL were identified based on relative QβC ranking in Experiment 1. In this regard, the most informative RIL in a mapping population are those in the lower and upper tails of a phenotypic distribution (Bernardo 2002). Thus, the five RIL possessing the highest and lowest QβC from each location (WI and CA) representing 25% of the populations were selected for phenotypic reevaluation to confirm putative QTL controlling QβC. The RIL subset, parental lines, and 'Sol Dorado' (SD) and 'Esteem' (ES) were planted and evaluated at DREC and HES during the spring and summer of 2006, respectively (Experiment 2). The experimental design at both locations was a RCBD, consisting of four blocks with eight plants per plot. Planting culture and plant density in each location was the same as described in Experiment 1.

Data collection

Although parents and RIL differ in flowering time (Zalapa et al. 2007a), the length of the harvesting period between RIL overlapped sufficiently to allow for the identification of a period where mature fruits from each RIL could be harvested simultaneously. Three mature fruit at same stage, i.e., full-slip, within treatment plot and from different plants were harvested and sampled for QβC. Fruit mesocarp samples ($\sim$5.0 g fresh weight) were collected from freshly, transversely cut fruits, and stored at -80°C until carotene extraction, which was performed according to Simon and Wolff (1987). Sample lyophilization and QβC was accomplished by processing each block (based on experimental design) at one time to minimize experimental error. QβC was determined by reverse phase high-performance liquid chromatography (HPLC) using a standardized synthetic β-carotene curve (Simon and Wolff 1987).

Analysis of variance and estimation of heritabilities

Location data for each experiment were initially combined to perform analyses of variance (ANOVA) using the *Proc mixed covtest* method *type3* procedure of SAS (SAS 1999). Variance components were estimated employing restricted maximum likelihood (REML), and each variance estimate was tested for significance using the likelihood ratio statistic (Littell et al. 1996). The linear random effects model for such ANOVA was the following: $Y = \mu + L + B(L) + R + LxR + e$; where Y is the trait, μ is the common effect, L is the location effect, $B(L)$ is the block within location effect, R is the effect of the RIL, $L \times R$ is the location $\times$ RIL interaction, and e is the plot to plot variation within RIL. Analyses of the RIL were also performed by location, where phenotypic distributions for QβC in RIL were evaluated for normality by box plot analyses, and Shapiro–Wilk normality tests (Shapiro and Wilk 1956).

Best linear unbiased predictors (BLUPs; Bernardo 2002), standard errors (SE), and 95% confidence intervals (CIs) were estimated for each RIL using the *solution* option of the *random* statement of the *Proc mixed covtest* procedure in SAS (SAS Institute 1999). Best linear unbiased estimators (BLUEs) were also estimated for P_1, P_2, F_1, and commercial varieties using the *solution* option of the *model* statement of the *Proc mixed covtest* procedure (SAS Institute 1999). This procedure estimates fixed effect values from the raw data while making variable value adjustments during such estimations (de Leon et al. 2005).

In order to assess whether genotype $\times$ environment (G $\times$ E) interactions were due to trait magnitude changes between locations or changes in the direction of the response (i.e., RIL rank changes), Spearman (rank) correlation coefficients (r_s) were calculated using RIL data for β-carotene content across locations according to Yan and Rajcan (2003). When the correlation coefficient between data across locations was $r_s \leq 0.5$, G $\times$ E interactions were considered more likely to be due to RIL rank changes, and when $r_s \geq 0.5$, GxE interactions were considered more likely to be due to trait magnitude changes between locations.

Phenotypic correlations (r; $n = 81$) between QβC (Experiment 1 only), and the fruit yield and quality component traits evaluated by Zalapa et al. (2007b) and Paris et al. (2008) using the same RIL at the same locations employed herein were also calculated by location using the *Proc corr spearman* procedure of SAS (SAS Institute 1999).

The broad-sense heritabilities based on RIL BLUPs (h^2_{BS}) were calculated as $h^2_{BS} = (\sigma^2_R)/\sigma^2_{PR}$; where σ^2_R and σ^2_{PR} are the variance among RIL and phenotypic variance based on RIL BLUPs, respectively. The estimate of σ^2_{PR} was calculated as $\sigma^2_R + \sigma^2_{L\times R/b} + \sigma^2_{e/bl}$; where b, l, σ^2_R, $\sigma^2_{L\times R}$ and σ^2_e refer to the number of blocks, the number of locations, the variance among RIL, the variance due to location $\times$ RIL interactions, and the plot-to-plot variation within RIL, respectively (Falconer and Mackay 1996). The standard error (SE) of broad-sense heritabilities based on RIL BLUPs were calculated as SE $(h^2_{BS}) = [\mathrm{Var}(\sigma^2_R)]^{1/2}/\sigma^2_{RF}$.

Target genes, primer design, and PCR amplification of putative carotenoid structural and related genes

Genes and their abbreviations defined herein are listed in Table 1. Since a melon EST library was not available at

Table 1 Characteristic of candidate putative carotenoid structural and related genes evaluated in melon (*Cucumis melo* L.) RIL population derived from a cross between 'USDA 846-1' (P_1) and 'Top Mark' (P_2)

Enzyme name	Gene symbol	Sequence of degenerative primers	(°C)[a]	Fragment size (bp)			Accesion number of closest putative ortholog and % identity[b]	Melon EST database	Sequence of primers used to amplify fragment used for genotyping and sequencing	Annealing temperature (°C)	Fragment Size	Poliy-morphism
				Exon	Intron	Total						
Phytoene synthase	PS	F- ATIGAAGGRATGMGDATGGA R-GCYTCDATCTCRTCCARTAT	60	478	(2) 1034	1,512	DQ494214 (95)	MU10942	F-TGCTGGTACAGTTGGGTTGA R- TGAAAGACCTGCCTGTGCTA	60	672	SNP[d]
β-carotene hydroxylase	BOH-1	F- TGGGCVIGRTGGGCBCA R- ICCRTCGTGRACRAACATGTA	60	243	(2) 441	684	DQ157169 (83)	MU9511	F- TGGGCVIGRTGGGCBCA R-TCCCCAGTGATTAAACAAACA	60	523	SNP[d]
	BOH-2		60	243	(2) 356	599	DQ156907 (77)	–	F-TCGTTATGGGATATGCACGA R-TGGAGACCCACGTCATACAA	60	595	–
Lycopene β–cyclase	LycB	F1- GATGARTTTGARGCYATGGA R1- GGRTTRTAIGGITTRTCRTAYTG	52	320	0	320	DQ641127 (98)	MU9417	–	–	–	–
		F2- CARTAIGAIAARCCITAYAAYCC R2-ATRTCCATICCRAARCAGAAGAA	52	590	0	590	EF183522 (95)	MU254	–	–	–	–
Carotenoid isomerase	CRTISO	F- AATGCTACWAGATGGGAYAC R- GTTGTAAAWATGTGAAGWATATG	54	287	(2) 230	517	AB114667 (80)	MU4592	–	–	–	–
Zeaxanthin epoxidase	ZEP	F- AARATGCARTGGTAYGCATT R- GCATCRTCRTCYTCRAACCA	63	636	(6) 1414	~2100	DQ641126 (100)	MU7815	F-TCACATTGTCACACCAACCA R-AAACACCCCAATCAACCAGA	60	430	SNP[d]
Zeta carotene desaturase	ZCDS[c]	F- GTGGGATCCWGTTGCNTAYGC R-AYCTCYCTRCAHCCCCACC	60	192	(1) 91	283	AF372617 (81)	MU4107	–	–	–	–
Phytoene desaturase	PDS[c]	F-TGATWTTTGCWATGCCAARYAARCC R-CAYTGCATYGAMAGYTCRTCWG	60	150	(2) 549	700	ABM45860 (91)	MU9070	–	–	–	–
Violaxanthin de-epoxidase	VDE	F-TGTGGDGAYITGTTYGARAA R-TATCCATCCCADGCRTCRTT	54	472	(1) 768	1240	DQ233246 (77)	–	F-TGTACCCGACCCTTCTGTTC R-TTGGCTCATTTCGGTCCTAC	60	639	SNP[d]
Orange gene	Or	F-TGCTGATTGAGCCAGTATCG R-TGYTCRCTNGCCATWSCCAT	60	148	(1) 655	803	DQ482459.1 (59)	MU4014	–	–	–	–

[a] Annealing temperature of the primers

[b] Sequence similarity at protein level based on tblastx hits. Percent similarity is shown for longest matching stretch of best hit sequence labeled as a desired ortholog gene

[c] Degenerative primers were from previously publication (Just et al. 2007)

[d] SNP were genotype with direct sequence with the primers designed for that

project initiation, and only one structural carotenoid gene had been published, *phytoene synthase* (Karvouni et al. 1995), degenerative primers were designed for eight carotenoid structural genes. Homologous gene sequence information (nucleotide and protein) was obtained for several plants species (*Arabidopsis*, *Citrus*, *Daucus*, etc.) from the NCBI database, and then aligned using ClustalX (Thompson et al. 1997) to design 28 degenerative primers originating from the most conserved genomic regions examined. Published degenerative primers for some carotenoid structural genes (Just et al. 2007) were also evaluated for their utility in generating gene sequence information. Additionally, the complete protein and nucleotide sequences from the recently identified *Or* gene, which is associated with the accumulation of β-carotene in cauliflower (*Brassica oleracea*) (Lu et al. 2006), were aligned using ClustalX, and four degenerative primers were designed from highly conserved genomic regions.

Polymerase chain reactions (PCR) and PCR cycling conditions were performed according to Zalapa et al. (2007b), where annealing temperatures were adjusted depending on the primers set (Table 1). After gel electrophoresis, unique amplicons were physically isolated, purified using the Wizard SV Gel clean-up system (Promega Corp., Madison, WI, USA), sequenced via BigDye terminator chemistry, and then evaluated with other species homologous using the NCBI Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST). Putative genes were declared if the BLAST results contained matches in regions not covered by the original degenerative primers. Sequence polymorphisms between RIL parental lines were examined, and more specific primer sets flanking each polymorphism were then designed (Table 1).

Melon SSR and cucumber EST evaluation

Three hundred and eighty-four SSR markers (31, Chiba et al. 2003; 144, Ritchel et al. 2004;183, Fukino et al. 2007; 26, Gonzalo et al. 2005; Monforte, personal communication 2008), and 42 EST-SSR markers (Kong et al. 2006, 2007) were used to amplify parental DNA for polymorphism detection according to Zalapa et al. (2007b). Parental screen was performed using capillary electrophoresis employing fluorescent dUTP (ChromaTide Alexa Fluor 546-14-dUTP, Invitrogen, CA, USA) in the PCR reaction, and GeneMarker V1.5 software was used to determine alleles sizes (Soft Genetics LLC, 2005). RIL genotyping was performed using 4% (w/v) Metaphor agarose (Lonza, NJ, USA) or capillary electrophoresis, depending on differences in allele sizes.

One hundred and ninety-one cucumber (*Cucumis sativus* L.) EST primers (Johnson, unpublished) were evaluated for amplification in melon. Primers combinations that produced unique amplicons were purified using the Wizard SV Gel clean-up system (Promega Corp., Madison, WI, USA), and sequenced via BigDye terminator chemistry. Single nucleotide polymorphisms (SNP) between RIL parental lines were identified, and then utilized to develop cleaved amplified polymorphic sequences (CAPS) markers using appropriate restriction enzymes (RE). After performing specific primer-based PCR, 5 µl of the recovered amplicons were mixed with a 15-µl containing 1 U of RE and buffer supplied by manufacturer, and subsequently incubated for 2 h at temperatures that optimized RE. Digestion products were then evaluated using 1% (w/v) agarose.

Linkage map construction

The degree of segregation distortion associated with newly identified SSR and SNP markers was determined by marker data comparison against the expected 1:1 ratio for RIL using chi-square tests, where significant distortion was declared at $P < 0.01$ (Vuylsteke et al. 1999). Genotypic data originating from RIL analysis conducted herein were combined with previous map information (Zalapa et al. 2007b) to produce a matrix of 256 markers [104 SSR, 7 CAPS, 4 SNP, 140 dominant markers (104 RAPD, 29 AFLP, and 7 dominant SSR), and the *a* locus (andromonoecy)] for analysis.

A linkage map was constructed using MapMaker/EXP 3.0 (Lander et al. 1987), where markers were associated with the *group* command at LOD = 5.0 and a recombination frequency value of 0.20. Markers within a group were ordered using the *Order* command with LOD of 3.0. The remaining markers were the located with the *Try* command and the map order was re-tested using the *Ripple* command. Map distances were calculated with the Kosambi function. Some of the ordered groups were joined using a LOD = 4.0 or 3.0 depending on the recombinant distance between them, and the map order was then re-tested using the *Ripple* command.

QTL mapping

Composite interval mapping (Zeng 1993, 1994) was performed using Windows QTL Cartographer 2.5 (Wang et al. 2001–2004) with a walking speed of 1 cM and a window size from 0.5 to 5.0 cM; up to 12 maximum background marker loci were selected by stepwise forward regression to reduce background effects. A QTL was declared significant when the LOD score was higher than the LOD threshold calculated using 1,000 permutations for an experimental-wise (type I) error rate of $P = 0.05$.

Two-dimensional genome scans for detection of epistatic interactions were performed by employing the

Haley–Knott protocol (HK; Haley and Knott 1992) in R/qtl (Broman et al. 2003) which identifies putative epistatic interactions by pair-wise comparisons. The multi-point genotype probabilities for the HK analysis were calculated using the *Calc.genoprob* command with a step interval of 2 cM and error probability of 0.01. Two-dimensional genome scans calculated LOD scores for the full (two QTL plus interaction) and additive (two QTL but not interaction) model, which were then used to calculate interaction LOD scores by subtracting LOD full from LOD additive ($LOD_{INT} = LOD_{FULL} - LOD_{ADD}$). The LOD threshold for the interaction was determined by 1,000 permutations and an experimental-wise (type I) error rate higher of $P = 0.05$ for both experimental locations (Experiment 1).

Results

Analysis of variance and heritabilities estimate

The QβC in RIL population ranged from 8.1 to 22.3 μg/g in California, and 3.7 to 24.4 μg/g in the Wisconsin Experiment 1 (2005 evaluation). The RIL population was normally distributed in both locations, and the ANOVA indicated significant ($P \leq 0.001$) RIL main effects and environment × RIL interactions (data not presented). Results of variance component analyses (Table 2) mirrored those of the ANOVA [i.e., significant ($P \leq 0.01$) differences among RIL and environment × RIL interactions; Table 2] when analyses were conducted over and by location. RIL were also the largest variance component either in combined or independent location analyses. Spearman correlations (r_s) between environments indicated that the interaction between environment and RIL was due to changes in trait magnitude and changes in the direction of the response (i.e., RIL rank changes) in the locations examined ($r_s = 0.51$).

The h^2_{BS} for QβC was moderate with values ranging from 0.56 (CA) to 0.68 (WI) (Table 2). The h^2_{BS} in combined location analysis was 0.61. Regardless of location, all h^2_{BS} estimates were at least twice the value of their standard errors.

Parent and RIL comparisons

Given that parental performance differed between environments (Table 3) and significant RIL genotype × location interaction effects (Table 2) were detected over years and locations, data are hereafter presented by location. Although QβC in parental and F_1 fruits grown in California were similar, fruits harvested from 'Top-Mark' (17.9 μg/g) in Wisconsin contained, on average, more ($P < 0.05$) β-carotene than 'USDA 846-1' (11.3 μg/g) and F_1 progeny (11.8 μg/g), which were themselves similar (Table 3). These

Table 2 Variance components, percentage of variance component contribution to the total variance, and broad-sense heritabilities (h^2_{BF}) for β-carotene content (μg g^{-1}) in melon (*Cucumis melo* L.) recombinant inbred lines (RIL) derived from a cross between 'USDA 846-1' (P_1) and 'Top Mark' (P_2) grown at El Centro, CA and Hancock, WI, 2005

Source of variation	El Centro, CA		Hancock, WI	
	Variance component	Percent of total	Variance component	Percent of total
Block [B]	10.42 ± 11.7NS	36.0	1.54 ± 1.7NS	7.6
RIL	12.40 ± 2.6**	42.9	16.24 ± 2.9**	80.4
RIL × Block [RIL × B]	6.09 ± 1.2**	21.1	2.42 ± 1.0*	12.0
Total	28.91	20.20		
h^2_{BF}	0.56 ± 0.16	0.68 ± 0.17		

Source of variation	El Centro and Hancock	
	Variance component	Percent of total
Location [*L*]	0 ± 1.4NS	0.0
Block (Location) [*B*(*L*)]	5.86 ± 4.5NS	27.4
RIL	9.18 ± 2.2**	29.6
RIL × Location [RIL × *L*]	6.33 ± 1.4**	43.0
Total	21.37	
h^2_{BF}	0.61 ± 0.14	
(r_s)	0.51**	

NS not significant

*, ** The effect is significant at $P \leq 0.05$ and $P \leq 0.01$

Table 3 Best linear unbiased estimations (BLUE's) for β-carotene content (μg g^{-1}) of commercial melon (*Cucumis melo* L.) hybrids 'Sol Dorado', 'Esteem', and line 'USDA 846-1' (P_1), 'Top Mark' (P_2), and F_1 progeny, and best linear unbiased predictions (BLUP's) for RIL population and related statistics as evaluated at Hancock, WI and El Centro, CA, 2005 and 2006

Hancock, WI.				El Centro, CA			
Cultigen		2005[a]	2006[b]	Cultigen		2005[a]	2006[b]
Sol Dorado		19.6	16.9	Sol Dorado		10.7	13.7
Esteem		11.3	10.7	Esteem		12.4	8.8
Top-Mark		17.9 a	11.4 a	Top-Mark		13.28 a	10.47 a
USDA 846		11.3 b	8.6 b	USDA 846		15.30 a	10.80 a
F_1		11.8 b	10.6 b	F_1		12.57 a	9.88 a
RIL's		13.8 ± 0.9	11.4 ± 1.8	RIL's		14.1 ± 1.8	12.4 ± 1.3
CI (95%) lower		10.1	5.6	CI (95%) lower		5.8	8.3
CI (95%) upper		17.5	17.3	CI (95%) upper		22.3	16.5
Highest	RIL-049[c]	24.4	20.5	Highest	RIL-049[c]	22.3	22.9
	RIL-045	23.5	15.1		RIL-064	19.7	12.3
	RIL-024	23.0	16.9		RIL-067	19.7	10.43
	RIL-124	22.8	16.3		RIL-144	18.9	11.4
	RIL-066	21.0	20.4		RIL-028	18.2	14.8
Lowest	RIL-122[c]	3.7	6.0	Lowest	RIL-122[c]	8.1	6.8
	RIL-075[c]	5.7	3.3		RIL-075[c]	8.8	6.3
	RIL-063	6.4	10.7		RIL-061	8.8	9.3
	RIL-047[c]	8.2	6.5		RIL-174	10.4	8.7
	RIL-147	9.1	6.4		RIL-047[c]	10.5	9.5
r_s across years		0.84**		r_s across years		0.78**	

[a] Evaluation of all recombinant inbred lines ($n = 81$)

[b] Evaluation of sub-set of recombinant inbred lines ($n = 20$)

[c] RIL that transgressed mean of P_1, or P_2 and were consistent across location

Values are amount of β-carotene content (μg g^{-1}) in the fresh flesh tissue of the fruit, extracted according to Simon and Wolff (1987), and quantified by HPLC analysis

Values in columns followed with the same letter are not significantly different at 0.05 significance level

*, ** The effect is significant at $P \leq 0.05$ and $P \leq 0.01$, respectively

differences and similarities and attending GxE interactions were confirmed in Experiment 2 (Table 3).

The mean QβC performance of 'Top-Mark' (17.9 μg/g) was significantly different ($P < 0.05$) from that of the RIL population (13.8 μg/g) in Wisconsin (BLUE vs. BLUP CI's comparisons (Table 3). The location RIL BLUP QβC values (μg/g) were similar across years [WI = 13.8 ± 0.9, CA = 14.1 ± 1.8 (Experiment 1); WI = 11.4 ± 1.8, CA = 12.4 ± 1.3 (Experiment 2)]. The lowest QβC values in subset RIL were higher in California (average = 9.32 μg/g) than in Wisconsin (average = 6.62 μg/g) regardless of growing season. Twenty-three RIL transgressed the mean performance of either parent across locations (8 RIL with higher and 15 RIL with lower QβC than parents in WI and CA) (data not presented). Similarly, fruit of six RIL had higher QβC than either commercial variety ('Sol Dorado' and 'Esteem'), whose values were either higher or lower than the average of the RIL population depending on location and year. Although the BLUP QβC values across years indicated that six RIL (RIL-169, RIL-49, RIL-122, RIL-75, RIL-61, and RIL-47) performed similarly in both locations, RIL producing fruit having lower QβC (<10 μg/g) were less sensitive to environmental effects than their high β-carotene (>18 μg/g) producing counterparts. Nevertheless, results indicated that RIL performed consistently in each environment [i.e., no rank changes among subset RIL (high vs. low) in Experiments 1 and 2; Table 3].

Significant ($P \leq 0.05$) phenotypic correlations were detected between QβC and some previously evaluated traits (Zalapa et al. 2007b; Paris et al. 2008). Fruit weight (FW) and average fruit weight (AWF) were positively correlated with QβC in Wisconsin ($r = 0.29$ and $r = 0.26$; respectively), but not in California. Similarly, mesocarp pressure (MP, i.e., fruit firmness) was negatively correlated with QβC only in Wisconsin ($r = -0.41$), and seed cavity to fruit diameter ratio (CD) was positively correlated with QβC in California ($r = 0.23$), but not in Wisconsin.

Carotenoid related genes

Genomic sequences from eight structural carotenoid genes and one carotenoid related gene were characterized using degenerative primers (Table 1). Gene sequence information regarding *BOH-2* and *VDE* is not currently present in the only publicly released EST melon library (International Cucurbit Genomic Initiatives (ICuGI); access: July 2008, http://www.icugi.org). However, since this library is solely composed of partial gene sequences and given the amplicons derived from the *LyCB* and *Or* sequence generated herein originated from a unique and different protein region, the partial sequence information obtained was considered informative. In support of this contention, single nucleotide polymorphisms (SNP) in four carotenoids genes (*PS*, *BOH-1*, *ZEP*, and *VDE*; Table 1) were identified in structural elements of introns, and were, in turn, used for RIL-based gene mapping.

The degenerative primers for the β-carotene hydroxylase (*BOH*) gene produced two amplicons [684 and 599 bp (base pair)] (data not presented). The posterior sequence of both amplicons was indicative of gene duplication, where inherent size differences resulted from intron sequence variation. Although genomic sequence alignments were not possible (i.e., intron sequences were too degenerate), the partial nucleotide and protein sequence of the exon could be aligned after intron deletion. The large and small sequence fragments of *BOH* were designated gene *BOH-1* and *BOH-2*, respectively. The nucleotide sequence of melon EST accession MU9511 was found to have considerable homology with *BOH-1*, and gene-specific primers were designed using the intron sequence of each gene. Subsequently, single nucleotide polymorphisms were identified for *BOH-1*, but not for *BOH-2*.

SSR evaluation and cucumber EST

The parental screening identified 65 polymorphic SSR markers which were identified from SSR arrays published by Chiba et al. (2003; 6), Ritchel et al. (2004; 17), Fukino et al. (2007; 27), Kong et al. (2006, 2007; 7), and Gonzalo et al. (2005; 6, personal communication; Monforte 2008; 2). These SSR, in conjunction with 39 previously identified polymorphic SSR in this RIL population (Zalapa et al. 2007b), allowed the use of 104 allele-variable SSR markers in map construction. While about 61% (64 SRR) of this SSR marker pool have previously been placed on various melon maps (Perin et al. 2002a; Silberstein et al. 2003; Gonzalo et al. 2005; Zalapa et al. 2007b; Fukino et al. 2008), 40 markers are unique to the map construction herein. Regardless of marker origin, no segregation distortion was detected in this SSR marker array as evaluated by RIL chi-square segregation analyses.

Fig. 1 Linkage map and locations of quantitative trait loci associated with β-carotene content estimated in melon (*Cucumis melo* L.) recombinant inbred lines (81) derived from a cross of 'USDA 846-1' × 'Top Mark'. Linkage numbers are designated according to Perin et al. (2002a), and yield component QTL are designated using the backbone map of Zalapa et al. (2007b), and quality QTL are according to Paris et al. (2008). Italicized numbers (*1-15*), underlined numbers (1–12) and, roman numerals (I–XII) correspond to linkage groups in Zalapa et al. 2007b, Gonzalo et al. 2005 and Fukino et al. 2008, respectively. SSR markers are in *bold*, EST-SSR markers are italicized and in bold, EST-Cucumber (*Cucumis sativus* L.) are in *bold* and *underline*, Carotenoid related genes are *italicized*, *bold* and *underlined* (see Table 1), and *fn*, *fw*, and *scc* designate fruit number, fruit weight, and total soluble solids QTL, respectively, *W* refer to QTL identified using Wisconsin data independently, *C* refer to QTL identified in California data independently, *W–C* refer to QTL identified using Wisconsin and California data collectively, *Markers or groups of markers joint at LOD 4, **Markers or groups of markers joint at LOD 3, Aligned of unjoined LG were based on syntentic analyses employing common SSR markers with Gonzalo et al. (2005) and Fukino et al. (2008)

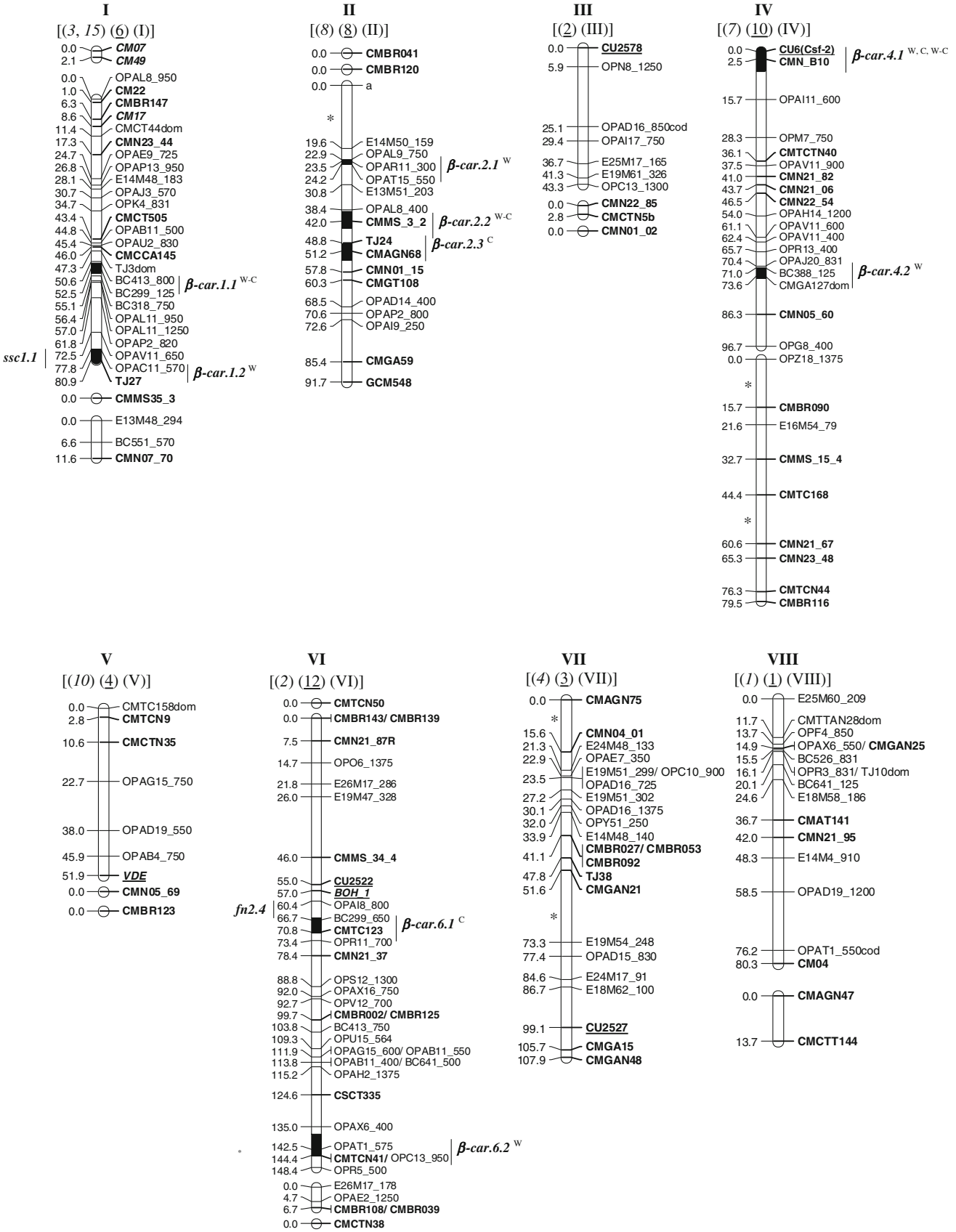
Fifty-three cucumber EST primers produced unique amplicons in melon, where SNP were identified in nine (CU2477, CU2527, CU2557, CU2522, CU6, CU2578, CU468, CU340, and CU2484) of these primers (~17%). Two cucumber EST markers (CU2477 and CU468) were not used in marker construction since segregation distortion was detected in RIL chi-square analysis. Comparative analysis of the nine polymorphic EST-Cucumber sequences identified that CU2557, CU2522, CU6, CU2578, CU468, CU340 and CU2484 were homologous to EST-Melon MU4718, MU12036, MU10410, MU16153, MU5734, MU3626, MU15536, respectively.

Linkage map construction

The 256 markers [104 SSR, 7 CAPS, 4 SNP, 140 dominant markers, and one morphological trait (*a*)] employed for the linkage analysis allowed for the construction of an initial map consisting of 21 linkage groups (LG). These LG were subsequently relegated to 12 linkage groups based upon SSR markers that were common to previously published melon genetic maps (Perin et al. 2002a; Gonzalo et al. 2005; Zalapa et al. 2007b; Fukino et al. 2008; Fig. 1). Similarly, eight SSR markers that remained unlinked were positioned in their corresponding LG using previously published melon maps. The resulting map spanned 1180.2 cM, with a mean marker interval of 4.6 cM, where the largest distance between any two markers was 22.3 cM (CMTCN19 locus and OPS12_570; LG X).

QTL analysis

Composite interval mapping detected eight QTL, three in California and five in Wisconsin (Table 4; Fig. 1). These QTL were distributed over four LG such that one was


I
[(3, 15) (6) (I)]
0.0 CM07
2.1 CM49
0.0 OPAL8_950
1.0 CM22
6.3 CMBR147
8.6 CM17
11.4 CMCT44dom
17.3 CMN23_44
24.7 OPAE9_725
26.8 OPAP13_950
28.1 E14M48_183
30.7 OPAJ3_570
34.7 OPK4_831
43.4 CMCT505
44.8 OPAB11_500
45.4 OPAU2_830
46.0 CMCCA145
47.3 TJ3dom
50.6 BC413_800 β-car.1.1 W-C
52.5 BC299_125
55.1 BC318_750
56.4 OPAL11_950
57.0 OPAL11_1250
61.8 OPAP2_820
ssc1.1 72.5 OPAV11_650
77.8 OPAC11_570 β-car.1.2 W
80.9 TJ27
0.0 CMMS35_3
0.0 E13M48_294
6.6 BC551_570
11.6 CMN07_70
II
[(8) (8) (II)]
0.0 CMBR041
0.0 CMBR120
0.0 a
19.6 E14M50_159
22.9 OPAL9_750
23.5 OPAR11_300 β-car.2.1 W
24.2 OPAT15_550
30.8 E13M51_203
38.4 OPAL8_400
42.0 CMMS_3_2 β-car.2.2 W-C
48.8 TJ24
51.2 CMAGN68 β-car.2.3 C
57.8 CMN01_15
60.3 CMGT108
68.5 OPAD14_400
70.6 OPAP2_800
72.6 OPAI9_250
85.4 CMGA59
91.7 GCM548
III
[(2) (III)]
0.0 CU2578
5.9 OPN8_1250
25.1 OPAD16_850cod
29.4 OPAI17_750
36.7 E25M17_165
41.3 E19M61_326
43.3 OPC13_1300
0.0 CMN22_85
2.8 CMCTN5b
0.0 CMN01_02
IV
[(7) (10) (IV)]
0.0 CU6(Csf-2)
2.5 CMN_B10 β-car.4.1 W, C, W-C
15.7 OPAI11_600
28.3 OPM7_750
36.1 CMTCTN40
37.5 OPAV11_900
41.0 CMN21_82
43.7 CMN21_06
46.5 CMN22_54
54.0 OPAH14_1200
61.1 OPAV11_600
62.4 OPAV11_400
65.7 OPR13_400
70.4 OPAJ20_831
71.0 BC388_125 β-car.4.2 W
73.6 CMGA127dom
86.3 CMN05_60
96.7 OPG8_400
0.0 OPZ18_1375
15.7 CMBR090
21.6 E16M54_79
32.7 CMMS_15_4
44.4 CMTC168
60.6 CMN21_67
65.3 CMN23_48
76.3 CMTCN44
79.5 CMBR116
V
[(10) (4) (V)]
0.0 CMTC158dom
2.8 CMTCN9
10.6 CMCTN35
22.7 OPAG15_750
38.0 OPAD19_550
45.9 OPAB4_750
51.9 VDE
0.0 CMN05_69
0.0 CMBR123
VI
[(2) (12) (VI)]
0.0 CMTCN50
0.0 CMBR143/ CMBR139
7.5 CMN21_87R
14.7 OPO6_1375
21.8 E26M17_286
26.0 E19M47_328
46.0 CMMS_34_4
55.0 CU2522
57.0 BOH_1
fn2.4 60.4 OPAI8_800
66.7 BC299_650 β-car.6.1 C
70.8 CMTC123
73.4 OPR11_700
78.4 CMN21_37
88.8 OPS12_1300
92.0 OPAX16_750
92.7 OPV12_700
99.7 CMBR002/ CMBR125
103.8 BC413_750
109.3 OPU15_564
111.9 OPAG15_600/ OPAB11_550
113.8 OPAB11_400/ BC641_500
115.2 OPAH2_1375
124.6 CSCT335
135.0 OPAX6_400
142.5 OPAT1_575 β-car.6.2 W
144.4 CMTCN41/ OPC13_950
148.4 OPR5_500
0.0 E26M17_178
4.7 OPAE2_1250
6.7 CMBR108/ CMBR039
0.0 CMCTN38
VII
[(4) (3) (VII)]
0.0 CMAGN75
15.6 CMN04_01
21.3 E24M48_133
22.9 OPAE7_350
23.5 E19M51_299/ OPC10_900
OPAD16_725
27.2 E19M51_302
30.1 OPAD16_1375
32.0 OPY51_250
33.9 E14M48_140
41.1 CMBR027/ CMBR053
CMBR092
47.8 TJ38
51.6 CMGAN21
73.3 E19M54_248
77.4 OPAD15_830
84.6 E24M17_91
86.7 E18M62_100
99.1 CU2527
105.7 CMGA15
107.9 CMGAN48
VIII
[(1) (1) (VIII)]
0.0 E25M60_209
11.7 CMTTAN28dom
13.7 OPF4_850
14.9 OPAX6_550/ CMGAN25
15.5 BC526_831
16.1 OPR3_831/ TJ10dom
20.1 BC641_125
24.6 E18M58_186
36.7 CMAT141
42.0 CMN21_95
48.3 E14M4_910
58.5 OPAD19_1200
76.2 OPAT1_550cod
80.3 CM04
0.0 CMAGN47
13.7 CMCTT144

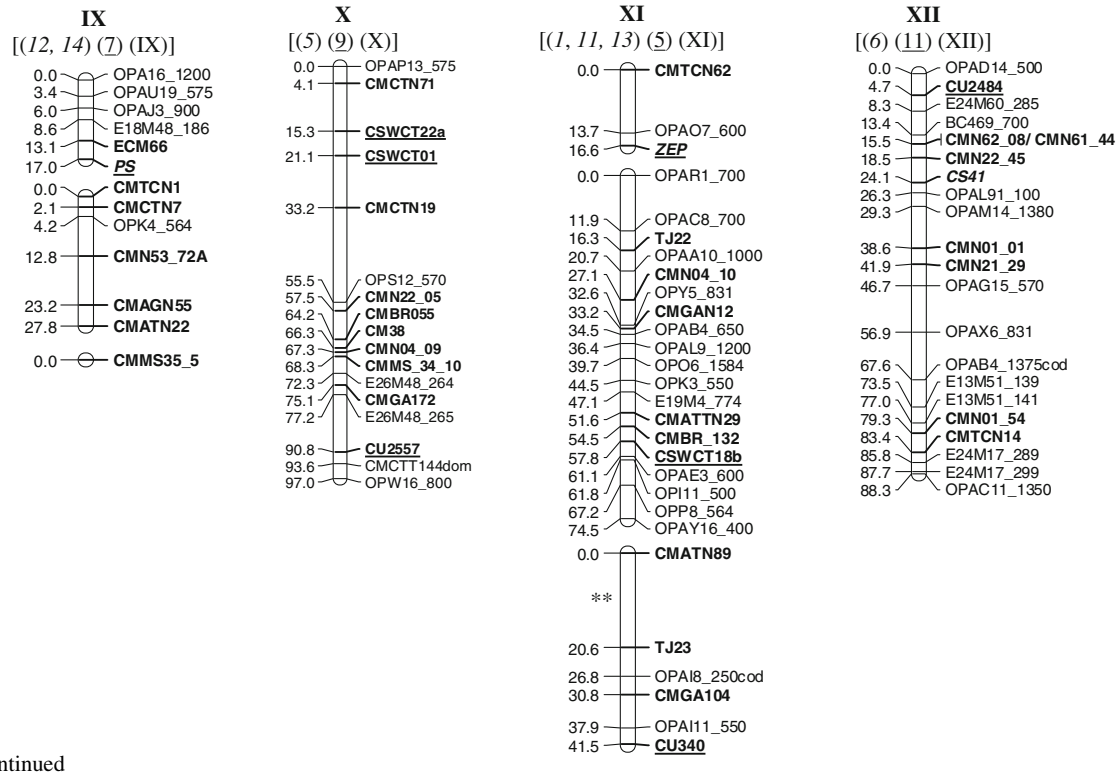


Fig. 1 continued

located in LG I, two in LG II, three LG IV, and two in LG VI. Additionally, one location-independent QTL was detected in LG IV (*β-car.4.1*), totaling 7 Q*β*C-associated QTL. Three QTL detected in California and four detected in Wisconsin were defined above the mapping threshold ($P \leq 0.001$; LOD > 4.0 and 4.3 for locations, respectively). The proportion of the phenotypic variance explained by a single QTL (R^2) ranged from 8.0% (*β-car.4.2*) to 31.0% (*β-car.4.1*). Two QTL (*β-car.4.1* and, *β-car.2.2*) detected in California must be considered as major QTL ($R^2 > 20\%$), and three QTL detected in Wisconsin had R^2 values between 12.0 and 14.0%. The direction of allelic effects varied depending on specific Q*β*C QTL, where the effect on Q*β*C was increased by some QTL alleles (*β-car.6.1*, *β-car.4.1*, *β-car.2.1*, and *β-car.2.3*) contributed by line 'USDA 846-1' or increased by QTL (*β-car.6.2*, *β-car.1.2* and, *β-car.4.2*) resident in 'Top Mark'.

Composite interval mapping also detected three QTL using Wisconsin and California collectively (Table 4; Fig. 1). These QTL were distributed over three LG (LG I, LG II, and LG IV) as defined by the mapping threshold ($P \leq 0.001$; LOD > 4.0), and thus must be considered major QTL ($R^2 > 20\%$). One QTL detected on LG I (*βcar.1.1*) is located 25 cM apart from QTL *β-car.1.2*, which was detected in Wisconsin. Similarly, a QTL detected in LG II (*β-car.2.2*) resides near QTL *βcar.2.1* and QTL *β-car.2.3* as detected in Wisconsin and California, respectively. Likewise, the third QTL was positioned on LG IV at about the same position as the independent location QTL *β-car.4.1*, thus it consider the same QTL. Because of the stability of expression of *β-car.4.1* across growing locations, this QTL should be preferred for MAS.

Two-dimensional epistasis genome scans

The evaluation of 496 pair-wise genomic combinations identified three and four putative epistatic interactions (LOD > 3) in Wisconsin and California, respectively (data not presented). Since these interactions were location dependent with LOD values lower than the established LOD threshold [LOD > 4.4 ($P > 0.05$)], they were not considered remarkable.

Discussion

Recently, considerable effort has been placed on the identification of QTL associated with carotenoid pathways and biosynthetic genes in tomatoes (*Lycopersicon* spp.; Fulton et al. 2000), pepper (*Capsicum* spp.; Throup et al. 2000; Huh et al. 2001), carrots (*Daucus* spp.; Santos and Simon 2002; Just et al. 2007), maize (*Zea* spp.; Wong et al. 2004), and wheat (*Triticum* spp.; Pozniak et al. 2007).

Table 4 Quantitative trait loci (QTL), linkage group position (LG), associated logarithm of odd (LOD), percentage of explained phenotypic variation (R^2), and additive effects for β-carotene content estimated in a population of melon (*Cucumis melo* L.) recombinant inbred lines (81) derived from a cross of line 'USDA 846-1' × 'Top Mark' evaluated in El Centro, CA and Hancock, WI in 2005

Linkage group	QTL[a]	Location	Position (cM)	Nearest marker locus[b]	LOD	R^2 (%)	Additive effect[c]
I	*β-car.1.2*	WI	77.8	OPAC11_570	6.06	12.0	−1.52
II	*β-car.2.1*	WI	20.6	OPAR11_300	6.40	14.0	1.57
IV	*β-car.4.1*	WI	0.0	CU6 (*csf-2*)	6.33	13.0	1.49
IV	*β-car.4.2*	WI	72.0	BC388_125	3.96	8.0	−1.79
VI	*β-car.6.2*	WI	142.5	OPAT01_575	4.62	9.0	−1.33
II	*β-car.2.3*	CA	52.2	CMAGN68	6.82	20.0	1.96
IV	*β-car.4.1*	CA	5.5	CMN_B10	9.01	31.0	1.92
VI	*β-car.6.1*	CA	66.7	BC299_650	4.71	11.0	1.78
I	*β-car.1.1*	WI-CA	52.5	BC299_125	9.24	20.0	−3.62
II	*β-car.2.2*	WI-CA	43.1	CMMS_3_2	8.18	20.0	1.32
IV	*β-car.4.1*	WI-CA	0.0	CU6 (*csf-2*)	9.69	22.0	1.31

QTL analysis was performed using data by location independently (e.g., WI or CA) and collectively (e.g., WI-CA)

[a] QTL designated by abbreviated trait name, linkage group name, and QTL number

[b] Nearest marker to peak of the detected QTL

[c] Additive effect as obtained from a composite interval mapping (CIM) model resident in QTL cartographer (Wang et al. 2001–2004) and represent the effect from 'USDA 846-1'

Although these pathways may relate to health-related aspects and the mode of β-carotene accumulation in melon, the genetic analysis of carotenoids in this species is in its infancy largely because of the lack of appropriate test populations. Recently, melon mapping populations have been developed using exotic PI accessions and elite varieties differing in mesocarp color: white versus green, orange versus green (Perin et al. 2002b; Monforte et al. 2004). These populations allowed for the study of fruit color inheritance, but not to study QβC. In contrast, although the parents of the RIL used herein typifying US Western Shipping market type commercial melon did not differ significantly in orange mesocarp pigmentation, the derived RIL segregated widely for QβC (Table 3). Since the RIL used vary in mesocarp color and QβC, this study represents the first step towards understanding the genetics of QβC and carotenoid-related genes in melon.

Environmental effects on quantity of β-carotene

Even though the effect of environmental factors (i.e., temperature, humidity, and soil) influencing QβC has been described for some vegetable species (e.g., muskmelon, Lester and Eischen 1995; sweetpotato, Manrique and Hermann 2001), the G × E interactions associated with QβC in melon are not well documented. In the present study, locations differences were likely due, in large part, to dramatic disparities in growing environments (i.e., temperature and humidity). For instance, the comparatively higher temperatures during fruit set, development, and maturation in California likely promoted rapid β-carotene accumulation and rapid fruit development. In fact, comparative analysis of RIL across locations (Table 3), reveal that QβC was in general lower in WI than in CA (e.g., RIL-122, RIL-75, and RIL-047; Table 3). Although, GxE interactions (i.e., ranking inconsistencies across location within years) were detected in Experiment 1, QβC consistency for high/low RIL within locations was confirmed in Experiment 2 (RIL ranking; $r_{s\ \text{across year}} = 0.78$ and 0.84, CA and WI, respectively; Table 3). These results indicate that selection to increase QβC in specific environments will be achievable. However, although extensive location testing might be required, it may be possible to identify and select genotypes possessing a stable QβC across environments (e.g., RIL-49; Table 3). Since reliable QTL effects across locations (experiments) are a prerequisite for exploiting QTL in breeding programs (Bernardo 2002), the consistent within-location RIL performance differences described herein (Experiments 1 and 2) suggests that high performance RIL can be used as donor parents in backcrossing to elite germplasm with lower QβC to enhance carotene content in derived progeny. Certainly the location-independent QβC–QTL detected herein should be considered for marker-assisted selection (MAS) to introduce alleles conditioning high QβC.

Linkage map and SSR relationships

Moderately saturated melon genetic maps exist for melon (Baudracco-Arnas and Pitrat 1996; Perin et al. 2002a; Gonzalo et al. 2005), and those incorporating SSR markers have been proposed for map merging experiments (Gonzalo

et al. 2005). The Zalapa et al. (2007b; nomenclature by Perin et al. 2002a) backbone-map used herein consists predominantly of dominant markers (1,116 cM over 15 LG with a mean marker interval of 5.9 cM). Thus, since dominant markers tend to over-estimate the total length of genetic maps (Mackay 2001), the improved map construction described herein sought to eliminate dominant markers (10 RAPD, and 4 AFLP) that were difficult to position, while integrating an additional 65 SSR, 7 CAPS, and four SNP markers to increase marker density (mean marker interval 4.6 cM; map length = 1,180 cM; Fig. 1). The introduction of additional markers in this revised map undoubtedly facilitated a reduction in LG number to the expected 12 by joining resident small, independent LG (Fig. 1).

Genome homology allows for the comparative analysis of cucurbit mapping populations where common QTL for horticultural traits have been defined (Staub et al. 2007). SSR markers, in fact, were used by Danin-Poleg et al. (2000) to define colinearity among three melon maps (Baudracco-Arnas and Pitrat 1996; Danin-Poleg et al. 1997; Oliver et al. 2001). However, such intra-species comparisons may be complicated by inadequate numbers of syntenic markers (Moore et al. 1993). At least 60–70 polymorphic SSR markers are required to define three to four common markers per LG for map merging in melon (Danin-Poleg et al. 2000). The 104 SSR markers resident in our revised RIL-based melon map incorporates 64 SSR markers common to maps of Gonzalo et al. (2005; 97 SSRs) and, Fukino et al. (2008; 124 SSRs), and thus will allow for inter-map synteny/colinearity and QTL comparisons such as those defined in cucumber (Staub et al. 2007).

Carotenoid genes and quantity of β-carotene

None of the QβC-associated QTL identified herein closely aligned with the four mapped carotenoid genes, except *β-car.6.1* (LG VI), which was ~10 cM from *BOH-1* gene (Fig. 1). While the associated QTL alleles had a positive additive effect on QβC (Table 4), *BOH-1* alleles had a negative additive effect, thus indicating that *BOH-1* gene is likely not associated with this QTL. The lack of associations between QβC–QTL and structural carotenoid gene detected herein mirrors observations in tomato and carrot (Fulton et al. 2000; Santos and Simon 2002; Just et al. 2007). Nevertheless, comparative map analysis using the common SSR identified in this LG suggests that the QTL *β-car.6.1* and *β-car.6.2* are syntenic with orange flesh color QTL identified by Monforte et al. (2004).

At least 19 genes have been identified in the carotenoid pathway (Cunningham and Gantt 1998). Also, recently identified genes [*Or* (cauliflower) and *DEETIOLATED1* (tomato)] associated with carotenoid accumulation were found to be unrelated to the carotenoid biosynthesis pathway (Lu et al. 2006; Davuluri et al. 2005). The location independent QTL, *β-car.4.1* (Calif; $R^2 = 31\%$ LOD = 9.01 and Wisc; $R^2 = 13.0\%$, LOD = 6.33), aligned with the gene *Csf-2* (cucumber EST CU6) identified in cucumber (Suyama et al. 1999). This gene is highly expressed during cucumber fruit development, and was found in a melon EST library developed from mature fruits. Although their function is currently unknown, they may be related to QβC. If true, then the map location of other genes associated or not associated with QβC should be aligned with the QβC–QTL defined herein in an effort to confirm potential genetic relationships. Moreover, the genomic sequence information defined herein might be used to locate such QβC associated genes in other melon market class mapping populations.

Breeding strategies for quantity of β-carotene

Six QTL identified herein explained a significant amount of the observed variation for QβC (four QTL $R^2 > 10\%$, and two QTL > 20%; Table 4), and have potential for use in MAS. Nevertheless, the importance of G × E interactions associated with these QTL (Experiments 1 and 2; Table 3) suggests that MAS must be environment specific.

Identification and characterization of correlative and epistatic effects associated with yield and quality components in melon is critical to their strategic deployment in MAS (Zalapa et al. 2007b; Paris et al. 2008). Even though consistent location-independent epistatic interactions were not detected, such interactions are undoubtedly important in QβC. Classical populations (e.g., RIL) typically used to map QTL, are often ineffective in detecting epistatic effects (Causse et al. 2007). Therefore, the development and comparative analysis of nearly isogenic lines differing in QβC might be prescriptive for defining such effects in this melon population.

Albeit relatively low, the positive correlation between QβC and seed cavity to fruit diameter ratio (CD; $r = 0.23$) identified in California should be considered during breeding to improve genotypes, since high QβC and smaller CD are both commercially desirable. Rigorous selection (high-selection intensity) for increased QβC during the creation of new lines may result in a concomitant undesirable increase in CD value. The location-dependent QTL *β-car.6.1* ($R^2 = 11.0\%$, LOD = 4.71, Table 4) is in the same genomic region (LG VI, Fig. 1) as a QTL for fruit number (*fn2.4*; $R^2 = 21.0\%$; LOD = 11.86; Zalapa et al. 2007b), where both QTL have positive additive effects for increased QβC and fruit number. Thus, these results and information regarding the two major QTL (*β-car.4.1*, $R^2 = 31.0\%$, LOD = 9.01; *β-car.2.3*, $R^2 = 20.0\%$, LOD = 6.82) identified in California (Table 4) suggest that high fruit number and high QβC are likely achievable using MAS specific to California environments.

Marker-assisted selection has been effective for increasing gain from selection (ΔG) for yield components in cucumber, but its successful deployment depended on a knowledge of source/sink relationships, G × E and epistatic interactions (Fazio et al. 2003; Fan et al. 2006). In these cases, appreciable ΔG was detected when selection was practiced on some traits using QTL that explained relatively small portions of the phenotypic variation (R^2 = 5–20%). Given that the QβC QTL identified herein in Wisconsin did not explain substantial amounts of the phenotypic variation (R^2 = <20%; Table 4) and moderately low negative correlations were detected between QβC and other economically important traits, MAS maybe ineffective in facilitating dramatic ΔG for QβC in this population and environment. For instance, the negative correlation between QβC and MP ($r = -0.41$) must be considered during breeding since selection for increased QβC could negatively change this important fruit quality parameter (i.e., soft fruit). Nevertheless, the horticulturally important positive correlation between QβC and AWF ($r = 0.26$) might be exploited since selection could theoretically be employed to increase QβC in large fruit sizes without significant reductions in yield. Likewise, QβC-QTL *β-car.1.2* (R^2 = 12.0%, LOD = 6.06, Table 4) mapped close to a QTL for fruit soluble solid (i.e., sugars) content [(*ssc1.1*), R^2 = 10%; LOD = 6.0, Paris et al., 2008], but have opposite phenotypic effects. Thus, selection for positive QβC alleles contributed by 'Top Mark' may result in a concomitant reduction in fruit total soluble solid content. Fine mapping of this region may, however, aid in the identification of recombinant genotypes which carry the positive allele for QβC without association with the negative allele(s) for soluble solid content.

The development of improved, high yielding melon varieties that bear high quality fruit is difficult (Paris et al. 2008). The lack of genetic information relating to epistatic effects between yield, quality and, nutrition components limits the effective use of germplasm and the deployment of efficient methodologies during melon breeding. Nevertheless, the RIL populations used herein, having been phenotyped for yield (Zalapa et al. 2007b), and quality (Paris et al. 2008), allow for in depth studies of epistatic effects. Moreover, RIL with high yield, quality and nutritional value (i.e., high QβC) identified herein, could be used as donor parents in strategic backcrossing to develop novel germplasm.

References

Bartley GE, Scolnik PA (1995) Plant carotenoids: pigments for photoprotection, attraction, and human health. Plant Cell 7: 1027–1038

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

Bernardo R (2002) Mapping quantitative trait loci. In: Bernardo R (ed) Breeding for quantitative traits in plants. Stemma Press, Woodbury, pp 277–300

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Causse M, Chaïb J, Lecomte L, Buret M, Frédéric H (2007) Both additivity and epistasis control the genetic variation for fruit quality traits in tomoto. Theor Appl Genet 115:429–442

Chiba N, Suwabe K, Nunome T, Hirai M (2003) Development of mircosatellite markers in melon (*Cucumis melo* L.) and their application to major cucurbit crops. Breed Sci 53:21–27

Cunningham FX, Gantt E (1998) Genes and enzymes of carotenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 49:557–583

Danin-Poleg Y, Paris HS, Cohen S, Rabinowithc HD, Karchi Z (1997) Oligogenic inheritance of resistance to zucchini yellow mosaic virus in melons. Euphytica 93:331–337

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

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

Davuluri GR, van Tuinen A, Fraser PD, Manfredonia A, Newman R, Burgess D, Brummell DA, King SR, Palys J, Uhlig J et al (2005) Fruit-specific RNAi-mediated suppression of DET1 enhances carotenoid and flavonoid content in tomatoes. Nat Biotechnol 23:890–895

de Leon N, Coors JG, Kaeppler SM, Rosa GJM (2005) Genetic control of prolificacy and related traits in the golden glow maize population: I phenotypic evaluation. Crop Sci 45:1361–1369

Demming-Adams B, Adams WW (2002) Antioxidants in photosynthesis and human nutrition. Science 298:2149–2153

Eduardo I, Arus P, Monforte AJ (2007) Estimating the genetic architecture of fruit quality traits in melon using a genomic library of near isogenic lines. J Amer Soc Hort Sci 132:80–89

Falconer DS, Mackay TF (1996) Introduction to quantitative genetics, 4th edn. Longman Group, London

Fan Z, Robbins MD, Staub JE (2006) Population development by phenotypic selection with subsequent marker-assisted selection for line extraction in cucumber (*Cucumis sativus* L.). Theor Appl Genet 112:843–855

Fazio G, Chung SM, Staub JE (2003) Comparative analysis of response to phenotypic and marker-assisted selection for multiple lateral branching in cucumber (*Cucumis sativus* L.). Theor Appl Genet 107:875–883

Fukino N, Ohara T, Monforte A, Sugiyama M, Sakata Y, Kunihisa M, Matsumoto S (2008) Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (*Cucumis melo* L.). TAG (in press)

Fukino N, Sakata Y, Kunihisa M, Matsumoto S (2007) Characterization of novel simple sequence repeat (SSR) markers for melon (*Cucumis melo* L.) and their use for genotype identification. J Hortic Sci Biotechnol 82:330–334

Fulton TM, Grandillo S, Beck-Bunn T, Fridman E, Frampton A, Loper J, Petiard V, Uhlig J, Zamir D, Tanksley SD (2000) Advanced backcross QTL analysis of *Lycopersicon esculentum* × *Lycopersicum parviflorum* cross. Theor Appl Genet 100:1025–1042

Giovannucci E (2002) Lycopene and protaste cancer risk. Methodological consideration in the epidemiology literature. Pure Appl Chem 74:1427–1434

Gonzalo MJ, Oliver M, Garcia-Mas J, Monforte A, Dolcet-Sanjuan R, Katzir N, Arús P, Monforte AJ (2005) Development of a

consensus map of melon (*Cucumis melo* L.) based on high-quality markers (RFLPs and SSRs) using F2 and double-haploid line populations. Theor Appl Genet 110:802–811

Gross J (1987) Carotenoids. In: Schweigert BS (ed) Pigments in fruits. Academic Press, London, pp 87–186

Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. J Heredity 69:315–324

Hirschberg J (2001) Carotenoid biosynthesis in flowering plants. Curr Opin Plant Biol 4:210–218

Huh JH, Kang BC, Nahm SH, Kim S, Ha KS, Lee MH, Kim BD (2001) A candidate gene approach identified phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp.). Theor Appl Genet 102:524–530

Just BJ, Santos CAF, Fonseca MEN, Boiteux LS, Oloizia BB, Simon PW (2007) Carotenoid biosynthesis structural genes in carrots (*Daucus carota*): Isolation, sequence characterization, single nucleotide polymorphism (SNP) markers and genome mapping. Theor Appl Genet 114:693–704

Karvouni Z, John I, Taylor JE, Watson CF, Turner AJ, Grierson D (1995) Isolation and characterization of a melon cDNA clone encoding phytoene synthase. Plant Mol Biol 27:1153–1162

Kong Q, Xiang C, Yu Z, Zhang Z, Liu F, Peng C, Peng X (2007) Mining and charactering microsatellites in *Cucumis melo* expressed sequence tags from sequence database. Mol Ecol Notes 7:281–283

Kong Q, Xiang C, Yu Z (2006) Development of EST-SSR in *Cucumis sativus* from sequence database. Mol Ecol Notes 6:1234–1236

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

Lester GE, Eischen F (1995) Beta-carotene content of postharvest orange-fleshed muskmelon fruit: Effect of cultivar, growing location and size. Plant Food Hum Nutr 49:191–197

Liou PC, Chang YM, Hsu WS, Cheng YH, Chang HR, Hsiao CH (1998) Construction of a linkage map in *Cucumis melo* (L.) using random amplified polymorphic DNA markers. In: Drew RA (ed) Proceedings of the international symposium in biotechnology: troplical and subtropical species, pp 123–131

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute Inc., Cary

Lu S, Van Eck J, Zhou X, Lopez AB, O'Halloran M, Cosman KM, Conlin BJ, Paolillo DJ, Garvin DF, Vrebalov J, Kochian LV, Küpper H, Earle ED, Cao J, Li L (2006) The cauliflower *Or* gene encodes a DNA-J cysteine-rich domain-containing protein that mediates high levels of β-carotene accumulation. The Plant Cell 18:3594–3605

Mackay TFC (2001) The genetic architecture of quantitative traits. Ann Rev Genet 35:303–339

Manrique K, Hermann M (2001) Effect of GxE interaction on root yield and beta-carotene content of selected sweetpotato (*Ipomoea batatas* (L) Lam.) varities and breeding clones. CIP Program Report 1999–2000, pp 281–287

Mares-Perlman JA, Millen AE, Ficek TL, Hankinson SE (2002) The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease. J Nutr 132:518S–524S

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

Moore G, Gale MD, Kurata N, Flawell R (1993) Molecular analysis of small grain genomes: current status and prospects. Biotechnology (New York) 11:584–589

Munger HL, Robinson RW (1991) Nomenclature of *Cucumis melo* L. Cucurbit Genet Coop Rep 14:43–44

Navazio JP (1994) Utilization of high carotene cucumber germplasm for genetic improvement of nutritional quality. PhD Thesis, University of Wisconsin, Madison

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

Paris KM, 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 and elite US Western Shipping germplasm. Mol breed, doi 10.1007/S11032-008-9185-3

Perin C, Hagen LS, de Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2002a) 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 (2002b) Genetic control of fruit shape acts prior to anthesis in melon (*Cucumis melo* L). Mol Genet Genomics 266:933–941

Pozniak CJ, Knox RE, Clarke FR, Clarke JM (2007) Identification of QTL and association of a phytoene synthase gene with endosperm colour in durum wheat. Theor Appl Genet 114: 525–537

Ritchel PS, de Lima-Lins TC, Lourenco-Tristan R, Cortopasi-Buso GS, Buso JA, Ferreiram ME (2004) Development of microsatellite marker from an enriched genomic library for genetic analysis of melon (*Cucumis melo* L.). BMC Plant Biol 4. http://www.Biomedcentral.com/I471-2229/4/9

Römer S, Fraser PD (2005) Recent advances in carotenoid biosynthesis, regulation and manipulation. Planta 221:305–308

Royal Horticultural Society (2005) The Royal Horticultural Society Colour Chart. The Royal Horticultural Society, London

Santos CAF, Simon PW (2002) QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrots roots. Mol Genet Genomics 268:122–129

SAS Institute (1999) SAS version 8.02 for windows. SAS Institute Inc. , Cary

Schwartz SH, Quin X, Zeevaart JD (2003) Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes and enzymes. Plant Physiol 131:1591–1601

Shapiro SS, Wilk MB (1956) An analysis of variance test for normality (complete samples). Biometrika 52:591–611

Silberstein L, Kovalski I, Brotman Y, Perin C, Dogimont C, Pitrat M, Klingler J, Thompson G, Portnoy V, Katzir N, Perl-Treves R (2003) Linkage map of *Cucumis melo* including phenotypic traits and sequence-characterized genes. Genome 46:761–773

Simon PW, Wolff XY (1987) Carotenes in typical and dark orange carrots. J Agri Food Chem 35:1017–1022

Staub JE, Sun Z, Chung SM, Lower RL (2007) Evidence for colinearity among genetic linkage maps in cucumber. HortScience 42:20–27

Suyama T, Yamada K, Mori H, Takeno K, Yamaki S (1999) Cloning cDNAs for genes preferentially expressed during fruit growth in cucumber. J Am Soc Hort Sci 124:136–139

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Throup TA, Tanyolac B, Livingstone KD, Popovsky S, Paran I, Jahn M (2000) Candidate gene analysis of organ pigmentation loci in the Solanaceae. Proc Natl Acad Sci USA 97:11192–11197

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

Wang S, Basten CJ, Zeng ZB (2001–2004) Windows QTL Cartographer 2.0. Department of Statistics, North Carolina State University, Raleigh. (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

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

Wong JC, Lambert RJ, Wurtzel ET, Roncherford TJ (2004) QTL and candidate genes phytoene synthase and ζ-carotene desaturase associated with the accumulation of carotenoids in maize. Theor Appl Genet 108:349–359

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

Zalapa (2005) Inheritance and mapping of plant architecture and fruit yield in melon (*Cucumis melo* L). PhD Dissertation, University of Wisconsin, Madison

Zalapa JE, Staub JE, McCreight JD (2007a) Variance component analysis of plant architectural traits and fruit yield in melon. Euphytica: on line December 2007

Zalapa JE, Staub JE, McCreight JD, Chung SM, Cuevas HE (2007b) 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

Zeng ZB (1993) Theoretical basis of separation of multiple linked gene effects on mapping quantitative trait loci. Proc Natl Acad Sci USA 90:10972–10976

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468