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ORIGINAL PAPER

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Identification of quantitative trait loci involved in fruit quality traits in melon (*Cucumis melo* L.)

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Abstract Two populations [an F_2 and a set of 77 double haploid lines (DHLs)] developed from a cross between a 'Piel de Sapo' cultivar (PS) and the exotic Korean accession PI 161375 were used to detect QTLs involved in melon fruit quality traits: earliness (EA), fruit shape (FS), fruit weight (FW) and sugar content (SSC); and loci involved in the colour traits: external colour (ECOL) and flesh colour (FC). High variation was found, showing transgressive segregations for all traits. The highest correlation among experiments was observed for FS and the lowest for FW and SSC. Correlations among traits within experiments were, in general, not significant. QTL analysis, performed by Composite Interval Mapping, allowed the detection of nine QTLs for EA, eight for FS, six for FW and five for SSC. Major QTLs (R^2>25%) were detected for all traits. QTLs for different traits were no clearly co-localised, suggesting low pleiotropic effects at QTLs. Sixty-one per cent of them were detected in two or more experiments. QTLs for FS were detected in more trials than QTLs for FW and SSC, confirming that FS is under highly hereditable polygenic control. ECOL segregated as yellow:green in both experimental populations. The genetic control of ECOL was found to be complex, probably involving more than two loci with epistatic interactions. One of these loci was mapped on linkage group 9, but the other loci could not be clearly resolved. FC segregated as white:green:orange. The locus responsible for the green FC was mapped on linkage group 1, and it was proposed to correspond to the previously described locus *gf*. The genetic control of orange FC was complex: two loci in linkage groups 2 and 12 were associated with orange flesh, but larger population sizes would be necessary to elucidate completely the genetic control of orange flesh in this cross. Exotic alleles from PI161375 showed beneficial effects on EA, FW and SSC, indicating the usefulness of PI 161375 as a new source of genetic variability to improve European and American cultivars.

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Introduction

Melon (*Cucumis melo* L.) is an economically important species of the Cucurbitaceae family. This species has been divided in two subspecies according to the hairiness of the ovary: *C. melo ssp. melo*, with pilose or lanate ovaries, and *C. melo ssp. agrestis*, with sericeous ovaries (Jeffrey 1980). Currently, melon has a worldwide distribution with high phenotypic variation. For instance, melon fruit shape ranges from oblong to extremely elongated, fruit weight from a few grams to several kilograms, and flesh taste from bitter to very sweet (Kirkbride 1993; Stepansky et al. 1999). Also, high levels of genetic variability have been detected using molecular markers (Stepansky et al. 1999; Mliki et al. 2001; Akashi et al. 2002). Recently, Monforte et al. (2003) found an average of 6.3 alleles per microsatellite locus in an array of 27 melon genotypes. Altogether, these data confirm that *C. melo* is one of the most diverse species of the genus *Cucumis*, although the genetic control of its phenotypic variation is largely unknown. Over 100 genes involved on morphological traits and disease resistance have been reported (Pitrat 1998), and 20 of them have been recently mapped (Périn et al. 2002a). These mapped genes should represent only a very small fraction of the genes underlying the phenotypic variation observed in

melon germplasm. Most of the variation could be due to a multiple allelic variability at a large number of quantitative trait loci (QTLs). Thorough analysis of QTLs requires detailed molecular marker linkage maps (Tanksley 1993), which are now being developed (Oliver et al. 2001; Périn et al. 2002a), allowing the first reports of QTL analysis in melon. For example, Périn et al. (2002b) identified QTLs involved in fruit and flower shape using two recombinant inbred populations of melon.

QTL analysis can be used as a strategy to mine genes or QTL alleles in exotic germplasm and transfer them to the elite modern cultivars, allowing an efficient management of the genetic diversity available in natural populations, wild species and germplasm banks (Tanksley and McCouch 1997). This strategy has been applied successfully in tomato (Eshed and Zamir 1995; Tanksley et al. 1996; Monforte et al. 2001), rice (Xiao et al. 1998) and soybean (Concibido et al. 2003). Sexual crosses among *C. melo* and wild *Cucumis* species do not produce fertile hybrids (Chen and Adelberg 2000), but genetic variability studies have demonstrated that African, Indian and Oriental germplasm can be considered as exotic germplasm for Occidental cultivars (Stepansky et al. 1999; Mliki et al. 2001; Monforte et al. 2003). Melon exotic germplasm has been used to search for resistance genes to transfer to Occidental cultivars, but the potential of that germplasm as source of new QTL alleles with favourable effects on fruit quality has not been investigated thoroughly.

In the current report, we have studied the genetic control of fruit traits by QTL analysis in F_2 and double haploid line (DHL) populations derived from the same cross between a Spanish 'Piel de Sapo' cultivar and the exotic Korean accession PI161375. The objective of this work is to present preliminary data about the architecture of quantitative traits in melon, as well as mining QTL alleles with favourable effects on fruit quality traits from an exotic genotype.

Materials and methods

Experimental populations and linkage map

F_2 plants and DHLs were obtained from the cross between the Korean accession 'Shongwan Charmi' PI 161375 (PI) and a 'Piel de Sapo' cultivar (PS) provided by 'La Mayora' research station (Málaga, Spain) and Semillas Fitó S. A. (Barcelona, Spain), respectively. The F_2 population consisted of the 93 plants (Oliver 2001) maintained in vitro on an MS solid medium (Murashige and Skoog 1962). This population was used by Oliver et al. (2001) to construct a reference linkage map for melon. The methodology developed for carnation (*Dianthus caryophyllus* L.) (Dolcet-Sanjuan et al. 2001) was adapted and used in generating the melon DHL population (detailed elsewhere). Briefly, 77 lines were developed by in situ-induced parthenogenesis through pollination by Co^{60} gamma-irradiated pollen, in vitro rescue of parthenogenic embryos, in vitro chromosome-doubling by colchicine treatment, and self-pollination of acclimated plants. A total of 107 evenly distributed molecular markers and the morphological marker *pentamerous* (*p*) were used to construct a linkage map with the DHLs following Oliver et al. (2001). Linkage maps of F_2 and DHLs were merged for graphic display purposes with JoinMap 3.0 (Van Ooijen and Voorrips 2001) with the 'Combine Groups for Map Integration' function.

Field experiments and characters measured

Ninety-three F_2 genotypes were evaluated during the spring of 1997 and 1998 in the greenhouses owned by Semillas Fitó S. A. located in Premia de Mar (Barcelona, Spain). The trials were named 'F2.97' and 'F2.98', respectively. In both years, cuttings of the F_2 plants maintained in vitro were acclimated, five replicates of each plant were transferred to the greenhouse, planted at 0.25-m spacing among plants with a completely randomised design and drip-irrigated. DHLs were evaluated in four trials: greenhouse in Cabrils (Barcelona, Spain) during the spring of 2000 (Cab00), greenhouse in Premia de Mar during 2000 (Pre00), greenhouse in Cabrils during 2002 (Cab02) and field trial in Zaragoza (Spain) during 2002 (Za02). In the DHL evaluations in the greenhouse, five replicates of each DHL were randomised with five to ten replicates of each parental and grown in the greenhouse as the F_2 population. For the field experiment Za02, three plots of five plants for each DHL and parentals were randomised in the field, flowers were open-pollinated and five fruits from each plot were harvested. Flowers were hand-pollinated in all greenhouse experiments.

The agronomic evaluation included the following traits: earliness (EA) (the number of days since the pollination day to harvest in the greenhouse trials in the Za02 open field trial, EA was recorded as the days from planting to harvest), fruit weight (FW), fruit shape (FS) (the ratio between fruit length and fruit maximum diameter) and soluble solids concentration (SSC) (measured as °Brix with a hand refractometer from a homogenized of melon flesh). Colour traits were measured categorically: external colour (ECOL) as green or yellow and flesh colour (FC) as green, white and orange. Means, standard deviations, trait distributions and correlations among traits and among trials were calculated with SYSTAT 5.03 for Windows.

QTL analysis

QTLs were analysed by composite interval mapping (Zeng 1993, 1994) using Windows QTL Cartographer 2.0 (Basten et al. 1994; Basten et al. 2002). Each trait and location was treated separately. QTLs were declared significant if the corresponding LOD score met at least one of the two criteria: (1) in one location the LOD score was higher than the LOD threshold calculated for an experiment-wise error type I error rate α=0.05 calculated empirically by a permutation test (Churchill and Doerge 1994) with QTL Cartographer (average LOD threshold = 2.8), and (2) in at least two locations the LOD was higher than 2.0. Additive (a) effects, dominance (d) effects and per cent of phenotypic variance explained by the QTL (R^2) were estimated with Windows QTL Cartographer at highest probability peaks. Positive additive effect indicates that PI 161375 alleles improve the trait. QTL positions were estimated with a 2-LOD confidence interval surrounding the maximum LOD peak. QTLs affecting the same trait detected in different trials were interpreted to be the same QTL when the 2-LOD confidence interval of their positions overlapped. Confidence intervals of the position for QTLs detected in two or more experiments were defined by the larger interval calculated in each experiment.

Analysis of FC

ECOL was recorded as "1" for yellow and "0" for green colour. FC was divided in two variables: green flesh colour (GFC), recorded as "1" for green and "0" for white, and orange flesh colour (OFC) recorded as "1" for orange and "0" for green or white. Associations of markers and colour traits were studied with contingency tables and the statistical significance established by Fisher's exact test.

Markers were declared significantly associated to colour traits if the probability of test for the contingency table was $P<0.01$ in one population or $P<0.05$ in the two experimental populations. When the segregation of colour fitted with the segregation of a single locus, colour was mapped using MAPMAKER 3.0 (Lander et al. 1987).

Results

Phenotype analysis

Means, standard deviations and phenotypic range for each of the traits for parents and experimental populations among the different trials are given in Table 1. PS fruits were large, oval, sweet and white flesh-coloured. PI161375 fruits were smaller, pear-shaped, non-sweet and green flesh-coloured. The most important differences among parents were for FW, SSC and FC, and minor differences were observed in EA and FS. ECOL of both parents was green, although the colour intensity was different: dark green for PS and light green for PI161375. Wide variability was observed within experimental populations (F_2 and DHL) for all traits. FW, SSC, FS and EA showed continuous variation and were classified as quantitative traits, whereas ECOL and FC were classified as qualitative traits. Extreme phenotypes with much larger and/or lower values than any of the parents were observed for EA, FW, FS and SSC, indicating transgressive segregations. For example, FS in experimental populations ranged from near-perfect round (FS = 1) to extremely elongated (FS up to 3.18), in spite of the small differences between the parents. Other unexpected phenotypes also appeared within experimental populations,

Table 1 Means for parentals ['Piel de Sapo cultivar' (PS) and PI161375], experimental populations, standard deviations (SD) and range for the fruit quality traits in the trials

Trait[b]	Experiment	PS Mean	PI161375 Mean	Experimental population[a]		
				Mean	SD	Range
EA	Cab00	34.50	48.00	46.83	7.99	64–31
	Cab02	47.7	n.a.[c]	44.6	6.7	62–28
	Za02[d]	84.0	75.3	81.5	5.0	92–71
	F2.98	68.0	56.0	59.7	10.3	77–36
FW	Pre00	1,175.8	380.0	697.5	393.9	1,774–25
	Cab00	1,246.0	361.0	725.1	377.3	1,872–138
	Cab02	1,767.0	n.a.	1,009.0	475.3	2,676–106
	Za02	1,464.9	922.8	737.9	254.3	1,287–231
	F2.97	1,750.0	1,025.0	1,118.5	286.5	2,180–567
	F2.98	1,600.0	1,000.0	889.2	278.4	1,854–357
FS	Pre00	1.4	1.7	1.7	0.3	2.8–1
	Cab00	1.5	1.6	1.7	0.4	3.1–1
	Cab02	1.4	n.a.	1.6	0.4	2.7–0.9
	Za02	1.4	1.4	1.6	0.3	2.4–1.0
	F2.97	1.8	1.6	2.0	0.4	3.2–1.3
	F2.98	1.8	1.9	1.9	0.3	2.6–1.0
SSC	Pre00	13.1	4.0	7.9	2.2	12.6–3.2
	Cab00	11.5	6.7	9.4	2.8	16.6–4.4
	Cab02	11.9	n.a.	8.2	1.9	12.6–4.1
	Za02	10.5	8.8	10.1	1.7	14.6–6.8
	F2.97	12.1	8.1	9.2	1.9	12.7–5.2

[a] F2.97 and F2.98 experiments were carried out with the F_2 population and Pre00; Cab00, Cab02 and Za02 with the double hapolid lines (DHLs)
[b] Traits: *EA* earliness, *FS* fruit Shape, *FW* fruit weight, *SSC* soluble solid concentration
[c] Data not available
[d] EA scored as days from transplanting to harvest

Table 2 Correlations among experiments for the quantitative fruit traits. *Bold* numbers indicate $P<0.01$. Correlation for earliness could be studied only between Cab00 and Cab02 trials

FW					FS				
	Pre00	Cab00	Cab02	F2.97		Pre00	Cab00	Cab02	F2.97
Cab00	**0.56**	–	–	–	Cab00	**0.77**	–	–	–
Cab02	**0.41**	0.34	–	–	Cab02	**0.65**	**0.75**	–	–
Za02	**0.43**	**0.46**	**0.47**	**–**	Za02	**0.72**	**0.78**	**0.66**	**–**
F2.98	–	–	–	**0.45**	F2.98	–	–	–	**0.5**
SSC					EA				
	Pre00	Cab00	Cab02			Pre00	Cab00	Cab02	
Cab00	**0.54**	–	–	–	Cab00	–	–	–	–
Cab02	**0.64**	0.36	–	–	Cab02	–	**0.47**	–	–
Za02	**0.51**	0.34	**0.65**	**–**	Za02	–	–	–	–

Table 3 QTL analysis of quantitative fruit traits. QTLs are named by an abbreviation of the trait followed by the linkage group number and a QTL number. The markers flanking the most likely position of the QTLs, LOD score statistic and estimates of the genetic effects at QTL (R^2 = proportion of phenotypic variance explained by the QTL, a = additive effect being positive when PI 161375 improved the trait, d = dominance deviation) are given for each trial

Trait	QTL	Trial	Flanking markers	LOD	R^2	a	d
EA	*ea1.1*	Cab00	MC68–Aox2	6.95	0.41	7.90	–
	ea1.2	Cab00	MC11–MC78	4.06	0.22	–5.47	–
	ea1.3	Za02	CMAT141–MC138	2.53	0.10	1.82	–
		F2.98	CMAT141–MC138	4.30	0.12	2.92	–5.87
	ea2.1	Za02	MC296–MC365	3.95	0.16	2.95	–
		F2.98	MC296–MC365	4.62	0.13	4.70	4.70
	ea9.1	F2.98	MC149–MC17	6.75	0.22	–5.65	4.46
	ea9.2	Cab02	CMGA172–MC112	5.82	0.32	4.00	–
		Za02	MC112–MC67	7.07	0.30	2.82	–
	ea10.1	Cab02	CM122–MC219	4.13	0.22	5.31	–
	ea12.1	Cab00	MC8–MC21	5.33	0.30	4.59	–
		F2.98	MC226–MC8	2.48	0.06	3.84	0.21
	ea12.2	F2.98	MC21–MC224	3.80	0.14	5.17	2.64
SSC	*ssc1.1*	Cab02	CMAT141–MC138	2.17	0.07	–0.67	–
		Za02	CMAT141–MC138	3.84	0.20	–0.82	–
		F2.98	MC11–MC78	3.25	0.10	–0.92	0.63
	ssc2.1	Cab02	MC54–MC296	4.35	0.16	1.13	–
		F2.98	MC54–MC296	4.10	0.10	0.23	1.24
	ssc4.1	Cab02	MC233–CMAT35	4.41	0.17	–1.67	–
		F2.98	MC233–CMAT35	3.42	0.19	–1.36	0.66
	ssc4.2	Pre00	MRGH63–CMTAA166	5.86	0.30	–1.24	–
	ssc8.1	Pre00	CMGA108–MC273	2.75	0.11	–0.84	–
		Cab02	CMGA108–MC273	2.95	0.12	–0.76	–
		ZA02	CMGA108–MC273	4.89	0.25	–2.48	–
		F2.98	CMGA108–MC273	4.62	0.16	–1.07	0.27
FW	*fw3.1*	Cab00	CSAT425B–CM139	4.28	0.23	–193.12	–
	fw4.1	Pre00	CMAT35–MC256	2.29	0.08	117.06	–
		F2.97	MC233–CMAT35	2.33	0.10	111.00	117.00
		F2.98	MC233–CMAT35	3.22	0.12	119.00	43.00
	fw5.1	Za02	CMTC160–MC264	4.02	0.30	176.83	–
	fw5.2	Pre00	MC231–MC349	5.99	0.34	287.53	–
		Cab02	MC231–MC349	2.55	0.13	171.39	–
		Za02	MC375–MC231	3.04	0.15	296.84	–
	fw12.1	F2.97	MC226–MC8	2.67	0.11	–155.15	109.43
		F2.98	MC226–MC8	2.81	0.10	74.72	145.64
	fw12.2	Cab00	MC8–MC21	2.40	0.13	–143.10	–
		F2.97	MC8–MC21	2.46	0.10	–135.00	44.00
		F2.98	MC8–MC21	2.73	0.10	–105.00	133.00
FS	*fs1.1*	Pre00	Aox2–MC356	3.77	0.16	0.13	–
		Za02	MC11–MC78	4.27	0.15	0.13	–
		F2.97	Aox2–MC356	3.20	0.09	0.17	0.06
	fs3.1	F2.97	MC253–CSAT425B	6.50	0.19	0.28	0.01
		F2.98	MC311–MC253	2.10	0.08	0.10	0.17
	fs5.1	F2.97	MC375–MC231	3.84	0.10	–0.12	–0.03
	fs6.1	F2.97	MC279–MC134	3.70	0.13	–0.21	0.00
	fs6.2	Cab00	CSWCT11–MC294	2.87	0.18	0.23	–
	fs7.1	Cab00	CMTC47–CM98	4.81	0.33	0.31	–
		Pre00	CMTC47–CM98	2.36	0.10	0.10	–
	fs9.1	Cab00	CMGA172–MC112	2.05	0.10	0.15	–
		Cab02	CMGA172–MC112	2.92	0.17	0.16	–
		Za02	CMGA172–MC112	3.76	0.13	0.10	–
		F2.98	MC149–MC17	2.77	0.11	0.15	0.10
	fs11.1	Cab00	MC132–MC320	3.36	0.20	–0.21	–
		Pre00	MC132–MC320	5.27	0.26	–0.16	–
		Cab02	MC330–CSAT425A	2.39	0.12	–0.14	–
		Za02	NC330–CSAT425A	5.99	0.24	–0.14	–
		F2.97	MC320–MC330	5.37	0.15	–0.20	0.11
		F2.98	MC320–MC330	3.81	0.16	–0.11	0.21

such as melons with yellow skin and others with orange flesh.

Table 2 shows the correlations among experiments for the quantitative traits. The highest correlations were observed for FS, being similar among all the experiments involving the DHL population (average correlation 0.72). Significant correlations for SSC and FW ranged from 0.4 to 0.6, with non-significant correlations for SSC between the Cab00 and Cab02, and Cab00 and Za02 trials and for FW between the Cab00 and Cab02 trials. The only significant correlations ($P<0.01$) between traits within an

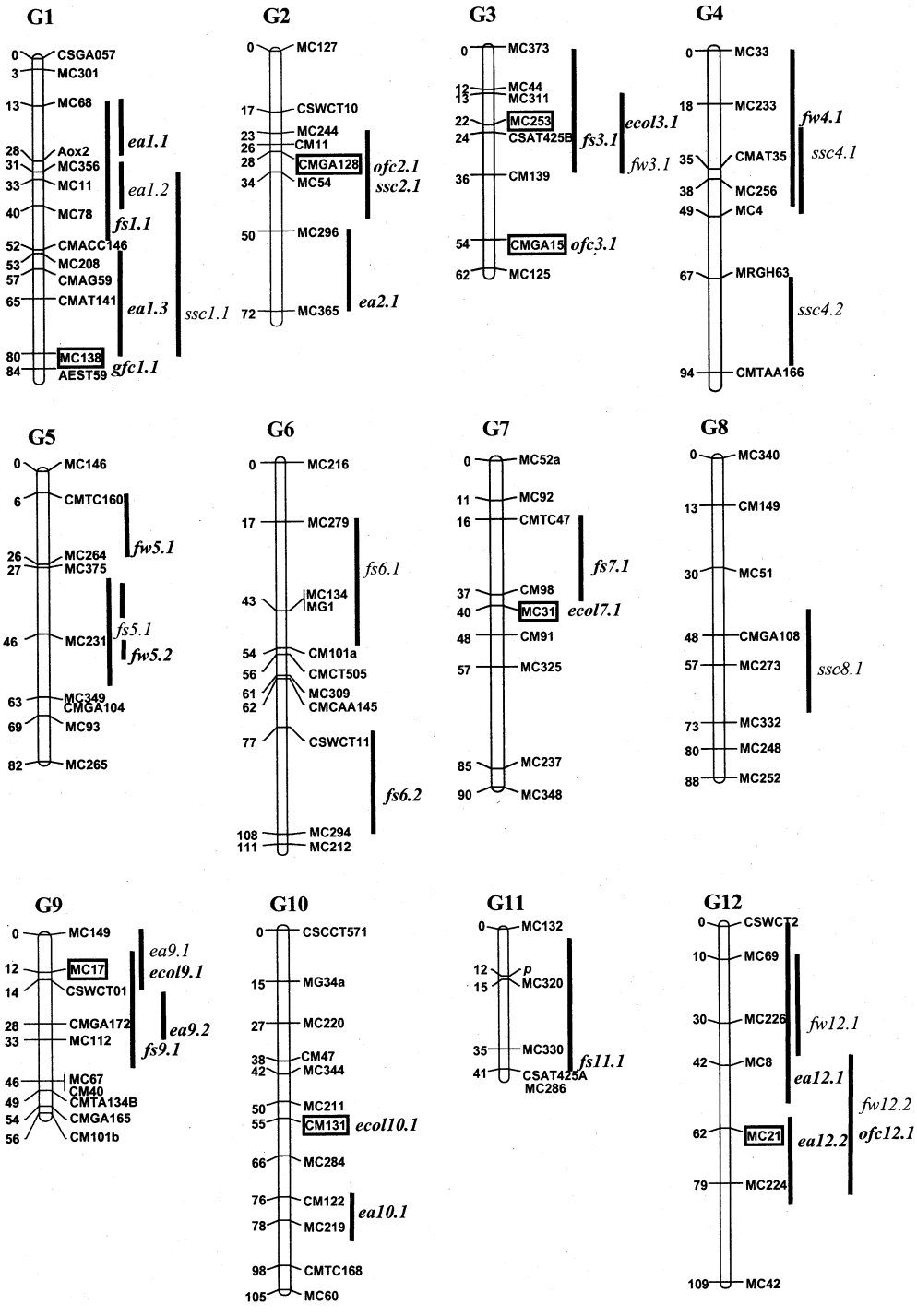

Fig. 1 Map locations of the QTLs involved in melon fruit traits. CentiMorgan distances based on the merging of DHL and F_2 maps are given on the *left* of each linkage group. QTLs are designated by *letter/number combinations* on the right of the linkage groups, the *location of the QTL name* indicates the position of the highest LOD score, and the *bar* the 2-LOD confidence interval. *Bold* QTLs signify that the PI161375 alleles had favourable effects or increased the trait. *Boxed markers* indicate significant association with colour traits

experiment were EA with SSC in the Za02 trial (r=0.39) and EA with FS in the F2.98 trial (r=0.42).

QTL mapping of fruit traits

Table 3 provides the list of the QTLs. Their most likely positions on the linkage map are shown in Fig. 1. Altogether, 28 QTLs were detected, ranging from five SSC QTLs to nine EA QTLs. The proportion of phenotypic variance explained by single QTL (R^2) ranged from 0.06 (*ea12.1* in the F2.98 trial) to 0.41 (*ea1.1* in the Cab00 trial). Major QTLs (R^2>0.25) were detected for all traits. The direction of the allelic effects at individual QTL was variable for all traits. For some QTLs (e.g. *fw12.2*, *fs11.1*, *ssc8.1*) the PS allele increased the traits, whereas for other QTLs (e.g. *fw5.1*, *fs9.1*, *ssc2.1*) the trait was increased by PI161375 alleles. Consequently, the direction of the allelic effects at some QTLs (e.g. *fw4.1*, *fw5.1*, *ssc2.1*) was the opposite of that expected according

Table 4 Contingency tables for the segregation of markers and external colour (*ECOL*) and orange flesh colour (*OFC*) and abbreviation of the trait followed by the linkage group number and the number of locus within the linkage group. The marker with more significant association with the colour trait in the linkage group is shown with the probability (*P*) of the Fisher exact test

Trait	Locus	Marker	*P*	Population	Colour phenotypes			
					PIPI[a]	PSPS[b]	PIPI	PSPS
					Yellow		Green	
ECOL	*ecol3.1*	MC253	<0.00001	DHL	0	11	26	8
	ecol7.1	MC31	0.01	F_2	14	1	13	11
	ecol9.1	MC17	<0.0001	F_2	0	14	16	9
	ecol10.1	MC17	<0.0001	DHL	0	14	23	16
		CM131	0.009	F_2	12	2	12	18
					Orange		Green or white	
OFC	*ofc2.1*	CMGA128	0.004	F_2	5	0	7	20
			0.04	DHL	6	0	21	21
	ofc3.1	CMGA15	0.007	DHL	8	0	25	25
	ofc12.1	MC21	0.05	F2	4	0	14	15
			0.01	DHL	4	0	14	24

[a] Number of homozygote genotypes for the PI161375 alleles
[b] Number of homozygote genotypes for the PS alleles

to parent phenotypes. Interestingly, QTL alleles from PI161375 improving agronomic interesting traits were detected (i.e. *ea1.2*, *ea9.1*, *fw4.1*, *fw5.1*, *ssc2.1*).

Seventeen QTLs (61%) were detected in at least two experiments. The repeatability of QTL detection on the trait ranged from 80% of SSC QTLs detected in two or more trials to 44% for EA QTLs. Of 19 QTLs involved in the fruit quantitative traits FW, SSC and FS, 13 (68%) were detected in at least two experiments. *Fs9.1*, and *fs11* were detected in at least four trials. Twelve of 18 QTLs (67%) detected in the F_2 population were also detected in at least one DHL trial. FS QTLs were detected in an average of 2.5 trials, SSC QTLs in an average of 2.4 trials, FW QTLs in an average of 2.16 trials and EA QTLs in an average of 1.44 trials. QTLs detected in two or more trials usually mapped in the same or neighbour marker interval, except for *fs1.1*, *fs9.1*, *ssc1.1*. In those cases, the 2-LOD confidence interval calculated in each trial overlapped, so they were considered to be the same QTL. The direction of additive effects was the same for all QTLs detected in several trials, except for *fw12.1*, where the additive effects were not significant in the F2.98 trial, although in both F2.97 and F2.98 trials the dominant component was important. These results reinforce the accuracy of our QTL mapping results.

Fruit colour

Categories of ECOL and FC were studied for linkage as single loci. Occasional discrepancies on colour evaluation in some DHL trials were observed (data not shown) that could be due to genotype-by-environment interactions, or the difficulty in assessing the exact ripening stage of the fruits at harvest, leading to errors in the evaluations. To reduce phenotyping errors, data from all the trials were pooled, and lines which scored differently in two locations were discarded from the analysis. ECOL segregated 29:57 (yellow:green) in the F_2 population, fitting a single locus segregation (1:3, χ^2=3.44, n.s.) but the segregation was 15:40 (yellow:green) in the DHL population (1:1, χ^2=11.36, P<0.001). The association of marker MC17 on linkage group (G) 9 with ECOL segregation was significant in both F_2 and DHLs (Table 4), defining a proposed locus involved in ECOL: *ecol.9.1* (Fig. 1). Visual inspection of genotypes of F_2 plants and DHLs showed that every genotype displaying yellow ECOL lacked PI161375 alleles at marker MC17 of G 9, although not all plants carrying PS alleles at this locus had yellow ECOL. Three other markers (MC253 on G 3, MC31 on G 7 and CM131 on G 10) also showed significant association with ECOL, but only in one experimental population. The 14 DHLs with yellow ECOL had PS alleles at both markers: MC253 on G 3 and MC17 on G 9 and only 2 DHLs with PS alleles at both markers had green ECOL.

The segregation of FC (white:green:orange) was 50:25:9 in the F_2 population and 32:19:8 in the pooled DHL data. Discarding the orange flesh fruits, the segregation of the GFC fitted the segregation of a single recessive locus (1:3 in the F_2, χ^2=2.77 P>0.5; 1:1 in the DHLs, χ^2=3.3 P>0.05). GFC was mapped as a single locus on G 1 with MAPMAKER 3.0 in both populations, and the locus was named *gfc1.1* (Fig. 1).

OFC did segregate as a single locus (1:3 in the F_2, χ^2=15.14 P<0.001; 1:1 in the DHLs, χ^2=33.38 P<0.0001). Association of MC21 on G 12 and CMGA128 on G 2 with the segregation of OFC in both populations was significant (Table 4). Visual inspection of genotypes showed that lines displaying OFC were homozygous for PI161375 or heterozygous (Table 4). The putative loci were named *ofc.2.1* and *ofc.12.1*, although the allelic combinations of these two loci could not explain completely the OFC segregation. Furthermore, the CMGA15 marker on G 3 also showed significant association in the DHL population; every line displaying OFC had PI161375 alleles at that locus, defining a putative third locus: *ofc3.1*. The segregation of *gfc.1.1* was independent of the orange flesh segregation in both populations.

Discussion

Genetic architecture of quantitative traits in melon

Taking into account the results from all the trials, five to nine QTLs were detected among the fruit quantitative traits studied (EA, FW, FS, SSC), which is within the range of most QTL analysis in plants (Kearsey and Farquhar 1998). All QTLs showed important effects (R^2>10%) in at least one trial, and the detection of major QTLs (R^2>25%) was common in all traits, as expected from the limitations of the experimental design. Probably a large number of minor QTLs remained undetected. These results suggest that the differences in fruit characteristics between PS and PI161375 genotypes are due to a large number of loci.

QTLs were localized on all linkage groups, although QTLs detected on two or more trials were not localized on G 6 and G 10. QTLs for different traits did not show clear co-localization, corresponding with the observed lack of correlations among traits, suggesting that the QTLs in general had low, if any, pleiotropic effects. Negative correlation between SSC and FW, as well as co-localization of SSC and FW QTLs with opposite effects, have been extensively reported in tomato (Emery and Munger 1970; Paterson et al. 1991; Tanksley et al. 1996; Monforte et al. 2001; Saliba-Colombani et al. 2001). Whether that correlation is due to pleiotropic effects of the same QTLs or to tightly linked QTLs is currently still under debate. The reasons supporting the first hypothesis are based on the fact that tomato fruits grow by cell expansion, which may be accompanied by increasing water content and, consequently, decreasing soluble solid content in large-fruited genotypes. Additionally, the accumulation of sugars could be more limited by the available photosynthetic resources in large-fruited genotypes (Stevens 1986). Negative correlation between SSC and FW makes the use of small-fruited tomato wild germplasm to enhance SSC of large-fruited tomato cultivars difficult (Chen et al. 1999). In contrast, significant correlation between these traits was not found in melon and only two QTLs (*fw4.1* and *ssc4.1*) were detected in the same linkage group, revealing a different relationship between these developmental processes in melon as compared with tomato. In agreement with our results, Higashi et al. (1999) found that the differences in FW between two melon cultivars were determined by the differences in cell number in the early stages of fruit growth, with no differences in cell size after the cell-enlargement phase of fruit development. The differences in SSC observed in our experimental populations are probably not due to differential increasing of water content in cells.

Périn et al. (2002b) reported six FS QTLs in two populations of recombinant inbred lines. Comparing the position of the QTLs reported by those authors with the position presented in the current report and taking into account the co-linearity between both genetic maps (Périn et al. 2002a), it can be deduced that four QTLs have been detected in common: *fs1.1*, *fs.5.1*, *fs6.1*or *fs6.2* and *fs11.1* may correspond to *fs8.1* or *fs8.2*, *fs11.1*, *fs1.1* and *fs12.1*, respectively, in Périn et al. (2002b). Interestingly, *fs11.1* (*fs12.1* in Périn et al. 2002b) was detected in all experiments including PI161375 as exotic parent in both studies. *Fs11.1* maps on the region where the gene *p* (which controls the carpel number) lies (Oliver et al. 2001), reinforcing the hypothesis that *p* is a candidate gene for *fs11.1* (Périn et al. 2002b).

EA was the trait with lower QTL detection repeatability among the fruit quantitative traits. However, the analysis may have been confounded by being scored differently in the Za02 trial and not being scored in the Pre00 and F2.97 trials, so only three trials were fully comparable. For the other fruit quantitative traits, FW, SSC and FS, 68% of the QTLs were detected in at least two experiments. FS QTLs were generally detected in more trials (for example, *fs11.1* was detected in all trials), most FW QTLs were detected in three trials, whereas SSC QTLs were usually detected in only two trials. These results suggest that fewer genotype × environment (G × E) interactions occur at FS QTLs than at FW and SSC QTLs, consistent with the differences observed in correlations in the trials, being larger correlations for FS than the correlations for FW and SSC. Low G × E for FS in melon was also observed by Périn et al. (2002b). Similarly, in several advanced backcross progenies of tomato, FS QTLs have been detected more frequently in independent trials than FW and SSC QTLs (Tanksley et al. 1996; Fulton et al. 1997; Bernacchi et al. 1998; Fulton et al. 2000). Altogether, these results suggest that FS is under highly hereditable polygenic control, with few environmental interactions in both species; so, by extension, this kind of genetic control may be common to other fruit-bearing species. Given these results, it seems reasonable to suggest that some of the genes controlling FS in melon and tomato are the same. The current progress on cloning of the FS QTLs in tomato (Van der Knaap and Tanksley 2001; Liu et al. 2002; Van der Knaap et al. 2002), as well as FW (Frary et al. 2000) and sugar accumulation (Fridman et al. 2000) may provide a good source of candidate genes for these characters in melon.

Genetic control of fruit colour in melon

The inheritance of melon ECOL has not been clearly elucidated. Some authors proposed a single locus control (Parthasarathy and Sambandam 1981), whereas others suggested a polygenic control (Whitaker and Davis 1962). The monogenic hypothesis was rejected in our population; ECOL seemed to be controlled by more than two loci with epistatic interactions. We propose *ecol9.1* as one of the loci involved in ECOL. A second locus (*ecol3.1*) was detected only in the DHL trials; all yellow ECOL lines had PS alleles in that region of G 3. This locus was not detected in the F_2 population, which may be explained by the different genetic structure of the DHL and F_2 populations where dominant × additive or dominant × dominant interactions may occur, producing genetic

interactions that may affect ECOL. However, the interaction of these two loci could not fully explain the observed segregation of ECOL; interaction with other loci seems to be necessary for developing the yellow ECOL. The interaction could not be limited to a unique third locus, otherwise a segregation of three epistatic loci (1:15) would have been observed in the DHLs. Other loci could be involved in the interaction, such as *ecol7.1* and *ecol10.1*, detected in the F_2 population.

Melon FC has been proposed to be controlled by two genes: green flesh (*gf*, Hughes 1948) and white flesh (*wf*, Iman et al. 1972). Theses genes interact epistatically: *wf*$^+$-/*gf*$^+$- and *wf*$^+$-/*gfgf* allelic combinations have orange flesh, *wfwf*/*gf*$^+$- white flesh and *wfwf*/*gfgf* green flesh (Clayberg 1992). According to this model, PS (white flesh) would have the combination *wfwf*/*gf*$^+$*gf*$^+$ and PI161375 (green flesh) *wfwf*/*gfgf*. In our experimental population green flesh segregated as a single recessive locus, in agreement with the above model. We propose that *gfc1.1* on the bottom of G 1 corresponds to *gf*. Périn et al. (2002a) reported the mapping of *gf* on their linkage group IX, corresponding to our G 7. In our opinion, this inconsistency between their data and the current report could be due to a different interpretation of the genotypes at FC loci of the parents. Périn et al. (2002a) studied a RIL population from a cross between 'Véndrantais' (cantalupensis type, orange flesh) and PI 161375 (green flesh). In accordance with the above model, the genotype of 'Véndrantais' could be either *wf* $^+$*wf*$^+$/*gf*$^+$*gf*$^+$ or *wf*$^+$ *wf*$^+$/*gfgf*. If the genotype of 'Véndrantais' was *wf*$^+$*wf*$^+$/*gf* $^+$ *gf* $^+$, then a segregation 2:1:1 of orange (*wf* $^+$*wf*$^+$/*gf* $^+$*gf*$^+$, *wf*$^+$*wf*$^+$/*gfgf*), white (*wfwf*/*gf*$^+$*gf*$^+$) and green (*wfwf*/*gfgf*) FC would have been expected in that population. However, Périn et al. (2002a) described the segregation of FC as a single recessive gene. If, however, Véndrantais is *wf* $^+$ *wf* $^+$/*gfgf*, the segregation of FC in that population would be 1:1 orange (*wf*$^+$*wf*$^+$/*gfgf*), green (*wfwf*/*gfgf*), fitting the segregation observed by those authors. Therefore, we suggest that, assuming the two-loci model, Périn et al. (2002a) could have mapped *wf* instead of *gf*. The genetic combination of alleles at FC in our population allows us to map *gf* on G 1, whereas *wf* would map on G 7, linked to CMCT47.

The segregation of OFC in our populations did not fit the previous two-loci model. Segregation of markers linked to FC genes (MC138 on G 1 and CMTC47 on G 7) was independent of the segregation of OFC, indicating that in the current cross, OFC must be under different genetic control involving loci not reported previously. The genetic control is complex: *ofc2.1* and *ofc12.1* are consistent candidates as both regions were associated with OFC in both experimental populations, and are proposed as new loci involved in the control of melon FC. Larger population sizes are necessary to determine whether *ofc3.1* and other loci are also involved in OFC.

Use of exotic germplasm in melon breeding

Detection of QTL alleles from wild species with favourable effects from an agronomical perspective has been reported in tomato (Eshed and Zamir 1995; Tanksley et al. 1996; Monforte et al. 2001), rice (Xiao et al. 1998) and soybean (Concibido et al. 2003), among other species. In the current report, we demonstrate that, despite its inferior horticultural characteristics compared with PS, PI 161375 contains QTL alleles with favourable effects on important fruit quality traits: EA (seven alleles), FW (three alleles), SSC (one allele), newly discovered genes affecting ECOL and FC and alleles modifying FS from round to elongated. The lack of correlation among traits and co-localization of QTLs affecting different traits suggest that the introgression of QTLs from PI161375 to PS could be carried out with low genetic drag. QTLs must be validated and their effects estimated in the PS genetic background by the construction of NILs with PI 161375 QTL alleles in the PS genetic background. The construction of a set of NILs covering all the PI 161375 genome is currently under progress (Monforte et al. 2002) and its analysis will help better to estimate the impact of using exotic germplasm to improve the fruit quality of European and American cultivars, as this report demonstrates that favourable QTL alleles could be mined from exotic melon germplasm.

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