

# Shaping melons: agronomic and genetic characterization of QTLs that modify melon fruit morphology

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**Abstract** The consistency of quantitative trait locus (QTL) effects among genetic backgrounds is a key factor for introgressing QTLs from initial mapping experiments into applied breeding programs. We have selected four QTLs (*fs6.4*, *fw4.3*, *fw4.4* and *fw8.1*) involved in melon fruit morphology that had previously been detected in a collection of introgression lines derived from the cross between a Spanish cultivar, “Piel de Sapo,” and the Korean accession PI161375 (Songwan Charmi). Introgression lines harboring these QTLs were crossed with an array of melon inbred lines representative of the most important cultivar types. Hybrids of the introgression and inbred lines, with the appropriate controls, were evaluated in replicated agronomic trials. The effects of the QTLs

were consistent among the different genetic backgrounds, demonstrating the utility of these QTLs for applied breeding programs in modifying melon fruit morphology. Three QTLs, *fw4.4*, *fs6.4* and *fs12.1* were subjected to further study in order to map them more accurately by substitution mapping using a new set of introgression lines with recombination events within the QTL chromosome region. The position of the QTLs was narrowed down to 36–5 cM, depending on the QTL. The results presented in the current study set the basis for the use of these QTLs in applied breeding programs and for the molecular characterization of the genes underlying them.

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## Introduction

Melon (*Cucumis melo* L.) fruit are remarkably variable among cultivars, landraces and wild accessions. Melon fruit size ranges from a few tens of grams to several kilograms, and the fruit shape varies from globular to extremely elongated (Stepansky et al. 1999; Monforte et al. 2005). Melon fruit morphology is an important quality trait as it is one of the first characteristics noted by consumers. The fruit of cultivars belonging to different market classes should fit within a range of morphology parameters. For example, cantaloupes should be medium size and round, whereas “Piel de Sapo” cultivars should be oval and larger.

The genetic control of melon fruit morphology has been studied recently by quantitative trait locus (QTL) analysis (Perin et al. 2002a; Monforte et al. 2004; Eduardo et al. 2007; Zalapa et al. 2007; Paris et al. 2008; Fernandez-Silva et al. 2009). Two major genes have pleiotropic effects on fruit shape (*a* controlling sex expression and *p* controlling carpel number), but several independent QTLs with variable effects have also been described. According to these

works, fruit size and shape show moderate to high heritability and some QTLs have quite consistent effects, making them good targets to be exploited in melon breeding.

QTLs are usually detected in a specific genetic background. From an applied breeding point of view, the QTL effects must be consistent among the diverse genetic backgrounds that breeders use in their programs. However, the overall effect of a given QTL may not remain the same in a background other than the one in which it was identified. This feature is known as QTL by genetic background interaction (QTL  $\times$  GB). This interaction may be very important and may be one of the reasons why marker-assisted selection for QTLs is still not routinely implemented in breeding programs. For example, Steele et al. (2006) introgressed QTLs controlling rice root traits from an upland *japonica* cultivar into an upland *indica*. The QTLs had been detected in previous mapping populations, which differed in the recurrent rice cultivar. After several rounds of marker-assisted introgression, only one QTL maintained its effects on the new genetic background. The best documented example of QTL  $\times$  GB is most likely the series of marker-assisted introgression experiments studying QTLs controlling fruit quality traits (Lecomte et al. 2004; Chaib et al. 2006, 2007; Causse et al. 2007), where 50% of the studied QTLs were stable across genetic backgrounds, while the rest showed important QTL  $\times$  GB interactions. There are other examples where QTLs were transferred to different genetic backgrounds (Monforte et al. 2001; Gur and Zamir 2004). The former examples show the necessity of testing QTL  $\times$  GB interactions before implementing the marker-assisted introgression of QTLs into different genetic backgrounds.

The resolution of QTL mapping using the common populations is usually coarse: the confidence interval of the position is usually longer than 20 cM. The management of chromosome fragments of this size in breeding is problematic because of the linkage drag effects of other genes included within the QTL region and the possible recombination between markers and the QTL that diminishes the efficiency of marker-assisted selection. Paterson et al. (1990) demonstrated that substitution mapping using introgression lines with recombinant introgressions in the QTL region is a powerful approach to mapping QTLs more precisely. Using this approach, linkage drag has been reduced in some works (Monforte et al. 2001) and, QTLs have been cloned (Frery et al. 2000).

Eduardo et al. (2005) developed a set of near-isogenic lines (NILs) derived from the cross between the cultivar “Piel de Sapo” (PS) and the Korean cultivar “Shongwan Charmi” PI 161375 (SC), each having a unique marker-defined introgression of SC into the PS background. Using this NIL collection, Eduardo et al. (2007) described several

QTLs involved in melon fruit morphology with consistent effects across environments. After this work, QTLs were named using an abbreviator of the trait (i.e., FS for fruit shape, FW for fruit weight) and the code of the introgression line from where they were defined (i.e., QTL *fw4.3* is a fruit weight QTL detected in the melon introgression line SC4-3). Four were selected to be introgressed into other cultivar types: the QTL involved in fruit shape, *fs6.4* (that is also represented in the introgression line SC6-3, Moreno et al. 2008), and the QTLs involved in fruit size, *fw4.3*, *fw4.4* and *fw8.1* (represented in the NILs SC4-3, SC4-4 and SC8-1, respectively). The objectives of the present work are to study QTL  $\times$  GB interactions and to more accurately map some of these QTLs in order to transfer them more efficiently into breeding programs and facilitate future projects for their molecular characterization. NILs SC6-3, SC4-3, SC4-4 and SC8-1 (Electronic Supplementary Material, Figure S1) were selected for QTL  $\times$  GB experiments using an array of melon inbred lines belonging to different commercially important market classes. The QTLs *fw4.4*, *fs6.4* and *fs12.1* were also selected to increase their mapping resolution by substitution mapping (the latter is represented in the NIL SC12-1 and was not selected for the QTL  $\times$  GB experiment because it is recessive according to Fernandez-Silva et al. 2009).

## Materials and methods

### DNA extraction and molecular markers

DNA was extracted from young leaves using the modified CTAB method (Doyle and Doyle 1990). The simple sequence repeat (SSR) markers used in the current study were developed previously and were genotyped as described below (Gonzalo et al. 2005; Fernandez-Silva et al. 2008). In brief, SSRs were visualized using a LI-COR 4200 IR<sup>2</sup> sequencer (Li-Cor Inc., Lincoln, NE, USA). The labeling of PCR products was performed during the PCR reaction. A 20-mer nucleotide sequence from the M13 cloning vector (CACGACGTTGTAAAACGACC) was attached to the 5-end of the forward primers. PCR reactions were performed in a final volume of 15  $\mu$ l with 1 $\times$  Taq buffer [10 mM Tris-HCl, 50 mM KCl, 0.001% gelatine (pH 8.3)], 1.5–3.5 mM MgCl<sub>2</sub>, 166  $\mu$ M dNTPs, 2 U Taq DNA polymerase, 2 pmol of forward and reverse primers and 0.66 pmol of IRD700- or IRD800-labeled oligonucleotide with the 20-mer M13 sequence and 20 ng DNA. The cycling conditions were as follows: an initial cycle at 94°C for 1 min, followed by 35 cycles at 94°C, 30 s, 40–60°C, 30 s and 72°C, 1 min, and a final cycle at 72°C for 5 min.

## Plant material

The melon inbreds GAL, CAN, AMA, PS2, PS3 and the recurrent parent PS were kindly provided by Semillas Fitó (Spain) and the cultivar “Vedrantais” (VED) by Michel Pitrat (INRA, France). PS, PS2 and PS3 are “Piel de Sapo” types, AMA is “Amarillo” (yellow canary) type (all these previous cultivars are included in the cultivar group *inodorus*, Robinson and Decker-Walters 1997) CAN and VED are “cantaloupe” types and GAL is a “Galia” type (both included in the cultivar group *cantalupensis*). Other accessions were also used as references for the genetic variability analysis: PI 124112 (INB), PI 385966 (EIN), PI 161375 (SC), Ames24297 (TRI) and PI 435288 (FLEX), obtained previously from the North Central Regional Plant Introduction Station (Ames, IA, USA). NILs SC4-3, SC4-4, SC4-5, SC6-1, SC6-2, SC6-3, SC6-4, SC6-5, SC8-1 and SC12-1 are introgression lines with a single marker-defined introgression from SC into the PS background previously developed by Eduardo et al. (2005). NILs with shorter introgressions (hereafter referred to as “subNILs”) were obtained by selecting recombinant F<sub>2</sub> plants generated from the cross between PS and SC4-4 (for SC4-4-1, SC4-4-2, SC4-4-3 and SC4-4-4), or SC6-4 (for SC6-4-4 and SC6-4-3) or SC12-1 (SC12-1-53 and SC12-1-153), respectively, using the appropriate flanking markers. Recombinant plants were selected and selfed and F<sub>3</sub> plants were scored with appropriate markers to fix the recombinant chromosomes and to determine the exact point of recombination. Similarly, subNILs SC12-1-99 and SC12-1-55 were developed previously by Essafi et al. (2009).

## Genetic variability analysis

The following SSR markers were used to assess genetic variability between melon inbred lines: CMAT35, CMCTN35, CMCTN38, CMTCN41, CMAGN75, CMAGN79, TJ2, TJ31, ECM81, ECM88, ECM122 and ECM230, which are polymorphic among melon germplasm (Monforte et al. 2003, 2005; Gonzalo et al. 2005; Fernandez-Silva et al. 2008). Nei et al. (1983) genetic distances were calculated with Powermarker 3.5 (Liu and Muse 2005) and the Neighbor-Joining (NJ) tree was plotted with MEGA 4.0 (Kumar et al. 2008).

## Genetic background interaction experiments

Hybrids of PS, SC4-3, SC4-4, SC6-3 and SC8-1 with the array of elite inbreds (AMA, CAN, GAL, PS, PS2, PS3 and VED) were produced at the Semillas Fitó, S.A. facilities (Table 1). SC6-3 was chosen instead of SC6-4 because the latter also increased fruit weight, whereas SC6-3 did not show any such effects (Moreno et al. 2008). The hybrids

**Table 1** Crosses performed to produce the hybrids for QTL × genetic background experiments

Male parent	Female parent	Market type	Cultivar group
PS	AMA	Yellow Canary	<i>Inodorus</i>
SC4-3	CAN	Cantaloupe	<i>Cantalupensis</i>
SC4-4	GAL	Galia	<i>Cantalupensis</i>
SC6-3	PS	Piel de Sapo	<i>Inodorus</i>
SC8-1	PS2	Piel de Sapo	<i>Inodorus</i>
	PS3	Piel de Sapo	<i>Inodorus</i>
	VED	Cantaloupe	<i>Cantalupensis</i>

PS is the recurrent parent used to introgress the chromosomal regions from the Korean accessions PI161375 represented in the NILs SC4-3, SC4-4, SC6-3 and SC8-1 that contain QTLs modifying fruit morphology. The market type and cultivar group for each genotype used as female parent is also shown

were evaluated for fruit shape (FS, defined as the ratio of the maximum fruit diameter to maximum fruit length) and fruit weight (FW) in field trials in Zaragoza (Spain) and Cabrils (Spain). In Zaragoza, plants were grown in an open field with drip irrigation, whereas in Cabrils plants were grown in a plastic greenhouse with drip irrigation. For each hybrid/genotype five plots of three plants each were randomized in the field, and 1–8 fruits per plot were harvested. All the hybrids were evaluated during 2007 in Cabrils, whereas in Zaragoza, the experiment was divided into two parts: SC4-4 and SC6-4 hybrids were evaluated in 2007, and SC4-3 and SC8-1 were evaluated in 2008 due to field space limitations. The hybrid SC6-3 × GAL could not be evaluated and the hybrids with VED were evaluated only in Zaragoza due to seed limitations and low germination.

## Substitution mapping experiments

Much like in the above experiments, five plots of three plants for SC4-4, SC6-4 and their respective-related NILs (see above) and 20 plots of three PS control plants were randomized in fields in Zaragoza and Cabrils during 2006. The SC12-1-related NILs were evaluated in the same way during 2008 at both locations.

## Data analysis

All statistical analyses were performed with JMP 5.1 2. for Windows (SAS Institute, Cary, NC, USA). For the genetic background experiment, the effect of the introgression (I), genetic background (GB) and location (L) were investigated by multifactor analysis of variance (ANOVA) and their significance by an *F* test. The means of the hybrids of inbreds and the PS control were compared with a Fisher Least Significant Difference test at  $P < 0.05$ . For the substitution mapping experiment, the means of the NILs in

each experiment were compared with the PS control using Dunnett's contrast (Dunnett 1955) with Type-I error  $\alpha \leq 0.05$ .

## Results

### Molecular and phenotypic variability between parent lines

Six inbred lines representing four important melon market classes ("Amarillo," "Piel de Sapo," "Galia" and "Cantaloupe") were selected to produce hybrids with the recurrent PS parent and NILs. We studied the molecular genetic variability between them with a set of 12 polymorphic SSR markers, all inbreds showed a unique SSR profile except the PS–PS3 pair. The genetic relationships were as expected according to Monforte et al. (2003): "Piel de Sapo" (PS) inbreds clustered together close to "Amarillo" (AMA) (all of them are within *inodorus* cultivar group) and separated from "Galia" (GAL), "Cantaloupe" (CAN) and "Vedrantais" (VED) (all of them are within *cantalupensis* cultivar group), confirming that the selected inbreds are a genetically diverse sample of cultivated melons. The NJ tree based on Nei et al. (1983) genetic distances is depicted in Figure S2.

The fruit phenotype of the hybrids of PS and inbreds also showed remarkable differences (Figure S3). Hybrids of PS and PS2, PS3 and AMA showed a typical "Piel de Sapo" phenotype (i.e., oval shape, green external color and white flesh), whereas hybrids of PS and GAL, CAN and VED exhibited very different phenotypes (for example, white or yellow external color or orange flesh). Remarkable differences were observed for the traits under study: with regard to fruit weight (FW) the hybrids with PS ranged from medium (1.25 kg) to large (3.36 kg) sizes and fruit shape (FS) from nearly round (FS = 1.10) to elongated (FS = 1.50) shapes (Fig. 1). The effect of the different genetic backgrounds was significant for both traits, FS and FW (Fig. 1; Table 2).

### QTL effects and their interaction with location and genetic background

The introgression presented in NIL SC6-3 had significant effects on FS and FW (Fig. 1; Table 2). On average, the fruit of hybrids with the SC6-3 introgression were rounder (the mean FS index was 9.5 and 7.0% lower among hybrids of inbreds with SC6-3 than among the hybrids of inbreds and PS, in Cabrils and Zaragoza, respectively), and smaller (a mean FW reduction of 13.3 and 12.7% in Cabrils and Zaragoza, respectively) than the respective hybrids of PS.

In a previous report, SC6-4 fruits were larger than those of PS (Eduardo et al. 2007), so the reduction of FW observed in some SC6-3 hybrids must be caused by a different QTL than that of SC6-4, as was described initially. Moreover, these effects on FW were more evident in other genetic backgrounds apart from the recurrent PS. Interactions between introgression (I) and location (I\*L) and genetic backgrounds (I\*GB) were not significant for both traits. Therefore, the effect of the QTL is consistent across locations and genetic backgrounds, confirming the QTL for FS previously detected with NILs SC6-4 and SC6-3 (Eduardo et al. 2007; Moreno et al. 2008; Fernandez-Silva et al. 2009).

The introgression of NIL SC4-3 showed significant effects (I) on FW, which also confirmed the QTL detected in previous experiments (Eduardo et al. 2007). The introgression from SC decreased FW in all GB, and significant differences in effect magnitude across GB (I\*GB) were not observed (Fig. 1; Table 2). Significant I\*L interaction was detected, which may be due to the differences in magnitude of the SC4-3 introgression effects between locations. The mean effect of SC4-3 on FW ranged between a 32% reduction in Cabrils to 18.6% in Zaragoza.

The introgression of NIL SC4-4 also had a significant effect (I): the mean reduction of FW ranged from 16.5 to 13.1% in Cabrils and Zaragoza, respectively. Neither I\*GB nor I\*L interaction effects were observed, which demonstrates the consistency of the QTL effects. Finally, the SC8-1 introgression displayed significant effects on FW (I), with a 20 and 18.6% reduction in Cabrils and Zaragoza, respectively. No significant I\*GB or I\*L interactions were observed, which confirms the consistency of the QTL effect.

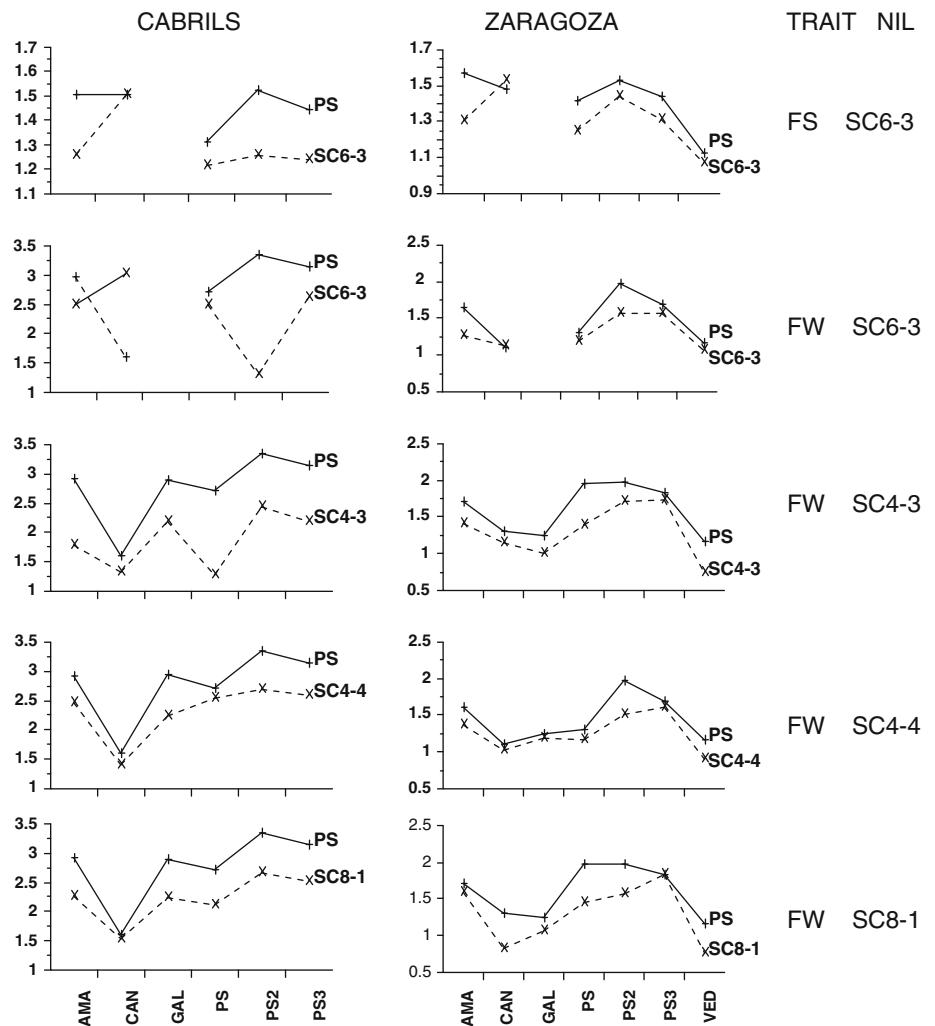
### Substitution mapping of QTLs involved in fruit morphology

We developed new sets of recombinant lines (subNILs) with shorter introgressions from SC4-4, SC6-3 and SC12-1 to estimate the QTL positions more accurately.

#### SC4-4

Four newly developed subNILs (SC4-4-1 to SC4-4-4) as well as NILs SC4-4 and SC4-5 were compared with the recurrent PS (Fig. 2). All NILs and subNILs, except SC4-4-2, showed a significant decrease in FW compared to PS. The introgressions of that set of NILs share an introgression between CMAGN73 and CMCTN6 (19 cM), and so *fw4.4* was assigned to that marker interval (Fig. 2). NILs harboring the SC allele of *fw4.4* showed a mean reduction in FW of 28–30% (Cabrils-Zaragoza) compared to PS.

**Fig. 1** Means of hybrids of inbred lines and PS (black lines) and the NILs (dashed lines) used in the current report. The NILs used for crossing with inbreds (AMA, CAN, GAL, PS, PS2, PS3 and VED on the bottom) are indicated on the right column together with the trait under study (FS fruit shape, FW fruit weight). The means of the hybrids between PS and the NILs for the traits are depicted in the central diagrams for both locations. The connecting lines are drawn only to compare the shape of the lines between PS and NIL means; solid lines connect hybrids with PS recurrent genotype and dashed lines connect hybrids with the NILs, they do not depict any kind of relationship between inbred lines. Means for the hybrids with GAL for NIL SC6-3 are not shown due to insufficient data



**Table 2** Significance of the introgression (I), genetic background (GB), location (L) effects and their respective interactions (I\*GB, I\*L, GB\*L) from multifactor ANOVA for the traits fruit shape (FS) and fruit weight (FW)

NIL	Trait	I	GB	L	I*GB	I*L	GB*L	Mean PS hybrids	Mean NIL hybrids
SC6-3	FS	<0.001	<0.001	<0.05			<0.01	1.44	1.35
SC6-3	FW	<0.001	<0.001	<0.001				2.05	1.81
SC4-3	FW	<0.001	<0.001	<0.001		<0.001	<0.001	2.2	1.63
SC4-4	FW	<0.001	<0.001	<0.001			<0.01	1.96	1.64
SC8-1	FW	<0.001	<0.001	<0.001			<0.001	2.05	1.76

The introgression is indicated in the NIL row. Empty cells indicate  $P > 0.05$ . Mean of the hybrids with the recurrent parent PS and the NILs over genetic backgrounds and locations are indicated on the last columns. FS is the ratio between maximum fruit length and maximum fruit diameter and FW is the average fruit weight in kg

**SC6-4**

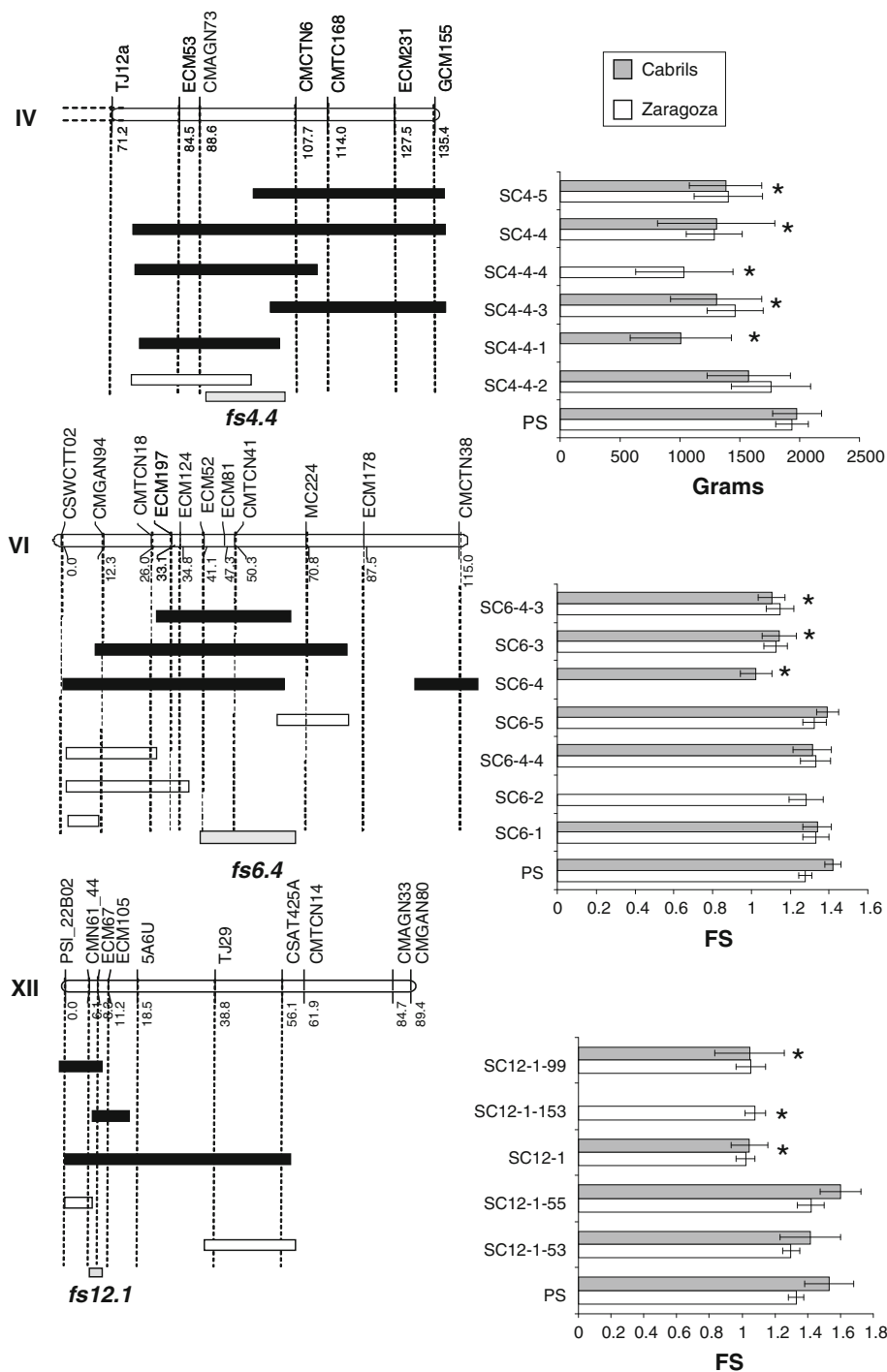
Two new subNILs (SC6-4-3 and SC6-4-4) were developed from SC6-4, whereas the remaining ones (SC6-1, SC6-2, SC6-3, SC6-4 and SC6-5) were previously developed by Eduardo et al. (2005). Only NILs and subNILs sharing introgressions around the CMCTN41 marker in the central region of the linkage group exhibited round-shaped fruits. Thus, the QTL named *fs6.4* was assigned between MC224

and ECM124 (36 cM, Fig. 2). The SC alleles in *fs6.4* produced a mean reduction of 23% and 11% of FS in Cabrils and Zaragoza, respectively.

**SC12-1**

The NIL SC12-1 and subNILs SC12-1-99 and SC12-1-153 produced round melons. These NILs share an SC introgression between CMN61\_44 and ECM105 (5 cM).

**Fig. 2** Substitution mapping of the QTLs *fw4.4*, *fs6.4* and *fs12.1*. For each QTL, the linkage group number is shown on the left in Roman numerals according to Perin et al. (2002b), the molecular marker linkage map of the QTL region according to Fernandez-Silva et al. (2008) and the extension of the introgressions for the NILs and subNILs. On the right, the means and 95% confidence interval for each NIL in the two locations; those means marked with asterisks are significantly different from control PS according to Dunnet’s test ( $P < 0.05$ ). On the bottom, the deduced position of the QTLs. Black introgression bars highlight means significantly different from the control. For some NILs, data were missing in one location. In those cases a single bar is shown



The QTL *fs12.1* was consequently mapped within that region (Fig. 2). The fruit from those NILs had five carpels, whereas subNILs SC12-1-55 and SC12-1-53, which produced more elongated fruit, had three carpels (data not shown). The mean reduction of FS by the SC allele of *fs12.1* varied between 31 and 18% in Cabrils and Zaragoza, respectively.

**Discussion**

Consistency of QTL effect across genetic backgrounds

These results show that the current set of QTLs have stable effects across a number of genetically distinct melon genetic backgrounds, belonging to different important

market classes. Reverse effects were not observed, in contrast to other studies (Pumfrey et al. 2007; Septiningsih et al. 2003; Thomson et al. 2003).

QTL  $\times$  GB interactions have been investigated using different approaches. The most common of these are: the study of differential QTL detection in segregating populations sharing a common parent, or the differences between hybrids of selected QTL-NILs and an array of genotypes. The differential detection of QTLs among segregating populations could be due to real interactions, but sampling effects may also be important (Melchinger et al. 1998; Schon et al. 2004; Xu 2003). QTL  $\times$  GB interactions have been extensively reported when studying segregating populations, especially for yield-related traits (Liao et al. 2001; Concibido et al. 2003; Mihaljevic et al. 2004; Villalta et al. 2007; Kang et al. 2009; Li et al. 2009). The consistency of QTL effects across genetic backgrounds may be dependent on the genetic architecture of the trait. QTL  $\times$  GB interactions are usually more important for traits than yield, as they are controlled by a large number of QTLs with minor effects (Bernardo 2008).

The study of QTL  $\times$  GB interactions using NILs has the advantage of reducing genetic complexity, because only one QTL is segregating, which permits the detection of a larger number of QTLs and the obtention of better estimates of their effects, as well as the interactions between them (Eshed and Zamir 1995, 1996) in the appropriate genetic background. QTL  $\times$  GB interactions have indeed been found in some cases. Some examples of these would be: rice panicle size traits using reciprocal introgression lines (Mei et al. 2006), rice root traits (Steele et al. 2006) and tomato fruit acidity and sucrose content and texture (Chaib et al. 2006, 2007). Nevertheless, in all these cases there were also some individual QTLs that did not show interactions. In other reports, a substantial number of QTLs have not shown any interaction with genetic background. In most cases, the QTLs were involved in fruit morphology or highly heritable QTLs, such as soluble solid concentration in tomato (Monforte et al. 2001; Chaib et al. 2006). In the current report, we have also focused on highly heritable fruit morphology QTLs (Eduardo et al. 2007). In these cases, it may be argued, following Bernardo's (2008) rationale, that these traits are under a simpler (although polygenic) genetic control compared to yield, and as a consequence the QTL  $\times$  GB interaction is less important. Gur and Zamir (2004) pyramided three yield QTLs from *Solanum pennellii* into a processing tomato cultivar and tested their effects on several genetic backgrounds. The QTLs did not show QTL  $\times$  GB interactions, which demonstrates that it is also possible to find stable QTLs across genetic backgrounds even for very complex traits. Therefore, the QTL–NIL approach is adequate for defining QTLs with consistent effects across environments and

genetic backgrounds, which are suitable to be introduced into breeding programs.

The effect of the genetic background may be due to either allelic variation of the QTL under study or to epistatic interactions with other loci. Discrimination between the two hypotheses is not always possible. Buckler et al. (2009), using the maize Nested Association Mapping population, found very significant variation of QTL allelic effects but low epistasis, which would support the first hypothesis. Chaib et al. (2006) also attributed the lack of consistency in one tomato fruit firmness QTL to allelic variation at the locus of interest because the effects were not observed in backgrounds that yielded firmer fruit. We have not found genetic background interactions in our QTL sample, which suggests that there are no allelic differences at the studied QTLs among the inbred lines. That hypothesis is reasonable if we consider the large genetic distance between inbreds and the donor parent SC, i.e., we are introgressing new allelic variability that was not present among the elite inbreds.

#### Substitution mapping of fruit morphology QTLs

Substitution mapping is a powerful approach to fine-mapping QTLs. The resolution depends on both the number and distribution of recombinant events and marker density in the characterization of recombination points. We were able to map melon fruit morphology QTLs within 22 (*fw4.4*), 36 (*fs6.4*) and 5 (*fs12.1*) cM intervals, considerably reducing the previous resolution obtained by Eduardo et al. (2007) (65 cM for *fs4.4*, nearly the whole linkage group VI for *fs6.4* and 56 cM for *fs12.1*). The main limitation for increasing the map resolution was the lack of markers in the regions where the QTLs are located. Several recombination events were produced within the QTL intervals. Although medium density linkage maps of melon have been developed relatively recently (Oliver et al. 2001; Perin et al. 2002b; Gonzalo et al. 2005; Fernandez-Silva et al. 2008; Fukino et al. 2008; Deleu et al. 2009), a high-resolution map is still not available. Currently, all the genetic maps have been integrated within the International Cucurbit Genomics Initiative (<http://www.icugi.org>). This new high-resolution map will help to increase the mapping resolution of the QTLs reported in the current study.

Due to the poor resolution of common QTL studies, the genetic basis of a single QTL could be due to several linked genes. Currently, an important number of QTLs have been cloned (Alonso-Blanco et al. 2009). In most cases, these QTLs have large effects and a single gene was found to control each one, for example, *fw2.2* in tomato (Frary et al. 2000), *Q* in wheat (Simons et al. 2006), *sh4* in rice (Li et al. 2006) and *Vgt1* in maize (Salvi et al. 2007). In contrast, other QTLs are more complex; QTLs for yield in tomato

(Monforte and Tanksley 2000; Monforte et al. 2001) or for flowering time in rice (Thomson et al. 2003) have finally been resolved as two different QTLs each at a genetic scale (i.e., the distance between the QTLs was several cM), whereas in other cases the QTLs were very tightly linked, and could only be resolved by high-resolution mapping, like the case of a growth rate QTL in *Arabidopsis thaliana* (Mitchell-Olds and Schmitt 2006). The QTLs involved in melon fruit morphology studied in the current study still behave as a single locus at the current mapping resolution. Even though the resolution is not sufficient to repudiate the hypothesis that several genes may be underlying any of those QTLs, the simplest hypothesis at this moment is that each one is controlled by a single locus.

The QTL *fs12.1* was mapped with a higher resolution than *fw4.4* and *fs6.4* at the distal region of LG XII. NILs and subNILs with the round QTL allele also exhibit fruit with five carpels, in contrast with wild-type fruit which have three carpels. Carpel number is controlled by the previously defined gene *p* (*pentamerous*) and has pleiotropic effects on fruit shape, i.e., five-carpel fruit are rounder than three-carpel fruit (Perin et al. 2002a; Monforte et al. 2004). The fact that we could not break the linkage between carpel number and fruit shape in our substitution mapping experiment supports the previous results. The distal part of the linkage group harbors other agronomical genes of interest: *nsv*, which confers resistance to the Melon Necrotic Spot Virus (Nieto et al. 2006) and *cmv1*, which confers resistance to the Cucumber Mosaic Virus (Essafi et al. 2009). Five carpels is an undesirable trait because it causes fruit to have an empty cavity of greater volume, making it important to break the linkage between this trait and the resistance genes. According to our results, *p* is tightly linked to ECM67 at the top of LG XII (Fig. 2), in a different position than *nsv*, which in turn is tightly linked to 5A6U (an SSR marker located in the same BAC clone as *nsv*). However, we could not break the linkage between *p* and *cmv1* as both map in the same interval (Essafi et al. 2009). Higher resolution mapping is therefore necessary to break that linkage in order to be able to transfer *cmv1* to cultivars avoiding the linkage drag produced by *p*.

## Conclusions

The QTLs selected in the current study (*fs6.4*, *fw4.3*, *fw4.4* and *fw8.1*) had consistent effects across several environments and genetic backgrounds, which validates the previous results (Eduardo et al. 2007; Moreno et al. 2008; Fernandez-Silva et al. 2009). These QTLs are a suitable choice for modifying fruit morphology in different melon cultivars belonging to different commercial market classes. The mapping resolution of two QTLs (*fw4.4* and *fs6.4*) and *fs12.1* was increased by substitution mapping. The results

are compatible with the hypothesis that each QTL is controlled by a single independent locus, and establish the basis for the subsequent high-resolution mapping and/or cloning of the genes underlying them.

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