Agronomical, genetical and developmental characterization of fs6.4: a Quantitative Trait Locus controlling melon fruit shape

E. Moreno1, I. Fernández-Silva1, I. Eduardo1, A. Mascarell2, J.M. Álvarez3, A. Caño2, and A.J. Monforte1*

1 IRTA Centre de Recerca en Agrigenómica (CSIC-IRTA-UAB). Carretera de Cabriols Km 2. 08348 Cabriols, Spain
2 IBMB-CSIC. Centre de Recerca en Agrigenómica (CSIC-IRTA-UAB) Jordi Girona 18-26, Barcelona 08034, Spain
3 Centro de Investigación y Tecnología Agroalimentaria de Aragón. Apartado 727. 50080 Zaragoza, Spain
* Corresponding author e-mail: Antonio.Monforte@irta.es

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Abstract

The genetic control of melon fruit shape is complex. Recently, several Quantitative Trait Loci (QTLs) affecting melon fruit shape have been identified using an introgression line population derived from the cross between a Cucumis melo ‘Piel de Sapo’ cultivar and the Korean accession PI 161375 ‘Songwan Charmí’ (SC). One of these QTLs, fs6.4, was selected for further genetical and developmental characterization. The SC allele of fs6.4 induces round fruit by limiting the longitudinal development of the fruit. Its mode of gene action is largely additive, showing low interactions with environment, genetic background or when combined with other fruit shape QTLs. Its chromosomal position could be narrowed to the central part of Linkage Group VI, breaking the linkage with a fruit weight QTL located in the same linkage group. Flower development analysis showed that the QTL determines the fruit shape in the very first stages of the flower development, just few hours after the appearance of the flower bud. These results set up the basis for the molecular characterization of the gene underlying fs6.4.

INTRODUCTION

Melon germplasm exhibits a high range of fruit shape, defined as the ratio length/diameter (LD), from globular (LD≈ 1) to elongated (LD> 5) (Stepansky et al. 1999; Monforte et al. 2005). Fruit shape is one of the external attributes that consumers appreciate. For example, Cantaloup and Galia cultivar types should be round, whereas Piel de Sapo types should be oval. Consumers would reject fruit that do not fit the expected shape according their cultivar type. Thus, fruit shape is an important trait in melon breeding.

Two genes involved in major morphological traits (a controlling sex type and p controlling carpel number) have been associated to variation in fruit shape, although a number of QTLs independent of these genes has been also detected (Périn et al. 2002; Monforte et al. 2004). Therefore, fruit shape is under a polygenic genetic control in melon as it has been described in tomato (Grandillo et al. 1999).

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101
Recently, Eduardo et al. (2005) constructed a collection of introgression lines (Near Isogenic Lines, NIL) from the cross between the Spanish cultivar ‘Piel de Sapo’ (PS) and the Korean accession PI 161375 ‘Songwan Charmi’ (SC). Each NIL contains a unique, independent introgression, defined by molecular markers, from SC into the PS genome. The introgressions represented in this NIL set covers most of the SC genome. Eduardo et al. (2007) performed the first agronomical evaluation of a selected core from this NIL collection. One of the NILs, SC6-4, yield larger and rounder fruit than PS. The introgression of SC6-4 expands most of the LG VI, so the effects on fruit size and shape would most likely be caused by different linked QTLs. The QTL for fruit shape, thereafter fs6.4, was selected for further study to incorporate it in future breeding programs and dissect the molecular basis of fruit shape variation.

The successful introduction of QTLs into breeding programs requires a high resolution of the QTL position and to estimate the interactions QTL by environment, QTL by QTL and QTL by genetic background (Monforte et al. 2001). The objectives of the present work are to map more precisely the position of fs6-4, mainly to break the linkage between the fruit size and shape QTLs on LG VI, estimate its mode of gene action, interactions with other QTLs and different genetic background, and determine the development stage when the fruit shape is determined.

MATERIAL AND METHODS

Plant material

NILs SC6-1, SC6-2, SC6-3, SC6-4, SC6-5 carrying different introgressions from SC into the PS genetic background (see Eduardo et al. 2005 for further details) were used to fine map fs6-4. SC6-4 was crossed with PS to produce the SC6-4F1 hybrid to estimate the mode of gene action, and with SC8-3, SC10-2 and SC12-1 (NILs carrying other fruit shape QTLs, see Eduardo et al. 2007) to study their interaction with fs6-4. These NILs were also crossed with PS to develop their respective hybrids (SC8-3F1, SC9-1F1 and SC12-1F1). PS and SC6-3 were also crossed with an array of cultivars, four of them from Semillas Fitó [two ‘Piel de Sapo’ (PS-1 and PS-2), ‘Amarillo’ (AMA) and ‘Cantaloup’ (CANT) types] and the cantaloupe-type cultivar ‘Vedrantais’ (VED) to investigate the interaction of fs6-4 with different genetic backgrounds.

Greenhouse and Field experiments

Fine mapping, estimation of gene action and interaction with other QTLs experiments were carried out in a greenhouse (Cabrils, Spain) during different years. Ten to fifteen plants from each genotype were completely randomized, grown in peat bags with 0.25 m spacing between plants, drip irrigated and hand pollinated, allowing only one fruit per plant. The genetic background experiment was performed in a field in Zaragoza, Spain, where five plots of three plants each for each hybrid of SC6-3 and PS with the different genetic backgrounds were randomized in the field. Flowers were open-pollinated.

For all experiments, fruit shape was defined as the ratio maximum length/maximum diameter of mature fruit (LD). In the fine mapping experiment, fruit weight (FW) was also measured.
Data analysis
All statistical analyses were performed with JMP (version 5.1.2 for Windows, SAS Institute, Cary, NC). Details of each statistical procedure can be obtained from Monforte et al. (2001). Briefly, the position of fs6-4 was estimated by substitution mapping comparing the mean of each NIL with the recurrent parent PS using the Dunnet contrast (Dunnet 1955). The dominance deviation was evaluated by the contrast 2*SC6-4F1–(SC6-4+PS). The dominance/additive ratio was calculated as d/a = (SC6-4F1-(PS+ SC6-4)/2)/(SC6-4-PS)/2. Interaction of fs6-4 with other QTLs or different genetic backgrounds was estimated by two-way analysis of variance.

Flower development experiments
Fresh female flowers were harvested at five different stages, every day from the appearance of flower bud to anthesis. Flowers were fixed in 3.7% formaldehyde (FAA) solution and dehydrated with ethanol and Histoclear® solution. Samples were then gradually embedded in molten paraffin. Longitudinal and radial sections of the flowers were done at 6 µm using a Supercut 2050 microtome (Reichert-Jung) and placed in microscopy slides, stained with a toluidine blue solution and visualized using an Axiophot microscope.

RESULTS AND DISCUSSION
Fine mapping of fs6-4
Graphic genotype and comparison of means with PS for each NIL are depicted in Figure 1. Only SC6-3 and SC6-4 showed significant effects on LD, whereas SC6-1, SC6-2 and SC6-3 showed effects on FW. Thus, FW and LD are controlled by different linked QTLs: fw6-4 and fs6-4, being fw6-4 in the distal part of the LG VI and fs6-4 in its central part. SC6-3 contained only fs6-4 but not fw6-4 within its SC introgression, consequently this NIL was chosen for future studies. These results provide the starting point for future molecular cloning of genes involved in melon fruit size and shape as it has been achieved in tomato (Frary et al. 2000; Liu et al. 2002).

Gene mode action of fs6-4 and interaction with other QTLs
Fruit shape values of SC6-4, PS and their hybrid SC6-4F1, together with the estimate of the QTL genetic effects are shown in Table 1. The mode of action of the fs6-4 gene was additive (significant additive effects but not significant dominant effects). The effect on the shape was due to a decrease of the fruit length, as no significant effects were observed on fruit diameter.

Three NILs including other fruit shape QTLs (SC8-3 and SC10-2 yielding elongated fruit and SC12-1 yielding round fruit, Eduardo et al. 2007) were selected to study the interaction of fs6-4 with other QTLs. Significant interaction was only observed in the cross with SC8-3 (Fig. 2). The fruit from the hybrid SC8-3xSC6-4 were 14% rounder than expected. This epistasis may indicate these QTLs are affecting the same developmental pathway (Coupland 1995), whereas the additive effects observed in the hybrids SC10-2xSC6C6-4 and SC12-1xSC6-4 would indicate that those QTLs would act on different pathways.
Figure 1. Delineation of the introgressed segments from SC in black and position of the QTLs *fw6-4* and *fs6-4*. The markers used by defining the introgressions and the genetic map of LG VI are according Gonzalo et al. (2005). Means for Fruit Weight in grams (FW) and ratio fruit length/diameter (LD) for the PS recurrent parent and NILs are shown on the right. Statistically significant differences between NILs and PS (P<0.05) are marked as (*).

Table 1. Fruit shape trait (LD is the ratio between fruit length and diameter) means and standard deviations for the recurrent genotype ‘Piel de Sapo’ (PS), the NIL SC6-4 and their respective hybrid (SC6-4F1). Estimations of additive effect (a), dominance deviation (d) and mode of gene action (d/a) are also shown. (*) indicates significant effects at p<0.05.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fruit Length (cm)</th>
<th>Fruit Diameter (cm)</th>
<th>LD</th>
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<tr>
<td>Genotype</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PS</td>
<td>18.92 ± 1.40</td>
<td>15.23 ± 0.49</td>
<td>1.24 ± 0.06</td>
</tr>
<tr>
<td>SC6-4</td>
<td>15.62 ± 2.02</td>
<td>16.08 ± 0.60</td>
<td>0.96 ± 0.08</td>
</tr>
<tr>
<td>SC6-4F1</td>
<td>17.25 ± 1.07</td>
<td>15.97 ± 0.62</td>
<td>1.07 ± 0.05</td>
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</table>

Genetic effects

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<thead>
<tr>
<th></th>
<th>a</th>
<th>d</th>
<th>d/a</th>
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<tbody>
<tr>
<td>additive</td>
<td>1.65*</td>
<td>-0.02</td>
<td>-0.01</td>
</tr>
<tr>
<td>d</td>
<td>-0.42</td>
<td>0.32</td>
<td>-0.76</td>
</tr>
<tr>
<td>d/a</td>
<td>0.14*</td>
<td>-0.03</td>
<td>-0.19</td>
</tr>
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</table>
**Genetic background interactions**

Several elite genotypes belonging to economical important market cultivars were chosen as testers for this experiment. The interaction of *fs6-4* with the different genetic backgrounds was significant (*p*<0.001). Figure 3 shows the LD means of the different hybrids tested. The QTL *fs6-4* reduced LD in all genetic backgrounds, except of CANT. The magnitude of the effect also varied among genetic backgrounds, being more important in PS and AMA background. It is noteworthy that *fs6-4* showed significant effects on VED background, even though VED fruit are already round. QTL by genomic background interactions may be important, making difficult to transfer QTLs from one background to other (Lecompte et al. 2004). The *fs6-4* effects among genetic background were quite consistent, showing just little variation; most of this variation was on the magnitude of the effect. Therefore, this QTL is a good candidate to be transfer to a large number of genetic backgrounds and modify the fruit shape of cultivars belonging to different melon types.

**Figure 2.** Interaction between SC6-4 and SC8-3 effects on fruit length/diameter ratio (LD). PS, SC6-4 x PS, SC8-3 x PS and SC8-3 x SC6-4 indicate the LD mean from the recurrent parent ‘Piel de Sapo’, its hybrid with SC6-4, SC8-3 and the cross between SC8-3 and SC6-4, respectively. The dash line indicate the expected LD mean for SC8-3 x SC6-4 according with the phenotypes of the hybrid SC6-4 x PS and SC8-3 x PS.
Figure 3. Fruit length/diameter ratio (LD) means for the crosses of the SC6-3 and PS with different genotypes (PS-1 and PS-2 are ‘Piel de Sapo’ type, AMA is ‘Amarillo’ type, CANT and VED are ‘Cantaloup’ type).

**Flower development experiments**

Melon fruit shape is determined prior to anthesis (Périn et al. 2002). Consequently, SC6-4 ovaries are rounder than PS ovaries (Eduardo et al. 2005), thus, the fruit shape is determined at some point during the flower development. Five flower developmental stages were defined at regular times between the appearance of the flower bud to anthesis. The shape of ovaries harvested at flower bud appearance was practically equal between PS and SC6-4. However, significant differences were observed between ovaries harvested one day after the appearance of the flower bud. These differences were maintained during the flower development until anthesis. Consequently, fs6.4 effects were clearly observed within the first hours after the appearance of the flower bud, demonstrating that melon fruit shape is determined very early during the flower development. Therefore, the characterization of the cellular and molecular processes involved in the determination of fruit shape should be studied right just after the appearance of the flower bud.
The major loci controlling the variation of fruit shape in tomato have been identified (Tanksley et al. 2004), some of them have been already cloned (Liu et al. 2002) and the cloning of additional ones is expected to occur in the very next future (van der Knaap et al. 2004). These genes will be an important source of candidate genes for melon fruit shape QTLs. Additionally, comparative development studies with other models species will asses whether the same process and genes are controlling variation of fruit morphology (Paran and van der Knaap 2007). The possible finding of common genes controlling fruit shape among species would facilitate the manipulation of fruit morphology in any crop species by the breeders. Furthermore, the comparison between of fruit bearing and non-fruit bearing crop species flower development would be also of interest to understand the evolution of fleshy fruit.

CONCLUSIONS

In the current report, we present a multidisciplinary characterization of the fruit shape QTL $fs6-4$. The genetic effects of this QTL are largely additive: its effects are consistent across environments, different genetic backgrounds and when combined with other fruit shape QTL. This reliability of QTL effects has allowed us to start a fine mapping experiment and set up the basis for its future map-based cloning. We have determined the flower developmental stage where the fruit shape is defined, this result will be very useful to compare better with other model species studies and discriminate among possible candidate genes.

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