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REVIEW PAPER

The genetic basis of fruit morphology in horticultural crops: lessons from tomato and melon

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Abstract

Fruits represent an important part of the human diet and show extensive variation in size and shape between and within cultivated species. The genetic basis of such variation has been studied most extensively in tomato, where currently six quantitative trait loci (QTLs) involving these traits have been fine-mapped and the genes underlying the QTLs identified. The genes responsible for the cloned QTLs belong to families with a few to many members. *FASCIATED* is encoded by a member of the YABBY family, *CNR*/*FW2.2* by a member of the Cell Number Regulator family, *SlKLUH*/*FW3.2* by a cytochrome P450 of the 78A class (CYP78A), *LOCULE NUMBER* by a member of the WOX family including WUSCHEL, *OVATE* by a member of the Ovate Family Proteins (OFP), and *SUN* by a member of the IQ domain family. A high portion of the history and current diversity in fruit morphology among tomato cultivars can be explained by modifications at four of these cloned QTLs. In melon, a number of QTLs involved in fruit morphology have been mapped, but the molecular basis for these QTLs is unknown. In the present review, we examine the current knowledge on the molecular basis of fruit morphology in tomato and transfer that information in order to define candidate genes of melon fruit shape and size QTLs. We hypothesize that different members of the gene families identified in tomato may have a role in the regulation of fruit morphology in other species. We anchored the published melon QTL map on the genome sequence and identified the melon family members of the six cloned tomato QTLs in the genome. We investigated the co-localization of melon fruit morphology QTLs and the candidate genes. We found that QTLs for fruit weight co-localized frequently with members of the *CNR*/*FW2.2* and *KLUH*/*FW3.2* families, as well as co-localizations between OFP family members and fruit-shape QTLs, making this family the most suitable to explain fruit shape variation among melon accessions.

Key words: Candidate gene, domestication, mapping, QTL, shape, size.

Introduction

Fruits provide a means of plant reproduction and dispersal, and are the hallmarks of the angiosperm lifestyle. Development of flowers and fruit has been attributed to the success of angiosperm during evolution as exemplified by a great diversity in species found around the globe. Moreover, fruits are a critical food source. Depending on use, a fruit is labelled as vegetable or as fruit, and collectively provide many essential nutrients and minerals that are required for a balanced diet. Fruit development initiates with the formation of a flower from the floral meristem. A prototypical floral meristem will give rise

Abbreviations: CNR, cell number regulator; FAS, FASCIATED; FS, fruit shape; FW, fruit weight; OFP, Ovate Family Protein; LC, locule number; NIL, near-isogenic Line; QTL, quantitative trait locus; SEM, scanning electron microscopy; SOV, suppressor of ovate mutation.

to four whorls: the sepal, petals, stamen, and pistil. The stamen provides the male reproductive structures giving rise to pollen. The pistil provides the female reproductive structure giving rise to the ovules within the ovary. At the time of flower opening, or anthesis, pollen will land on the stigma of the pistil and germinate, and the pollen tube will grow through the style towards the ovules. Fertilization of the ovules marks the beginning of fruit development. Signals from the fertilized ovules and developing seed will initiate growth of the ovary walls. Fruit development generally follows the Gillaspy *et al.* (1993) model. The initial stage is marked by increases in cell division, followed by cell expansion. Once at full size, the ripening process is initiated which is highlighted by major biochemical changes in the maturing fruit. Depending on the plant species, the ripening process is typically associated with dramatic changes in colour, aroma, and fruit structure. The ripening process is regulated mainly by the hormone ethylene in climacteric fruits (Giovannoni, 2004; Gapper *et al.*, 2013), whereas other hormones such as brassinosteroids (Symons *et al.*, 2006), auxins, and abscisic acid (Jia *et al.*, 2011) seem to have an role in non-climacteric fruit ripening, although a general model for this type of ripening is still under debate (Symons *et al.*, 2012).

Fruit development is critical for dispersal of species in natural settings. Birds and rodents may carry the fruit over long distances and distribute the seeds away from the mother plant. Large fruit is not advantageous for dispersal in the wild. For human consumption, however, large fruit of different dimensions is required. In general, all domesticated fruit and vegetables carry fruit of larger size than typically found in the wild. Also, fruit dimensions vary such that some fruits are flat, ribbed, oblate or long, in addition to round. Examples of different morphologies among cultivated fruits can be found, from the very small (blackberries, blueberries) to giant (pumpkins), and from oblate (Saturn peaches) to extremely elongated (cucumbers, snake melons).

This review focuses on the fruit of two domesticated species: tomato (*Solanum lycopersicum* L.) and melon (*Cucumis melo* L.). Cultivars from both species show a huge fruit morphological diversity (Figs 1 and 2) that is under the control of a large number of genetic loci (Grandillo *et al.*, 1999; Diaz *et al.*, 2011; Xu *et al.*, 2013). For tomato, some of the genes underlying these quantitative trait loci (QTLs) have been cloned, whereas for melon the underlying genes have remained elusive. The purpose of the present review is to summarize the current knowledge on the molecular genetic basis

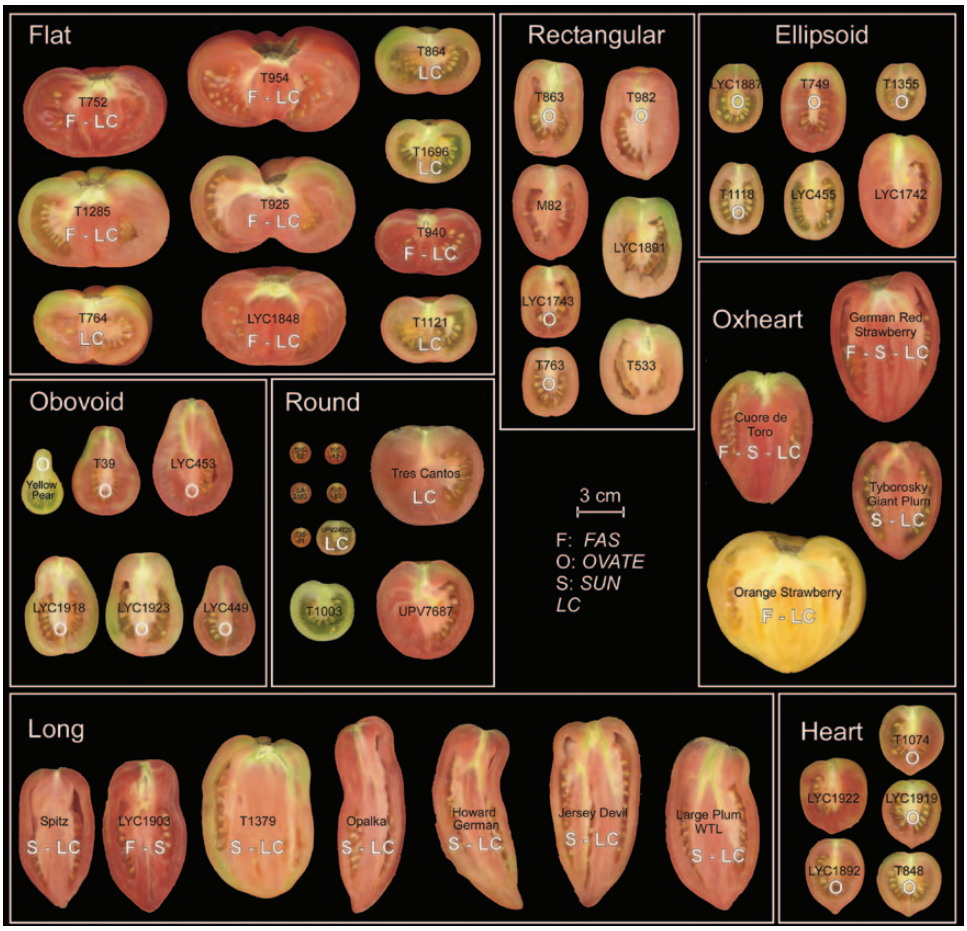

Fig. 1. Diversity in tomato fruit shapes. The shape categories are defined according to Rodriguez *et al.* (2011). Each fruit is identified by variety name (information available at http://solgenomics.net/ and Rodriguez *et al.*, 2011) and the presence of the variant allele of *SUN*, *OVATE*, *FAS* (abbreviated as S, O, and F, respectively) and/or *LC*. Figure reprinted from Rodriguez GR, Munos S, Anderson C, Sim SC, Michel A, Causse M, Gardener BBM, Francis D, van der Knaap E. 2011. Distribution of SUN, OVATE, LC, and FAS in the tomato germplasm and the relationship to fruit shape diversity. *Plant Physiology* 156, 275–285, www.plantphysiology.org. Copyright American Society of Plant Physiology. (This figure is available in colour at *JXB* online.)

Fig. 2. Representative fruits of several melon (*Cucumis melo*) cultivar groups according Pitrat (2008): (A) *inodorus* (Piel de Sapo); (B) *conomon* (Shiro Uri Okayama); (C) momordica (PI124112); (D) *chate* (Carosello Barese); (E) *dudaim* (Queen Anne's pocket melon); (F) *acidulous* (TGR-1551); (G) *makuwa* (Ginsen Makuwa); (H) *ameri* (Kizil Uruk); (I) *cantalupensis* (Vedrantais); (J) *reticulatus* (Dulce); (K) *flexuosus* (Arya); (L) *tibish* (Tibish); (M) *chinensis* (Songwhan Charmi), and (N) wild melon (trigonus). (This figure is available in colour at *JXB* online.)

of fruit morphology in tomato and to transfer this information to melon in order to investigate whether the variation of Cucurbitaceae fruit morphology may be due to orthologous genes found in tomato as well as those belonging to the same gene families.

Tomato domestication and diversification

The history of tomato domestication has become clearer in recent years (Blanca *et al.*, 2012). Extensive genetic characterization of 272 accessions using Tomato Illumina Bead Chips (Illumina, San Diego, California, USA) developed by the SolCAP project (Hamilton *et al.*, 2012; Sim *et al.*, 2012) containing 7414 single-nucleotide polymorphism markers led to the hypothesis that tomato was probably domesticated in two waves: from *Solanum pimpinellifolium* to *S. lycopersicum* var. *cerasiforme* in Ecuador and Northern Peru; and later from S. *lycopersicum* var. *cerasiforme* to *S. lycopersicum* var. *lycopersicum* in Central America (Blanca *et al.*, 2012). After the discovery of America by Christopher Columbus, tomatoes travelled from Mexico to Europe and the rest of the world resulting in additional improvements of fruit characters. Fruit from S. *pimpinellifolium* are small and round, weighing approximately 1 g. Fruit from *S. lycopersicum* var. *cerasiforme* are generally larger than those of *S. pimpinellifolium* (10–30 g), and some accessions exhibit an oval or a flat fruit shape in addition to the classical round shapes. Fruits from *S. lycopersicum* var. *lycopersicum* are much larger (up to 1 kg) and highly diverse in shape (Rodriguez *et al.*, 2011) (Fig. 1). Classification of tomato shapes using the software program Tomato Analyzer (Brewer *et al.*, 2006) led to the identification of eight shape categories that are found in cultivated tomato: flat, ellipsoid, rectangular, oxheart, heart, long, obovoid, and round (Rodriguez *et al.*, 2011). Much of this variation can be explained by four genes that each play a dominant role in the regulation of fruit morphology (see below).

Melon domestication and diversification

Recent molecular phylogenies indicate that the origin of the species was most likely in Asia or Australia, and that *C. melo* reached Africa at a later date (Kocyan *et al.*, 2007; Renner *et al.*, 2007; Schaefer *et al.*, 2009; Sebastian *et al.*, 2010). Wild melon fruits are small (3–6 cm diameter, weighing less than 50 g), round or oval with a very thin and bitter-tasting mesocarp (Fig. 2). Wild melons may be found in East and West Africa, and Central India (Roy *et al.*, 2012). Melon may have been domesticated in Africa or Asia or in both locations independently (Esquinas-Alcazar and Gulick, 1983, Bates and Robinson, 1995), probably for its seeds rich in proteins and lipids for the production of flour (Pitrat 2008). The development of the fruit mesocarp, and thus the edible flesh, occurred

after the initial domestication stage. The species underwent an extensive process of diversification, where Central Asia and the Mediterranean basin represent the primary and secondary centres of diversity, respectively. This diversification led to a huge diversity in fruit characteristics among the cultivars. Regarding fruit morphology, the size varies from very small (less than 100 g), small (100–400 g), medium (400 g to 1 kg), and large (1–5 kg) to very large (more than 4 kg, up to 10 kg), and fruit shape varies from slightly flat, ellipsoid, obovoid, round, and long to extremely long (Fig. 2). Other fruit traits such as rind colour, flesh content and colour, sweetness, sourness, aromatic compounds, and climacteric behaviour also show an impressive variability within the species (Stepansky *et al.*, 1999; Nuñez-Palenius *et al.*, 2008; Pitrat, 2008; Burger *et al.*, 2006; Fernandez-Trujillo *et al.* 2011).

Melon is divided into two subspecies based on the ovary's hairiness: ssp. *melo* with long hairs (this subspecies is found from India to Europe and America), and ssp. *agrestis* with short hairs (this subspecies is found from India to Japan) (Jeffrey, 1980). The Occidental melon cultivars (cantaloupe, galia, honeydew, Western shippers, 'Piel de Sapo', and Christmas melon) belong to ssp. *melo*. The intraspecific melon classification has been revised several times, with the most recent clustering proposed by Pitrat (2008), who suggested 15 groups or *varietas* namely *chinensis*, *makuwa*, *momordica*, *conomon*, and *acidulus* belonging to ssp *agrestis*, and *chate*, *flexuosus*, *tibish*, *adana*, *ameri*, *cantalupensis*, *chandalak*, *reticulatus*, *inodorus*, and *dudaim* belonging to ssp. *melo.* The African and Asian wild melons are not listed in this classification, although they are generally referred to as *agrestis*.

The fruit morphology diversity corresponds to the Pitrat classification: *conomon* melons are long and medium sized (hundreds of grams to 1 kg); *makuwa* are flat to round, ellipsoid, and obovoid, and of medium size; *chinensis* are obovoid and of medium size; *momordica* are flat to long and of medium size; *acidulus* are ellipsoid and are small to medium in size; *tibish* are ellipsoid or obovoid and small; *chate* are round to ellipsoid and middle-sized; *flexuosus* are extremely long (up to 2 m), sometimes serpentine and very large (up to 10 kg); *cantalupensis* are flat to ellipsoid, medium to large (more than 1 kg) in size; *reticulatus* are round to ellipsoid and medium to large in size; *ameri* are ellipsoid and long and medium to large in size; *inodorus* are round to ellipsoid and large; *dudaim* are round and small; and the wild melons *agrestis* are round to ellipsoid and very small (50 g).

Fruit development in tomato and melon

Tomato fruit are classified as berries. The fruit develops from the ovary after fertilization of the ovules. The walls of the ovary (called the valves in *Arabidopsis thaliana* L.) become the pericarp comprising the largest part of the fruit. The pericarp surrounds the locules that contain the placenta and the seeds. Like its immediate ancestor, *S. pimpinellifolium* L. from which tomato was domesticated, the fruit are red. However, cultivated types also carry fruit that is yellow, pink, or orange. Nearly all cultivated tomato fruit are produced by selfing. Distant relatives, such as *Solanum pennellii* Correll and *Solanum habrochaites* D. Knapp & D. M. Spooner, carry green fruit and are typically self-incompatible, requiring cross-pollination with pollen from plants that carry a compatible allele at the S locus. Moreover within the tomato clade, crosses using distant wild relatives can only be made by using cultivated tomato as the female parent, a process called unilateral incongruity (Bedinger *et al.*, 2011). Thus, genetic exchanges among distant relatives usually occur only under controlled conditions but have been critically important for the introgression of useful traits into modern tomato such as disease resistance and fruit quality traits.

Tomato produces perfect flowers that contain five sepals, five petals, five stamen, and two carpels (locules). After successful fertilization of the ovules, the pericarp, columella, and placenta tissues expand mainly by enhanced cell divisions for 5–10 d after pollination. This stage is followed by cell enlargement, which results in dramatic increases in fruit mass (Gillaspy *et al.*, 1993). Domestication of tomato resulted in larger fruit of different dimensions including drastic changes in locule number (Paran and van der Knaap, 2007; Rodriguez *et al.*, 2011). The basis of these changes in fruit dimensions are probably caused by changes in organ patterning and may include changes in cell-division planes and rates (Wu *et al.*, 2011).

Melon fruit share common aspects with tomato as well as other features that are unique. In contrast to tomato, melon plants may bear different combinations of flower types: monoecious (male and female flowers), andromonoecious (male and hermaphrodite flowers), and gynoecious (only female flowers). Wild melons and landraces from the centres of domestication are monoecious, whereas most modern cultivars are andromonoecious, suggesting that the mutation that led to andromonoecy was produced after the domestication. Obviously, monoecious cultivars need cross-pollination. Yet andromonoecious cultivars also need cross-pollination as the amount of pollen produced by the stamens from the hermaphroditic flower is usually not sufficient to fertilize all the ovules.

Melon fruits are classified as a pepo, i.e. a modified berry with a hard rind and soft fleshy mesocarp inside. A central cavity harbours usually three locules (although some cultivars have five locules), derived from the carpels where the seeds are located in the proximal–distal direction. The rind develops from the inferior ovary and the edible flesh from mesocarp tissue. In melon, fruit development follows the same phases as tomato.

In order to understand better the early development of melon fruit, we used histological analysis and scanning electron microscopy (SEM) to characterize female flower anatomy at different developmental stages in the 'Piel de Sapo' cultivar. Female floral organs are composed of two outer whorls with five sepals and five petals, which constitute the perianth. The innermost whorl represents the female reproductive organ and contains three fused carpels, which form the gynoecium. Figure 3A shows a longitudinal view of a mature flower revealing the inferior location of the ovary in

the gynoecium. The SEM images of a mature flower show a median longitudinal section of an ovary in the lateral and basal parts (Fig. 3B, C). The ovary wall (pericarp) is composed by the outer exocarp and the mesocarp, separated by vascular bundles (Fig. 3B). The inner pericarp tissues enclose the endocarp, which include the placenta with the ovules as the female gametophyte (Fig. 3B). In the basal part of the ovary, vascular tissue connects the ovary to the pedicel (Fig. 3C). Under optimum growth conditions, female flower development takes approximately 5 d from floral bud appearance by the naked eye until anthesis, which generally lasts a day. After ovule fertilization, the fruit grows rapidly for 3 weeks, after which the growth rate slows down to a certain pace that is typical of the cultivar. In large-fruit cultivars, fruit growth may be maintained until harvest, whereas in small-fruit cultivars, growth stops a few weeks before harvest (Higashi *et al.*, 1999). Figure 4A depicts the longitudinal and equatorial growth of fruits from the Spanish 'Piel de Sapo' cultivar after anthesis showing a high growth rate for 2 weeks after anthesis and slower growth rates thereafter.

In tomato, depending on the QTL, changes in fruit morphology such as size and shape manifest themselves either before or after anthesis (van der Knaap and Tanksley, 2001; Chakrabarti *et al.*, 2013). In melon, a high correlation has been reported between ovary and mature fruit morphology (Perin *et al.*, 2002; Eduardo *et al.*, 2007), indicating that the fruit shape is predominantly determined pre-anthesis in this species. The elongated shape is generally also highly correlated with the length of the fruit but not with the diameter (Monforte *et al.*, 2004; Eduardo *et al.*, 2007), suggesting that

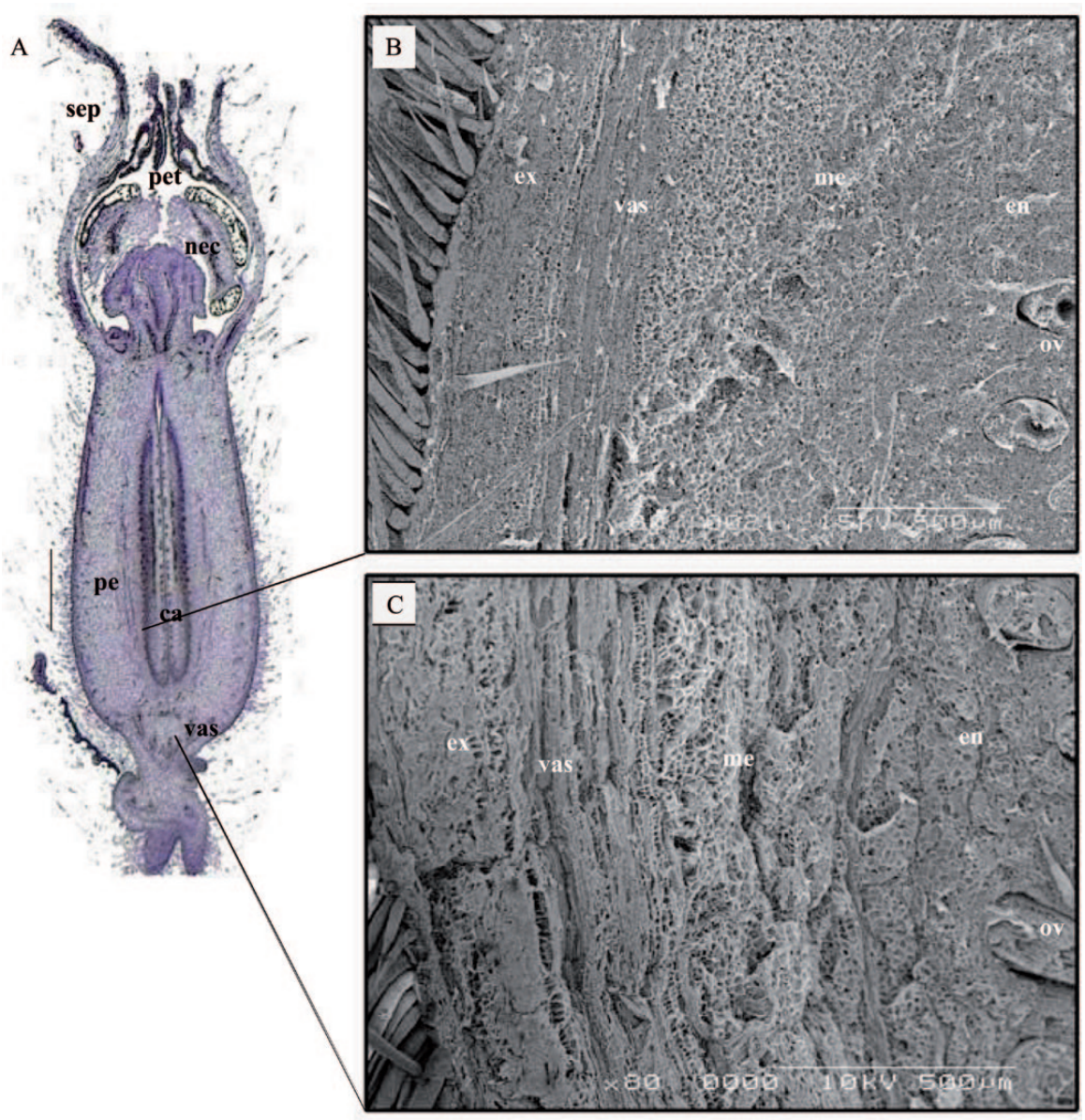

Fig. 3. Morphological characterization of 'Piel de Sapo' female flowers. (A) Histological longitudinal section of a melon female flower. The different floral organs and tissues are distinguished: sepals (sep), petals (pet), nectaries (nec), pericarp (pe), carpels (ca), and vasculature (vas). (B, C) SEM images of the lateral (B) and basal part (C) of the ovary, displaying a detailed view of the inner cellular organization. Different tissues and parts are clearly visible: exocarp (ex), vasculature (vas), mesocarp (me), endocarp (en), and ovules (ov). (This figure is available in colour at *JXB* online.)

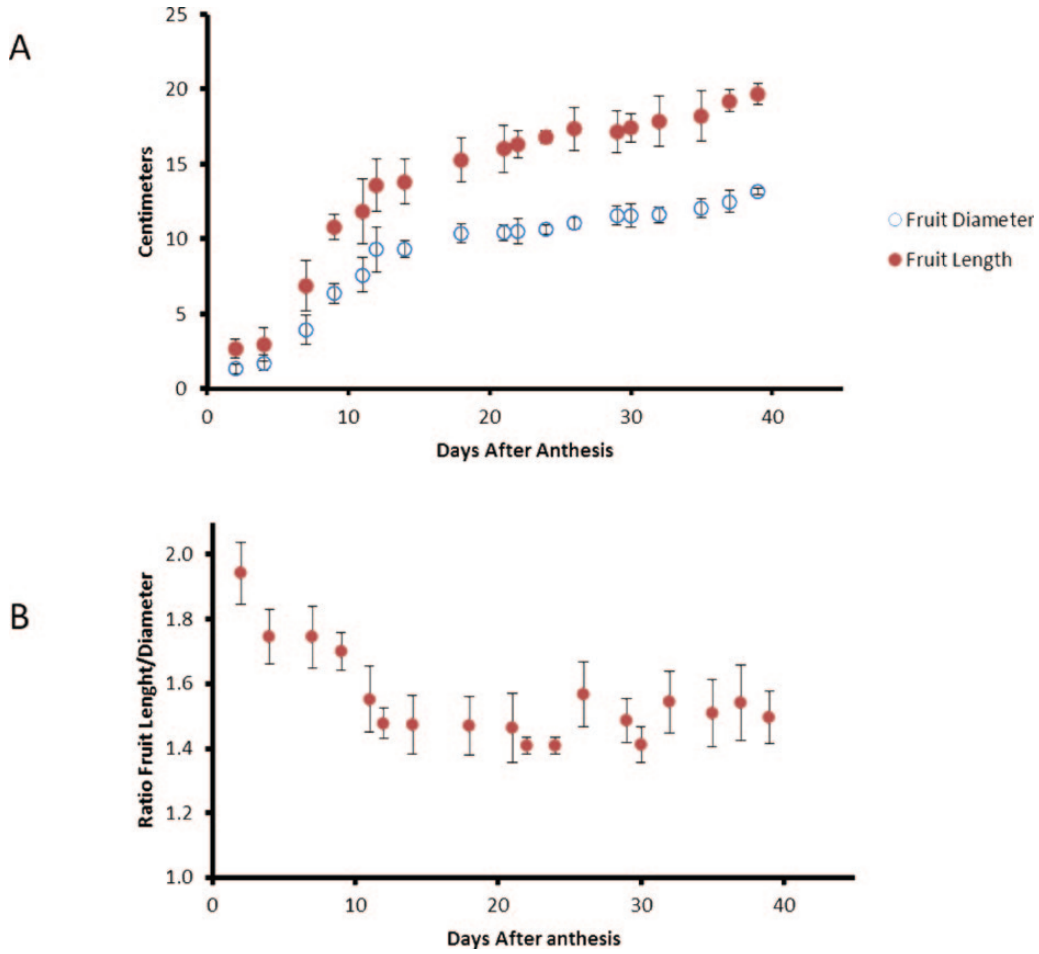

Fig. 4. Growth of melon fruits from the 'Piel de Sapo' variety through fruit development. (A) Longitudinal and equatorial diameters. (B) Ratio of fruit length:diameter. (This figure is available in colour at *JXB* online.)

longitudinal growth is the major factor of the final shape (round or elongated). The 'Piel de Sapo' cultivar shows a higher fruit shape index in the ovary than in the mature fruit, which decreases as consequence of increasing of equatorial diameter by mesocarp growth during fruit development; the final shape is achieved at around 15 d post-anthesis (Fig. 4B).

Molecular genetic basis of fruit shape and size variation in tomato

The first fruit size QTL that was cloned is *CNR*/*FW2.2*, encoding a member of the Cell Number Regulator (CNR) family (Frary *et al.*, 2000; Guo *et al.*, 2010; Guo and Simmons, 2011). Fruit size increases occur after anthesis and predominantly in the placenta tissues of the developing tomato fruit (Cong *et al.*, 2002; Liu *et al.*, 2003). Increases in fruit size occur before anthesis, as the ovaries of the near-isogenic lines (NILs) carrying the large-fruited allele already exhibit a larger size compared with the small-fruited NIL (Frary *et al.*, 2000). The second cloned fruit size QTL is *SlKLUH*/*FW3.2*, encoding a member of a subfamily of cytochrome P450 A78 class (CYP78A) and the orthologue of KLUH (Chakrabarti *et al.*, 2013). Increased fruit size is manifested after anthesis, and the tissues that are most significantly enlarged are the pericarp and septa areas. Cell size is not altered but instead the large-fruited NILs show two extra cell layers in the pericarp, implying that *SlKLUH* affects cell division. Concomitant with the increase in cell layers, there is a delay in ripening of approximately 4 d (Chakrabarti *et al.*, 2013). The yield per plant is not altered, i.e. larger-fruited lines will not result in higher fruit weight per plant. This is most likely due to the reduced number of side shoots and side shoot lengths found in the large-fruited lines, thereby offsetting the increase in fruit weight (Chakrabarti *et al.*, 2013). Thus, *SlKLUH* has a pleiotropic effect on side shoot growth.

Changes in tomato fruit shape are contributed to a large extent by mutations in four genes: *SUN* and *OVATE* regulating fruit elongation, and *LOCULE NUMBER* (*LC*) and *FASCIATED* (*FAS*) regulating locule number and flat fruit shape (Rodriguez *et al.*, 2011). *SUN* encodes a protein that is a member of the IQ domain family (Abel *et al.*, 2005; Xiao *et al.*, 2008). This family is characterized by a calmodulin-binding domain, suggesting a role of this protein in calcium signalling. Overexpression of *SUN* leads to very elongated parthenocarpic fruit, twisted stems and leaf rachis, and changes in leaf serration (Wu *et al.*, 2011). These phenotypes led to another assumption that the auxin pathway might be perturbed as a result of overexpression of *SUN*, but no direct links to this hormone have been found. Fruit weight does not

vary between the NILs that differ for *SUN*. Instead, the shape is determined by increased cell number in the proximal–distal direction and decreased cell number in the medial–lateral direction of the fruit (Wu *et al.*, 2011). The most dramatic effect on fruit shape mediated by *SUN* occurs during the early stages of fruit development. However, patterning is most likely established before anthesis, as ovary shape and cell number changes are already slightly different before fruit set in the *SUN* NILs (Xiao *et al.*, 2008; Wu *et al.*, 2011).

OVATE encodes a protein in the Ovate Family Protein (OFP) and is thought to negatively regulate transcription of target genes (Liu *et al.*, 2002; Hackbusch *et al.*, 2005). Although not significant, the NILs with the *ovate* mutation carry fruit of slightly lower weight compared with those of the wild type (Clevenger, 2012). Thus, fruit elongation is not the result of continued growth along the proximal–distal axis. Instead, the increase in fruit elongation is due to cell proliferation in the proximal region of the developing ovary (S. Wu and E. van der Knaap, unpublished data). In *ovate* NILs, the ovary shape is very elongated at the time of anthesis and gradually becomes less elongated during fruit development (van der Knaap and Tanksley, 2001; Clevenger, 2012). These data show that fruit shape patterning by *OVATE* is established well before anthesis.

FAS encodes a protein that is a member of the YABBY family regulating organ polarity (Cong *et al.*, 2008), whereas *LC* is probably encoded by the orthologue of the *A. thaliana* gene *WUSCHEL*, which is a member of the WOX family, involved in regulation of meristem size (Muños *et al.*, 2011). It is likely that locule number is determined very early in floral development, although this has not been examined further.

In addition to these known genes, other loci controlling fruit shape and size have been fine-mapped in recent years. These include *fw11.3* controlling fruit weight. *fw11.3* maps very close to but is not allelic with *FAS* (Huang and van der Knaap, 2011). Additional loci controlling fruit shape include two suppressors of the *ovate* mutation (SOVs) located on chromosomes 10 and 11 (Rodriguez *et al.*, 2013) and *fs8.1* mapping near the centromere of chromosome 8 (Clevenger, 2012). *sov1* on chromosome 10 has recently been fine-mapped to two candidate genes (H.J. Kim and E. van der Knaap, unpublished data), whereas *fs8.1*, despite its centromeric location, has been confined to a 3 Mb region comprising 122 candidate genes (Clevenger, 2012)

Genetic basis of fruit shape and size variation in melon

During the last decade, the genetic basis of melon fruit morphology has been investigated in several studies (Perin *et al.*, 2002; Monforte *et al.*, 2004; Eduardo *et al.*, 2007; Zalapa *et al.*, 2007; Paris *et al.*, 2008; Harel-Beja *et al.*, 2010; Diaz *et al.*, 2011). Fernandez-Silva *et al.* (2010) confirmed three of these QTLs (*FSQC6.4*, *FSQC12.1*, and *FWQ4.4*) Recently, Tomason *et al.* (2013) reported markers associated with fruit morphology by association mapping. The germplasm used in the aforementioned studies included modern cultivars and landraces from both subspecies (Supplementary Fig. S1 and Supplementary Table S1 available at *JXB* online) but not wild relatives. Thus, the QTLs are likely to correspond to genes involved in cultivar diversification and not domestication.

Melon fruit morphology QTLs are anchored onto genetics maps. To anchor them onto the physical map and the melon genome, we retrieved the sequences of the closest linked markers from the International Cucurbit Genomics Initiative (ICuGI) data base (http://www.icugi.org) or from the results of Fernandez-Silva *et al.* (2008) and used BLAST (basic local alignment search tool) to compare them against the pseudo-chromosomes of version 3.5 of the melon genome (Garcia-Mas *et al.*, 2012) available at https://melonomics.net/. In cases where only primer sequences were available, we used the forward and reverse primers and checked whether the positions in the genome were comparable with the size of the product from PCR amplification. In general, the BLAST results were consistent with the expected result. If the results were not consistent, the marker was replaced with a nearby marker based on the consensus map (Diaz *et al.*, 2011). The position of the QTLs anchored in the genome is depicted in Fig. 5.

Definition of melon fruit shape QTLs

The genetic basis of fruit shape was investigated in six independent populations and one association mapping study (Table 1). Forty-two QTLs (Diaz *et al.*, 2011) and nine associations (Tomason *et al.*, 2013) were correlated with fruit shape. The fruit shape QTLs were located in 14 regions throughout the genome, assuming that when two QTLs from different studies are located in the same region, they represent the same QTL. Nine regions harboured QTLs that were detected in two or more studies using different germplasm sources, and five regions harboured QTLs from three studies (Fig. 5). The latter QTLs represent the best candidates to underlie the most important fruit shape diversification in the melon germplasm and are defined as Meta-QTL.

Chromosome 1, FSMQ1

A meta-QTL located in the distal part of the chromosome was mapped as a classical QTL (A, J, P, Q, and X according to Table 1) and in an association mapping study, explaining up to 31 % of phenotypic variance. The allele from the ssp. *agrestis* parent created elongated fruits.

Chromosome 2, FSMQ2

This meta-QTL was detected in the following crosses: C, J, N, P, and Q with strong additive effects and explaining a high proportion of the phenotypic variance (up to 52%). In most populations, this QTL co-localized with the gene *a* that controls sex determination in female flower. The *a* gene was found to encode a 1-aminoacyclopropane-1-carboxylic acid synthase protein, designed CmACS-7 (Boualem *et al.*, 2008). The andromonoecious phenotype is due to a loss of CMACS-7 enzymatic activity in the developing flower. The presence of stamens in female flowers restricts longitudinal ovary growth, resulting in a pleiotropic effect on fruit shape, and by a reduction of fruit elongation (Monforte *et al.*, 2005; Abdelmohsin and Pitrat, 2008).

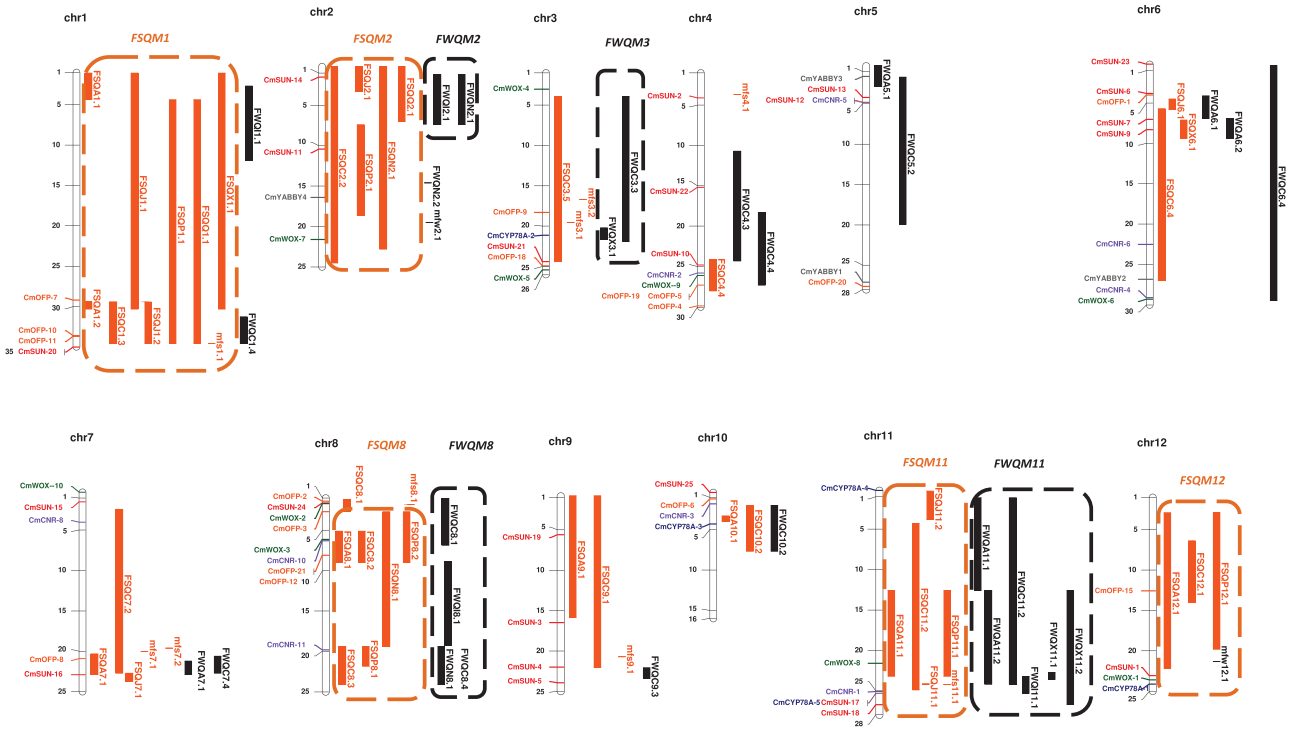

Fig. 5. Melon fruit morphology QTLs and members of *SUN* (red), *OFP* (orange), *CNR* (purple), *YABBY* (grey), *WUSCHEL* (green), and *KLUH* (blue) gene families located on the melon chromosomes. On the left of each chromosome, the distance in Mb and the melon gene member (with a 'Cm' prefix) are shown. The fruit morphology QTLs (FS, fruit shape in orange, and FW, fruit weight in black, using the terminology of Diaz *et al.*, 2011) are located on the right with a bar indicating the confidence interval of their position. The markers associated with fruit morphology are coded in lower case with the prefix 'm'. The meta-QTLs (FSQM and FWQM) were defined from QTLs detected in at least three or two independent experiments for FS and FW, respectively.

Chromosome 8, FSMQ8

The proximal and distal regions of this chromosome were associated with fruit morphology QTLs. This meta-QTL, detected in populations A–C, N, and P, explains up to 22% of the variation and can increase fruit length by as much as 50%. The *agrestis* allele controls the extreme elongation.

Chromosome 11, FSQM11

This meta-QTL was detected in three independent populations (A–C, P, and Q), and also in an association study. The effect of this locus is 17% and the *agrestis* allele results in fruit elongation.

Chromosome 12, FSQM12

This meta-QTL was detected in three populations (A, C and P) derived from the Korean accession PI161375. This QTL is probably due to mutations in the *p* locus (pentamerous) controlling carpel number. The fruit of most melon varieties have three carpels, and only a few accessions (including PI161375) produce fruits with five carpels. An increase in carpel number results in flat fruit in tomato (Rodriguez *et al.*, 2011), and carpel number may therefore control the shape in melon as well. Analysis of NILs differing in the *p* alleles has shown that fruit with five carpels usually have a larger internal cavity, resulting in increased fruit diameter, and therefore causing a rounder shape (Eduardo *et al.*, 2007; Obando *et al.*, 2008). The ssp. *agrestis* allele results in round fruit with up to 29% phenotypic variance.

Definition of melon fruit weight QTLs

The genetic basis of fruit weight (FW) was investigated in four independent populations (Supplementary Table S1 available at *JXB* online) and one association mapping study (Tomason *et al.*, 2013). A total of 27 fruit weight QTLs were compiled by Diaz *et al.* (2011) in addition to three loci that were associated with fruit weight (Fig. 5) (Tomason *et al.*, 2013). QTLs and associations with fruit weight were located on all chromosomes. In general, the occurrence of the same QTL across different populations was lower than for fruit shape QTL. Only two loci, on chromosomes 8 and 11, were found in three different populations. Loci on chromosomes 2 and 3 were associated with fruit weight in two populations, whereas the remaining QTL were detected in one population only (QTLs FWQA and FWQC were detected in different populations from the same cross); therefore, four meta-QTLs were defined as follows.

Chromosome 2, FWMQ2

This was detected in two populations (I and N), explaining up to 43 % of the variance. This QTL co-segregates with the *a* gene, suggesting that the sex expression gene has effects on fruit size as well as fruit shape (see above for *FSMQ2*).

Chromosome 3, FWMQ3

This was detected in two populations (C and X), although the phenotypic variance was not consistent in replicated trials within experiments (Eduardo *et al.*, 2007).

Chromosome 8, FWMQ8

This was detected in three populations (C, I, and N), in the same region as the fruit shape QTLs. Eduardo *et al.* (2007) showed that the fruit weight QTL is located in an introgression that lacks the fruit shape QTL. In addition, the USDA864-1×Top Mark population only segregates for the fruit weight QTL. Therefore, *FSMQ8* and *FWMQ8* are probably independent loci. In these two last examples, this QTL had a large effect (14%) by reducing fruit weight by up to 40%.

Chromosome 11, FWMQ11

This was detected in three populations (A, W, and X) with a very large effect of up to 34% of the variance. *FWMQ11* and *FWMQ8* appeared to be the most consistent QTLs across different populations, and independent of fruit shape.

Co-localization of members of YABBY (FAS), OVATE, CNR (FW2.2), SUN, WOX (LOCULE NUMBER), and CYP78A/KLUH (FW3.2) gene families with melon QTLs

The genes underlying the cloned tomato fruit morphology QTLs are part of gene families comprising up to 34 members, as in the case of *SUN* (Huang *et al.*, 2013). Putative orthologues of these genes have been proposed as candidates genes for QTLs involved in fruit morphology in other species such as pepper (Tsaballa *et al.*, 2011; Chakrabarti *et al.*, 2013) and cherry (De Franceschi *et al.*, 2013), suggesting that the variation in fruit morphology in different taxa could be controlled by genes belonging to members of certain ancestral gene families. If so, any member from a gene family could be involved in the variation of fruit morphology in different species. Therefore, the search for candidate genes by comparative genomics should not be reduced to the identification of closest orthologues of known causative genes but to the analysis of the whole gene family. Following this rationale, we decided to analyse the gene families of the cloned tomato fruit morphology QTLs in the melon genome and evaluate their potential as candidate genes in this species.

The protein sequences of tomato *SUN*, *OFP*, and *YABBY* gene families were obtained from Huang *et al.* (2013); *CNR* and *WOX* were retrieved from the Sol Genomics Network (http://solgenomics.net); and *A. thaliana* DNA coding sequences of the CYP78A subfamily of P450 corresponding to the tomato KLUH/FW3.2 from http://www.arabidopsis.org/ (Supplementary Table S2 available at *JXB* online). BLASTX and BLASTP searches were performed against the predicted melon protein sequences (https://melonomics.net/), the first three hits of the BLAST and BLASTP searches were retained (*E* value ≤1–e10) and the melon genes were located in the melon pseudo-chromosomes v.CM_3.5.

A total of 24 members of the *SUN* (*CmSUN*), 21 of the *OFP* (*CmOFP*), five of the *YABBY* (*CmYABBY*), nine of the *CNR* (*CmCNR*), five of the *KLUH*/*CYP78A* (*CmCYP78A*), and 10 of the *WOX* (*CmWOX*) families were identified in melon (Supplementary Table S3 available at *JXB* online). The position on the melon pseudo-chromosomes could be assigned to all of them, except for four members of the *CmOFP* and one of the *CmYABBY* families. As expected, the genes were distributed throughout the melon genome, ranging from as few as four on chromosomes 1, 2, and 9 to as many as 12 on chromosomes 6 and 8 (Fig. 5). Moderate to low clustering was observed among members of the same family. The most important clustering was identified for *CmOFP* with two clusters of genes on chromosomes 1 and 4. Only one cluster for *CmSUN* and *CmCNR* was observed on chromosome 6 and no clusters for the *CmCYP78A*, *CmYABBY*, and *CmWOX* families. This modest clustering contrasts with the high clustering observed in tomato for certain *OFP* members (Huang *et al.*, 2013) and in peach for certain *CNR* members (De Franceschi *et al.*, 2013).

In tomato, the fruit shape and locule number genes *FASCIATED* and *LC* also control fruit size. Therefore, instead of controlling carpel number, which is rarely changed in melon, these genes may play a role in fruit size instead of fruit shape. Some of the candidate morphology genes mapped within fruit morphology QTLs. *CmYABBY* members showed a modest level of co-mapping with the fruit weight QTLs. Most of the co-localizing QTLs were observed in only one population, except for *FWMQ2*. However, this QTL is most likely controlled by the *CmACS-7* gene and not by *CmYABBY*. Therefore, YABBY is probably not controlling size or shape variation among melon varieties. *CmWOX* members co-mapped with five fruit weight QTLs, with the most interesting co-localizations on chromosomes 8 and 11, which were detected in three to five populations. *CmCNR* members co-mapped with five fruit weight QTLs, most of which were detected in a single population, except for the meta-QTLs *FWQM8* and *FWQM11*. Members of the *CmCYP78A* family co-mapped with four fruit weight QTLs, including *FWMQ11*; in other words, members of the *CmWOX*, *CmCNR*, and *CmCYP78A* families mapped within the two most stable across-population fruit weight QTLs (*FWQM8*A and *FWQM11*). For the family of genes that control tomato shape exclusively, *CmSUN* members co-mapped with eight fruit shape QTLs and most of them were detected in a single population, except for the meta-QTLs *FSQM2* and *FSQM11*. *CmOFP* members co-mapped with seven fruit shape QTLs. Remarkably, several members of this family co-localized with the *FSMQ1* and *FSMQ8* QTLs. Although final conclusions cannot be drawn from this analysis, the pattern of co-mapping of the different gene family members with melon fruit morphology QTLs allows us to suggest the plausible candidate genes at these loci. Thus, the gene family *CmYABBY* probably has a low impact on melon fruit morphology diversity. *CmCNR*, *CmCYP78A*, and *CmWOX* members co-mapped at high frequency with fruit weight QTLs, and so these families are good candidates for the diversification of fruit weight. *CmSUN* members also showed intriguing co-localizations with fruit shape QTLs, although in fewer populations than the *CmOFP* members. Remarkably, members of this last family co-mapped frequently with fruit shape QTLs detected in single and several independent experiments. Therefore, we hypothesize that members of this gene family are probably the most important in explaining the diversification of fruit shape among melon varieties.

Perspectives

In recent years, significant progress has been made in uncovering the molecular and genetic bases of tomato fruit morphology, and the role of some genes in tomato domestication is being revealed. From the list of nine key loci controlling fruit morphology in tomato proposed by Tanksley (2004), six have been cloned. Models of the evolution of fruit shape variation from domestication to cultivar diversification and the origin of the mutations causing the phenotypic variation have been proposed (Rodriguez *et al.*, 2011; Chakrabarti *et al.*, 2013).

In melon, ancient fruit shape and size diversification has been noted among Central and East Asia melon accessions belonging to the *agrestis* subspecies group, suggesting the existence of many alleles for morphological diversity (Dhillon *et al.*, 2007; Yi *et al.*, 2009; Fergany *et al.*, 2011; Roy *et al.*, 2012). In contrast to tomato, elongated shapes are much more frequent than round shapes among melon accessions, especially in wild and ancient landraces, as described above. The botanical groups cultivated in Occidental countries generally show less variation within groups. In addition, a reduction in fruit elongation is noted because fruits are generally ellipsoid to perfectly round and slightly flat (as typified by *cantalupensis* or *reticulatus*). Fruit weight is also larger, probably due to more intense breeding efforts leading to fixation of alleles resulting in rounder and larger fruits from middle-sized elongated melons that are found more commonly at the primary centres of diversity.

Most of the Central Asia accessions are monoecious, and most of the detected QTL alleles from that germplasm result in long fruit. On the other hand, Occidental and Far-Oriental cultivars are andromonoecious, and the selection of this mutation on the *a* gene eliminated the pleiotropic effects on fruit shape, which may have been one of the major steps facilitating the rise in importance of round fruit cultivars. Other QTLs that were probably also important in the process are *FSMQ1*, *FSMQ8*, and *FSMQ11*. A combination and fixation of the andromonoecious allele of *a* with these last QTLs may have led to the modern round melon cultivars. *FSMQ12* may not be important in melon shape diversification, as only a few *chinensis* cultivars carry the mutation leading to five carpels, a trait that is entirely absent in Occidental cultivars.

With respect to melon fruit weight QTLs, relatively few were found across many populations. This may mean that, among varieties, a number of different genes lead to increases in melon fruit size. Also, none of the studies was aimed at identifying QTLs from wild×domesticated populations, which is in contrast to tomato where all the cloned shape and size variation QTLs were identified in wide crosses. Thus, the identified tomato fruit weight QTLs may have originated early during domestication and are now fixed in most of the large-fruited cultivated tomatoes. In melon, the intervariety populations may therefore highlight the existence of many fruit size genes, some that may have originated early during domestication but are still segregating in the cultivated pool. Regardless of this, more research is warranted to confirm that the two most consistent meta-QTLs described here (*FWMQ8* and *FWMQ11*) are important in the fruit weight diversification of melon cultivars.

The release of the tomato genome (The Tomato Consortium, 2012) and knowledge about the major fruit shape and size genes will lead to faster identification and fine-mapping of additional QTLs in tomato (Rodriguez *et al.*, 2013; E. Illa and E. van der Knaap, unpublished data). Crosses between accessions can be made based on the knowledge of the distribution of the known genes. For example, a mutation in *OVATE* nearly always leads to an elongated shape. However, two out of 368 accessions carried round fruit, despite carrying the mutant allele of *OVATE* (Rodriguez *et al.*, 2011). Populations were developed that segregated for shape but not at the known *ovate* locus. Mapping indeed confirmed the existence of two QTLs that suppress the *OVATE* mutation (Rodriguez *et al.*, 2013). Fine-mapping is underway, which undoubtedly will lead to further insights into how *OVATE* regulates fruit shape.

Regarding melon, the number of studies and resolution of the QTLs were not sufficient to provide a solid hypothesis on the molecular basis of the variation in fruit morphology. Studies of populations derived from crosses between wild and cultivated melons have not been conducted. Therefore, the variation only comprises aspects related to cultivar diversification. Nevertheless, in the current work, we identified four genomic regions (*FSQM1*, *FSQM2*, *FSQM8*, and *FSMQ11*) for fruit shape and two for fruit weight (*FWQM8* and *FWQM11*) that are good candidates to harbour genes responsible for much of the variation in fruit morphology among cultivars. The study of a larger number of populations from different parents in addition to association mapping with a large germplasm collection would confirm the importance of the proposed regions. The recent publication of melon genome sequence (Garcia-Mas *et al.*, 2012) will certainly accelerate the identification of the genes underlying the fruit morphology genetic control in this species.

The comparative genomics of members of gene families involved in fruit morphology in tomato with the melon QTLs suggested the possibility that common genes are involved in fruit morphology variation in both species (*OFP*, *CNR*, *CYP78A*/*KLUH*, *SUN*, mand *WOX*), but this situation is not as evident for the *YABBY* gene family. This difference can be explained by the similarities and differences in fruit architecture among species. In tomato, *YABBY* genes act mainly on locule number; mutations in tomato results in larger and flat-shaped fruit. However, little variation in locule number is observed among melon cultivars, and therefore those genes may not be expected to control melon fruit morphology. The only exception is the *p* gene, which controls carpel number in melon. Higher carpel number is found only in a small fraction of melon cultivars that are of Far-East origin. Nevertheless, no members of the *YABBY* or *WOX* family were found in the chromosome 12 region where the *p* gene maps. The *OFP*, *SUN*, *CNR*, and *CYP78A*/*KLUH* genes may be regulators of cell division. Therefore, it might be expected that variation of these genes would also cause variation in the organ where they are acting, independently of the organ anatomy, i.e. these genes may be considered as general regulators of cell number and patterning across different plant species.

Interestingly, cultivar diversification in tomato has led to the development of a subset of varieties with long shapes, in

contrast to the round fruit of wild and intermediate tomato species. However, in melon, there was a tendency, especially in Occidental regions, to develop cultivars with rounder fruits from highly elongated fruits. Thus, in tomato, the alleles selected from genes such as *SUN* and *OFP* may be considered enhancers of elongated shape, whereas, in melon, the alleles would function to repress elongated shape.

In summary, we have found several candidate genes for melon morphology based on the cloned tomato QTLs. Future fine-mapping and cloning of those melon QTLs will elucidate whether the same gene families are involved in the variation of fruit morphology in both species.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Table S1. Melon crosses where QTLs for fruit morphology (FS, fruit shape; FW, fruit weight) have been described. The horticultural groups are according to Pitrat (2008). The population types are recombinant inbred lines (RILs), double haploid lines (DHLs), F2, and near-isogenic lines (NILs). Crosses and QTLs are coded according Diaz *et al.* (2011).

Supplementary Table S2. Protein sequences of *YABBY*, *OVATE*, *CNR*, *SUN*, and *WOX*, and coding DNA sequences of *CYP78A-P450* gene families used to find the putative orthologue melon genes by BLASTP and BLASTX analysis.

Supplementary Table S3. Mapping of genes from the families *YABBY*, *OVATE*, *CNR*, *SUN*, *WOX*, and *CYP78A* on the melon genome. Only the best hit is shown for each melon gene, coded according to https://melonomics.net/ and the probability of the hit (*P*) is also expressed. The chromosome and the position of the genes are according to the melon pseudo-chromosomes v.3.5 (Garcia-Mas *et al.*, 2012).

Supplementary Fig. S1. Neighbour-joining tree based on the genetic distances of Nei *et al.* (1983) calculated from the allele frequencies of 697 single-nucleotide polymorphism markers in 71 melon accessions belonging to the 13 botanical groups within *Cucumis melo* described by Pitrat (2008) and the wild melon *agrestis* (adapted from Esteras *et al.*, 2013). Crosses carried out to date involving genotypes from the same or different groups are named according to Supplementary Table S1.

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References

Abdelmohsin ME, Pitrat M. 2008. Pleiotropic effect of sex expression on fruit shape in melon. In: Pitrat M, ed. *9th EUCARPIA meeting on genetics and breeding of Cucurbitaceae*. Avignon, France, 551–555.

Abel S, Savchenko T, Levy M. 2005. Genome-wide comparative analysis of the IQD gene families in *Arabidopsis thaliana* and *Oryza sativa*. *BMC Evolutionary Biology* **5**.

Bates DM, Robinson RW. 1995 Cucumbers, melons and water-melons. In: Smartt J, Simmonds eds. *Evolution of crop plants*. Longman, Essex, UK, 89–96.

Bedinger PA, Chetelat RT, McClure B, *et al.*. 2011. Interspecific reproductive barriers in the tomato clade: opportunities to decipher mechanisms of reproductive isolation. *Sexual Plant Reproduction* **24,** 171–187.

Blanca J, Canizares J, Cordero L, Pascual L, Diez MJ, Nuez F. 2012. Variation revealed by SNP genotyping and morphology provides insight into the origin of the tomato. *PLOS One* **7,** e48198.

Boualem A, Fergany M, Fernandez R, *et al.* 2008. A conserved mutation in an ethylene biosynthesis enzyme leads to andromonoecy in melons. *Science* **321,** 836–838.

Brewer MT, Lang LX, Fujimura K, Dujmovic N, Gray S, van der Knaap E. 2006. Development of a controlled vocabulary and software application to analyze fruit shape variation in tomato and other plant species. *Plant Physiology* **141,** 15–25.

Burger Y, Sa'ar U, Paris HS., Lewinsohn E, Katzir N, Tadmor Y, Schaffer AA. 2006. Genetic variability for valuable fruit quality traits in *Cucumis melo*. *Israel Journal of Plant Sciences* **54,** 233–242.

Chakrabarti M, Zhang N, Sauvage C, *et al*. 2013. A cytochrome P450 regulates a domestication trait incultivated tomato. *Proceedings of the National Academy of Sciences, USA* **110,** 17125–17130.

Clevenger J. 2012. Metabolic and genomic analysis of elongated fruit shape in tomato (*Solanum lycopersicum*). M.S. thesis dissertation, Ohio State University, OH, USA.

Cong B, Barrero LS, Tanksley SD. 2008. Regulatory change in YABBY-like transcription factor led to evolution of extreme fruit size during tomato domestication. *Nature Genetics* **40,** 800–804.

Cong B, Liu JP, Tanksley SD. 2002. Natural alleles at a tomato fruit size quantitative trait locus differ by heterochronic regulatory mutations. *Proceedings of the National Academy of Sciences, USA* **99,** 13606–13611.

De Franceschi P, Stegmeir T, Cabrera A, *et al.* 2013. Cell number regulator genes in *Prunus* provide candidate genes for the control of fruit size in sweet and sour cherry. *Molecular Breeding* **32,** 311–326.

Dhillon NPS, Ranjana R, Singh K, Eduardo I, Monforte AJ, Pitrat M, Dhillon NK, Singh PP. 2007. Diversity among landraces of Indian snapmelon (*Cucumis melo* var. momordica). *Genetic Resources and Crop Evolution* **54,** 1267–1283.

Diaz A, Fergany M, Formisano G, *et al.* 2011. A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (*Cucumis melo* L.). *BMC Plant Biology* **11,** 111.

Eduardo I, Arus P, Monforte AJ, Obando J, Fernandez-Trujillo JP, Martinez JA, Alarcon AL, Alvarez JM, van der Knaap E. 2007. Estimating the genetic architecture of fruit quality traits in melon using a genomic library of near isogenic lines. *Journal of the American Society for Horticultural Science* **132,** 80–89.

Esquinas-Alcazar JT, Gulick PJ. 1983. *Genetic resources of Cucurbitaceae—a global report*. International Board for Plant Genetic Research, Rome, Italy.

Esteras E, Formisano G, Roig C, *et al.* 2013. SNP genotyping in melons: genetic variation, population structure, and linkage disequilibrium. *Theoretical and Applied Genetics* **126,** 1285–1303.

Fang L, Yong X, Yue Z, Di C, Jian-ming F, Shao-gui G, Guo-yi G, Hongping Y, Ming-zhu W, Hai-ying. 2009. Construction of permanent genetic map and comparative analysis of Xinjiang Hami melon (*Cucumis melo* L. ssp. melo. convar. ameri (Pang.) Greb). *Acta Horticiculturae Sinica* **36,** 1767–1774.

Fergany M, Kaur B, Monforte AJ, Pitrat M, Rys C, Lecoq H, Dhillon NPS, Dhaliwal SS. 2011. Variation in melon (*Cucumis melo*) landraces adapted to the humid tropics of southern India. *Genetic Resources and Crop Evolution* **58,** 225–243.

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

Fernandez-Silva I, Moreno E, Essafi A, Fergany M, Garcia-Mas J, Martin-Hernandez AM, Alvarez JM, Monforte AJ. 2010. Shaping

melons: agronomic and genetic characterization of QTLs that modify melon fruit morphology. *Theoretical and Applied Genetics* **121,** 931–940.

Fernandez-Trujillo JP, Picó B, Garcia-Mas J, Álvarez JM, Monforte AJ. 2011. Breeding for fruit quality in melón. In: Jenks MA, Bebeli PJ, eds. *Breeding for fruit quality*. John Wiley & Sons, 261–278.

Frary A, Nesbitt TC, Grandillo S, van der Knaap E, Cong B, Liu JP, Meller J, Elber R, Alpert KB, Tanksley SD. 2000. *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. *Science* **289,** 85–88.

Gapper NE, McQuinn RP, Giovannoni JJ. 2013. Molecular and genetic regulation of fruit ripening. *Plant Molecular Biology* **82,** 575–591.

Garcia-Mas J, Benjak A, Sanseverino W, *et al.* 2012. The genome of melon (*Cucumis melo* L.). *Proceedings of the National Academy of Sciences, USA* **109,** 11872–11877.

Gillaspy G, Bendavid H, Gruissem W. 1993. Fruits—a developmental perspective. *Plant Cell* **5,** 1439–1451.

Giovannoni JJ. 2004. Genetic regulation of fruit development and ripening. *Plant Cell* **16,** S170–S180.

Grandillo S, Ku HM, Tanksley SD. 1999. Identifying the loci responsible for natural variation in fruit size and shape in tomato. *Theoretical and Applied Genetics* **99,** 978–987.

Guo M, Rupe MA, Dieter JA, Zou JJ, Spielbauer D, Duncan KE, Howard RJ, Hou ZL, Simmons CR. 2010. *Cell Number Regulator1* affects plant and organ size in maize: implications for crop yield enhancement and heterosis. *Plant Cell* **22,** 1057–1073.

Guo M, Simmons CR. 2011. Cell number counts—the *fw2.2* and *CNR* genes and implications for controlling plant fruit and organ size. *Plant Science* **181,** 1–7.

Hackbusch J, Richter K, Muller J, Salamini F, Uhrig JF. 2005. A central role of *Arabidopsis thaliana* ovate family proteins in networking and subcellular localization of 3-aa loop extension homeodomain proteins. *Proceedings of the National Academy of Sciences, USA* **102,** 4908–4912.

Hamilton JP, Sim S-C, Stoffel K, Van Deynze A, Buell CR, Francis DM. 2012. Single nucleotide polymorphism discovery in cultivated tomato via sequencing by synthesis. *Plant Genome* **5,** 17–29.

Harel-Beja R, Tzuri G, Portnoy V, *et al.* 2010. A genetic map of melon highly enriched with fruit quality QTLs and EST markers, including sugar and carotenoid metabolism genes. *Theoretical and Applied Genetics* **121,** 511–533.

Higashi K, Hosoya K, Ezura H. 1999. Histological analysis of fruit development between two melon (*Cucumis melo* L. *reticulatus*) genotypes setting a different size of fruit. *Journal of Experimental Botany* **50,** 1593–1597.

Huang ZJ, van der Knaap E. 2011. Tomato fruit weight 11.3 maps close to fasciated on the bottom of chromosome 11. *Theoretical and Applied Genetics* **123,** 465–474.

Huang ZJ, Van Houten J, Gonzalez G, Xiao H, van der Knaap E. 2013. Genome-wide identification, phylogeny and expression analysis of *SUN*, *OFP* and *YABBY* gene family in tomato. *Molecular Genetics and Genomics* **288,** 111–129.

Jeffrey C. 1980. A review of the Cucurbitaceae. *Botanical Journal of the Linnean Society* **81,** 233–247.

Jia H-F, Chai Y-M, Li C-L, Lu D, Luo J-J, Qin L, Shen Y-Y. 2011. Abscisic acid plays an important role in the regulation of strawberry fruit ripening. *Plant Physiology* **157,** 188–199.

Kocyan A, Zhang LB, Schaefer H, Renner SS. 2007. A multi-locus chloroplast phylogeny for the Cucurbitaceae and its implications for character evolution and classification. *Molecular Phylogenetics and Evolution* **44,** 553–577.

Liu JP, Cong B, Tanksley SD. 2003. Generation and analysis of an artificial gene dosage series in tomato to study the mechanisms by which the cloned quantitative trait locus *fw2.2* controls fruit size. *Plant Physiology* **132,** 292–299.

Liu JP, Van Eck J, Cong B, Tanksley SD. 2002. A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. *Proceedings of the National Academy of Sciences, USA* **99,** 13302–13306.

Monforte AJ, Eduardo I, Abad S, Arus P. 2005. Inheritance mode of fruit traits in melon: heterosis for fruit shape and its correlation with genetic distance. *Euphytica* **144,** 31–38.

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.). *Theoretical and Applied Genetics* **108,** 750–758.

Muños S, Ranc N, Botton E, *et al.* 2011. Increase in tomato locule number is controlled by two SNPs located near WUSCHEL. *Plant Physiology* **156,** 2244–2254.

Nei M, Tajima F, Tateno Y. 1983 Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data. *Journal of Molecular Evolution* **19**:153–170.

Nuñez-Palenius HG, Gomez-Lim M, Ochoa-Alejo N, Grumet R, Lester G, Cantliffe DJ. 2008. Melon fruits: genetic diversity, physiology, and biotechnology features. *Critical Reviews in Biotechnology* **28,** 13–55.

Obando J, Fernandez-Trujillo JP, Martinez JA, Alarcon AL, Eduardo I, Arus P, Monforte AJ. 2008. Identification of melon fruit quality quantitative trait loci using near-isogenic lines. *Journal of the American Society for Horticultural Science* **133,** 139–151.

Paran I, van der Knaap E. 2007. Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *Journal of Experimental Botany* **58,** 3841–3852.

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

Perin C, Hagen LS, Giovinazzo N, Besombes D, Dogimont C, Pitrat M. 2002. Genetic control of fruit shape acts prior to anthesis in melon (*Cucumis melo* L.). *Molecular Genetics and Genomics* **266,** 933–941.

Pitrat M. 2008. Melon (*Cucumis melo* L.). In: Prohens J, Nuez F, eds. *Handbook of crop breeding, Vol. I: Vegetables*. Springer, New York, 283–315.

Renner SS, Schaefer H, Kocyan A. 2007. Phylogenetics of *Cucumis* (Cucurbitaceae): cucumber (*C. sativus*) belongs in an Asian/Australian clade far from melon (*C. melo*). *BMC Evolutionary Biology* **7,** 58.

Rodriguez GR, Kim HJ, van der Knaap E. 2013. Mapping of two suppressors of OVATE (sov) loci in tomato. *Heredity* **111,** 256–264.

Rodriguez GR, Munos S, Anderson C, Sim SC, Michel A, Causse M, Gardener BBM, Francis D, van der Knaap E. 2011. Distribution of SUN, OVATE, LC, and FAS in the tomato germplasm and the relationship to fruit shape diversity. *Plant Physiology* **156,** 275–285.

Roy A, Bal SS, Fergany M, Kaur S, Singh H, Malik AA, Singh J, Monforte AJ, Dhillon NPS. 2012. Wild melon diversity in India (Punjab State). *Genetic Resources and Crop Evolution* **59,** 755–767.

Schaefer H, Heibl C, Renner SS. 2009. Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. *Proceedings of the Royal Society B—Biological Sciences* **276,** 843–851.

Sebastian P, Schaefer H, Telford IRH, Renner SS. 2010. Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Sciences, USA* **107,** 14269–14273.

Sim SC, Durstewitz G, Plieske J, *et al.* 2012. Development of a large SNP genotyping array and generation of high-density genetic maps in tomato. *PLOS One* **7,** e40563.

Stepansky A, Kovalski I, Perl-Treves R. 1999. Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. *Plant Systematics and Evolution* **217,** 313–332.

Symons GM, Chua YJ, Ross JJ, Quittenden LJ, Davies NW, Reid JB. 2012. Hormonal changes during non-climacteric ripening in strawberry. *Journal of Experimental Botany* **63,** 4741–4750.

Symons GM, Davies C, Shavrukov Y, Dry IB, Reid JB, Thomas MR. 2006. Grapes on steroids. Brassinosteroids are involved in grape berry ripening. *Plant Physiology* **140,** 150–158.

Tanksley SD. 2004. The genetic, developmental, and molecular bases of fruit size and shape variation in tomato. *Plant Cell* **16,** S181–S189.

The Tomato Genome Consortium. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **485,** 635–641.

Tomason Y, Nimmakayala P, Levi A, Reddy UK. 2013. Map-based molecular diversity, linkage disequilibrium and association mapping of fruit traits in melon. *Molecular Breeding* **31,** 829–841.

Tsaballa A, Pasentsis K, Darzentas N, Tsaftaris AS. 2011. Multiple evidence for the role of an *Ovate*-like gene in determining fruit shape in pepper. *BMC Plant Biology* **11,** 46.

van der Knaap E, Tanksley SD. 2001. Identification and characterization of a novel locus controlling early fruit development in tomato. *Theoretical and Applied Genetics* **103,** 353–358.

Wu S, Xiao H, Cabrera A, Meulia T, van der Knaap E. 2011. SUN regulates vegetative and reproductive organ shape by changing cell division patterns. *Plant Physiology* **157,** 1175–1186.

Xiao H, Jiang N, Schaffner E, Stockinger EJ, van der Knaap E. 2008. A retrotransposon-mediated gene duplication underlies morphological variation of tomato fruit. *Science* **319,** 1527–1530.

Xu J, Ranc N, Muños S, Rolland S, Bouchet JP, Desplat N, Le Paslier MC, Liang Y, Brunel D, Causse M. 2013. Association mapping for fruit quality traits in cultivated tomato and wild related species. *Theoretical and Applied Genetics* **126,** 567–581.

Yi SS, Akashi Y, Tanaka K, Cho TT, Khaing MT, Yoshino H, Nishida H, Yamamoto T, Win K, Kato K. 2009. Molecular analysis of genetic diversity in melon landraces (*Cucumis melo* L.) from Myanmar and their relationship with melon germplasm from East and South Asia. *Genetic Resources and Crop Evolution* **56,** 1149–1161.

Zalapa JE, Staub JE, McCreight JD, Chung SM, Cuevas H. 2007. Detection of QTL for yield-related traits using recombinant inbred lines derived from exotic and elite US Western Shipping melon germplasm. *Theoretical and Applied Genetics* **114,** 1185–1201.