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Genetic control of fruit shape acts prior to anthesis in melon (*Cucumis melo* L.)

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Abstract Genetic control of fruit shape in *Cucumis melo* was studied using QTL analysis in two Recombinant Inbred (RI) populations consisting of 163 and 63 individuals, respectively, obtained by crossing the same round-fruited parent with two different elongated-fruit lines. Fruit shape is mainly explained by fruit length in these two populations. Most QTLs for fruit shape and ovary shape detected were found to co-segregate, thus demonstrating early control of fruit shape during ovary development. A high level of correlation between fruit shape and ovary shape was also found in 14 unrelated genetic lines, a finding which suggests that control of fruit shape by gene(s) active early in the ovary is a general feature in *C. melo*. Two major flower genes, *a* (*monoecious*) and *p* (*pentamerous*), were shown to have major effects on fruit shape. Major tightly linked QTLs for fruit and ovary shape were found close to the *a* and *p* genes, probably reflecting their pleiotropic effect on fruit shape. Moreover, one of the two QTLs detected in the Védrantais × PI 414723 population was also found in the Védrantais × PI 161375 population. Variation of fruit shape in melon could be due to variations having quantitative effects on a large set of genes that are probably involved in ovary development.

Keywords Melon · *Cucumis melo* · Fruit shape · Quantitative Trait Loci (QTLs) · Fruit development

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Introduction

Fruit is one of the latest organs to have evolved within the plant kingdom. The fruits of the Angiosperms show a broad range of phenotypic variation. This may be linked to their biological functions of seed maturation and dispersion. In addition, the domestication process, which started with the agrarian cultures of 10,000 years ago, has lead to great differences in fruit shape and size between wild and cultivated forms of fruit-bearing species like tomato, or cucurbits. For instance, in *Cucumis melo* L., fruits as short as 4 cm long (*C. melo* var. agrestis) and as long as 2 m (*C. melo* var. flexuosus), attaining weights of between 50 g and more than 15 kg (a 300-fold variation in size) are known (Naudin 1859).

As early studies showed, fruit development is under strict genetic control (Sinnot 1945; Gillaspy et al. 1993). Sometimes, evidence for oligogenic control of fruit shape was found (Kaiser 1935). Thus, Kaiser (1935), in a single F_2 population of *C. annum*, described monogenic, dominant control by a *round*-like gene. For *Lycopersicon* species, some genes like *o* (*ovate*) (Yeager 1937) and *pear shape* (Ku et al. 1999), which may be an allele of the *ovate* locus, and *Abg* (Chetelat and Rick 1999) were found to have major effects on fruit shape. Sinnot (1927) described digenic control of fruit shape in *Cucurbita pepo*. However, polygenic control of fruit shape is most frequently found, as in *Cucumis sativus* (Serquen et al. 1997), *C. melo* (Stino et al. 1958; Lippert and Hall 1982; Kalb and Davis 1984), *Lycopersicon* (Grandillo and Tanksley 1995; Tanksley et al. 1996), and *Capsicum* (Kaiser 1935; Khambanonda 1950). The development of molecular markers with the help of QTL detection methodology has afforded an opportunity to adopt a new and more systematic approach to answering these old questions. QTLs for fruit shape and size have now been detected in various species (Grandillo and Tanksley 1995; Tanksley et al. 1996; Serquen et al. 1997; Bernacchi et al. 1998; see also Grandillo et al. 1999, for a review on fruit-shape and fruit-weight genes in tomato).

Bernacchi et al. (1998) discovered two major QTLs, *fs8.1* and *fs2.1*, and seven additional QTLs of lesser effect in a 315-member BC2 population derived from a cross between *L. esculentum* and *L. pimpinellifolium*.

Indirect evidence for relationships between the development of ovary shape and fruit shape has been provided by correlation studies. A high correlation between ovary shape and ripe fruit shape was found by Sinnot and Kaiser in *Lycopersicon* and *Cucurbita* (Sinnot and Kaiser 1934; Kaiser 1935; Sinnot 1944). More recently, Grandillo et al. (1996) described a high correlation between fruit and ovary shape in near-isogenic lines for a major tomato fruit-shape QTL (*fs8.1*), and demonstrated that *fs8.1* sets the tomato shape well before anthesis (Ku et al. 2000b). These correlations between fruit shape and ovary shape found in several species suggest a pleiotropic effect of one QTL or close linkage between two different QTLs.

In the work reported here, we have studied the genetic control of fruit shape and ovary shape in *C. melo*, and investigated the link between fruit shape development and ovary shape. We used two Recombinant Inbred (RI) populations obtained by crossing a common round-fruited parent with two elongate-fruited parents, a type of genetic material that is particularly well-adapted for the evaluation of complex phenotypic characters (Burr and Burr 1991). As we have constructed quasi-saturated molecular maps from these both populations, QTLs can be accurately localized. We tried to answer three questions regarding fruit-shape development. (1) Is fruit shape under the pleiotropic control of ovary-shape development genes? (2) Is this pleiotropic control found in different varieties? (3) Is variation in fruit shape due to allelic differences in a small set of genes?

Materials and methods

Recombinant Inbred Lines (RILs)

The Védrantais × PI 161375 population (Ved161) was established as follows. Two homozygous lines, Védrantais, a French line (Vilmorin) with round fruits, and PI 161375, a Korean line with oblong fruits, were crossed using Védrantais as the female parent to produce an F1 hybrid. One hundred and ninety eight F6/F7 RILs were derived by single-seed descendance from the F2 through five/six selfing generations without conscious selection. A subset of 110 RILs from this population was used to study the genetic control of fruit shape.

Plants were grown under a plastic tunnel at Montfavet (Southern France). Two plants of each RI line were planted per plot. In the summer of 1997, three blocks of plots with the 110 RI plants were grown. Two blocks were grown in 1998. Placement of plots within blocks was completely randomized. Blocks correspond to different planting dates. Fruit shape and ovary shape were evaluated in each trial over the 2 years. Two fruits per plant were analyzed for fruit shape and five flowers were examined for ovary shape.

In order to test the cross specificity of fruit-shape QTLs, we used a second RI population, the Védrantais × PI 414723 population (Ved414) consisting of 63 F6/F7 lines from a cross between Védrantais and PI 414723, a monoecious Indian line with more elongated fruits than PI 161375. Two plants were sown per plot in each block during the summer of 1999 at Montfavet. Fruit shape was evaluated for each harvested fruit.

Near-isogenic lines for the *andromonecious* gene and genetically unrelated melon lines

Near isogenic lines for the gene *a* (*andromonoecious*) designated as Isomono a (allele a = *andromonoecious*) and Isomono m (allele a^+ = *monoecious*) were developed by six successive backcrosses of the cv. Alpha (Tézier, monoecious) with Charentais T as the recurrent andromonoecious line. These two genotypes belong to the Charentais type.

Fourteen homozygous, genetically unrelated, lines with very different fruit shapes were used to check the phenotypic correlation between fruit shape and ovary shape (Table 1). Three plants of each unrelated and near-isogenic line were cultivated under a plastic tunnel during the summer of 1998. Ovaries were measured on each plant (3–14 flowers per plant) and mature fruits (1–4 per plant) were measured for fruit shape.

Phenotypic evaluation of qualitative traits

The *andromonoecious* gene (symbol *a*) controls the presence (*vs.* absence) of stamens in female flowers that are hermaphroditic (*vs.* female) (Rosa 1928; Poole and Grimball 1939). Védrantais and PI 161375 are andromonoecious, PI 414723 is monoecious and the gene *a* segregates only in the Ved414 population. The character "empty cavity" (at maturity with placenta separation) is controlled by one dominant gene (*Ec*), which is present in PI 414723 but not in Védrantais nor PI161375 (Périn et al. 1999), and segregates only in the Ved414 population. The character "five placentas", controlled by the *pentamerous* gene (symbol *p*), is present in PI 161375, whereas Védrantais and PI 414723 have three placentas (Rosa 1928; Baudracco-Arnas and Pitrat 1996). The gene *p* segregates only in the Ved161 population. The *andromonoecious* and *pentamerous* genes were screened on the basis of three flowers per plants and the *Empty cavity* gene was screened on at least three fruits per plant.

Phenotypic evaluation of quantitative traits

Newly developed female flowers (1–4 flowers per plant) were used to evaluate ovary shape in each plant grown under plastic tunnels. All measurements were performed with a caliper square. For each flower, we determined the ovary length (ovl; peduncle to petal insertion) and the ovary width (ovw; halfway between the peduncle and petal insertions). Ovary shape (ovs) was calculated as the ratio of ovary length to ovary diameter (width). Mature fruit shape (fs) was measured as the ratio of the polar diameter (peduncle to blossom end), referred to as fruit length (fl), to the equatorial width of the fruit (fw) (measured halfway between the peduncle and blossom end).

QTL analysis

Two framework maps were constructed from the Ved161 and Ved414 crosses, based on the segregation of 460 and 318 markers, respectively, mainly IMAs and AFLPs (Périn et al. 2001). The maps could be compared on the basis of 116 common markers comprising 106 co-migrating AFLP/IMA, five SSR and five phenotypic markers in a composite map (Périn et al. 2001). Two framework maps with 106 and 156 markers, respectively, were extracted from the Ved161 and Ved414 map for QTL detection based on the following conditions.

1) Framework maps were built with high-confidence markers, i.e. markers ordered with a LOD score support higher than 2.0.
2) Unskewed markers with as few as possible missing data were chosen for QTL analysis.

Table 1 Means and standard deviation of the fruit and ovary dimensions for 14 genetically unrelated lines

Genotype	Ovary length (mm)	Ovary width (mm)	Ovary shape	Fruit length (mm)	Fruit width (mm)	Fruit shape
Sucrin de Tours	10.9 ± 1.1	8.9 ± 0.9	1.2 ± 0.1	88.2 ± 11.5	152.2 ± 51.4	0.6 ± 0.2
MR-1	11.5 ± 0.7	9.3 ± 0.6	1.2 ± 0.1	102.3 ± 13.9	136.5 ± 14.9	0.8 ± 0.1
Charentais T	13.6 ± 1.0	8.7 ± 0.9	1.6 ± 0.1	122.6 ± 12.4	134.0 ± 8.2	0.9 ± 0.1
Ogen	14.0 ± 1.0	8.9 ± 0.7	1.6 ± 0.1	127.7 ± 19.6	132.9 ± 20.9	1.0 ± 0.1
Hale's Best Jumbo	14.2 ± 2.3	8.4 ± 1.3	1.7 ± 0.1	148.5 ± 12.0	146.0 ± 11.3	1.0 ± 0.0
Honey Dew	15.5 ± 1.3	8.4 ± 1.1	1.9 ± 0.1	160.0 ± 14.2	156.2 ± 17.8	1.0 ± 0.1
Amarillo ALV140	19.5 ± 2.0	9.9 ± 0.7	2.0 ± 0.1	188.7 ± 18.7	156.3 ± 25.0	1.2 ± 0.2
Cantaloup d'Alger	19.5 ± 1.8	9.3 ± 0.8	2.1 ± 0.1	203.3 ± 12.6	157.0 ± 6.2	1.3 ± 0.1
Top Mark	15.0 ± 1.5	8.1 ± 0.7	1.9 ± 0.2	149.0 ± 39.1	117.0 ± 25.1	1.3 ± 0.3
Ogon 9	14.0 ± 1.2	6.8 ± 0.6	2.0 ± 0.1	119.6 ± 23.1	78.8 ± 13.4	1.5 ± 0.2
PI 414723	21.9 ± 0.4	6.5 ± 0.6	3.4 ± 0.3	276.3 ± 39.7	114.0 ± 9.7	2.4 ± 0.2
Faizabadi	24.1 ± 3.1	6.3 ± 0.5	3.8 ± 0.3	262.4 ± 17.6	92.0 ± 11.3	2.9 ± 0.1
Adjour	31.1 ± 2.2	6.5 ± 0.5	4.8 ± 0.5	436.3 ± 130.8	101.8 ± 29.4	4.3 ± 0.5
Fakouss	35.0 ± 4.0	5.4 ± 0.2	6.5 ± 0.7	726.7 ± 90.7	106.7 ± 7.6	6.8 ± 1.1

3) With the exception of the three phenotypic markers, *andromonoecious*, *pentamerous* and *Empty cavity*, all the markers used for QTL detection were AFLP or IMA markers.

In our two Recombinant Inbred populations, both dominant and codominant markers can be mapped with the same precision (for a review, see Burr and Burr 1991). Moreover, our replicate progeny provide powerful tools for the mapping of QTLs (Causse et al. 1995; Goldman et al. 1995; Nandi et al. 1997).

Statistical analysis

Variance components and adjusted means were estimated for each trait by using analysis of variance [GLM and LSMEANS procedures in the program package provided by the SAS Institute (Cary, N.C.) 1989]. Normality was tested with a Shapiro-Wilk w test. Pearson correlation and basic statistical analysis were performed using the S-Plus v3.0 software package. QTL detection was performed with QTLCartographer software (Zeng 1994) for both populations, using three methods: single-factor analysis of variance, simple interval mapping (IM; Lander and Bostein 1989) and composite interval mapping (CIM; Zeng 1994). A forward-backward-stepwise procedure was performed to choose cofactors for CIM. Five cofactors and a window size of 10 cM were chosen. A permutation test with 1000 permutations was performed with QTLCartographer and LOD thresholds of 2.36 and 2.56 were obtained for IM and CIM, respectively, corresponding to an individual significance level of 0.001. These thresholds were used in the first round of QTL detection. To minimize the number of type-I errors leading to false positives, we retained significant association of a QTL within an interval only when: (1) the QTL was detected in both years with a LOD score between 2.36 and 3.0, or (2) the QTL was detected in only one year with a LOD score higher than 3.0. QTLs detected were mapped based on their positions on the composite map of the interval giving the highest LOD score in CIM or IM.

Correspondence between QTLs in different crosses and for different characters

In order to check the probability of QTL co-segregation for two different traits, we used a statistical test developed by Paterson et al. (1995). Markers that were common to the two maps (Périn et al. 2001) were used to compare the map positions of QTLs. We used a hypergeometric distribution law for correspondence between two QTL sets from two different crosses and/or characters. For n intervals, the probability p that m QTLs were shared between two sets of, respectively, r and s QTLs (s less than r) only by chance is given by the following equation derived from the hypergeometric law:

$$p = \frac{\binom{r}{m}\binom{n-r}{s-m}}{\binom{n}{2}}$$

We assumed that the average genome interval size within which we tested the correspondence between QTLs was about 25 cM. The number of intervals to be compared was $n = 60$ (1500 cM divided by 25).

Results

Genetically unrelated lines of melon

A large range of variation was observed among the 14 genetically unrelated lines studied for ovl, ovs, fl and fs. We found a three-fold and an 11-fold variation respectively, for ovl and fs between the line with the most elongated fruit, Fakouss, and that with the flattest fruit, Sucrin de Tours (Table 1). The ranges for fw and ovw were smaller (about a two-fold difference was observed).

These developmental traits were significantly correlated among the unrelated lines (Table 2). A significant negative correlation was observed between ovw and ovl ($r = -0.72$) and between ovw and fl ($r = -0.72$), i.e. between width and length for fruit and ovary (Table 2). The high correlation between length and shape demonstrates the predominant role of fl in determining fs; the correlation between ovl and ovs was 0.97 and between fl and fs was 0.98. The correlation between fs and ovs was also very high ($r = 0.99$), suggesting a correlation of fs and ovs within melon species. The shape of both the ovary and the fruit depends mainly on the length (highly significant positive correlation) and not on the width (negative correlation). Ovary shape and fruit shape are highly correlated (0.99) as are ovary and fruit length (0.95).

Near-isogenic lines for the *andromonoecious* gene

A significant difference in ovl, ovs, fl and fs was found between Isomono m (monoecious) and Isomono a

Table 2 Phenotypic correlations (*r*) among genetically unrelated lines estimated from means

Parameter[a]	Ovary length	Ovary width	Ovary shape	Fruit length	Fruit width
Ovary width	–0.72				
Ovary shape	0.97	–0.84			
Fruit length	0.95	–0.72	0.97		
Fruit width	NS	0.83	NS	NS	
Fruit shape	0.94	–0.81	0.99	0.98	NS

[a]Correlations that were statistically significant ($P < 0.05$) by the Pearson test are listed. NS, not significant

(andromoecious) (Fig. 1). Increases in fs and ovs seemed mainly due to the effect on ovl and fl and not on ovw and fw.

Variation in the Védrantais × PI 161375 population (Ved161)

Védrantais fruits are nearly spherical (ovs = 1.66 and fs = 0.99) with three carpels, whereas PI 161375 has pear-shaped fruits (ovs = 2.13, fs = 1.36) with five carpels. The F1 hybrid fruit were elongate (ovs = 2.4, fs = 1.34) with three carpels, demonstrating the dominance of the large-fruit-shape and 3-carpel characters (Rosa 1928). All the traits were characterized by continuous variation with an approximately normal distribution (data not shown). Values for ovary shape ranged from 1.2 to 3.2 and fs ranged from 0.71 to 2.19 among the 110 RILs, with transgressions for high and low values (data not shown). No significant differences were found between the two parents for ovw and fw, but large variations were found in the RI population.

Significant block effects were found for each character, except for fs in 1997, reflecting an effect of different transplantation dates for each block (data not shown). Highly significant genetic effects were found for each character. A strong phenotypic correlation was found between fs and ovs ($r = 0.88$ in 1997 and 0.82 in 1998), of the same order of magnitude as in genetically unrelated lines. This suggested possible pleiotropic control of both characters. Moreover, fl and ovl explained most of the variation in fs and ovs in the RI population (phenotypic correlations between fl and fs of 0.81 in 1997 and 0.73 in 1998; and between ovl and ovs of 0.79 in 1997 and 1998).

Variation in the Védrantais × PI 414723 population (Ved414)

Average values for fs were higher than in the Ved161 due to the greater difference between Védrantais and PI 414723 for fs and fl than between Védrantais and PI 161375. Transgressions were also observed for high and low fs values, as in the Ved161 population. Results for the Ved414 population (data not shown) were very similar to those for the Ved161 population, with a non significant correlation between fl and fw ($r = 0.23$) and a high correlation between fs and fl ($r = 0.88$).

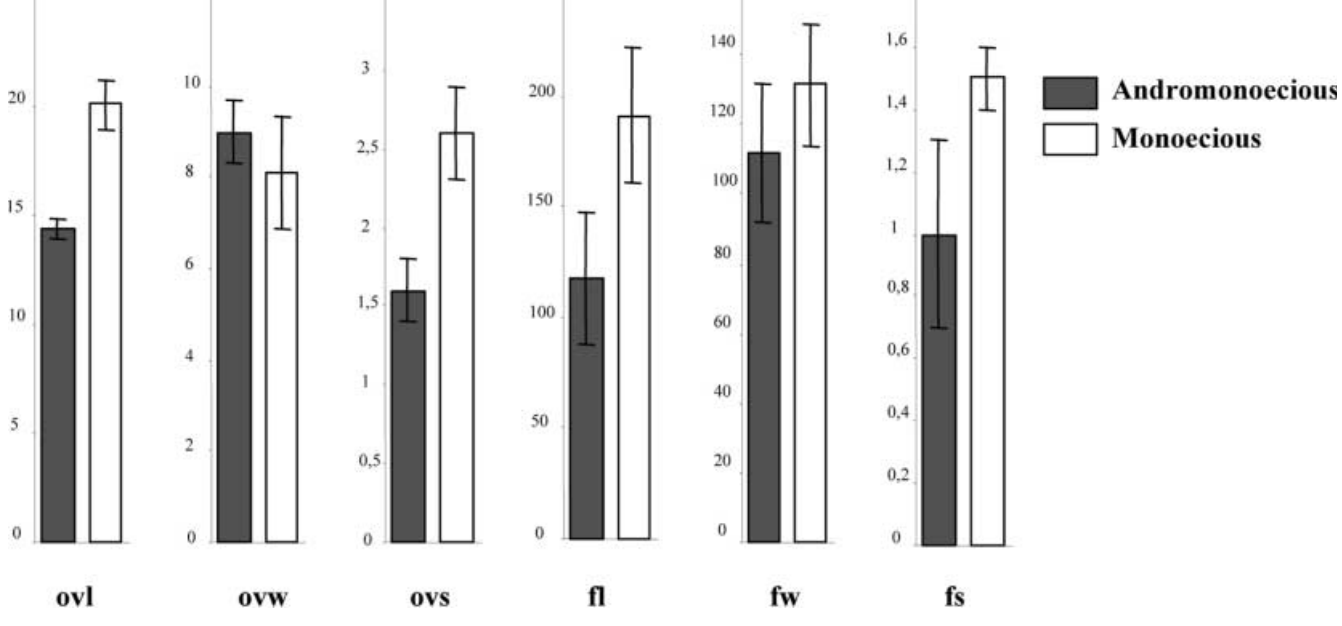

Fig. 1 Ovary and fruit traits of near-isogenic lines for the *a* gene. Data for near-isogenic lines carrying the andromonoecious (*a*) allele are indicated by the *shaded bars* and those bearing the monoecious allele (a^+) are indicated by the *open bars*. Among the six traits measured, three (ovary length, ovary shape and fruit shape), were found to differ significantly between the near-isogenic lines ovl, ovary length; ovw, ovary width; ovs, ovary shape; fl, fruit length; fw, fruit width; fs, fruit shape. All traits were evaluated in mm

QTL detection in the Védrantais × PI 161375 population (Ved161): fruit shape

Fifteen QTLs for fruit traits were detected, of which eight were detected in both years (53%) (Table 3, Fig. 2). Five of the six fs QTLs were detected in both years, demonstrating the low influence of environment on this character. In contrast, none of the QTLs for fw were recovered in both years. All four fl QTLs were found in the same genomic regions as fs QTLs. The probability that four fl QTLs would co-localize with four of the six fs QTLs only by chance is $P = 3.1 \times 10^{-5}$. This was not surprising, given the high correlation found between the two traits in the RI population. The QTL *fw12.1* was the only fw QTL found in common with a fs QTL, *fs12.1* (Fig. 3). They were both tightly linked to *p* (*pentamerous*), controlling flower carpel number (Fig. 3). Hence, fruit shape seems to be mainly due to genes involved in fruit length development but not in fruit width – with the exception of *fs12.1*. The overall R^2 value for fruit length explained more than 90% of the phenotypic variation.

QTL detection in the Védrantais × PI 161375 population (Ved161): ovary shape

Nineteen QTLs for ovary traits were detected, of which 17 were found in both years (85%) (Table 4, Fig. 2). Among eight ovs QTLs detected, four co-localized with ovl QTLs (*ovs2.2* and *ovl2.2*, *ovs7.1* and *ovl7.1*, *ovs8.2* and *ovl8.2*, *ovs9.1* and *ovl9.1*) and one with an ovw QTL (*ovs12.1* and *ovw12.1*). The probability that four ovs QTLs would cosegregate with four of the six ovl QTLs is $P = 3.1 \times 10^{-5}$. Ovary shape seemed to be mostly under the control of ovary length genes or common length/width genes, as in the case of fruit shape. The overall R^2 value for ovary shape explained more than 90% of the phenotypic variation. A major QTL, *ovs12.1*, with a phenotypic variation explained (PVE) value of 22.9%, was found near *p* (*pentamerous*) and co-segregated with a major ovary width QTL, *ovw12.1*, with a PVE of 32.2%. The *p* gene may modify, through a pleiotropic effect, ovary shape, and subsequently fruit shape through ovary width and fruit width, by increasing the size of the ovary cavity.

Fruit-shape and ovary-shape QTLs in Ved161

Six fs QTLs were detected on five different linkage groups, and eight ovs QTLs were detected on seven linkage groups. Five fs QTLs co-segregated with five ovs QTLs (*fs1.1* and *ovs1.1*, *fs2.2* and *ovs2.2*, *fs8.1* and *ovs8.1*, *fs8.2* and *ovs8.2*, *fs12.1* and *ovs12.1*). The probability that five QTLs for fs would co-localize with five of the eight ovs QTLs only by chance is $P = 1 \times 10^{-5}$. This confirmed the effect of ovary-shape genes on fruit shape, acting mainly through ovary length and fruit length.

QTL detection in the Védrantais × PI 414723 population (Ved414): fruit

Seven QTLs were found that mapped to six linkage groups (Table 5, Fig. 2). The efficiency of QTL detection in the Ved414 population was lower due to the fact that (1) fewer individuals were used for QTL detection (63 RI); (2) only 70% of the genome was covered; and (3) and only one year of data was used. Together, these limitations meant that only major QTLs with strong

Table 3 QTLs detected for fruit traits in the Recombinant Inbred population Ved161

Trait	Description	QTL[a]	Year		Detection method		Position[b]	PVE(%)[c]	LOD[c]
			1997	1998	IM	CIM			
fl	Fruit length	*fl1.1*	Yes	Yes	Yes	Yes	E42/M51_2–E42/M35_16a	13.7	3.24
		fl5.1	Yes	No	No	Yes	E46/M35_14–H36/M45_3	14.3	3.5
		fl8.1	Yes	Yes	Yes	Yes	E42/M31_36–H33/M43_25	23.7	5.95
		fl8.2	Yes	Yes	Yes	Yes	E42/M31_39–E40/M34_4	22.4	3.99
fw	Fruit width	*fw2.1*	Yes	No	No	Yes	AT_2500–B_1100	18	4.1
		fw4.1	Yes	No	Yes	Yes	E42/M31_18–Y_1600	13.4	3.06
		fw7.1	No	Yes	Yes	Yes	E42/M35_14–H36/M42_15	14.1	2.98[a]
		fw9.1	Yes	No	No	Yes	E42/M31_6–E46/M48_7	20.9	4.62
		fw12.1	Yes	No	Yes	Yes	E33/M40_18–H36/M41_8	19.8	4.47
fs	Fruit shape	*fs1.1*	Yes	Yes	Yes	Yes	E42/M51_2–E42/M35_16a	12.1	2.47
		fs2.1	Yes	Yes	Yes	Yes	H33/M43_1–E38/M43_20	19.5	4.76
		fs8.1	Yes	Yes	Yes	No	H36/M45_13–E42/M31_36	15.2	3.24
		fs8.2	Yes	Yes	Yes	Yes	E42/M31_39–E40/M34_4	19.2	3.99
		fs11.1	No	Yes	Yes	Yes	E43/M44_23–E40/M34_11	15.4	3.35[d]
		fs12.1	Yes	Yes	Yes	Yes	E33/M40_18–H36/M41_8	29	7.16

[a]QTLs were named for the trait affected and the linkage group number

[b]QTL position is given according to the most probable position determined by QTLCartographer and the composite map position of molecular markers used for QTL detection (Périn et al. 2001)

[c]If the QTL was detected by composite interval mapping (CIM) and interval mapping (IM), data for CIM [i.e. LOD score, QTL position, phenotypic variation explained (PVE)] are indicated. When the QTL was detected only by IM, the IM data were used to estimate these parameters

[d]QTL detected only by IM

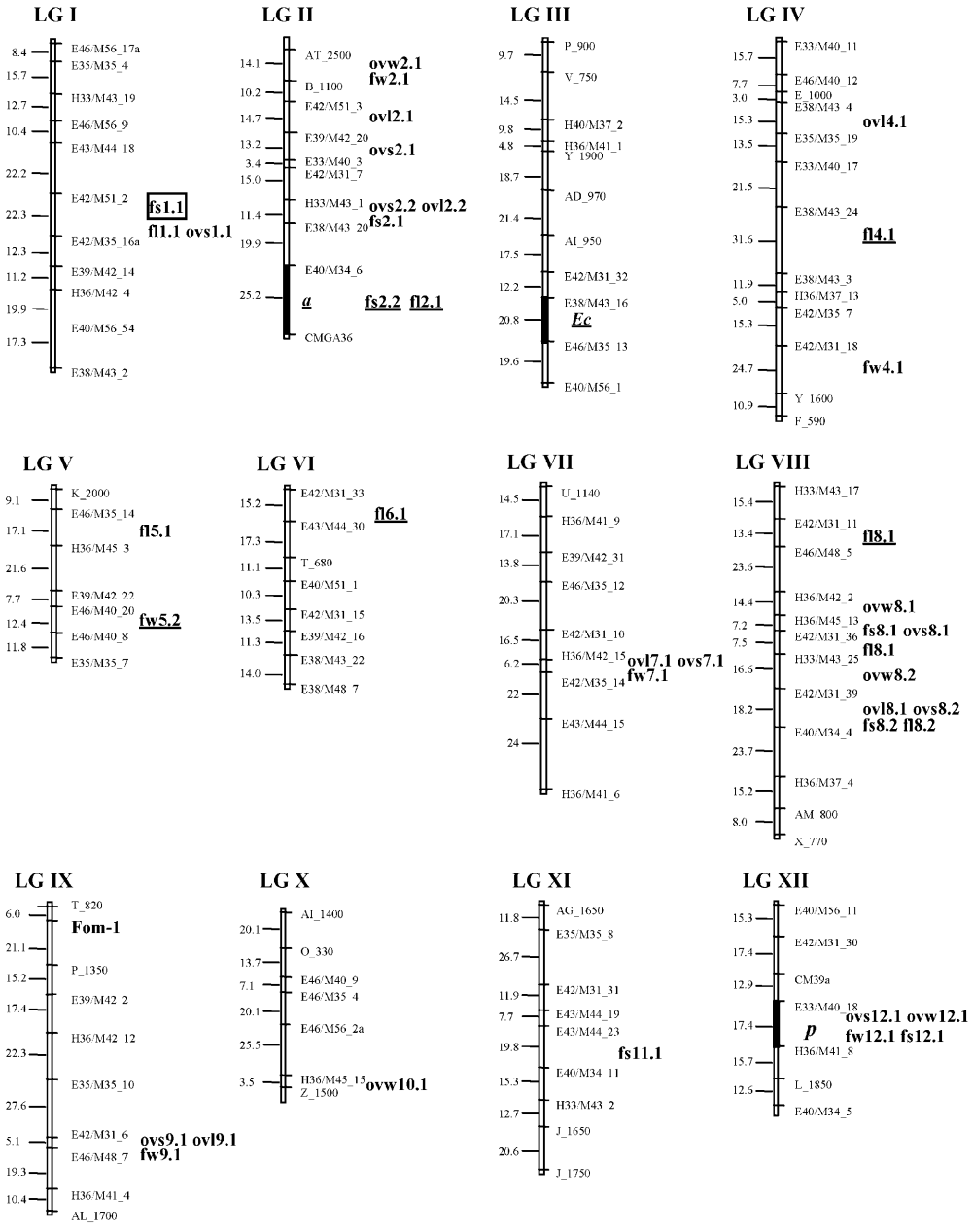

Fig. 2 Mapping of QTLs for ovary and fruit traits in the RI populations Ved161 and Ved414. Each linkage group name (LG) was assigned according to the nomenclature of Périn et al. (2001). Molecular markers used for QTL detection in the Ved161 population are indicated on the *right* of the LG, and genetic distances estimated according to the composite map distance are indicated on the *left*. The *black boxes* indicate map positions of known genes that are supposed to influence fruit and ovary shape (*a*, *p* and *Ec* genes). QTLs detected only in the Ved161 population are indicated in *bold*, QTLs detected only in Ved414 are *underlined* and QTLs detected in both populations are *boxed*

phenotypic effects were detected, and only in genomic regions covered by the molecular map.

Two fs QTLs were found on the two linkage groups I and II. On linkage group II, one fl QTL (*fl2.1*) co-localized with one fs QTL, *fs2.2* (Table 5). The overall R^2 value, probably over-estimated, explained more than 90% of the phenotypic variation for fruit shape. This character appears to be under oligogenic control, even if the number of true QTLs has been under-estimated due to the small size of our population and the strict detection probability level used. A major fruit-shape QTL, *fs2.2*, with a PVE = 52.2%, was found near the sexual-type gene *a* (*andromonoecious*) and co-segregated with *fl2.1*. This QTL could be responsible for the major variation in fruit length and fruit shape.

Correspondence between QTLs in different crosses and/or for different characters: fruit shape QTLs in the two RI populations

Védrantais was the common round parent of the two RI populations and allowed us to check allelic effects of QTLs detected in progeny obtained from crosses with two unrelated and genetically very distant lines, PI 161375 and PI 414723. More than 100 markers common to the two maps allowed comparison of map positions of fruit-shape QTLs. A total of eight QTLs were detected for fs in the two RI populations, six for Ved161 and two for Ved414 (Tables 3 and 5, Fig. 2). On linkage group I, *fs1.1*, for which the allele from Védrantais increased fruit length and fruit shape, was common to both populations

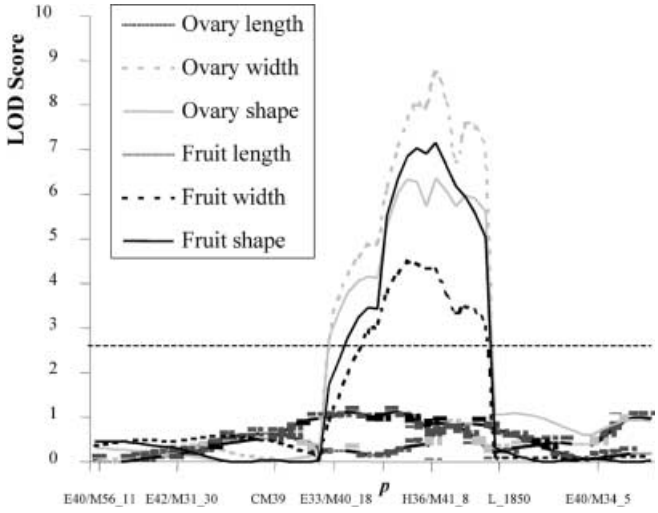

Fig. 3 Mapping of QTLs for ovary and fruit characters near the locus *p* (*pentamerous*) on linkage group XII in the Ved161 population. Major QTLs for fruit shape, ovary shape and fruit width were found near *p* by CIM, but no QTL was found for fruit length or ovary length. A pleiotropic effect of gene *p* through ovary width could be responsible for the effect on fruit shape detected

and explained the appearance of fs-transgressing RI lines. *fs1.1* had phenotypic effects in the same direction in the two populations, but with greater effects in the Ved414 population. All other fs QTLs seemed to be specific to the Ved161 or Ved414 population, even though it is possible that we missed minor QTLs in the smaller of our populations, Ved414.

Correspondence between QTLs in different crosses and/or for different characters: fruit-shape QTLs and *a* and *p* genes

Two major genes, *a* and *p*, having major effects on ovary shape were mapped, respectively, in the Ved414 and the Ved161 populations. The *a* gene effect acted through ovary and fruit length (Fig. 2), whereas the *p* gene effect acted mainly through ovary and fruit width (Figs. 2 and 3). Major fruit-shape QTLs co-localized with them: *fs2.2* co-segregated with *a* and *fs12.1* co-segregated with *p* (Fig. 3). The major effect of the gene *a* on fruit and ovary length, fruit and ovary shape, but not on fruit and ovary width, was also demonstrated with our near-isogenic lines (Fig. 1). Hence, the *a* gene appears to act mainly on ovary/fruit length and the *p* gene on ovary/fruit width.

Discussion

Fruit shape is under polygenic control, and shows very little environmental effect. Polygenic control was found in both crosses described here. Eight fs QTLs were detected in the two populations and one was found to be common to both. These QTLs were responsible for more than 90% of the phenotypic variation in both populations. Among the six fs QTLs in the Ved161 population, five were detected in both years. Our results demonstrated little environmental or genotype × environment influence on fruit shape. Several studies on tomato and cucumber have demonstrated high heritability for this trait (Serquen et al.

Table 4 QTLs for ovary traits detected in the Recombinant Inbred population Ved161

Trait	Description	QTL[a]	Year		Detection method		Position[b]	PVE (%)[c]	LOD[c]
			1997	1998	IM	CIM			
ovl	Ovary length	*ovl2.1*	Yes	Yes	Yes	Yes	E42/M51_3–E39/M42_20	12.3	2.4
		ovl2.2	Yes	Yes	Yes	Yes	H33/M43_1–E38/M43_20	21.9	5.87
		ovl4.1	Yes	Yes	Yes	Yes	E38/M43_4–E35/M35_19	16.7	2.82
		ovl7.1	Yes	No	No	Yes	E42/M35_14–H36/M42_15	15.6	3.52
		ovl8.1	Yes	Yes	Yes	Yes	E42/M31_39–E40/M34_4	27.4	4.03
		ovl9.1	Yes	Yes	Yes	Yes	E42/M31_6–E46/M48_7	23.1	5.71
ovw	Ovary width	*ovw2.1*	Yes	Yes	Yes	Yes	AT-2500–B_1100	12.3	3.07
		ovw8.1	Yes	Yes	Yes	Yes	H36/M42_2–H36/M45_13	26.3	4.95
		ovw8.2	Yes	Yes	Yes	No	H33/M43_25–E42/M31_39	14.1	3.05[d]
		ovw10.1	Yes	Yes	Yes	Yes	H36/M45_15–Z_1500	13.7	2.85
		ovw12.1	Yes	Yes	Yes	Yes	E33/M40_18–H36/M41_8	32.2	8.72
ovs	Ovary shape	*ovs1.1*	No	Yes	Yes	Yes	E42/M51_2–E42/M35_16a	20.3	3.34
		ovs2.1	Yes	Yes	Yes	No	E39/M42_20–E33/M40_3	11.4	2.33[d]
		ovs2.2	Yes	Yes	Yes	Yes	H33/M43_1–E38/M43_20	17.6	3.1
		ovs7.1	Yes	Yes	No	Yes	E42/M35_14–H36/M42_15	20	3.34
		ovs8.1	Yes	Yes	Yes	No	H36/M45_13–E42/M31_36	23	6.04[d]
		ovs8.2	Yes	Yes	Yes	Yes	E42/M31_39–E40/M34_4	30.4	6.26
		ovs9.1	Yes	Yes	Yes	Yes	E42/M31_6–E46/M48_7	11.8	2.57
		ovs12.1	Yes	Yes	Yes	Yes	E33/M40_18–H36/M41_8	22.9	6.38

[a]QTLs were named for the trait affected and the linkage group number

[b]QTL position is given according to the most probable position determined by QTLCartographer and the composite map position of molecular markers used for QTL detection (Périn et al. 2001)

[c]If the QTL was detected by composite interval mapping (CIM) and interval mapping (IM), data for CIM [i.e. LOD score, QTL position, phenotypic variation explained (PVE)] are indicated. When the QTL was detected only by IM, the IM data were used to estimate these parameters

[d]QTL detected only by IM

Table 5 QTLs for fruit traits detected in the Recombinant Inbred population Ved414

Trait	Description	QTL[a]	Detection method		Position[b]	PVE (%)[c]	LOD[c]
			IM	CIM			
fl	Fruit length	*fl2.1*	Yes	Yes	E40/M34_6–CMGA36	46.7	5.05
		fl4.1	No	Yes	E38/M43_24–E38/M43_3	28	3.34
		fl6.1	No	Yes	E43/M44_30–E42/M31_33	54.1	4.6
		fl8.1	No	Yes	E42/M31_11–E46/M48_5	32	3.66
fw	Fruit width	*fw5.2*	No	Yes	E46/M40_20–E46/M40_8	43.1	5.55
fs	Fruit shape	*fs1.1*	Yes	Yes	E42/M51_2–E42/M35_16a	31	3.74
		fs2.2	No	Yes	E40/M34_6–CMGA36	52.2	5.46

[a]QTLs were named for the trait affected and the linkage group number

[b]QTL position is given according to the most probable position determined by QTLCartographer and the composite map position of molecular markers used for QTL detection (Périn et al. 2001)

[c]If the QTL was detected by composite interval mapping (CIM) and interval mapping (IM), data for CIM [i.e. LOD score, QTL position, phenotypic variation explained (PVE)] are indicated. When the QTL was detected only by IM, the IM data were used to estimate these parameters

1997; Grandillo et al. 1999). In many species, fruit shape appears to be under the strict genetic control of several additive genes with small individual effects and little environmental influence, in addition to a few major QTLs (Grandillo et al. 1999). In melon, the large phenotypic variation for fruit shape seems to be under the control of a large number of fs QTLs mainly involved in the control of fruit length, as five fs QTLs among the six detected were linked to fl QTLs. In addition to these QTLs with moderate effect, two major QTLs with an R^2 value higher than 30% (*fs2.2*, *fs12.1*) were detected in the Ved414 and Ved161 populations, respectively, and were linked to major genes (*andromonoecious* and *pentamerous*).

Fruit shape is under the control of QTLs involved in early ovary development. Co-segregation of fs QTLs and ovs QTLs in the Ved161 population suggests a pleiotropic effect, or a tight linkage between QTLs of both types. Such a correlation was previously described for a major tomato fruit shape QTL (*fs8.1*) in a pair of near-isogenic lines (Grandillo et al. 1996). In *C. melo*, where we found that a strong correlation between fs and ovs was recovered even in unrelated lines (Table 2), pleiotropy seems more likely than linkage. Thus, to a large extent, genetic variation for fruit shape is the result of a combination of QTLs involved in ovary development/shape. Our work, together with the results of Grandillo et al. (1999) and Ku et al. (2000b), suggests that genetic and physiological control of fruit shape is probably similar in tomato and melon, and is determined well before anthesis, a hypothesis previously suggested by Sinnot and Kaiser (1934).

The genes *p* (*pentamerous*) in PI 161375 and a^+ (*monoecious*) in PI 414723 have a major effect on fruit shape and co-segregate with ovary fruit shape QTLs (Figs. 2 and 3). In *L. esculentum,* a gene similar to *p*, the *L* gene (*Locule number in ovary*), was also shown to have a major effect on fruit shape (Yeager 1937). Again, pleiotropy is the most likely explanation for co-segregation of ovary and fruit shape with *a* and *p* genes. A pleiotropic effect of the *a* gene was demonstrated following the reversal of sexual type from monoecious to andromonoecious by spraying with silver nitrate (Byers et al. 1972; Risser 1985). Reversal of sexual type induced a simultaneous shift in both ovary and fruit shape (Risser 1985). We have demonstrated that genes acting on ovary shape are mainly responsible for fruit shape variation in melon whether they are major genes or QTLs. Accordingly, we did not find any QTL for fruit and ovary shape close to the *Ec* gene (LG III), a major gene which increases fruit cavity size during ripening and acts after anthesis (Périn et al. 1999) (Fig. 2).

fs1.1 mapped to the same location in the two crosses, suggesting the possibility that fruit shape acquisition is also due to mutations with quantitative effects on a common set of genes. A major tomato fruit-shape QTL, *fs8.1*, was also found to be conserved across different *Lycopersicon* species (*L. pimpinellifolium*, *L. peruvianum* and *L. hirsutum*), and in all cases, the QTL allele of *fs8.1* which is responsible of fruit shape elongation came from *L. esculentum* (Grandillo et al. 1996, 1999). In our progenies, the QTL allele of *fs1.1* that is responsible for fruit elongation came also from our common parental line Vedrantais. Interestingly, orthologous QTLs for fruit shape (Grandillo et al. 1996, 1999) and size (Alpert et al. 1995) found in three different species of *Lycopersicon* tend to demonstrate that fruit development, like the emergence of other morphological innovations (maize/teosinte) during domestication (Doebley et al. 1995), are under the control of a few major QTLs, which may reflect general physiological mechanisms of fruit-shape development.

The isolation of fs QTLs, which is in progress in tomato (Ku et al. 1999, 2000a), will allow us to discover whether the same genes actually are involved in different fruit species. A major QTL for fruit size in tomato (*fw2.2*) has been successfully isolated by map-based cloning (Frary et al. 2000). Genes involved in determining such traits in several fruit species have either been conserved during evolution or derived through convergence of function. It should be possible using a candidate gene approach – mapping homologous or heterologous fs genes – to confirm or disprove these hypotheses. Co-segregation of candidate genes with fruit-shape QTLs will give the first answers. Moreover, the synteny among the Solanaceae (Tanksley et al. 1992; Livingstone et al. 1999) will help to elucidate whether

genes for fruit shape are common to pepper and tomato crops, despite the use of physiologically distinct controls. Molecular knowledge of these genes will yield an understanding of fruit shape acquisition in general, will allow molecular geneticists to develop new tools for the future molecular engineering of fruit, and will facilitate breeding for this quantitative character.

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References

Alpert KB, Grandillo S, Tanksley SD (1995) *fw 2.2*: a major QTL controlling fruit weight is common to both red- and green-fruited tomato species. Theor Appl Genet 91:994–1000

Baudracco-Arnas S, Pitrat M (1996) A genetic map of melon (*Cucumis melo* L.) with RFLP, RAPD, isozyme, disease resistance and morphological markers. Theor Appl Genet 93:57–64

Bernacchi D, Beck-Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance for *Lycopersicon hirsutum*. Theor Appl Genet 97:381–397

Burr B, Burr FA (1991) Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet 7:55–60

Byers RE, Baker LR, Sell HM, Herner RC, Dilley DR (1972) Ethylene: a natural regulator of sex expression of *Cucumis melo* L. Proc Natl Acad Sci USA 69:717–720

Causse M, Rocher JP, Henry AM, Charcosset A, Prioul JL, de Vienne D (1995) Genetic dissection of the relationship between carbon metabolism and early growth in maize, with emphasis on key-enzyme loci. Mol Breed 1:259–272

Chetelat RT, Rick CM (1999) TGRC stock lists. Tomato Genet Coop Rep 49:53–79

Doebley J, Stec A, Gustus C (1995) Teosinte *branched1* and the origin of maize: evidence for epistasis and the evolution of dominance. Genetics 141:333–346

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

Gillaspy G, Ben-David H, Gruissem W (1993) Fruits: a developmental perspective. Plant Cell 5:1439–1451

Goldman IL, Paran I, Zamir D (1995) Quantitative trait locus analysis of a recombinant inbred line population derived from a *Lycopersicon esculentum* × Lycopersicon cheesmanii cross. Theor Appl Genet 90:925–932

Grandillo S, Tanksley SD (1995) QTL analysis of horticultural traits differentiating the cultivated tomato from the closely related species *Lycopersicon pimpinellifolium*. Theor Appl Genet 92:935–951

Grandillo S, Ku H-M, Tanksley S (1996) Caracterisation of *fs8.1*, a major QTL influencing fruit shape in tomato. Mol Breed 2:251–259

Grandillo S, Ku HM, Tanksley SD (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99:978–987

Kaiser S (1935) The factors governing shape and size in Capsicum fruits; a genetic and developmental analysis. Bull Torr Bot Club 62:433–454

Kalb TJ, Davis DW (1984) Evaluation of combining ability, heterosis, and genetic variance for fruit quality characteristics in bush muskmelon. J Am Soc Hort Sci 109:411–415

Khambanonda I (1950) Quantitative inheritance of fruit size in red pepper (*Capsicum frutescens L.*). Genetics 35:322–343

Ku HM, Doganlar S, Chen KY, Tanksley SD (1999) The genetic basis of pear-shaped tomato fruit. Theor Appl Genet 9:844–850

Ku H, Doganlar S, Tanksley SD (2000a) High-resolution mapping of a major QTL responsible for pear-shaped tomato. In: Heller SR (ed) Plant and Animal Genome VIII (San Diego) Program and Abstracts Guide.

Ku H-M, Grandillo S, Tanksley SD (2000b) *fs8.1*, a major QTL, sets the pattern of tomato carpel shape well before anthesis. Theor Appl Genet 101:873–878

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185–199.

Lippert LF, Hall MO (1982) Heritabilities and correlations in muskmelon from parent-offspring regression analyses. J Am Soc Hort Sci 107:217–221

Livingstone KD, Lackney VK, Blauth JR, Wijk RV, Jahn MK (1999) Genome mapping in *Capsicum* and the evolution of genome structure in the Solanaceae. Genetics 152:1183–1202

Nandi S, Subudhi PK, Senadhira D, Manigbas NL, Sen-Mandi S, Huang N (1997) Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. Mol Gen Genet 255:1–8

Naudin C (1859) Essai d'une monographie des espèces et des variétés du genre *Cucumis*. Ann Sci Nat Ser Bot 11:5–87

Paterson AH, Lin Y, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu S, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1174–1717

Périn C, Dogimont C, Giovinnazzi N, Besombes D, Guitton L, Pitrat M (1999) Genetic control and linkages of some fruit characters in melon (*Cucumis melo* L.). Cucurbit Genet Coop 22:16–18

Périn C, Hagen LS, De Conto V, Katzir N, Danin-Poleg Y, Portnoy V, Baudracco-Arnas S, Chadoeuf J, Dogimont C, Pitrat M (2001) A reference map of *Cucumis melo* based on two recombinant inbred line populations. Theor Appl Genet, in press

Poole CF, Grimball PC (1939) Inheritance of new sex forms in *Cucumis melo* L. J Hered 30:21–25

Risser G (1984) Correlation between sex expression and fruit shape in musk melon (*Cucumis melo* L.). In: *Cucumis* and melons. Proceedings of the 3rd EUCARPIA Meeting, Plovdiv, Bulgaria, pp 100–103

Rosa JT (1928) The inheritance of flower types in *Cucumis* and *Citrullus*. Hilgardia 3:233–250

Serquen FC, Bacher J, Staub JE (1997) Mapping and QTL analysis of horticultural traits in a narrow cross in cucumber (*Cucumis sativus* L.) using random-amplified polymorphic DNA markers. Mol Breed 3:257–268

Sinnot EW (1927) A factorial analysis of certain shape characteristics in squash fruits. Amer Nat 1:333–343

Sinnot EW (1944) Cell polarity and the development of form in cucurbit fruits. Amer Jour Bot 31:388–391

Sinnot EW (1945) The relation of growth to size in cucurbit fruits. Amer J Bot 32:439–446

Sinnot EW, Kaiser S (1934) Two types of genetic control over the development of shape. Bull Torr Bot Club 61:1–7

Stino KR, Warid WA, Abdelfattah MA (1958) Inheritance studies of some fruit characters in the melon, *Cucumis melo* L. Bull Fac Agricult Cairo 215:3–24

Tanksley SD et al (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. Theor Appl Genet 92:213–224

Yeager AF (1937) Studies on the inheritance and development of fruit size and shape in the tomato. J Agri Res 55:141–152

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