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H.-M. Ku · S. Grandillo · S.D. Tanksley

fs8.1, a major QTL, sets the pattern of tomato carpel shape well before anthesis

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Abstract *fs8.1* is a major fruit-shape QTL differentiating fresh-market and processing tomatoes. Mature fruits from plants with the wild-type fresh-market alleles are round, whereas those with alleles from processing variety E6203 are elongated (sometimes referred to as blocky or square tomatoes). Fine mapping was undertaken to determine whether the effect is due to a single gene or several tightly linked genes. RAPD and RFLP linkage analysis, and substitution mapping of nearly isogenic lines (NILs) segregating for the 22.8 cM-TG176-CT92 interval at the top of chromosome 8 in tomato were used for high-resolution mapping. For the 1212 gametes screened in F_2 and F_3 families, it was determined that *fs8.1* maps as a single locus near the centromere of chromosome 8. A comparative developmental study of *fs8.1* NILs revealed that *fs8.1* alleles exert their effects on fruit shape early in carpel development at least 6 days before anthesis. Field evaluations of the NILs indicate that *fs8.1* affects not only fruit shape, fruit length, and fruit weight but also the number of flowers and fruits per inflorescence, and the harvest index. The date of first flower and fruit diameter were not significantly affected.

Key words Fruit shape · Map-based cloning · Centromere · Fruit development · QTL

Introduction

Fruit is defined morphologically as a mature ovary which can vary in type, ranging from legumes with a single carpel, to pineapples containing an entire inflorescence (Eames and MacDaniels 1925). Wild plants bear fruits which are usually small, inconspicuous, and adapted for seed dispersal. However, due to intense selection by humans in fruit-bearing crops, the carpels become greatly enlarged after fertilization and display a wide variety of sizes and shapes. Due to its quantitatively inherited nature, very little is known about how the transition of fruit development is coordinated temporally and spatially with floral development.

Relatively simple fruit shapes, such as round and elongated, are commonly found in taxonomically diverse species including tomato, pepper, eggplant, cucurbits and watermelon (Sinnott and Durham 1929; Sinnott and Kaiser 1934; Weetman 1937; Kano et al. 1957). However, little is known about the factors and possible mechanisms by which unique fruit shapes are established. Earlier in this century, many attempts were made to learn more about fruit-shape development through morphological studies. In pepper and eggplant, for example, fruit-shape determination was mainly associated with the growth of fruit after flowering in various dimensions (Kano et al. 1957). In squash, it was found that shape was established very early in the ovary primordia, and growth in various dimensions was almost constant after anthesis (Sinnott 1944). A strong correlation between the shapes of mature fruits and young ovaries was also reported for watermelon (Weetman 1937).

For tomato, genetic studies as early as the beginning of this century reported that a single recessive gene caused pear-shaped fruit (Hedrick and Booth 1907; Price and Drinkard 1908). This factor was later named *pr* (pyriform shape) or *o* (ovate shape) and placed on the first linkage group corresponding to chromosome 2 on the molecular map of Tanksley et al. (1992) (Jones 1917; Lindstrom 1926, 1927, 1929, 1932; MacArthur 1926; Young and MacArthur 1947). It was also proposed that an allele series exists for this locus with varying degrees of dominance (from dominant to recessive): oblate, round, and ovate (Lindstrom 1927). Other possible loci affecting fruit shape include *bk* (for beaked tomatoes with a sharp beak on the blossom end of fruit), *n* (for nipple-tip tomatoes), *f* (for fasciated fruit) and *lc* (for locule number) (Young and MacArthur 1947).

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H.-M. Ku · S. Grandillo · S.D. Tanksley (✉)
Department of Plant Breeding and Biometry, 252 Emerson Hall, Cornell University, Ithaca, NY 14853-1902, USA
e-mail: sdt4@cornell.edu
Tel: +1-607-255 1673, Fax: +1-(607)-255 6683

In the past ten years, *Arabidopsis thaliana* has been used as a model system for understanding plant growth and development; floral development has been an especially fruitful field (Howell 1998). However, since *Arabidopsis* has not been subjected to domestication, the lack of major phenotypic variation in *Arabidopsis* fruits (siliques) makes it difficult to use it as a model to study fruit development. Alternatively, the wide range of phenotypic variation among tomato fruits and well-established genome tools make tomato an idea model system to study the domestication process and the mechanisms controlling fruit development. Two well-characterized traits in tomato quantitative trait locus (QTL) studies are fruit weight and fruit shape. At least 28 fruit-weight and 11 fruit-shape QTLs have been identified and located on the tomato high-density molecular map (Grandillo et al. 1999).

Since little is known about the molecular mechanisms controlling fruit-shape variation, cloning and developmental studies of a major QTL controlling fruit shape in tomato, *fs8.1*, will open the door to understand the molecular basis of factors controlling fruit-shape characteristics. The objective of the present study was to map *fs8.1*, a major fruit-shape QTL, in a precise location as a prerequisite for map-based cloning and to determine the developmental timing at which the locus exerts its effects on shape.

Materials and methods

Plant materials

Nearly isogenic lines (NILs) (Tanksley 1993) homozygous for round (*Lycopersicon pimpinellifolium* LA1589=PM) fruit alleles (TA1198) versus elongated (*Lycopersicon esculentum* cv M82-1-7=E) fruit alleles (TA209) at the *fs8.1* QTL were developed in a previous study from a $BC4F_2$ population derived from a cross between *L. esculentum* cv M82-1-7 and *L. pimpinellifolium* (LA1589) (Grandillo et al. 1996). TA1198 contains homozygous PM alleles for the interval between RFLP markers TG176 and CT92 defining a 22.8-cM interval containing *fs8.1* on chromosome 8 in an otherwise homozygous *L. esculentum* background (see Fig. 1). A NIL heterozygous (E/PM) for the TG176-CT92 interval was selfed to generate a segregating population (hereafter called $NILF_2$). A total of 606 $NILF_2$ plants were screened with three markers from the interval (TG176, CD40, and CT92) and recombinant plants were selected. F_3 progeny from recombinant $NILF_2$s were screened with TG176, CD40, and CT92. At least two homozygous recombinants from each F_3 family (see Fig. 2) were transplanted to the greenhouse along with 15 non-recombinant controls (5 E/E, 5 E/PM, and 5 PM/PM). Two lines were excluded because of blossom-end rot on the fruits, and the remaining 25 homozygous F_3 recombinant plants were subjected to phenotypic evaluation (see Fig. 2). Five to ten mature fruits from each plant were used to collect fruit-shape index data (L/D) by measuring the ratio of longitudinal diameter (L) and equatorial diameter (D) as described in Grandillo et al. (1996).

RFLP and RAPD analysis

DNA extraction from tomato leaves was performed as reported in Fulton et al. (1995). Southern blotting and hybridization were as described in Bernatzky and Tanksley (1986). DNA was extracted from the two *fs8.1* NILs (TA1198 and TA209) and screened with 700 RAPD primers to detect additional polymorphic markers near *fs8.1* as described in Grandillo and Tanksley (1996).

Developmental study

The round-fruit-type NIL (TA1198) and elongated-fruit-type NIL (TA209) were used to study the fruit-shape difference in both pre-anthesis and post-anthesis stages. In order to collect the developing ovaries more precisely at pre-anthesis stages, flower buds of ten plants in each NIL were tagged and the size of the flower was recorded (both length and width) every day until anthesis. Flower-size (length and width) means were calculated from at least ten flowers and were plotted against the relative number of days pre-anthesis to estimate the relationship between flower size and the timing of ovary development (i.e., days before anthesis). Next, at least ten ovaries were collected from flowers estimated to be at 0, 3, 6, and 9 days pre-anthesis (based on flower size) and fixed for 24 h in FAA (4% formalin in acetic-alcohol), processed and embedded in paraffin and sectioned longitudinally 10-μ thick. Sass's modification of Mayer's Hemalum (Berlyn and Milcsche 1976) was used to stain the thin-sectioned ovaries. All of the sectioning and staining procedures were done as described in Berlyn and Milcsche (1976). The ovary and fruit-shape index was recorded as the ratio of ovary length to equatorial diameter. At least ten fruits of each genotype (TA1198, TA209) were collected at a series of

Table 1 Sequence information for RAPD markers in the *fs8.1* region

OP marker	Primer sequence
OP37	GACACGGACC
OP152	AAGAGGGCGT
OP176	TCCGTGCTGA
OP352	CCTTGACGCA
OP618	GGCTCATGTG
OP651	GTCACGTCCT

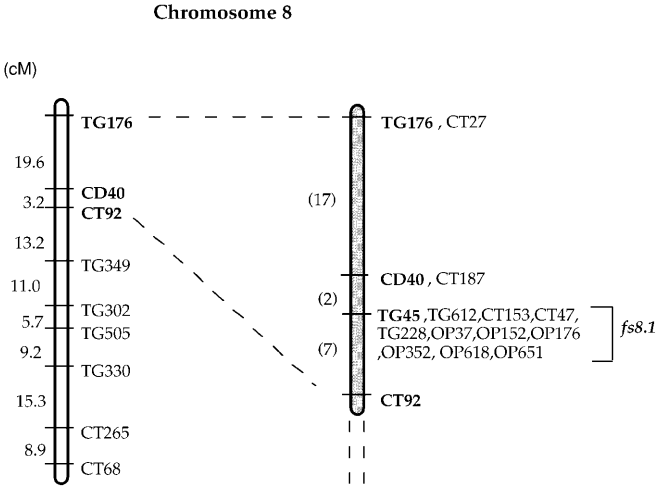

Fig. 1 Map of the *fs8.1* region. On the left is the molecular map of chromosome 8 (Tanksley et al. 1992), while the *numbers* to the left of the chromosome are the genetic distances (cM) between the markers selected for mapping in this study. On the right is an enlargement of the interval TG176–CT92 spanning the *fs8.1* region. *Numbers in parentheses* to the left of the TG176–CT92 segment indicate the number of recombinants identified from 606 $NILF_2$ plants derived from a cross between *L. esculentum* and *L. pimpinellifolium*

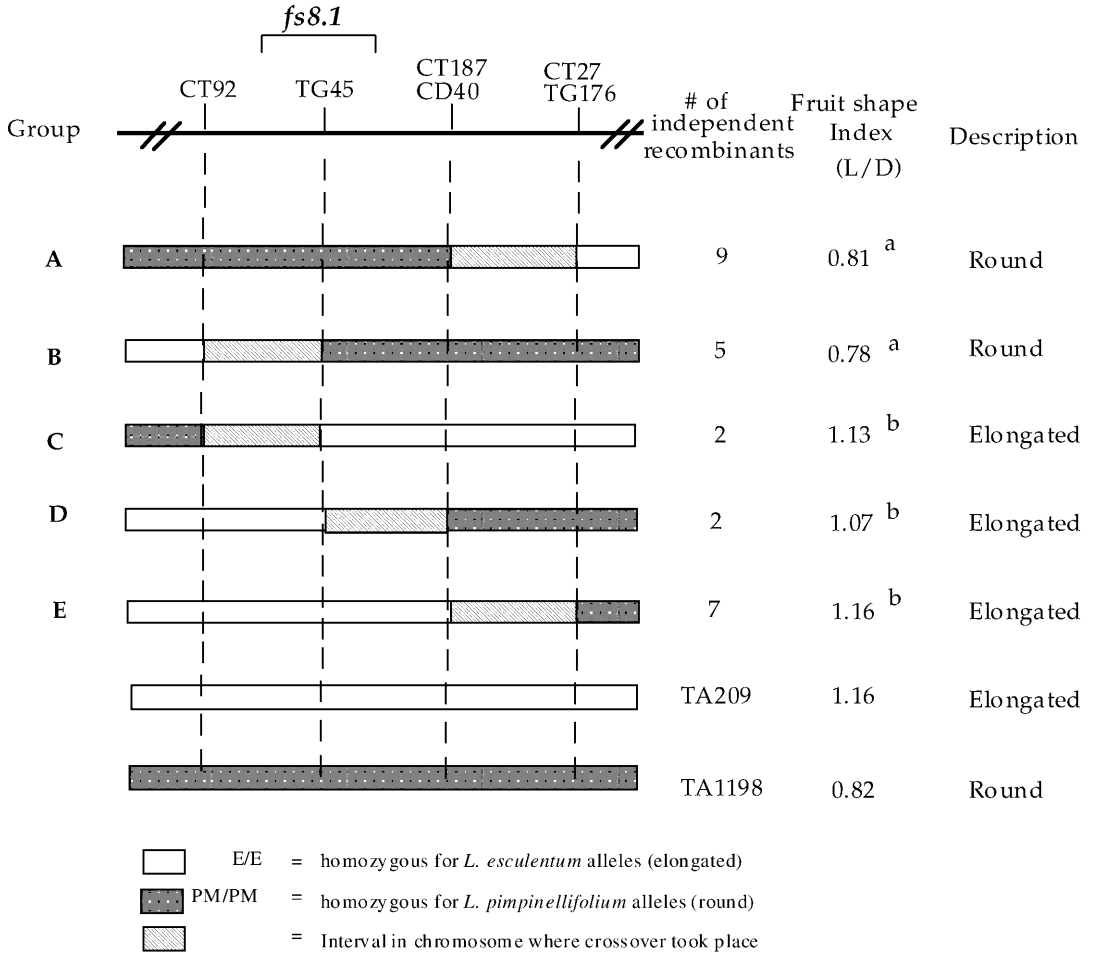

Fig. 2 Mean fruit-shape index (L/D) for homozygous recombinants in the *fs8.1* region on chromosome 8 in tomato**.** "The plants were categorized into five groups (*A*–*E*) based on their genotype. The numbers of independent recombinants are shown on the right of the genotype segments in each group. The four loci defining the genotypes of the recombinants are shown at the top of the figure. An "a" following the fruit-shape index indicates that the mean fruit shape of recombinants is significantly different from the control (TA209) at $P<0.001$; a "b" indicates that the mean fruit shape of recombinants is significantly different from the control (TA1198) at $P<0.001$

post-anthesis stages (5, 10, 15, 20, 25, 30, 35 days after anthesis). In addition, the means of both dimensions of ovaries and fruits for the entire developmental course were determined and plotted against the stages of developing ovaries and fruits using Cricket Graph III (version 1.5.2). JMP version 2.05 was used to apply an analysis of variance to the mean ovary and fruit-shape indices, and the length and width of NILs (TA209 and TA1198) at each pre-anthesis stage.

Field test

A total of 35 plants of each genotype (TA1198 and TA209) were transplanted to the field at Cornell University, Ithaca, New York, in the summer of 1997 in a completely randomized design. Data for the date of first flower, number of flowers and fruits per infloresence were recorded. In addition, the harvest index was obtained for each plant at harvest as the ratio of total fruit weight to the total biomass (total fruit weight plus the whole-plant weight) of each plant. At least ten fruits of each plant were used to determine the fruit-weight and fruit-shape index (L/D).

Statistical analysis

Linkage analysis was performed using MAPMAKER version 2.0 for Macintosh (Lander et al. 1987). Map units (cM) were obtained by applying the Kosambi (1944) function. JMP version 2.05 (SAS institute 1989) was used to apply an analysis of variance (ANOVA) to the mean fruit-shape index of recombinants and the means of control isogenic lines at $P<0.001$, and also the difference of flower size, ovary diameter, ovary length and ovary shape at pre-anthesis stages between TA209 and TA1198. In addition, ANOVA was performed to test the mean difference of TA1198 and TA209 on each trait in the field test. Genotype was used as a factor in the ANOVA analysis.

Results and discussion

To identify polymorphism between TA209 (elongated-fruit NILs) and TA1198 (round-fruit NILs) for *fs8.1*, a total of 700 RAPD primers were screened on the NILs. Of these primers, 78 produced bands that were polymorphic between TA209 and TA1198. Most of the polymorphic RAPD markers generated multiple bands when probed on Southern blots; however, six RAPD markers (Table 1) were derived from low-copy number DNA and were mapped onto the 27 recombinants from the $NILF_2$ population. In addition, 17 RFLP markers were available in the TG176–CT92 interval as described in Tanksley et al. (1992), but only ten of these markers showed poly-

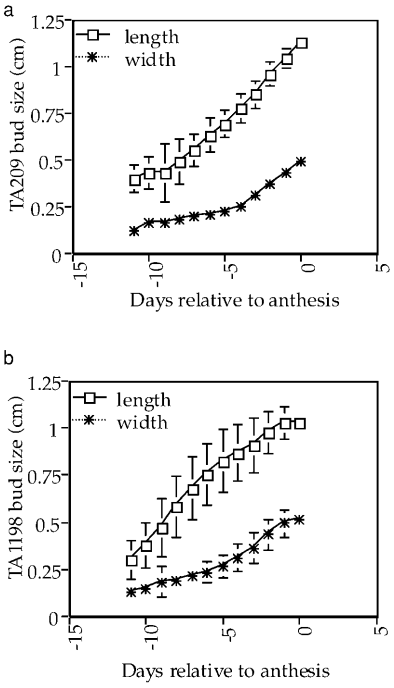

Fig. 3a, b The relationship between pre-anthesis stage and flower size (length and width). (*a*) The elongated fruit-type NILs (TA209), and (**b**) the round fruit-type NILs (TA1198)

morphism between the NILs and could be mapped on the recombinants. Twenty seven out of six hundred and six $NILF_2$s were found to be recombinant in the interval between TG176 and CT92. These recombinants were used for fine mapping with the aforementioned RFLP and RAPD markers. In total, 16 markers could be mapped to the TG176–CT92 interval containing *fs8.1* (Fig. 1).

QTL fine mapping

Recombinants were divided into five different classes based on their genotypes with respect to the markers in the TG176–CT92 interval (Fig. 2). Groups A and E show recombination between markers CD40 and TG176, groups B and C have recombination between markers TG45 and CT92, and group D has recombination between TG45 and CD40. All recombinants in groups C, D and E, containing the EE alleles for TG45 and cluster of markers (hereafter called the ”TG45 cluster”), showed a more elongated fruit-type and fruit-shape index values which were significantly different (at the $P<0.001$ level) from those for the round-fruit NIL control (TA1198). On the other hand, groups A and B showed round fruit and a significantly different fruit-shape index from those of the elongated fruit control (TA209) at the $P<0.001$ level. The phenotypic data of the 25 homozygous recombinants indicates that *fs8.1* segregates as a single locus which maps to the TG45 cluster (Figs. 1, 2). This result was consistent with the location of *fs8.1* near the centromere, as reported in a previous study (Grandillo et al. 1996). As reviewed in Frary et al. (1996), it was proposed that the centromeric effect or the

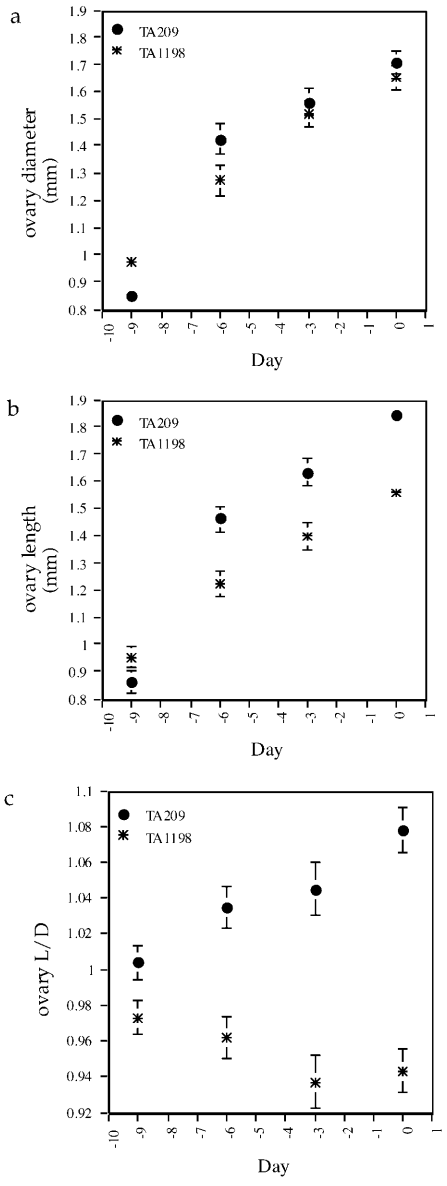

Fig. 4 (**a**) ovary length, (**b**) ovary equatorial diameter, and (**c**) ovary shape index (L/D) changes during pre-anthesis for elongated (TA209) and round (TA1198) fruit-type NILs. The *zero* on the x-axis marks anthesis, *negative values* indicate pre-anthesis stages in developing ovaries. Each *point* represents the mean value of at least five ovaries

presence of pericentric heterochromatin causes the suppression of recombination in the centromeric region. As a result, genetic distances of markers around the centromere may not be good predictors of physical distances (Frary et al. 1996; Grandillo et al. 1996). Therefore, the location of *fs8.1* near the chromosome-8 centromere may make it a difficult target for map-based cloning due to the reduced recombination frequency.

Developmental studies

The relationship between flower-bud size (length and width) and developmental stage (number of days before anthesis) was determined for both NILs (TA209 and

Table 2 The results of the ANOVA test for comparing ovary length, width and the shape index (L/D) between TA209 and TA1198 NILs at pre-anthesis stages

Trait	Genotype	Developmental stage of ovaries			
		9-days pre-anthesis	6-days pre-anthesis	3-days pre-anthesis	Anthesis
Ovary Shape Index	TA209	1.012	1.025	1.045	1.078
	TA1198	0.976	0.960	0.919	0.942
	P-value	NS	0.0099	0.0002	<0.0001
Ovary Length	TA209	0.861	1.461	1.633	1.841
	TA1198	0.95	1.223	1.399	1.555
	P-value	NS	0.0001	0.0002	<0.0001
Ovary Diameter	TA209	0.851	1.425	1.561	1.707
	TA1198	0.973	1.274	1.521	1.65
	P-value	NS	NS	NS	NS

NS means there was no significant difference (P=0.01)

Table 3 ANOVA test for difference in *fs8.1* NILs (TA209 and TA1198) for field traits at harvest: fruit width, fruit length, fruit-shape index, fruit weight, days to the first flower, number of flowers and fruits per inflorescence

Genotype	Type	Mature					First flowering day	No. flowers per inflorescence	No. fruits per inflorescence
		Fruit width	Fruit length	Fruit-shape index	Harvest index	Fruit weight			
TA209	Elongated	5.13	5.71	1.11	0.86	91.44	28.94	4.4	4.8
TA1198	Round	4.92	4.68	0.95	0.84	70.75	29.46	3.8	4.6
P-value		NS	<0.0001	<0.0001	<0.0001	<0.0001	NS	<0.0001	NS

NS means there was no significant difference (P=0.01)

TA1198). Using flower-bud size as an estimator of developmental stage, a plot of ovary dimensions was generated for pre-anthesis developmental stages (Fig. 3a and b). In addition, no significant differences were detected in flower size between the two NILs in this study. The ovary shape index (L/D) is shown to clearly differentiate TA209 and TA1198 well before anthesis (Fig. 4b), which suggests that *fs8.1* controls fruit shape through events occurring very early in carpel development. Although determination of ovary shape has been proposed to occur at the post-anthesis stages in some species (*e.g*., Sinnot and Kaiser 1934; pepper in Kaiser 1935; cucurbit in Sinnot 1936; and eggplant in Kano et al. 1957), in other species, including tomato (Houghtaling 1935), squash (Sinnott and Durham 1929; Sinnott and Kaiser 1934) and watermelon (Weetman 1937), fruit shape has been reported to be pre-determined at a very early stage of the ovary primordia. In addition, a highly significant correlation between ovary shape and fruit shape (r= 0.89; P<0.01) was recently shown for tomato (Grandillo et al. 1996), supporting the hypothesis that shape is largely determined during the early stages of ovary development. The results of the current study of *fs8.1* provide additional evidence to support this hypothesis (Fig. 4).

Furthermore, results from the current studies show that as early as 6 days pre-anthesis, ovary length is already significantly differentiated between TA209 and TA1198 (P<0.01): however, the diameter of the ovaries in both NILs is not significantly different (Table 2). This indicates that the two alleles of *fs8.1* are probably affecting growth in only one dimension (longitudinally). Field testing indicated that there was no difference between the NILs with respect to flowering date and fruit width (Table 3); however, the mean fruit weight, fruit length, harvest index, and number of fruits per inflorescence were significantly greater for TA209 than for TA1198. Taken together, these results suggest that *fs8.1* changes primarily the length of carpels during pre-anthesis, hence resulting in longer and larger mature fruit, which, in turn, increases total yield and gives a higher harvest-index ratio. The significant difference in the numbers of flowers (or fruits) per inflorescence in this study suggests that *fs8.1* has pleiotropic effects on these floral traits or that these traits might be affected by other genes that are tightly linked (<0.1 cM) to *fs8.1*.

To our knowledge this is the first time that a QTL for fruit shape has been fine-mapped and subjected to developmental studies in an isogenic background. Currently, it is not clear how many genes, and what molecular mechanisms, are involved in the determination of ovary and fruit shape in plants or when these factors exert their effect during the course of fruit development. A better understanding of the developmental and molecular basis of fruit-shape determination in tomato could be applicable to other domesticated plant species which display a similarly wide range of fruit sizes and shapes.

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