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A comparative analysis into the genetic bases of morphology in tomato varieties exhibiting elongated fruit shape

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Abstract Fruit shape is a quantitatively inherited character. In tomato, two major loci, *sun* and *ovate*, control fruit shape index, which is the ratio of fruit height over width. In this study, we measured many additional fruit shape features in three inter-specific F_2 populations using the software application Tomato Analyzer. These populations were derived from varieties carrying elongated fruit but for which the major shape loci differed. We compared the effect of the major fruit shape loci with overall shape, as well as with the distal and proximal end shape features in each population. *sun* and *ovate* represented the largest effect on fruit shape in the Howard German and Sausage F_2 populations, respectively. The largest effect QTL in the Rio Grande population carrying neither *sun* nor *ovate*, were *fs8.1* on chromosome 8 and *tri2.1/dblk2.1* on chromosome 2. These latter loci were also segregating in the other two populations, thus indicating common regions that control shape across the three populations. The phenotypic analyses showed that *sun* and *ovate* contributed to almost all aspects of shape such as the distal and proximal end features. In Rio Grande however, the largest effect QTL did not control all aspects of shape and the distal and proximal features were distinctly controlled in that population. Combined, our results implied that within the cultivated tomato germplasm pool the largest effect on elongated fruit shape was controlled by a combination of the loci *sun*, *ovate*, *fs8.1* and *tri2.1/dblk2.1*.

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Introduction

In contrast to the undomesticated relatives carrying spherical shaped fruit, cultivated tomato (*Solanum lycopersicum*) displays a large diversity in fruit morphology. The fruit shape of tomato varieties ranges from round to pear, heart, tapered, pointed and even bell pepper shaped. A common morphological feature distinguishing many cultivated varieties from undomesticated accessions is an elongated fruit shape. This attribute is often measured as the ratio of the fruit height to width and coined as "fruit shape index". With the development of a software program Tomato Analyzer, many additional fruit shape features, which are difficult to score by hand, are now accurately measured with this application. For example, the software precisely measures distal end angles at various positions along the boundary of the fruit allowing the researcher to distinguish the shape at this end from very pointed to round (Brewer et al. 2006). In addition, attributes such as triangular and heart shape, blockiness and angles at both proximal and distal end of the fruit are measured in segregating populations and these resulted in the identification of many QTLs controlling these attributes (Brewer et al. 2007). Thus, Tomato Analyzer provides the necessary tool for objective measurements of several fruit morphological features. As a

result, we are now well positioned to conduct reproducible and accurate phenotypic evaluations of the same attributes in different tomato populations. These results will allow us to compare the genetic bases of elongated fruit shape in the tomato germplasm in a consistent manner.

Previously, we conducted fruit morphological studies in three tomato populations derived from crosses between varieties carrying elongated tomato and a close wild relative *S. pimpinellifolium* LA1589 carrying round fruit (Brewer et al. 2007). In all the three populations, *sun* was the major locus controlling fruit elongation. Four genomic regions, on chromosomes 2, 3, 7 and 8, respectively, are found to control several shape attributes in all the three populations and/or explain the largest amount of variation within and between the populations. Other regions were found that control shape only in one population, which could explain the subtle differences in shape observed in the parental fruit (Brewer et al. 2007).

There is a tremendous amount of diversity in tomato fruit shapes especially among the heirloom types. These older open-pollinated varieties were handed down from generation to generation and are quite popular among home gardeners and organic farmers. The genetic basis of the morphological variation is not known for many of the old as well as modern varieties, however, it is anticipated that common and distinct loci as well as the interactions between the loci are likely to underlie the differences in morphology found in the germplasm. In addition to the major locus *sun*, another major locus that controls fruit elongation is *ovate*. Allelic variation at *ovate* typically conditions a pear-shaped and eccentric fruit in which the seeds are positioned towards the base of the organ compared to the central position of the seed in a round fruit (Ku et al. 1999; Liu et al. 2002; Van der Knaap et al. 2002). Tightly linked markers to these two shape loci allow us ascertain whether elongated shape is controlled by *sun*, *ovate*, both or neither in segregating F_2 populations. In addition, the F_2 populations can be studied for the control of other shape attributes, which may be common as well as unique in the studied varieties.

The main objectives of this project are to determine the genetic control of fruit shape in three tomato F_2 populations for which the major fruit shape locus differs. These three populations were derived from one cultivated type that harbors *sun* (Howard German), one that harbors *ovate* but the fruit is not pear-shaped like the previously studied varieties Yellow Pear and Long John (Sausage), and one that carries neither locus but exhibits an elongated fruit shape (Rio Grande). The goals were to identify common and unique loci that control shape in each population, and to elucidate the genetic basis of the elongated fruit shape in Rio Grande. In all, we seek to provide a comprehensive analysis of longitudinal shape variation in cultivated tomato varieties carrying oval shaped fruit, and to compare and contrast the loci identified.

Material and methods

Plant material

Three F_2 populations were constructed from crosses between one of three elongated *S. lycopersicum* cultivars (Howard German, Sausage and Rio Grande) and a wild species, *S. pimpinellifolium* accession LA1589 (Fig. 1). The data from the Howard German F_2 (HGF_2) population were reported by Brewer et al. (2007) and will only be used as comparison to the other two populations. The Sausage F_2 population (SAF_2) consisted of 106 plants grown in greenhouse during fall 2004. The 94 plants constituting the Rio Grande F_2 population (RGF_2) were grown in the same greenhouse in spring 2006. For all the three populations, eight representative fruit were harvested from each plant. Fruit were cut longitudinally and scanned at 300 dpi. Images were saved as JPEG files prior to phenotypic analyses with Tomato Analyzer as described in Brewer et al. (2006).

Phenotypic analysis

The Tomato Analyzer software program version 2.1.0.0, available at http://www.oardc.ohio-state.edu/vanderknaap/ was used for all phenotypic measurements. After making necessary adjustments to individual fruit in an image, analyses were conducted using the batch mode feature of the software application (Brewer et al. 2006). Fourteen attributes, segregating within populations by visual observation, were selected and analyzed. These attributes, listed in Table 1, included fruit shape attributes (fruit shape index,

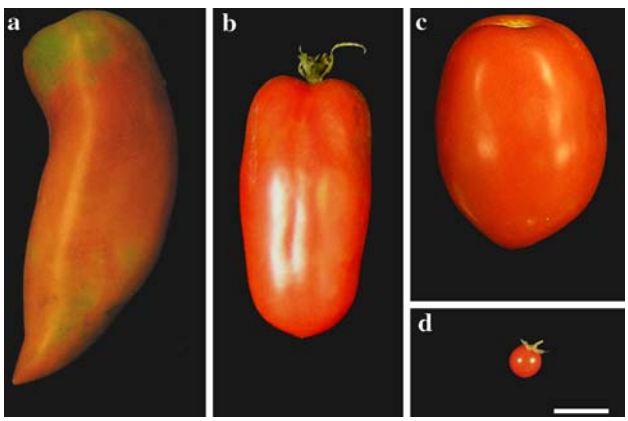

Fig. 1 Images of parental fruit. **a** *S. lycopersicum* cv. Howard German, **b** *S. lycopersicum* cv. Sausage, **c** *S. lycopersicum* cv. Rio Grande and **d** *S. pimpinellifolium* accession LA1589. *Size bar* represents 2 cm

Table 1 Mean fruit shape attribute values in Howard German, Sausage, Rio Grande and LA1589 parental fruit and the range of values in the respective F_2 populations

Trait category	Attribute[a]	Howard German	HGF_2	Sausage	SAF_2	Rio Grande	RGF_2	LA1589
Fruit shape	fs	2.27 (±0.23)	0.85–2.18	1.70 (±0.23)	0.85–1.50	1.35 (±0.04)	0.84–1.20	1.01 (±0.03)
	tri5	2.29 (±0.87)	1–3.07	1.43 (±0.31)	0.90–1.61	1.37 (±0.48)	1.08–2.35	1.11 (±0.16)
	tri20	1.70 (±0.29)	0.95–1.32	1.10 (±0.05)	0.85–1.17	1.17 (±0.02)	1.03–1.21	1.03 (±0.03)
	hrt	0.80 (±0.58)	0–1.24	0.69 (±0.26)	0–0.73	0.77 (±0.30)	0.11–0.71	0.07 (±0.06)
Distal fruit end shape	dblk5	0.31 (±0.06)	0.22–0.58	0.47 (±0.08)	0.35–0.54	0.39 (±0.03)	0.29–0.54	0.42 (±0.03)
	dblk20	0.62 (±0.112)	0.70–0.91	0.82 (±0.03)	0.76–0.86	0.76 (±0.01)	0.74–0.82	0.79 (±0.01)
	dan2	115 (±17)	94–296	165 (±19)	150–219	165 (±7)	144–205	171 (±8)
	dan5	61 (±18)	85–242	152 (±18)	136–184	145 (±4)	144–199	159 (±4)
	dan20	48 (±12)	53–121	54 (±14)	75–119	74 (±6)	83–119	110 (±6)
Proximal fruit end shape	pblk5	0.69 (±0.20)	0.43–0.68	0.66 (±0.07)	0.37–0.62	0.53 (±0.16)	0.53–0.60	0.46 (±0.04)
	pblk20	1.04 (±0.11)	0.78–0.96	0.90 (±0.02)	0.73–0.89	0.89 (±0.01)	0.84–0.92	0.82 (±0.02)
	psh	0.03 (±0.03)	0–0.06	0.03 (±0.01)	0–0.03	0.03 (±0.02)	0–0.03	0 (±0)
	pan	207 (±26)	176–209	207 (±7)	180–201	205 (±3)	180–200	180 (±0)
	piar	0.0041 (±0.0048)	0–0.014	0.0057 (±0.0024)	0–0.005	0.0051 (±0.001)	0–0.0052	0 (±0)

Values were obtained by Tomato Analyzer from four fruit of each parental type and eight fruit of each F_2 plant

[a] Trait acronyms associated with a number (tri, dblk, pblk) indicate the setting at which the width measurement was taken. For example, 5 = 5% (pblk), 95% (dblk) and both 5 and 95% (tri) from the proximal end. For distal end angle (dan), the number indicates the position along the boundary at which the slope was calculated. Values are given as the mean (±SD). The values observed in the F_2 represent the range of the average values obtained from the individual plants

triangle, and heart shape), distal fruit end shape attributes (blockiness and angle), and proximal fruit end shape attributes (blockiness, shoulder height, proximal angle, and indentation area). Fruit shape index (fs) was defined as the ratio of highest fruit height to widest width. Fruit shape triangle (tri) was measured as the ratio of the proximal end width to distal end width. The distal and proximal end widths were measured at two settings, 5 and 20% from both the distal and proximal ends of the fruit. Heart shape (hrt) is a function of the location of the maximum width, the shoulder height and the degree of tapering at the distal end. The angle of the distal fruit end (dan) was measured by determining the slope via regression along the boundary on both sides of the fruit. The angle was measured at the point where the lines intersected and was expressed in degrees, where 180° was flat, >180° was indented and <180° was pointed. The distal end angle was measured at three settings, which were 2, 5, and 20% from the tip of the fruit. Blockiness (dblk) was calculated as the ratio of the width close to the distal end of the fruit to the mid-width and was measured at both the 5 and 20% settings. Proximal end angle (pan), was measured where lines from the shoulder points to the site of the pedicel attachment intersect, where 180° is flat and >180° is concave. Blockiness at the proximal end of the fruit (pblk) was calculated as the ratio of the width closest to the proximal end of the fruit to the mid-width. The width closest to the proximal end was selected at 5 and 20% from the top of the fruit. The proximal end indentation area (piar) was measured as the ratio of the indentation area to the total fruit area. Shoulder height (psh) was calculated as the height of the shoulders of the fruit relative to the maximum fruit height. Additional details of the algorithms can be found in Brewer et al. (2006, 2007). The average values of the measurements were exported by Tomato Analyzer for further QTL analysis.

Genotypic analysis

Total genomic DNA was isolated from young leaves as described by Bernatzky and Tanksley (1986) and Fulton et al. (1995). The genetic maps were constructed with a combination of RFLP and PCR-based markers using MAPMAKER v3.0 and the Kosambi mapping function (Kosambi 1944; Lander et al. 1987). Additional information on RFLP and PCR-based markers, including map location and primer information can be found on the Solanaceae Genomics Network website (http://www.sgn.cornell.edu) and at http://www.tomatomap.net. The PCR-based markers were genotyped on CEQ8800 (Beckman Coulter) and Luminex200 (Luminex Corporation) at the Molecular and Cellular Imaging Center in Wooster, OH, USA. The molecular linkage maps for SAF_2 and RGF_2 contained 96 and 97 markers across the 12 tomato chromosomes, respectively. The maps spanned approximately 1,072 and 1,174 cM resulting in average marker distances of 11 and 12 cM, respectively for the SAF_2 and RGF_2 populations. The three populations showed distortion of segregation favorable for the *S. pimpinellifolium* allele at TG342 at the top of

chromosome 7 as previously reported (Brewer et al. 2007). In addition, segregation distortion was detected to be favorable for this allele at the top of chromosome 11 in both HGF_2 and SAF_2 populations near TG523, and on chromosome 12 near TG111. In the SAF_2 population, distortion was observed for the markers TG176 and SSR327 with an increase of the heterozygous class.

Statistical analysis

QTL analysis was performed by composite interval mapping (Zeng 1993, 1994) using model six with five marker cofactors selected by forward regression and a 10 cM window size, as implemented in Windows QTL Cartographer v2.5 (Wang et al. 2006). Permutation tests were conducted 1,000 times at a significance level of 0.05 to determine QTL threshold levels (Churchill and Doerge 1994). QTLs above the significance threshold determined by the permutation tests were considered significant. Additive and dominance effects and the fraction of the variance explained by the QTL (R^2) were estimated using Windows QTL Cartographer at highest probability peaks.

Results

Howard German (HG), Sausage (SA) and Rio Grande (RG) varieties carry elongated fruit that exhibited clear differences in morphology when compared to one another (Fig. 1). HG displayed an extremely elongated and pointed fruit at the distal end whereas SA carried rectangular and blocky fruit. RG fruit displayed a square fruit that was less elongated than the fruits from the other two varieties, and was slightly pointed at the distal end. The fruit of the wild relative LA1589 on the other hand was small and spherical with almost no noticeable shape features. We used Tomato Analyzer to measure 14 shape attributes on fruit from each parent (Table 1). For the attributes included in the fruit shape category, the most significant differences were found for fruit shape index. The largest fruit shape index was found in HG, followed by SA and RG with the smallest index found in LA1589. Furthermore, HG fruit tended to be more triangular shaped fruit than the other genotypes, which was reflected in the pointed and tapered fruit displayed by this variety. For the distal fruit end shape characters, distal end blockiness at 20% showed that HG was the least blocky (i.e. more tapered) whereas SA was the most blocky. The distal end angles at 2 and 5% from the tip of the fruit clearly differentiated HG from the other three genotypes, whereas the angle at 20% differentiated LA1589 from all the cultivated types. The distal end angle value at 20% reflected whether the fruit was elongated or round such that the smaller the angle, the more elongated the fruit. For the proximal end attributes, the values obtained for blockiness appeared to be largely similar amongst the cultivated genotypes. Also, the fruit of all the cultivated types were slightly indented at the proximal end compared to LA1589, which was reflected by the values for proximal angle and indentation area.

Genetic analysis of fruit shape attributes in three mapping populations

To determine the genetic basis for these similarities and differences, three F_2 populations derived from crosses between one cultivated parent shown in Fig. 1 and LA1589 were examined for variation in shape. Following the phenotypic analyses of the 14 shape attributes using Tomato Analyzer, we constructed genetic linkage maps and identified the QTL controlling the shape. Table 1 shows the range of the values found for each attribute in the populations, clearly demonstrating phenotypic variability for all 14 attributes.

A total of 20, 23 and 20 QTLs that controlled the 14 shape traits were identified in the HGF_2, SAF_2 and RGF_2, respectively (Tables 2, 3, 4). Overdominance was detected for only a few loci: *pblk2.1* and *pan2.1* in HGF_2 ($|D/A| > 1$, Table 2), and for *tri3.1*, *dblk5.1*, *dan2.1*, *dan5.1*, *pan3.1* in the RGF_2 (Table 4). When combining the overlapping locations, the mapping studies identified 8, 7 and 6 chromosomal regions in the HGF_2, SAF_2 and RGF_2 populations, respectively, that control at least one aspect of fruit shape (Tables 2, 3, 4; Fig. 2). QTLs that were found in all the three populations were located on the bottom of chromosome 2, the top of chromosome 8, and the bottom of chromosome 11. These regions are known to harbor fruit morphology QTL, notably *fw2.2* and *ovate* on chromosome 2, *fs8.1* on chromosome 8 and *f* on chromosome 11 (Fig. 2). The *fw2.2* locus controls fruit mass whereas *f* controls locule number (Frary et al. 2000; Barrero et al. 2006). In addition, population-specific regions were identified as well. We defined population-specific region when they harbor two or more overlapping QTL. HGF_2-specific regions were found on the top and bottom of chromosome 7, and on the top of chromosome 11. The analysis of a progeny test of the HG population confirmed the QTL controlling proximal end blockiness at 10 and 20% on the top of chromosome 11 (data not shown). SAF_2-specific regions were found on chromosome 1, and on the top of chromosome 12. RGF_2-specific regions were found on chromosome 3 and at the bottom of chromosome 5 (Fig. 2). In summary, when comparing the 13 fruit shape regions found in the three populations, we identified regions that were shared amongst the three populations whereas other regions carried population-specific shape QTL. The population-specific shape QTL may underlie genes that explain the subtle differences in shape seen in the parental fruit.

Table 2 List of QTL controlling fruit shape in the *S. lycopersicum* cv. Howard German × *S. pimpinellifolium* LA1589 F_2 population

Trait category	Attribute[a]	Permutation threshold[b]	Locus	Most significant marker	LOD	A^c	D^d	$R^{2\,e}$
Fruit shape	fs	3.8	*fs7.1*	COS103	34.4	0.30*	–	0.67
			fs8.1	TG176	5.4	0.08*	−0.04	0.07
	tri5							
	tri20	3.5	*tri11.2*	SSR80	3.7	−0.03	−0.03	0.14
	hrt	4.3	*hrt2.1*	TG337	4.5	0.06	−0.06	0.11
			hrt7.2	COS103	9.2	−0.11*	–	0.24
			hrt7.3	TG20	4.7	0.08*	−0.04	0.11
Distal end	dblk5	4.7	*dblk7.1*	COS103	6.7	−0.05*	–	0.32
	dblk20	3.9	*dblk7.1*	CT52	4.4	−0.03*	–	0.31
	dan2							
	dan5							
	dan20	3.7	*dan3.1*	TG242	5.6	−4.49*	−3.29	0.05
			dan7.2	COS103	37.2	−19.83*	–	0.7
			dan8.1	TG176	5.6	−5.54*	2.05	0.06
			dan11.1	TG36	3.7	−4.44*	−0.57	0.03
Proximal end	pblk5	3.6	*pblk2.2*	TG165	3.7	0.02*	0	0.11
			pblk2.1	TG537	5.7	0.02	−0.03*	0.16
	pblk20	3.4	*pblk2.1*	TG645	3.5	0.02*	0	0.12
	psh	5.4	*psh7.1*	COS103	10.5	−0.52*	–	0.3
	pan	3.7	*pan2.1*	TG537	3.7	1.23	−2.12*	0.09
			pan7.2	COS103	10.5	−3.47*	–	0.31
			pan7.3	TG20	3.7	2.12*	−1.1	0.11
	piar	7.1	*piar7.1*	COS103	7.6	−0.10*	–	0.21

[a] Trait acronyms associated with a number (tri, dblk, pblk) indicate the setting at which the width measurement was taken. For example, 5 = 5% (pblk), 95% (dblk) and both 5% and 95% (tri) from the proximal end. For dan, the number indicates the position along the boundary at which the slope was calculated. For example, 2 = 2% above the tip

[b] LOD threshold values for significant QTL by 1,000 permutations at $\alpha = 0.05$

[c] Additive effect. An asterisk (*) indicates a significant additive effect. A negative value indicates that an increase in the value of the attribute is due to the *S. pimpinellifolium* allele, and a positive value indicates that an increase in the value of the attribute is due to the *S. lycopersicum* allele

[d] Dominance effect. An asterisk (*) indicates a significant dominant effect. A negative value indicates that the *S. pimpinellifolium* allele is dominant and a positive value indicates that the *S. lycopersicum* allele is dominant. A dash (–) indicates that this value could not be accurately determined due to segregation distortion in this region

[e] Fraction of the phenotypic variance explained by the locus

Major fruit shape QTL

In two populations, HGF_2 and SAF_2, the largest fruit morphology QTL was found for the attributes fruit shape index and distal end angle at 20%. In the Howard German population, these traits were controlled by *sun* on chromosome 7 and *fs8.1* on chromosome 8, whereas in the Sausage population, these traits were controlled by *ovate* on chromosome 2, similar to HGF_2, *fs8.1* (Tables 2, 3; Fig. 2). Molecular analyses of the *sun* and *ovate* alleles indeed showed that HG carried the allele of *sun* that contributed to elongated fruit shape and not *ovate*, whereas Sausage carried the allele of *ovate* that contributed to elongated fruit shape and not *sun*. The *sun* QTL exhibited a LOD of 34.4 and explained 67% of the phenotypic variance for fruit shape index in the HGF_2 population. The *ovate* QTL exhibited a LOD of 20 and explained 41% of the phenotypic variance for fruit shape index in the SAF_2 population. The LOD and R^2 values for the distal end angle at 20% showed a similar trend as the fruit shape index in these populations. The locus *fs8.1* had a smaller effect on fruit shape index and distal end angle at 20% compared to *sun* and *ovate*, respectively, but nevertheless controlled fruit shape in these two populations (Tables 2, 3).

In the RGF_2 population, *sun* and *ovate* were not segregating (Table 4). Moreover, none of the traits were controlled by one major QTL that exhibited a substantial effect on the fruit shape in this population. Nevertheless, in the RGF_2 highly significant QTLs were detected for triangular shape at 5% and distal end blockiness at 5% and were

Table 3 List of QTL controlling fruit shape in the *S. lycopersicum* cv. Sausage × *S. pimpinellifolium* LA1589 F_2 population

Trait category	Attribute[a]	Permutation threshold[b]	Locus	Most significant marker	LOD	A^c	D^d	R^{2e}
Fruit shape	fs	7.5	*fs2.1*	TG645	20.0	0.16	−0.06	0.41
	tri5	3.5	*tri1.1*	SSR316	6.1	0.11	−0.05	0.21
	tri20	5.9	*tri2.2*	TG14	9.5	−0.02	0.02	0.36
	hrt	3.5	*hrt1.1*	SSR316	4.9	0.09	−0.03	0.11
			hrt2.1	TG645	7.8	−0.12	0.0004	0.20
Distal end	dblk5	3.5	*dblk2.1*	TG645	6.7	−0.02	0.01	0.18
			dblk11.1	TG36	4.9	0.02	−0.01	0.17
			dblk12.1	TG68	4.8	0.04	−0.01	0.16
	dblk20	3.7	*dblk11.1*	TG36	6.5	0.02	−0.01	0.20
	dan2							
	dan5	4.3	*dan2.2*	TG14	10.6	−3.8*	0.9*	0.44
	dan20	4.3	*dan2.1*	TG645	14.1	−8.7*	0.88*	0.30
			dan8.1	SSR327	7.0	−12.9*	2.72*	0.17
Proximal end	pblk5	3.5	*pblk1.1*	TG125	3.8	0.02	−0.01	0.11
			pblk2.1	TG645	16.4	−0.06	0.02	0.39
	pblk20	7.2	*pblk2.1*	TG645	14.3	−0.04	0.02	0.39
	psh	3.9	*psh1.1*	SSR316	5.0	0.42	−0.06	0.15
			psh2.1	TG645	7.2	−0.47	−0.04	0.20
	pan	3.5	*pan1.1*	SSR316	4.0	3.27*	−0.51*	0.13
			pan2.1	TG645	4.2	−2.91*	−0.59*	0.13
			pan12.1	TG68	3.7	2.91*	−2.87*	0.10
	piar	4.2	*piar1.1*	SSR316	4.7	−0.06	−0.02	0.14
			piar2.1	TG645	8.2	−0.08	−0.01	0.22
			piar9.1	TG291	4.5	−0.08	−0.06	0.17

[a] Trait acronyms associated with a number (tri, dblk, pblk) indicate the setting at which the width measurement was taken. For example, 5 = 5% (pblk), 95% (dblk) and both 5% and 95% (tri) from the proximal end. For dan, the number indicates the position along the boundary at which the slope was calculated. For example, 2 = 2% above the tip

[b] LOD threshold values for significant QTL by 1,000 permutations at $\alpha = 0.05$

[c,d,e] For descriptors, see Table 2 legend

controlled by an overlapping QTL close to *fw2.2* at a LOD of 9 and phenotypic variance of 25–35% explained by the locus. Similar to the HGF_2 and SAF_2 populations, fruit shape index and distal end angle at 20% was controlled by *fs8.1* in the RGF_2. The *fs8.1* locus exhibited a LOD of 6 and explained 29% of the variance for both the attributes in this population (Table 4). Fruit shape index was also controlled by a smaller QTL on chromosome 2, located between *ovate* and *fw2.2*. The *fw2.2* allele was segregating in the RGF_2 whereas *ovate* was not. Thus, the small effect fruit shape index, large effect triangular shape and distal end blockiness QTL found on chromosome 2 were either due to a pleiotropic effect of *fw2.2* or another linked gene.

One interesting aspect of fruit shape index in these three populations was indicated by the correlation of the traits. In the HGF_2 and SAF_2, fruit shape index was correlated to many other traits (Supplementary table S1). This was likely due to the major effect (high LOD and R^2) of *sun* and *ovate* loci respectively, in controlling fruit shape in these two populations. In the RGF_2 however, fruit shape index was not correlated to any of the attributes with the exception of distal end angle. This was likely due to the fact that the shape QTL were not of the same magnitude as *sun* and *ovate*, and suggested that the various shape attributes were controlled by distinct genes in the RGF_2 population. Moreover, since fruit shape index and triangular shape were not correlated (Supplementary table S1), it seemed unlikely that these traits were controlled by the same gene despite their QTL colocalization on chromosome 2.

QTL controlling shape attributes that differ between the cultivated parents

The major fruit shape differences between the parental varieties are exhibited by fruit shape index, which was controlled by a different major QTL, *sun*, *ovate* and *fs8.1* in the

Table 4 List of QTL controlling fruit shape in the *S. lycopersicum* cv. Rio Grande × *S. pimpinellifolium* LA1589 F_2 population

Trait category	Attribute[a]	Permutation threshold[b]	Locus	Most significant marker	LOD	A^c	D^d	R^{2e}
Fruit shape	fs	3.7	*fs2.1*	TG537	4.9	0.04	−0.02	0.19
			fs8.1	SSR327	6.0	0.06	−0.02	0.29
	tri5	3.3	*tri2.1*	fw2.2	9.0	0.13	−0.08	0.35
			tri3.1	T0581	4.1	0.05	0.09	0.13
	tri20	3.6	*tri2.1*	fw2.2	7.1	0.02	−0.01	0.26
	hrt	3.6	*hrt3.1*	TG134	4.1	0.08	0.03	0.15
Distal end	dblk5	3.7	*dblk2.1*	TG337	9.1	−0.02	0.01	0.25
			dblk5.1	LeOH73	6.4	−0.01	0.02	0.16
	dblk20	3.4	*dblk2.1*	TG337	4.8	−0.01	0.004	0.15
			dblk8.1	SSR327	4.1	0.01	0.004	0.13
	dan2	3.7	*dan2.1*	fw2.2	5.2	−5.53*	5.77*	0.19
	dan5	3.3	*dan2.1*	TG337	7.8	−5.22*	1.34*	0.21
			dan5.1	LeOH73	5.3	−2.73*	3.52*	0.13
	dan20	3.5	*dan2.1*	TG537	4.5	−3.68*	1.57*	0.16
			dan8.1	SSR327	7.0	−5.17*	2.63*	0.29
Proximal end	pblk5	3.6	*pblk3.1*	T0581	5.8	0.02	0.01	0.26
	pblk20	3.6	*pblk11.1*	CT55	4.2	0.01	0.01	0.18
	psh	3.6	*psh3.1*	TG134	5.2	0.48	0.23	0.19
	pan	3.6	*pan3.1*	LeOH223	4.2	−2.85*	3.20*	0.16
			pan3.2	TG134	5.3	3.23*	1.12*	0.20
	piar							

[a] Trait acronyms associated with a number (tri, dblk, pblk) indicate the setting at which the width measurement was taken. For example, 5 = 5% (pblk), 95% (dblk) and both 5% and 95% (tri) from the proximal end. For dan, the number indicates the position along the boundary at which the slope was calculated. For example, 2 = 2% above the tip

[b] LOD threshold values for significant QTL by 1,000 permutations at $\alpha = 0.05$

[c,d,e] For descriptors see Table 2

three populations, respectively. However, *fs8.1* was also a minor fruit shape index QTL in the HGF_2 population and a distal end angle at 20% QTL in the SAF_2 population (Tables 2, 3, 4). Other differences in the parental fruit were the degree of triangular shape, which in the three populations was controlled by different QTL: *tri11.2* in the HGF_2, *tri1.1* and *tri 2.2* in the SAF_2, and *tri2.1* and *tri 3.1* in RGF_2. With the exception of the latter two QTLs, the triangular shape QTL did not overlap with the largest fruit shape QTL in the respective populations and thus were not due to pleiotropic effects of the major shape QTL. On the other hand, for distal end blockiness, the region carrying the largest fruit shape index QTL was found to control this trait in each of the populations (Tables 2, 3, 4). In HGF_2 *dblk7.1* overlapped with *sun*, whereas *dblk2.1* in SAF_2 overlapped with *ovate*. However, in this latter population, distal end blockiness was also controlled by two other QTLs, *dblk11.1* and *dblk12.1* indicating that the control of this attribute was complex in this population. For the RGF_2 population, distal end blockiness was controlled by three QTL, two of which mapped to regions that contributed significantly to shape in this population, *dblk2.1* and *dblk8.1*. Lastly, for distal end angle at 2 and 5%, Howard German displayed the smallest angle compared to the other parental fruit (Table 1). Unfortunately, this attribute could not be mapped in the HGF_2 population suggesting the presence of small effect loci that were below the permutation threshold level. Indeed, QTL of significance just below the threshold level was found for distal end angle at 2 and 5% at both *sun* and *fs8.1* in this population (Brewer et al. 2007), which suggested that these loci control the shape of the very tip of the fruit.

The control of the three trait categories in different populations

The attributes were clustered in three categories: overall fruit shape, distal end shape and proximal end shape (Tables 1, 2, 3, 4). We determined whether different QTL controlled the shape features in these three categories in the populations. With the exception of triangular shape and proximal end blockiness, all traits that were scored in the

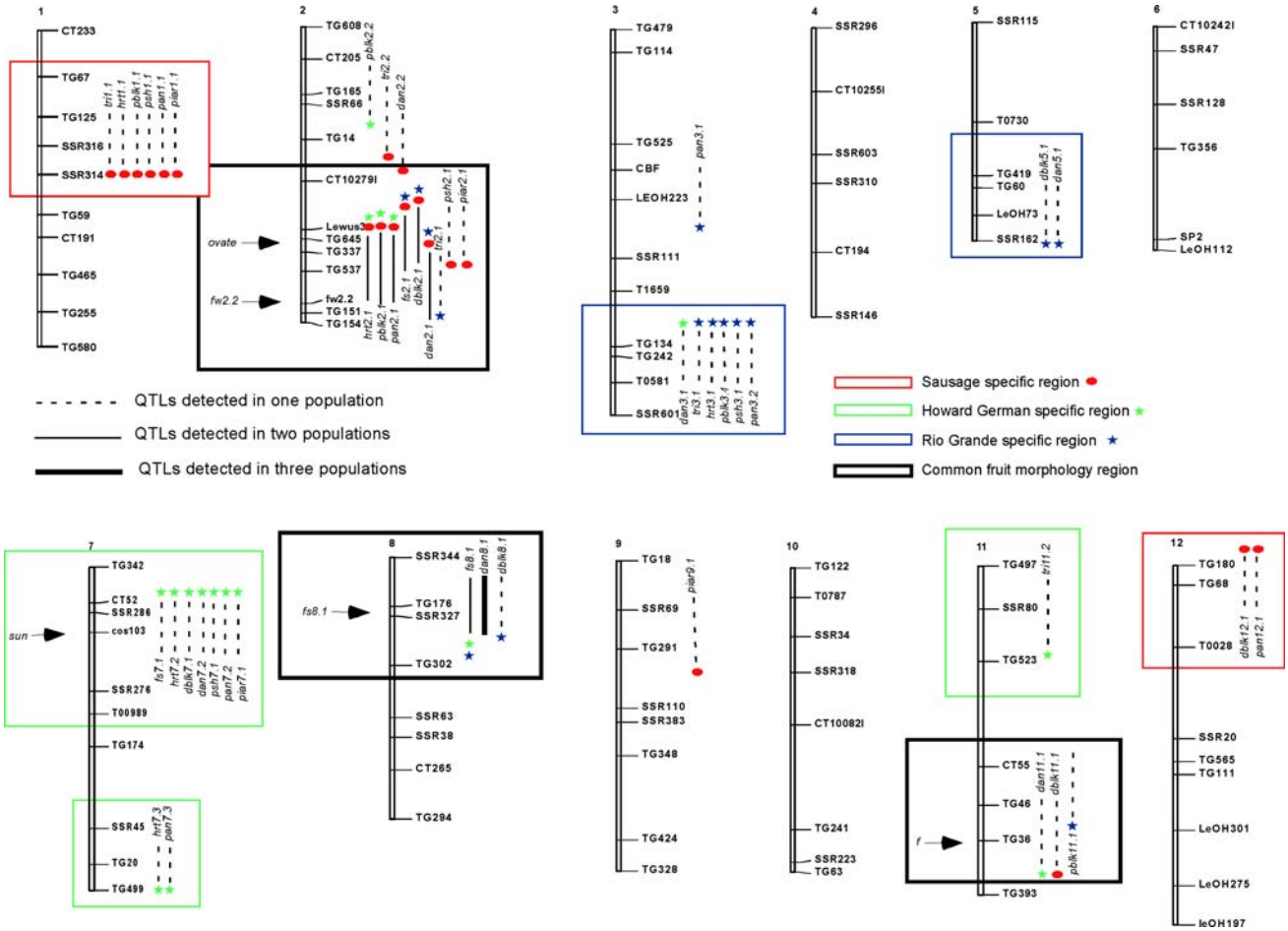

Fig. 2 QTL map position of 14 fruit shape attributes. Individual trait QTL, identified by composite interval analysis, is indicated to the right of each chromosome. QTL identified in all three populations are indicated by the *thick vertical line*; QTL identified in two populations are indicated by the *thin vertical line*; QTL identified only in one population are indicated by the *dashed line*. Population-specific regions and QTL are indicated by the *shaded box or symbols*, respectively. Known loci involved in fruit shape are indicated to the *left* of the corresponding chromosomes designated by an *arrow*

HGF_2 population were controlled, at least in part, by *sun* (Table 2). Similarly, with the exception of triangular shape, all traits that were scored in the SAF_2 were controlled in part by *ovate* (Table 3). This indicated that these major fruit shape loci controlled many aspects of shape. Therefore in essence, *sun* and *ovate* controlled both distal and proximal end shape categories in addition to overall fruit shape. On the other hand, in the RGF_2 population which did not show a QTL of the same magnitude as *sun* and *ovate*, fruit shape traits were controlled by multiple QTL. Overall fruit shape loci were found on chromosomes 2, 3, and 8. Distal end features on the other hand were controlled by loci on chromosomes 2, 5 and 8 and not on 3, whereas proximal end features were controlled by loci on chromosomes 3 and 11 and not on 2, 5 and 8 (Table 4). These results implied that the different parts of the fruit, the distal versus the proximal end, were controlled by different genes in the Rio Grande parent. This notion was further supported by the lack of correlation of fruit shape index and most other attributes (see above, Supplementary Table S1). The detection of minor QTL that controlled a specific aspect of shape in the RGF_2 population was probably due to the fact that RG did not carry the shape loci of extreme large effect, such as *sun* and *ovate*. Moreover, this result also implied that large effect QTL tended to control many attributes of shape because of their dominance in controlling overall morphology.

Discussion

In this study, we compared the genetic bases of longitudinal shape variation in three tomato populations. The common feature of these three populations was that one of the parental lines in each population carried elongated fruit (Fig. 1). However, the major fruit shape index QTL controlling fruit

elongation differed between the three populations. Prior research showed that HG carries *sun* (Brewer et al. 2007). The results from this research showed that SA carried *ovate* (Table 3). The *sun* and *ovate* loci exerted a major effect on fruit shape such that these loci controlled most of the attributes. It is likely, however, that these major loci masked the effect of other loci. The *ovate* and *sun* alleles were not present in RG and therefore, elongated fruit shape in this variety was differently controlled. The results from this study showed that fruit shape index was controlled by *fs8.1* and to a smaller extent by *fs2.1* (Table 4). Moreover, the major shape QTL controlling triangular shape and distal end blockiness mapped to chromosome 2 also, even though these QTL were not likely allelic with *fs2.1* because of the lack of correlation between these traits. On the other hand, an increase in triangular shape and a decrease in distal end blockiness signified a slightly tapered and elongated fruit and hence an increase in fruit shape index, suggesting allelism in the control of the three traits. The finding of *fs8.1* as a major fruit shape locus was not surprising considering that this locus is found in other studies (Grandillo et al. 1996; Ku et al. 2000). However, the lack of a marker for *fs8.1* required the undertaking of a linkage mapping study to unequivocally determine whether *fs8.1* is present in a given variety. Combined, the mapping studies demonstrated that the elongated allele of *fs8.1* was found in HG, SA and RG. In addition, this allele is also present in Banana Legs (BL), a variety carrying elongated fruit controlled by *sun* (Brewer et al. 2007) and E6203 a processing line carrying slightly elongated and blocky fruit (Grandillo et al. 1996). Thus it is reasonable to propose that the elongated allele of *fs8.1* is present in many elongated varieties. However, *fs8.1* also segregates in populations derived from round-fruited varieties such as Yellow Stuffer (Van der Knaap and Tanksley 2003). In this study, the *fs8.1* locus controls the uneven and segmented shape of this bell pepper tomato variety. Therefore, *fs8.1* appears to segregate in both round and elongated varieties and exhibits a pleiotropic role in fruit morphology.

The morphological analysis using Tomato Analyzer permitted us to measure the same fruit shape attributes in different tomato populations. The results showed three regions of the genome that were shared amongst the populations, chromosome 2, 8 and 11. In another study that used Tomato Analyzer for the phenotypic evaluation of fruit shape, the common regions were found at chromosomes 2, 3, 7 and 8 (Brewer et al. 2007). Therefore, the loci on chromosomes 2 and 8 were shared in both the studies. The shared region on chromosome 7 was unique to the aforementioned study, since all three populations were segregating at *sun*, and thus all showed significant QTL at that locus (Brewer et al. 2007). In this study, *sun* was only segregating in the HGF_2 and therefore represented a population-specific region when compared to the other two populations, SAF_2 and RGF_2. The shared locus on chromosome 3 in Brewer et al. (2007) was found to be population-specific in this study. However, the RGF_2 population-specific region on chromosome 3 also carried a distal end angle QTL present in the HGF_2 (Fig. 2). Moreover, the QTL *tri3.1* and *pblk3.1* segregate in the BLF_2, in addition to few other shapes QTL just below the threshold level of significance (data not shown; Brewer et al. 2007). Therefore, this chromosome 3 QTL was most likely segregating in several populations studied and thus was not specific to RG.

We noted that very few individual traits QTL overlapped between the three populations, with the exception of the shared regions on chromosomes 2 and 8 (solid lines in Fig. 2). The other regions feature populations-specific QTL, which would explain the lack of overlap of attribute-specific QTL across these three populations. When comparing the population-specific regions identified herein with those identified in other studies, some shape alleles were likely to be shared among the varieties. The SAF_2-specific region on chromosome 1 was also found in the BLF_2 (Brewer et al. 2007). The overlapping QTLs were *hrt1.1* and *psh1.1*. Interestingly, both varieties exhibited similar fruit shapes such as a blocky and squared proximal fruit shape even though the major shape QTL differ. Thus, the similarity in shape of BL and SA fruit may be controlled by a shared *hrt1.1* and *psh1.1* QTL, which would affect the proximal shape (Brewer et al. 2007; Fig. 1). In addition, the SAF_2 population-specific locus on chromosome 12 also controls shape in a HG backcross population (Brewer et al. 2007), although the attribute QTLs are not the same (*fs* and *dan* in HG; *dblk* and *pan* in SA). Nevertheless, the result suggests that a common shape QTL on chromosome 12 segregates in both SAF_2 and $HGBC_1$. The RGF_2-specific chromosome 5 QTL, *dblk5.1* overlapped with *dblk5.1* just below the threshold level in the HGF_2 population (Brewer et al. 2007). Thus, this QTL might also be shared amongst the HGF_2 and RGF_2. In summary, when comparing the 13 fruit shape regions found in the HGF_2, SAF_2, RGF_2 and the BLF_2 populations (Brewer et al. 2007 and this study), we identified regions that were shared among three to four populations and included the bottom of chromosome 2, the bottom of chromosome 3, top of chromosome 8 and bottom of chromosome 11. Additionally, other regions were shared only amongst two populations whereas few regions exhibited population-specific shape QTL. However, the lack of sufficient power to detect QTL caused by the low heritability may be the reason why some of the regions appear population-specific. Environmental effects are also likely to affect shape. Since the three populations were not grown at the same time, the differences in the environment could affect QTL detection.

By scoring fruit shape index and triangular shape in the segregating populations, we obtained highly significant

QTL that controlled elongated fruit shape in all three populations. These loci, in order of significance, are *sun* on chromosome 7, *ovate* on chromosome 2, *tri2.1/dblk2.1* on chromosome 2 and *fs8.1* on chromosome 8. It is very likely that these four loci are the key loci controlling elongated fruit shape in the germplasm. It is clear that the loci *ovate* and *sun* are segregating in some cultivated varieties and therefore represent loci that can be selected for improvement of the cultivated germplasm pool. On the other hand, *fs8.1* may be present in many if not in all varieties and thus may have been selected very early during domestication of tomato. The triangular shape, distal end blockiness and fruit shape index QTL found on chromosome 2 could overlap with *fw2.2* or represent distinct loci. Further fine mapping is needed to determine the linkage of these QTL with *fw2.2*. Thus the genetic control of elongated fruit shape in the tomato germplasm is largely due to a few major loci. However, the interactions between these major QTL and with minor QTL are likely to play an important role in the final shape displayed by each of the varieties.

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