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Single and joint effect of the basal region of chromosome 2 and centromeric region of chromosome 8 on morphological and fruit quality traits in tomato

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Abstract The goal of this study was to estimate the single and joint effect of the basal region of chromosome 2 and centromeric region of chromosome 8 on morphological and fruit quality traits in tomato (*Solanum lycopersicum*). The analysis was performed in a population derived from a cross between Rio Grande of *S. lycopersicum* and LA1589 of *S. pimpinellifolium* that segregates for both genomic regions. Four major QTLs were found on chromosome 2 and three on chromosome 8, all of them related with morphological traits. QTLs for fruit shape index, proximal fruit end angle and distal fruit end protrusion showed epistatic interaction. Both genomic regions (*fs2.1* and *fs8.1*) explained 62, 47 and 46 % of the phenotypic variability for fruit shape index, proximal fruit end angle and distal fruit end protrusion respectively. Minor QTLs were detected for other morphological and quality traits such as color, pH and fruit shelf life on chromosomes 2 and 8. Only single genomic region effects were found for quality trait. On the other hand, *fs2.1* and *fs8.1* regions control several fruit morphology attributes following a digenic linear additive model with epistatic interactions.

Keywords Epistasis · Fruit color · Fruit shape · *fs2.1* · *fs8.1* · Titratable acidity

Abbreviations

a/b	Color chroma index
ANOVA	Analysis of variance
ar	Fruit area
BC_1	First cycle of backcross
CAPS	Cleaved amplified polymorphic sequences
dan20	Distal fruit end angle
dblk20	Distal fruit end blockiness
f	Firmness
F_3	Third filial generation
fs I	Fruit shape index
fw	Fruit mass
H^2	Broad sense heritability
InDel	Insertion/deletion
L	Lightness color parameter
LL	Homozygous for Rio Grande alleles
LP	Heterozygous
pan20	Proximal fruit end angle

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pblk20	Proximal fruit end blockiness
pH	Hydrogen potential
PP	Homozygous for LA1589 alleles
QTL	Quantitative trait *loci*
S_1	First selfed generation
sl	Fruit shelf life
ssc	Soluble solid content
ta	Titratable acidity
tip	Distal fruit end protrusion
tri20	Fruit shape triangle

Introduction

Fruit shape in tomato defines the destination of the production and is recognized as being the second most important attribute for consumers after price (Simonne et al. 2006). Elongated tomatoes are usually used in processed or manufactured products while rounded fruits are most frequently used for fresh consumption. A large diversity of shapes are present in the cultivated tomato (*Solanum lycopersicum*) in contrast to wild genotypes which produce spherical and small-size fruits (Rodríguez et al. 2011). Four major QTLs have been associated with elongated fruits within the cultivated tomato germplasm: *ovate*, *sun*, *fs8.1* and *fs2.1* (Gonzalo and van der Knaap 2008). The *SUN* and *OVATE* genes have been cloned by positional mapping (Liu et al. 2002; Xiao et al. 2008) while the existence of the other two *loci* is known through mapping studies. Less than 10 *loci*, located in 7 of the 12 chromosomes, have been associated with most of the changes in the size and shape of tomato fruit (Tanksley 2004).

Fruit shape index (*fs I*, ratio of maximum height to maximum width) is an easy-measurement morphological character to differentiate some cultivars (Grandillo et al. 1999). The Rio Grande cultivar of *S. lycopersicum* carries the wild alleles at *SUN* and *OVATE* genes (Rodríguez et al. 2011) and the *fs I* morphological parameter is controlled by *fs8.1* and *fs2.1* (Gonzalo and van der Knaap 2008). Both *fs2.1* and *fs8.1* explained the 19.00 and 29.00 % respectively of the phenotypic variability of *fs I* in a F_2 population derived from a cross between Rio Grande and LA1589 of *S. pimpinellifolium* (Gonzalo and van der Knaap 2008). *fs8.1* is located close to the centromeric region of chromosome 8 and has been mapped in a region of 3.03 Mb (Sun et al. 2015). *fs8.1* exert their effect on fruit shape at least 6 days before anthesis, probably affecting fruit growth only in the longitudinal dimension (Ku et al. 2000). *fs2.1* is located in the distal region of chromosome 2 which shows a high-recombination frequency (Grandillo and Tanksley 1996; Lippman and Tanksley 2001). *fs2.1* and *fs8.1* were also detected in a backcross population derived from M82-1-7 of *S. lycopersicum* and LA1589 of *S. pimpinellifolium*. In that cross *fs8.1* explained the 27.40 % and *fs2.1* 5.70 % of the phenotypic variability for the trait (Grandillo and Tanksley 1996).

Several QTLs associated with fruit quality and yield traits have been identified in the same genomic regions that control fruit shape in Rio Grande (*fs8.1* and *fs2.1*). For example, the *fs8.1* region affects the number of flowers and fruits per inflorescence and the harvest index. These result suggest pleiotropic effects on these floral traits or may be that these traits could be affected by closely-linked genes (Ku et al. 2000). This same region has been associated with other traits such as fruit shelf life (Pereira da Costa et al. 2013), pericarp thickness (Grandillo and Tanksley 1996), carotene content and volatile compounds (Saliba-Colombani et al. 2001).

The *FW2.2* gene which controls fruit weight is located in the basal region of chromosome 2 (Frary et al. 2000). Also in this region, QTLs for fruit firmness, *pH*, soluble solid content (Bernacchi et al. 1998), *L*, *a* and *b* indexes of color, titratable acidity, sugar content, carotene content, volatile compounds (Saliba-Colombani et al. 2001) and pericarp thickness (Grandillo and Tanksley 1996) have been detected.

The goal of this work was to analyze the single and joint effect of genomic regions containing *fs2.1* and *fs8.1 QTL*s on morphological and fruit quality traits in a population derived from the cross between Rio Grande of *S. lycopersicum* and LA1589 of *S. pimpinellifolium*.

Materials and methods

Plant material

The commercial cultivar Rio Grande of *S. lycopersicum* and the wild accession LA1589 of *S. pimpinellifolium* were used as parental genotypes. The cultivated genotype has a large elongated fruit usually used for fresh

consumption and processed products and was obtained in a tomato-breeding program in the United States of America (http://www.solgenomics.net). The wild genotype, LA1589 has small rounded fruits and is originally from Peru (http://www.solgenomics.net). The molecular characterization of the F_2 population conducted by Gonzalo and van der Knaap (2008) allowed selecting that plant with higher proportion of cultivated tomato alleles covering the genome and as heterozygosity genotype in the regions spanning both the *fs.2.1* and *fs8.1 loci*. Using markers Lewus, TG337 and EP170/EP171 at chromosome 2 and TG176, TG45 and EP912/EP913 at chromosome 8 (Table 1), only one F_3 plant heterozygous at both genomic regions were backrossed to Rio Grande as the recurrent parent. Backcross and selfing cycles were done using forward (markers shown in Table 1) and background markers (along the genome) to obtain a F_3–BC_1–S_1 population segregating for distal end of chromosome 2 and centromeric region at chromosome 8.

Ten plants of each parental genotype and 128 plants of a segregating population F_3–BC_1–S_1 were transplanted into greenhouse at a distance of 1 m between rows and 40 cm between plants. Tomato plants were stringed vertically from a top-wire.

Phenotypic analysis

Six fruits from different trusses of each plant were longitudinally cut through the center, placed cut side-down on a scanner and scanned at 300 dots per inch (dpi). The fruit images were saved as jpeg files and imported into Tomato Analyzer 3.0 for automated phenotypic measurements (Rodríguez et al. 2010a). The analyzed attributes of each fruit were: fruit area (*ar*, in cm^2), fruit shape index (*fs I*, ratio of maximum height to maximum width), proximal fruit end blockiness (*pblk20*, ratio of the width at 20 % of the perimeter from the proximal end to width mid-height), distal fruit end blockiness (*dblk20*, ratio of the width at 20 % of the perimeter from the distal end to width mid-height), fruit shape triangle (*tri20*, ratio between proximal width and distal width both taken at 20 % of the perimeter from the proximal and distal end respectively), proximal fruit end angle (*pan20*, the angle between best-fit lines drawn at 20 % of the perimeter from the proximal end at both sides), distal fruit end angle (*dan20,* the angle between best-fit lines drawn at 20 % of the perimeter from the distal end at both sides) and the distal fruit end protrusion (*tip*, ratio of the area of the distal protrusion to the total area of the fruit, multiplied by 10).

Seven fruits per plant were harvested to assess fruit mass (*fw*, in grams) and shelf life (*sl*, in days). The *sl* trait was evaluated as the elapsed days between the harvest and the discard of the fruit. The harvested fruits were stored at 25 ± 3 °C on a shelf and were examined three times a week discarding those commercially unacceptable (Buescher et al. 1976; Schuelter et al. 2002). Another six fruit per plant were used to

Table 1 Molecular markers' description

Type	Name	Chr	Enzyme	RG allele (bp)	LA allele (bp)	Primer	Sequence 5′ –> 3′
InDel	Lewus	2	–	210	222	Forward	TGGAACTTTGGCTATGGAGAA
						Reverse	TGGTGAAGAAAATGTTGTTTTGAT
	EP170/EP171	2	–	169	179	Forward	CACATCTTACGATTATTGGGGTAA
						Reverse	TGTGCACACATCTTAACAAATCA
	EP912/EP913	8	–	180	195	Forward	TGATGTCACTGGGCATCTTC
						Reverse	GACAAATTCCTGAGCTTACTGC
CAPS	TG337	2	Hind III	1500	1000/500	Forward	GCAAAGCATCATCACCAATG
						Reverse	ATTATGGGCCACACGCAATA
	TG176	8	Rsa I	234	325	Forward	AGTAATAGCACTGCCCCACA
						Reverse	TTCGGCAAGTTTAGCCAAATA
	TG45	8	Dde I	98	78	Forward	AGCGGAACTTGTCATCCATC
						Reverse	TGAGTGGCCATTTTTAAATGCCTC

Chr chromosome, *InDel* insertion/deletion, *CAPS* cleaved amplified polymorphic sequence, *RG* Rio Grande of *S. lycopersicum, LA* LA1589 of *S. pimpinellifolium*

assess firmness (*f*) measured on the equatorial plane in two opposite areas of each fruit with a 0.10 cm^2 tip durometer (Durofel DFT100). Color was measured as the percentage of reflectance (*L*) and the chroma index (*a/b*) using a CR300 colorimeter. Soluble solids content (*ssc*, percentage of glucose plus fructose, in °Brix) and *pH* were measured from juice with a refractometer and a pH meter respectively. Titratable acidity (*ta*, grams of citric acid per 100 g of juice), was calculated as the necessary volume of sodium hydroxide to turn the pH of a 10 % m/m homogenate juice to 8.1.

Genetic analysis

Genomic DNA was extracted from young seedlings using the Wizard Genomic commercial kit (Promega, USA) following the manufacturer's protocol. InDel and CAPS molecular markers were used. The marker sequences and specific restriction endonucleases used are shown in Table 1. Electrophoresis of molecular markers TG176, TG45 and EP912/EP913 was conducted on 3 % w/v agarose gels, while TG337 was run on a 1 % w/v agarose gel. Gels were stained with ethidium bromide for visualization. PCR products for Lewus and EP170/EP171 were separated on 6 % w/v polyacrilamyde gel and visualized by silver staining procedure.

Statistical analysis

The normal distribution of evaluated traits in the parental genotypes and the segregating population was verified by the Shapiro–Wilk test (Shapiro and Wilk 1965). The *t* test (Snedecor 1964) or Kruskal–Wallis test (Kruskal and Wallis 1952) was applied for comparison of mean values in the parental genotypes. Pearson correlation coefficients were estimated among all traits.

In the segregating population, the broad sense heritability (H^2) of all traits was estimated through an ANOVA (Mariotti and Collavino 2014) and histograms of frequency were also performed.

The single point method (Tanksley 1993) was used to study the association between molecular markers and attributes. All these statistical analyses were carried out with InfoStat software Version 1.0 (Di Renzo et al. 2001). The d/a parameter of the additive-dominant model was calculated for those traits in which significant association was detected with one or more molecular markers and when significant differences between homozygous genotypes mean values was detected (Tanksley 1993). If complete dominance was not detected, a confidence interval of 95 % for the average of the heterozygous genotype was calculated. If the mid-point value was within the range of the interval of confidence, an additive gene action was assumed, otherwise partial dominance.

When both genomic region were associated with the same phenotypic trait, a digenic linear additive model was tested by a Chi square test (Snedecor 1964). The free software environment R (R Core Team 2014) was used to perform a regression analysis between the most significant markers and the associated attribute. Genotype mean value within each QTL were plotted in order to facilitate the data visualization. The average value (m) and additive effect (a_i) for a linear additive model were estimated for each trait. The expected phenotype values at different genotypes for a trait (E_{ph}) were estimated based on a linear additive model that includes the additive effect (a) in each genomic regions (*fs2.1* and *fs8.1*):

$$E_{ph} = m + a_{fs2.1} + a_{fs8.1}$$

A two-way analysis of variance (ANOVA) was used to assess the existence of epistatic interactions between genomic regions. For this analysis, one molecular marker per region was chosen for having the higher R^2 value for the trait. Orthogonal contrasts were applied to determine the type of genic interaction following the methodology proposed by Jana (1972).

Finally, a regression plot between the expected values and the observed phenotypic values was performed only for traits with significant epistatic effects. These statistical analyses were carried out with InfoStat software Version 1.0 (Di Renzo et al. 2001).

Results and discussion

Phenotypic analysis in the parental genotypes and the segregating population F_3–BC_1–S_1

Rio Grande exhibited significant differences in morphology when compared with LA1589. Fruits from RG were classified as "heart-shape" by Rodríguez et al. (2011) and clustered with accessions that show rectangular fruit and does not show the cultivated

alleles at *SUN*, *OVATE*, *FAS* and *LC* genes. In contrast, fruits from the wild parental genotypes LA1589 are small (less than 1 g weight) and spherical.

Significant differences ($P < 0.05$) between parents were found for each morphological trait and fruit quality traits except for *pH* (Table 2). Mean values for *ar*, *fs I*, *pblk20*, *tri20* and *tip* were higher in Rio Grande than in LA1589. These results can be explained by the size and shape of the cultivated fruit. Rio Grande has large elongated (*fs I* > 1.00) fruits which are also blocky-shaped at the upper side and sometimes have a tip at the lower end (*tip* value 0.01 ± 0.01). Mean values from the wild parent were higher than the mean values from the cultivated parent for *ta*, *ssc*, and *a/b*.

Several studies (Rodríguez et al. 2006, 2010b, Pratta et al. 2011, Pereira da Costa et al. 2013) have reported that some wild accessions (e.g., LA722 of *S. pimpinellifolium* or LA1385 *S. lycopersicum* var. *cerasiforme*) have better phenotypic values for quality traits such as *ssc* an *ta* than cultivated genotypes (e.g., cv. Caimanta of *S. lycopersicum*). Also, Georgelis et al. (2006) have reported that the PI 270248 accession of *S. lycopersicon* var. *cerasiforme* has approximately 43 % more of soluble solids than the 7833 accession of *S. lycopersicum*. All these reports demonstrated the superiority of the wild genotype for those traits compared with cultivated tomatoes. Rio Grande shows higher mean values for *sl*, *f* and *L* than LA1589 (Table 2). In contrast, other reports have shown that wild genotypes are usually more resistant to postharvest deterioration than cultivated genotypes (Rodríguez et al. 2006, 2010b, Pereira da Costa et al. 2013).

Phenotypic distributions for the evaluated traits in the segregating population are shown in the Fig. 1. Each distribution includes the mean value for both parental genotypes indicated by arrows. Most of traits showed normal distribution with exception for *tip* and *ta*. Some traits also showed transgressive segregation such as *pblk20*, *dblk20, tri20, tip, sl, f, pH and ta*

Table 2 Mean values of all analyzed traits in Rio Grande of *S. lycopersicum* and LA1589 of *S. pimpinellifolium*. Broad sense heritability values (in percentege) for all traits analyzed in the segregating population F_3–BC_1–S_1

Category	Trait	Rio Grande	LA1589	F_3–BC_1–S_1	
		Mean ± SE	Mean ± SE	% H^2 ± SE	*P* Value
Morphology	*ar*	25.25 ± 0.74[a]	0.75 ± 0.03[b]	87 ± 2	<0.0001
	fs I	1.35 ± 0.02[a]	1.01 ± 0.02[b]	73 ± 3	<0.0001
	pblk20	0.68 ± 0.04[a]	0.63 ± 0.01[b]	47 ± 3	<0.0001
	dblk20	0.55 ± 0.04[b]	0.59 ± 0.00[a]	54 ± 3	<0.0001
	tri20	1.26 ± 0.02[a]	1.08 ± 0.01[b]	41 ± 3	<0.0001
	pan20	87.15 ± 1.14[b]	110.54 ± 2.77[a]	68 ± 3	<0.0001
	dan20	81.99 ± 1.62[b]	104.99 ± 2.48[a]	59 ± 3	<0.0001
	tip	0.01 ± 0.01[a]	0.00 ± 0.00[b]	nc	
Quality	*fw*	76.44 ± 13.71[a]	0.59 ± 0.05[b]	80 ± 2	<0.0001
	sl	13.17 ± 3.17[a]	9.71 ± 0.54[b]	52 ± 2	<0.0001
	f	61.51 ± 3.24[a]	50.88 ± 1.03[b]	58 ± 3	<0.0001
	L	44.58 ± 1.14[a]	37.77 ± 0.29[b]	53 ± 3	<0.0001
	a/b	0.97 ± 0.06[b]	1.42 ± 0.03[a]	53 ± 3	<0.0001
	ssc	4.67 ± 0.22[b]	14.57 ± 3.07[a]	92 ± 3	<0.0001
	pH	4.71 ± 0.22[a]	4.75 ± 0.04[a]	77 ± 6	<0.0001
	ta	0.35 ± 0.09[b]	1.00 ± 0.27[a]	nc	

SE standard error, % H^2 broad sense heritability in percentage, *P-value* probability of type I error (α), *ar* fruit area, *fs I* fruit shape index I, *pblk20* proximal fruit end blockiness, *dblk20* distal fruit end blockiness, *tri20* fruit shape triangle, *pan20* proximal fruit end angle, *dan20* distal fruit end angle, *tip* distal fruit end protrusion, *fw* fruit weigh, *sl* fruit shelf life, *f* firmness, *L* color parameter, *a/b* chroma index, *ssc* soluble solid content, *ta* titatrable acidity, nc not calculated. Different letters indicate significant differences ($P < 0.05$)

(Fig. 1). The presence of complementary alleles between parents could explain the transgressive segregation found in this population for these traits. Genetic variability for all traits was demonstrated through % H^2 values which were all highly significant (Table 2). The highest % H^2 values for fruit morphology attributes were found for *ar* and *fs I* and for some fruit quality traits such as *fw*, *ssc* and *ta*. The % H^2 values for *tip* and *ta* were not calculated because they do not adjust to a normal distribution.

Significant ($P < 0.05$) phenotypic correlations were observed among morphological traits such as *fsI*, *dblk20*, *tri20*, *pan20*, *dan20* and *tip* (Table S1). An expected positive correlation was observed between *ar* and *fw* ($r = 0.92$). On the other hand, negative correlation between color parameters *L* and *a/b* was observed and between fruit area and soluble solids content.

Detection of QTLs and gene action (d/a)

A total of 17 QTLs were detected by mean of single point analysis. Eleven QTLs were located on chromosome 2 and six on chromosome 8 (Table 3). Major QTLs (% R^2 higher than 20 %) for *fs I*, *dblk20*, *tri20* and *tip* were detected on chromosome 2 and for *fs I*, *pan20* and *dan20* on chromosome 8. Minor QTLs (% R^2 lower than 20 %) for *ar*, *pblk20*, *pan20*, *dan20*, *pH*, *a/b* and *L* were found on chromosome 2 and three QTLs for *ar*, *tip* and *sl* on chromosome 8. Partial or complete dominance of the wild alleles was generally observed for morphological traits and an additive gene action for quality traits (Table 3).

Although both assessed genomic regions were associated with distal fruit end traits, the % R^2 values of QTLs in *fs2.1* were higher than those QTLs in *fs8.1*. The distal part of chromosome 2 has stronger associations with *tip* and *tri20* attributes, and the last attribute was significantly associated with molecular markers in *fs2.1* (Table 3). These results are consistent with those reported by Gonzalo and van der Knaap (2008) who found QTLs on chromosome 2 for *tri20*, *dblk20* and *dan20* in the F_2 population derived from Rio Grande and LA1589 cross. On the other hand, *fs8.1* seems control both proximal and distal morphological attributes because it has a highly significant effect on *pan20* and *dan20*. Ku et al. (2000) concluded that the region containing *fs8.1* affects fruit growth in the longitudinal dimension and in this work similar results were found.

Several minor QTLs were detected for fruit quality traits in this segregating population. A minor QTL on chromosome 8, which had been associated with EP912/EP913 marker, explained 5.62 % of the phenotypic variation observed for *sl* ($P = 0.03$; Table 3). The presence of the wild alleles improved this trait. As it was mentioned before, complementary long shelf life alleles carried by the parental genotypes could explain the transgressive segregation found in this population for this trait (Fig. 1). The presence of a QTL for fruit shelf life on chromosome 8 was also reported by Pereira da Costa et al. (2013) and explained the 18 % of the phenotypic variability observed in BC_1-S_1 families derived from the cross between Caimanta and LA722 of *S. pimpinellifolium*.

Another minor QTL for *pH* was detected on chromosome 2 (EP170/EP171 marker) and explained the 6.45 % of the observed phenotypic variance (Table 3). The d/a parameter could not be calculated for this trait because no difference was observed between homozygous mean values. Despite this fact, overdominance (mean value for the heterozygous genotype is higher than for both homozygous ones) can be seen for this trait in Table 3. This result agrees with that reported by Bernacchi et al. (1998), who detected a minor QTL on chromosome 2 that explained the 10 % of the phenotypic variability for *pH* in advanced backcross between *S. habrochaites* and *S. lycopersicum*. However, in that study the presence of *S. habrochaites* alleles reduces the mean value of the trait. Under the hypothesis that the same QTL for *pH* was detected at both studies, the differential behavior of wild alleles over the trait could be explained by allelic differences (*S. pimpinellifolium* or *S.habrochaites*) and their interactions with the respective genetic background.

Minor QTLs for color parameters L ($R^2 = 11.36$ %) and a/b ($R^2 = 7.61$ %) were detected on chromosome 2 associated with EP170/EP171 marker. An additive gene action was observed for these traits through the d/a parameter (Table 3).

The *FW2.2* gene on chromosome 2 that controls fruit weight was the first identified by positional mapping in tomato (Frary et al. 2000). Nesbitt and Tanksley (2002) after evaluating and comparing the coding sequences and gene promoters of different

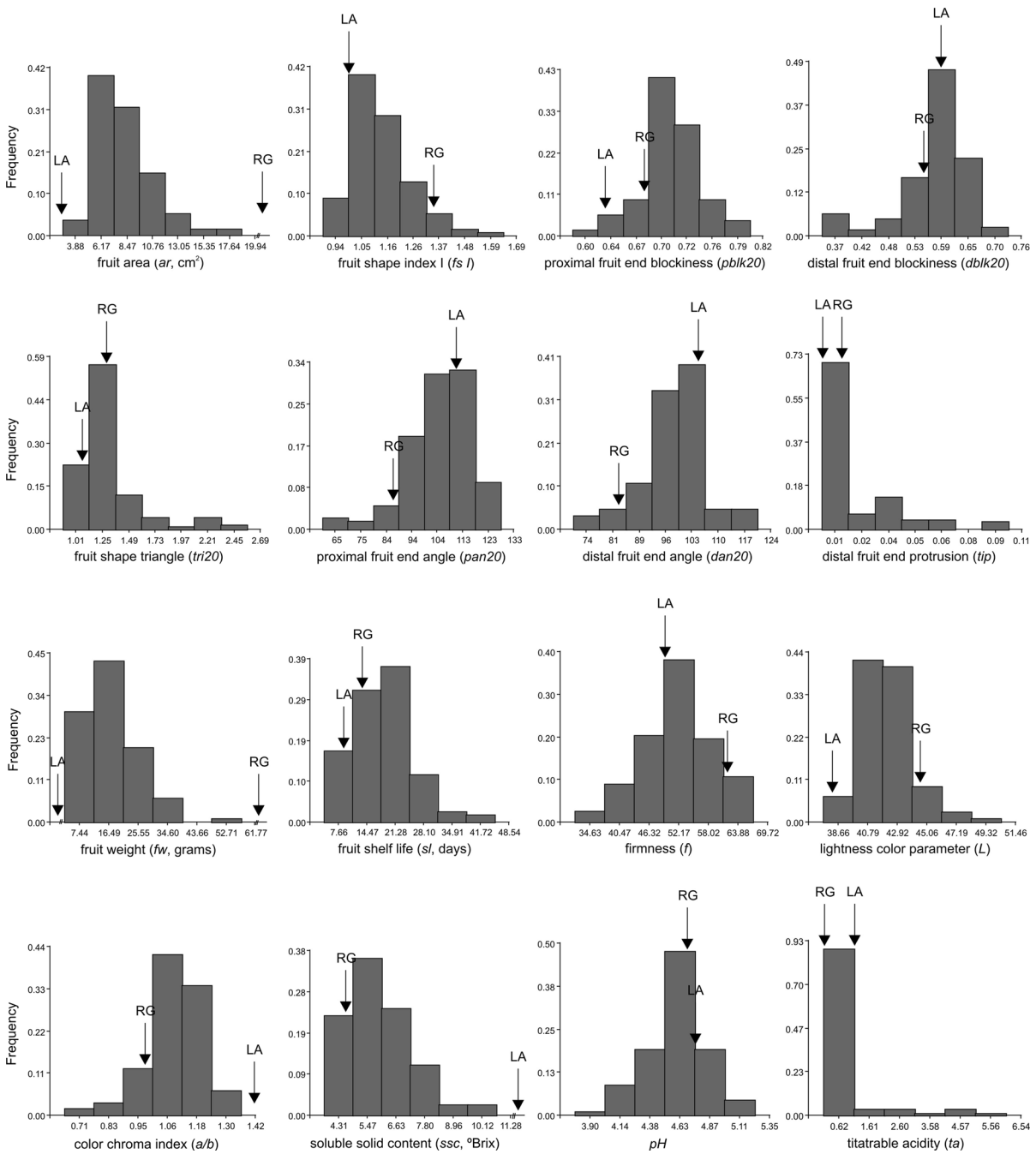

Fig. 1 Phenotypic distributions for fruit area (*area*, in cm^2), fruit shape index I (*fs I*), proximal fruit end blockiness (*pblk20*), distal fruit end blockiness (*dblk20*), fruit shape triangle (*tri20*), proximal fruit end angle (*pan20*, in °), distal fruit end angle (*dan20*, in °), distal fruit end protrusion (*tip*), fruit weight (*fw*, in g), fruit shelf life (*sl*, in days), firmness (*f*), lightness color parameter (*L*), color chroma index (*a/b*), soluble solid content (*ssc*, in °Brix), pH, and titratable acidity (*ta*, in g of citric acid per 100 g of tomato juice) in the segregating population F_3–BC_1–S_1

accessions of *S. lycopersicon*, *S. lycopersicon* var. c*erasiforme* and *S. pimpinellifolium*, concluded that *FW2.2* should be present in every cultivated tomato. In spite of the great difference among parents for fruit weight, the high genetic variability detected for this attribute in the analyzed segregating population and

Table 3 QTL analysis in the segregating population F_3–BC_1–S_1 for morphological and fruit quality traits

Category	Trait	Most significant marker	Chr.	P-value	% R^2	N	LL	n	LP	n	PP	d/a
Morphology	*ar*	EP170/EP171	2	0.01	6.80	40	9.14^{a}	55	7.79^{b}	29	7.44^{b}	cd. PP
		TG176	8	0.02	6.51	20	9.49^{a}	59	8.18^{ab}	44	7.44^{b}	ad
	fs I	TG337	2	<0.0001	29.69	44	1.22^{a}	52	1.09^{b}	28	1.06^{b}	cd. PP
		TG45	8	<0.0001	30.34	22	1.27^{a}	61	1.13^{b}	40	1.06^{c}	pd. PP
	pblk20	EP170/EP171	2	0.03	5.83	40	0.72^{a}	55	0.71^{ab}	29	0.70^{b}	ad
	dblk20	EP170/EP171	2	<0.0001	46.03	40	0.50^{b}	55	0.60^{a}	29	0.61^{a}	cd. PP
	tri20	EP170/EP171	2	<0.0001	47.89	40	1.58^{a}	55	1.19^{b}	29	1.15^{b}	cd. PP
	pan20	TG337	2	<0.0001	15.49	44	98.17^{b}	52	107.92^{a}	28	108.16^{a}	cd. PP
		TG45	8	<0.0001	32.82	22	90.49^{c}	61	104.76^{b}	40	110.88^{a}	pd. PP
	dan20	TG337	2	<0.005	10.00	44	94.41^{b}	52	99.87^{a}	28	100.56^{a}	cd. PP
		TG45	8	<0.0001	31.74	22	87.85^{c}	61	98.65^{b}	40	102.21^{a}	pd. PP
	tip	TG337	2	<0.0001	38.19	44	0.03^{a}	52	0.01^{b}	28	0.00^{b}	cd. PP
		TG45	8	<0.001	15.82	22	0.02^{a}	61	0.02^{a}	40	0.01^{b}	cd. LL
Quality	*sl*	EP912/EP913	8	0.03	5.62	23	15.07^{b}	63	18.42^{ab}	35	20.20^{a}	ad
	pH	EP170/EP171	2	0.03	6.45	34	4.54^{b}	50	4.65^{a}	28	4.53^{b}	nc
	L	EP170/EP171	2	<0.001	11.36	40	42.84^{a}	52	41.98^{b}	28	41.11^{c}	ad
	a/b	EP170/EP171	2	0.01	7.61	40	1.06^{b}	52	1.10^{ab}	28	1.15^{a}	ad

Chr. chromosome, *P-value* probability of type I error (α), % R^2 percentage of the phenotypic variability explained by the *locus, LL* homozygous for Rio Grande alleles, *LP* heterozygous, *PP* homozygous for LA1589 alleles, *ar* fruit area, *fs I* fruit shape index I, *pblk20* proximal fruit end blockiness, *dblk20* distal fruit end blockiness, *tri20* fruit shape triangle, *pan20* proximal fruit end angle, *dan20* distal fruit end angle, *tip* distal fruit end protrusion, *sl* fruit shelf life, *L* color parameter, *a/b* chroma index, nc not calculated, ad additivity, cd complete dominance, pd partial dominance. Different letters indicate significant differences ($P < 0.05$) according to a *t*-test

having a molecular marker (EP170/EP171) positioned on this gene (*FW2.2*), none QTL was identified for *fw* in the analyzed genomic region. The lack of association found in this report could be due to fixation of wild alleles at *loci* controlling the trait in the genetic background under study. This statement is based on previous reports that the region containing *FW2.2* has a high recombination rate (Grandillo and Tanksley 1996; Lippman and Tanksley 2001). Hence, this fact indicates that other genomic regions uncovered in this experiment underlie *fw* variation in the assayed segregating population.

Digenic linear additive model and epistasis

The highest % R^2 values were found for *ar*, *fs I*, *pan20*, *dan20* and *tip* (Table 3). These attributes were associated with almost all molecular markers at both studied genomics regions therefore were used to test a linear additive model and epistatic interactions. Only one molecular marker per region and trait (those with highest % R^2 in the one-way ANOVA) were selected to test the additive linear model and epistasis. The phenotypic values at those markers were used to estimate the m and a_i coefficients of the digenic linear additive model (Fig. A1 in supplementary material). Fruit area, *fs I* and *dan20* adjusted to the proposed model ($\chi^2 < 15.5$, $P > 0.05$) whereas *pan20* and *tip* did not ($\chi^2 > 15.51$, $P < 0.05$).

Epistasis or interaction between *loci* were significant ($P < 0.05$) for *fs I*, *pan20* and *tip* (Table 4). Mean values for each genotype at *fs2.1* were plotted in the x-axis against different genotypes at *fs8.1* in the y-axis (Fig. 2).

Epistasis occurs when differences in genotypic values at one *locus* vary depending on the genotype present at a second *locus* (Cheverud and Routman 1995). Using a two-way ANOVA and orthogonal contrast, it was found that additive gene actions were highly significant ($P < 0.0001$) at both *loci* and dominant gene action at *fs2.1* was slightly significant ($P = 0.03$; Table 5). Additive-by-additive epistatic

Table 4 Two-way ANOVA results for fruit shape index, proximal fruit end angle macro and distal fruit end protrusion

Trait	Molecular marker	F-value	P-value	% R^2
fs I	TG337	36.76	<0.0001	29.69
	TG45	28.62	<0.0001	30.34
	TG337 × TG45	3.27	0.01	61.55
pan20	TG337	12.17	<0.0001	15.49
	TG45	24.91	<0.0001	32.82
	TG337 × TG45	3.26	0.01	46.63
tip	TG337	37.70	<0.0001	38.19
	TG45	10.95	<0.001	15.82
	TG337 × TG45	4.04	<0.005	45.70

P-value probability of type I error (α), % R^2 percentage of the phenotypic variability explained by the *locus*, *fs I* fruit shape index I, *pan20* proximal fruit end angle, *tip* distal fruit end protrusion, TG337 molecular marker on chromosome 2, TG45 molecular marker on chromosome 8, TG337*TG45, interaction between chromosomes

interaction between *fs2.1* and *fs8.1* was significant for *fs I* ($P < 0.001$; Table 5). The significant individual effect and the interaction can be also visualized in Fig. 2.

An additive gene action was highly significant for *pan20* in both *loci* ($P < 0.0001$) and additive-by-additive epistasis was detected using orthogonal contrasts ($P = 0.02$; Table 5). However, dominant gene action in *fs2.1* ($P = 0.03$) and dominant-by-additive epistasis were also significant ($P = 0.01$; Table 5) for this trait. This result agrees with those obtained for *pan20* by the d/a parameter where complete dominance of wild alleles was observed in *fs2.1* (Table 3).

For *tip*, two major QTLs were detected on chromosomes 2 and 8 and the presence of cultivated alleles led to an increase in the average mean value of this trait (Table 3). An additive gene action was highly significant at both *loci* ($P < 0.001$) as the dominant effect in *fs2.1* ($P < 0.005$; Table 5). This result is consistent with those obtained for this trait by d/a parameter, where complete dominance of wild alleles was observed in *fs2.1* region (Table 3). Dominant-by-additive epistasis was observed between these *loci* using orthogonal contrasts ($P < 0.005$; Table 5).

Regression plots for traits with significant epistatic effects (*fs I*, *pan20,* and *tip*) are shown in Fig. 3. The dashed line in each graph represents the linear additive model for both genomic regions (*fs2.1* and *fs8.1*). The

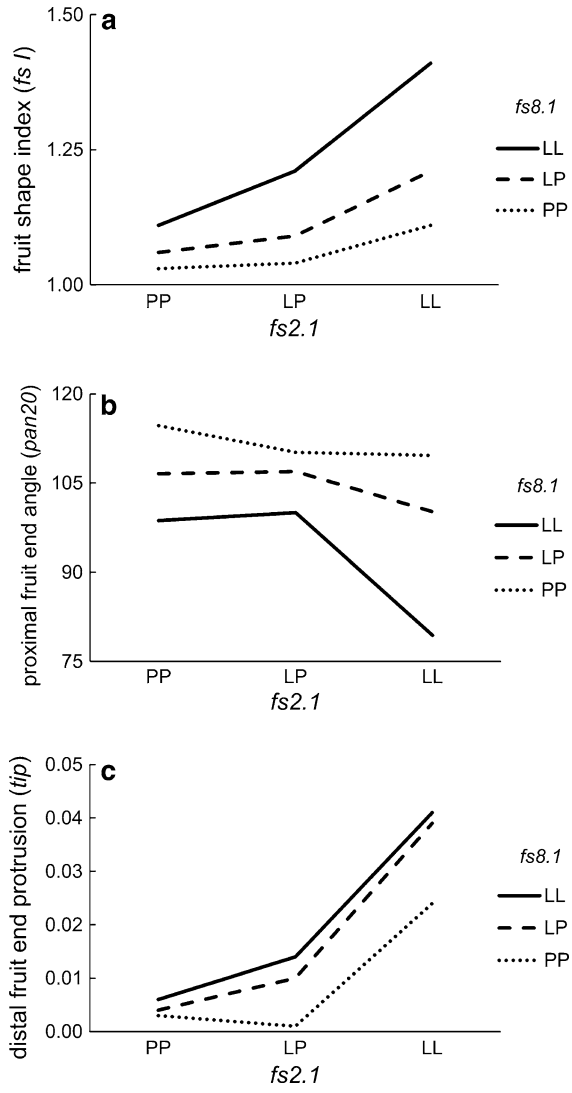

Fig. 2 Effects of interactions on fruit shape index I (*fs I*), proximal fruit end angle (*pan20*) and distal fruit end protrusion (*tip*) between the *loci* marked by TG337 (*fs2.1*) and TG45 (*fs8.1*). The vertical axis represents **a** fruit shape index (*fs I*), **b** proximal fruit end angle (*pan20*, in °) and **c** distal fruit end protrusion (*tip*). LL, LP and PP represents the three genotypes of each *locus*: *LL* homozygous for Rio Grande alleles, *LP* heterozygous, *PP* homozygous for LA1589 alleles

fs I trait adjusts to a digenic linear model which agrees with the two-way ANOVA results, i.e., the observed phenotype for the trait is explained by the individual additive effect of each *locus* and an additive–additive interaction. For *pan20* the genotypes PP_PP, LP_LL, LL_LP and LP_LP were the most divergent from the linear additive model. These results agree with those obtained in the two-way ANOVA (Table 4) and the

Table 5 Orthogonal contrast for fruit shape index, proximal fruit end angle macro and distal fruit end protrusion

Interaction	*fs I* P-value	*pan20* P-value	*tip* P-value
Additive *fs2.1*	<0.0001	<0.0001	<0.0001
Additive *fs8.1*	<0.0001	<0.0001	<0.001
Dominant *fs2.1*	0.03	0.01	<0.005
Dominant *fs8.1*	0.05	0.06	0.04
Additive *fs2.1*-by- additive *fs8.1*	<0.001	0.02	0.79
Additive *fs2.1*-by- dominant *fs8.1*	0.40	0.53	0.21
Dominant *fs2.1*-by- additive *fs8.1*	0.62	0.03	<0.005
Dominant *fs2.1*-by- dominant *fs8.1*	0.90	0.63	0.07

P-value probability of type I error (α), *fs I* fruit shape index I, *pan20* proximal fruit end angle, *tip* distal fruit end protrusion

orthogonal contrast for *pan20* (Table 5). For *tip*, genotypes carrying the heterozygous alleles at *fs2.1* (LP_PP, LP_LP and LP_LL) were the most divergent from the additive model followed for the genotypes carrying the cultivated homozygous alleles on *fs2.1* (LL_PP, LL_LP and LL_LL). An additive gene action was highly significant ($P < 0.001$) at both *loci*, a dominant effect in *fs2.1* was also significant ($P < 0.005$) and a dominant-by-additive epistasis was observed between these *loci* ($P < 0.005$). Summarizing, all deviations between the observed versus the expected values for each trait agrees with the two-way ANOVA (Table 4) and the orthogonal contrast results (Table 5).

According to the results of the two-way ANOVA, *fs2.1* and *fs8.1* together accounted for 61.55, 46.63 and 45.70 % of the phenotypic variability for *fs I*, *pan20* and *tip* respectively (Table 4). If we want to know the percentage of genetic variability that can be explained by *fs2.1* and *fs8.1* for the *fs I* trait we only have to do the rate between the % R^2 value (Table 4) and the % H^2 value (Table 2). Thus, the 84.31, 68.57 and 84.63 % of the genetic variability for *fs I*, *pan20* and *tip* respectively can be explained by these two QTLs (*fs2.1* and *fs8.1*).

In Fig. 4 discrepancies in fruit morphology can be visualized when different combinations of homozygous genotypes at *fs2.1* and *fs8.1* are present. Rounded morphology is recovered when wild alleles are present at homozygous state at both *loci* (*fs2.1* and *fs8.1*). The presence of cultivated alleles at only one *locus* (*fs2.1* or *fs8.1*) generate an intermediate morphology with an *fs I* mean value similar to that obtain for a double heterozygous plant (LP at *fs2.1* and LP at *fs8.1*, Fig. 2a). Finally, the most elongated fruit shape can be seen when cultivated alleles are present at homozygous state at both *loci*. In conclusion, these two *loci* have a synergistic effect over fruit shape index. The presence of wild alleles leads to an increase in *pan20*. Due to the highest % R^2 of *fs8.1* respect of *fs2.1*, the change of wild alleles for cultivated alleles at *fs2.1* do not generate a great change in the mean value of that trait (Fig. 4). In contrast, the presence of cultivated alleles at *fs8.1* generates a significant reduction in the mean value of *pan20*, being greater when cultivated alleles at homozygous state are present at both *loci*. A similar behavior can be seen for *tip*, but in this case the presence of cultivated alleles leads to an increase in the trait. At Fig. 4 we can see that this morphological trait is visible only when cultivated alleles are present at *fs2.1*, regardless of the genotype at *fs8.1*. This could be explained because of the differences at the % R^2 values, *fs2.1* explain more than twice of the phenotypic variance for the trait than *fs8.1* in this population.

Other studies revealed that epistasis may control a significant part of the genetic variation for quantitative traits in tomato. For example, Causse et al. (2007) when evaluating introgressed-lines derived from the cross between Cervil of *S. lycopersicum* var. cerasiforme and Levovil of *S. lycopersicum* found epistatic effects for fruit weight, locule number, firmness, soluble solids content, sugar content and titratable acidity. Lippman and Tanksley (2001) also detected a highly significant interaction between two QTLs *lcn2.1* and *lcn11.1* that control locule number.

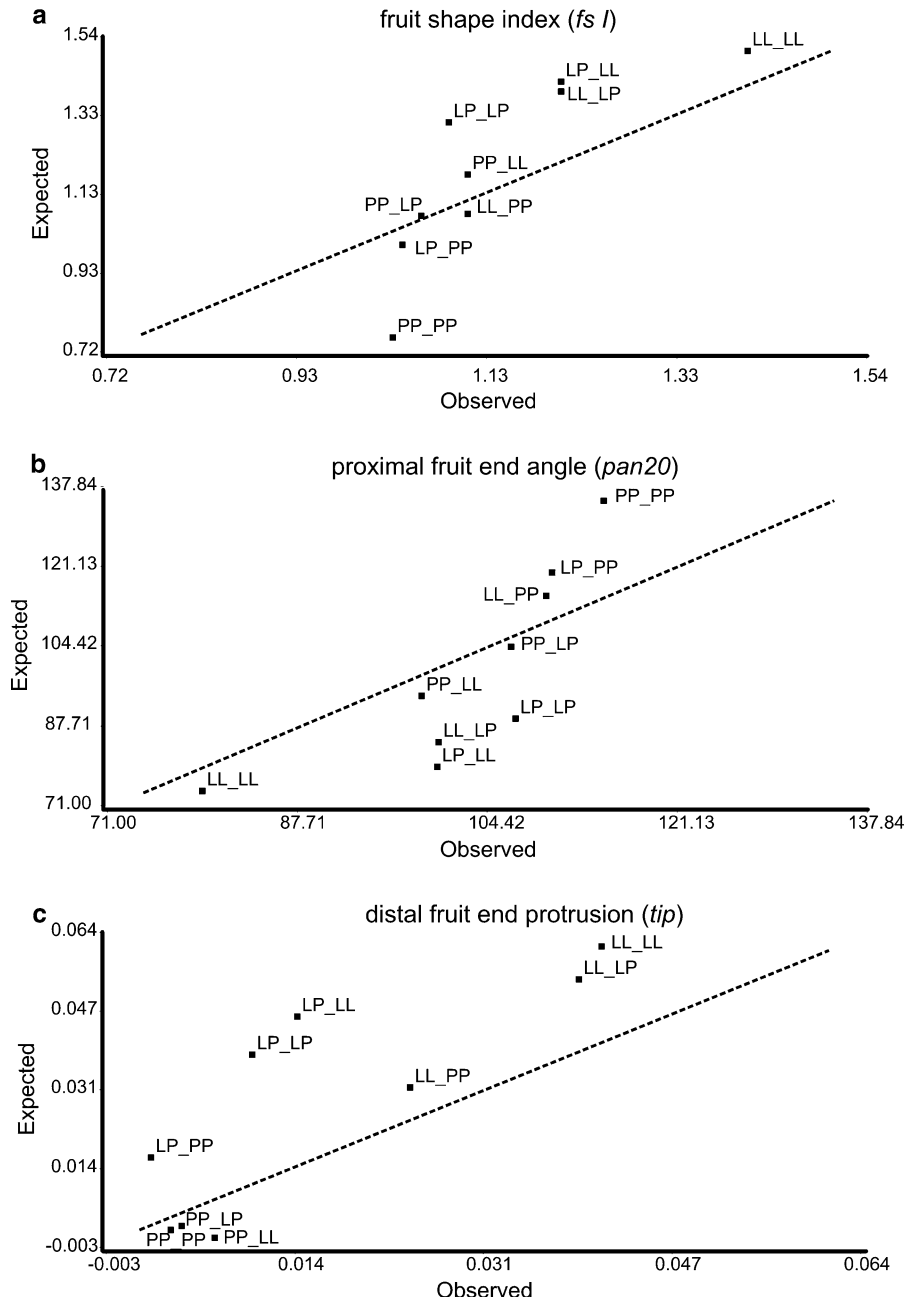

Fig. 3 Regression plots of expected versus observed phenotypic values for traits with significant epistatic effects. **a** Fruit shape index I (*fs I*). **b** Proximal fruit end angle (*pan20*). **c** Distal fruit end protrusion (*tip*). The *dashed lines* represent the expected values for the digenic linear additive model. Genotypes: *LL* homozygous for Rio Grande alleles, *LP* heterozygous, *PP* homozygous for LA1589 alleles. LL_LL, LL_LP, LL_PP, LP_LL, LP_LP, LP_PP, PP_LL, PP_LP and PP_PP represent all the different combinations for both *loci.* The first *two letters* indicates the genotype of the region *fs2.1* and the last two the genotype of the region *fs8.1*

Those results were obtained using an F_2 population derived from the cross between LA1589 of *S. pimpinellifolium* and Giant Heirloom of *S. lycopersicum*. The authors observed a disproportional increase in locule number when both *loci* were homozygous for Giant Heirloom alleles.

Conclusions

The individual effects of *fs2.1* and *fs8.1* on fruit morphological traits were validated in a different segregating population derived from the cross between cv. Rio Grande of *S. lycopersicum* and LA1589 of *S.*

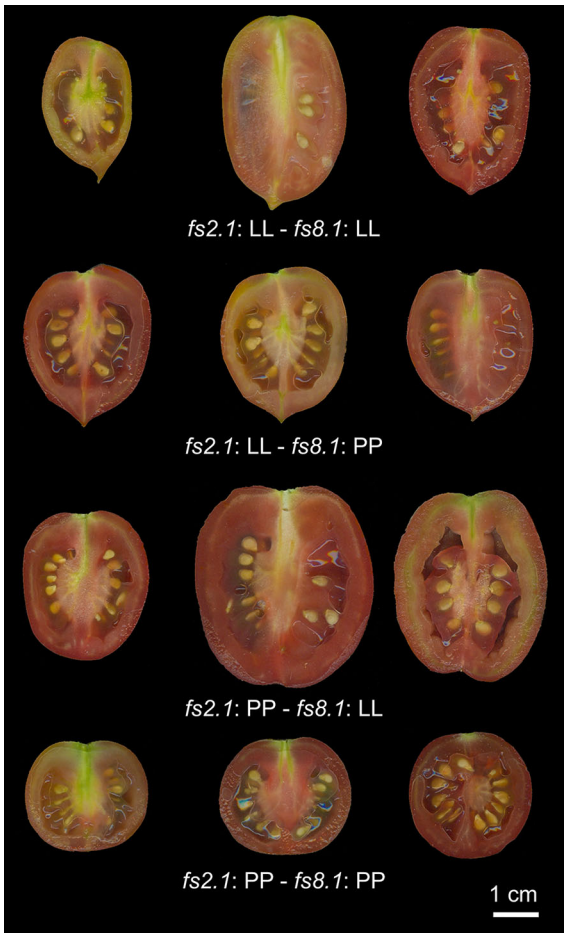

Fig. 4 Fruits from three representative plant for the combination of *loci fs2.1* and *fs8.1* at homozygous state in the segregating population F_3–BC_1–S_1. LL indicates the presence of cultivated alleles (Rio Grande) while PP indicates the presence of wild alleles (LA1589)

pimpinellifolium. Some reported QTLs for fruit quality traits such as *L*, *a/b*, *pH* and *sl* in distant tomato crosses were also confirmed in this segregating population. Further experiments should be done to define if QTLs of morphological traits have pleiotropic effect on fruit quality or they are different tightened linked *loci*. *fs2.1* and *fs8.1* do not share the control on the same fruit quality traits. On the other hand, these genomic regions control *ar*, *fs I* and *dan20* according to a digenic linear model and also interact in an epistatic way for *fs I*, *pan20* and *tip* traits to define the fruit morphology. The homozygous state at both *loci* (*fs2.1* and *fs8.1*) allows recover parental phenotypes whereas all other possible combinations interact to generate semi-elongated fruits.

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