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ORIGINAL PAPER

Tomato *fruit weight 11.3* maps close to *fasciated* on the bottom of chromosome 11

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Abstract Fruit weight is an important character in many crops. In tomato (*Solanum lycopersicum*), fruit weight is controlled by many loci, some of which have a major effect on the trait. *Fruit weight 11.3* (*fw11.3*) and *fasciated* (*fas*) have been mapped to the same region on chromosome 11. We sought to determine whether these loci represent alleles of the same or separate genes. We show that *fas* and *fw11.3* are not allelic and instead represent separate genes. The *fw11.3* locus was fine-mapped to a 149-kb region comprised of 22 predicted genes. Unlike most fruit weight loci, gene action at *fw11.3* indicates that the mutant allele is partially dominant over the wild allele. We also investigate the nature of the genome rearrangement at the *fas* locus and demonstrate that the mutation is due to a 294-kb inversion disrupting the *YABBY* gene known to underlie the *fas* locus.

Introduction

Fruit weight is an important selection criterion in the breeding programs of many fruit and vegetable crops. Domestication and selection of tomato (*Solanum lycopersicum*) led to dramatically increased fruit mass compared with fruit found in its wild relatives (Bai and Lindhout 2007; Paran and van der Knaap 2007). Tomato is also an excellent system for studying fleshy fruit development (Giovannoni 2001), and therefore genes controlling fruit size offer insights into basic plant developmental processes. Fruit weight is a quantitatively inherited character that is controlled by up to 28 quantitative trait loci (QTLs) (Grandillo et al. 1999). The loci *fw1.1*, *fw2.2*, *fw2.3*, *fw3.1*, *fw4.1* and *fw9.1* each explain more than 20% phenotypic variance in at least one independent study. In addition, *fw1.1*, *fw2.1*, *fw2.2*, *fw3.1*, *fw3.2*, and *fw11.3* have been identified in at least four independent studies (Grandillo et al. 1999). The aforementioned loci are referred to as major QTLs and account for the majority of fruit weight variation in the evolution of tomato from a small berry to a large fruit. Two loci, *locule number* (*lc*) and *fasciated* (*fas*), increase locule number and fruit width, often resulting in increased fruit weight (Grandillo et al. 1999; Lippman and Tanksley 2001; Causse et al. 2007).

To date, the genes for two tomato fruit size loci, *fw2.2* and *fas*, have been cloned (Frary et al. 2000; Cong et al. 2008). The large-fruited allele of *fw2.2* is thought to be fixed in cultivated tomato accessions, and thus it is believed the mutation arose early during domestication (Nesbitt and Tanksley 2002; Tanksley 2004). Histological studies suggest that FW2.2 protein is a regulator of cell division because of more cells in large compared with small fruits (Frary et al. 2000; Cong et al. 2002). Expression analyses have indicated that higher gene expression and different timing of expression correlate with fewer cells and smaller fruit (Cong et al. 2002). There are many *FW2.2*-related genes in other plant species. Some of the *FW2.2*-related genes are found to play similar roles in the regulation of organ cell number. In maize the overexpression of tomato *FW2.2*-like gene, *Cell Number Regulator 1* (*CNR1*), reduces plant and organ size by reducing cell

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number (Guo et al. 2010). In avocado, small fruit has fewer cells compared with normal-sized fruit. *Pafw2.2*, an *FW2.2*-like gene in avocado, is expressed at higher levels in small fruit compared with normal-sized fruit (Dahan et al. 2010). Greatly reduced expression of *GmFWL1*, a soybean *FW2.2*-like gene, significantly decreased nodule number and nuclear size (Libault et al. 2010) suggesting a role for *FW2.2*-like genes in organ initiation as well as organ size. The molecular function of FW2.2 and CRN1-like proteins is not well understood. However, based on protein localization at the plasma membrane (Cong and Tanksley 2006; Libault et al. 2010) and structural modeling (Guo et al. 2010), it has been suggested that their role in cell division is indirect and that this family of proteins might regulate transport of metals such as cadmium across membranes (Guo et al. 2010).

The main function of *fas* is to control locule number which affects fruit mass (Lippman and Tanksley 2001; Cong et al. 2008). Wild-type tomatoes carry fruit with 2–4 locules. However, certain accessions carry fruit of many locules, some of which are controlled by the *FAS* gene mutation (Cong et al. 2008; Rodriguez et al. 2011). This mutation also leads to increased floral organ number and fruit size (Lippman and Tanksley 2001; Barrero et al. 2006). It has been shown that a mutation in the *YABBY* gene underlies the *fas* locus (Cong et al. 2008). Furthermore, it has been postulated that the mutation is due to a 6- to 8-kb insertion in the first intron of *YABBY* resulting in down regulation of the gene (Cong et al. 2008). YABBY family members are regulators of organ polarity and the establishment of proper boundaries in meristems (Golz and Hudson 1999; Bowman 2000).

Cell number, cell size, and endoreduplication play important roles in the control of plant organ size such as the fruit (Cheniclet et al. 2005). *FW2.2* regulates fruit mass by altering cell number (Frary et al. 2000; Cong et al. 2002), and *FAS* regulates fruit mass by altering the boundaries within the floral meristem resulting in changes in locule number (Lippman and Tanksley 2001; Cong et al. 2008). To elucidate additional genes that were selected during domestication of tomato and to further understand the molecular mechanism that regulate tomato fruit size, it is important to isolate the genes underlying the other loci controlling fruit weight in this species. The *fw11.3* locus overlaps with *fas* on tomato chromosome 11 (Grandillo et al. 1999; Lippman and Tanksley 2001; van der Knaap and Tanksley 2003). Therefore, we hypothesized that *fw11.3* and *fas* were allelic and that *fw11.3* was the weaker allele of *fas*. In this study, the *fw11.3* locus was narrowed down to a ~149-kb region, which was 45 kb downstream of *fas* indicating that these loci were not allelic. Twenty-two predicted genes were found in the *fw11.3* target region. Moreover, the mutation at *fas* was not due to a 6- to 8-kb insertion but instead to a ~294-kb inversion in the first intron of *YABBY*, thereby disrupting the gene.

Materials and methods

Plant materials

Tomato seeds were obtained from the TGRC (LA0767, LA0925, LA1215, LA1589, LA1786, LA2452); Tomato Growers Supply Co (Howard German, Orange Strawberry, Yellow Stuffer, Zapotec Pinked Ribbed); Tomato Bob (Costoluto Genovese); Sunseeds (Sun1642); IPK Gatersleben (LYC281, LYC439, LYC444, LYC1823, LYC1848, LYC1917, LYC2456, T1708); MJ Gonzalo (UPV7637); Heinz (Heinz1706).

S. lycopersicum cv Howard German bears large and on average five locules per fruit as a result of the *lc* but not the *fas* mutation (Gonzalo et al. 2009; Rodriguez et al. 2011). We predicted that Howard German carried the cultivated allele of *fw11.3* because we found that QTLs associated with fruit shape coincided with the *fw11.3* locus (Brewer et al. 2007; Gonzalo and van der Knaap 2008). The rationale is that fruit shape is more pronounced in the large-fruited background and hence the effect is enhanced by a fruit weight QTL. A recombinant inbred line population $HGBC_1F_5$ was derived from a cross between Howard German and the wild species *S. pimpinellifolium* accession LA1589. The F_1 was backcrossed to Howard German and subsequent generations were self-pollinated in the field in Wooster, OH, USA, four times (Supplementary Fig. 1). One hundred forty-seven $HGBC_1F_5$ plants were evaluated in summer 2008 for the segregation of *fw11.3*. To confirm and map *fw11.3* to a defined interval, two recombinant plants, 09S11-1 and 09S12-12, were selected from 187 $HGBC_1F_3$ seedlings and self-pollinated in the greenhouse. These recombinant parents were chosen because other fruit size QTLs (*fw1.1*, *fw2.2*, *fw3.1*, *fw3.2,* and *fw4.1*) were fixed for the Howard German allele. From each recombinant parent, 11–12 homozygous recombinant and 11–12 homozygous non-recombinant progeny seedlings were selected and grown in the field in summer 2009. From each plant, 20 fruits were evaluated for fruit mass. To identify additional recombinants around *fw11.3*, 188 seedling progeny of 09S11-1 and 376 seedling progeny of 09S106-2 (selected from 09S11-1 and heterozygous for *fw11.3*) were screened with marker YABBY and EP1654 (Supplementary Table 1). A subset of the recombinant plants was selected for progeny test in the field in 2010. To determine the gene action of *fw11.3*, two heterozygous plants, 09S225-47 and 09S225-185, were selected from the progeny of 09S106-2. From the progeny of each plant, a set of homozygous plants carrying the Howard German allele

(EE), the LA1589 allele (PP), or heterozygous (EP) were selected for evaluation of fruit mass in the field in 2010.

To screen recombinants around the *fas* locus, we genotyped 1504 seedlings of an F_2 population derived from a tomato cultivar Orange Strawberry carrying the *fas* mutation (Rodriguez et al. 2011) crossed with LA1589, with markers EP1312, YABBY, and EP1057. The two EP markers were derived from the sequence of the Heinz1706 BAC clone HBa0323E19 carrying the *YABBY* gene. The progeny tests of four recombinants were conducted in the field or the greenhouse. From each plant, 20–30 fruits were evaluated for locule number.

Development of PCR-based markers

Putative SSR sequences in sequenced BACs or tomato whole genome scaffold sequences located on the bottom of chromosome 11 were identified by the microsatellite search tool, SSRHunter1.3 (Li and Wan 2005). PCR primers matching the flanking regions of these SSR sequences were designed using the PrimerSelect Lasergene program (DNASTAR Inc., Madison, WI, USA.). To develop the marker based on insertion/deletions (InDels) or single nucleotide polymorphism (SNP), PCR products from the parental lines were separately purified and sequenced. The sequence of PCR products were aligned using the software Sequencer 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA). Large InDels ($\geq$14 bp) and SNPs were used to develop primers flanking the InDel, cleaved amplified polymorphic sequences (CAPS) or derived cleaved amplified polymorphic sequence (dCAPS) (http://helix.wustl.edu/dcaps/dcaps.html; Neff et al. 2002) markers, respectively (Supplementary Table 1). The amplification of the markers was conducted using the following profile: 94°C 2 min; 35 cycles of 30 s at 94°C, 30 s at 52°C, and 30 s at 72°C; 5 min at 72°C. PCR products were separated on 3% agarose gel. All markers were mapped in silico to the tomato SL2.30ch11 (version 2.30).

RT-PCR experiment

Young flower buds (length <2 mm) were harvested from the progeny of 09S130-11 and were frozen in liquid nitrogen immediately. Total RNA was isolated using TRIzol Reagent (Invitrogen). Total RNA samples were treated with DNase I (New England Biolabs) to remove genomic DNA. The first strand of cDNA was synthesized from 5 μg of total RNA using SuperScript III Reverse Transcriptase (Invitrogen) and then diluted to 60 μl. For PCR, 2 μl cDNA sample was taken into a reaction volume of 30 μl using gene-specific primers (Supplementary Table 1). Reactions were performed with *Taq* Polymerase on the DNA Engine Tetrad 2 Peltier Thermal Cycler (BIORAD), with the following profile: 94°C 2 min; different cycles (*eIF4α* 21 cycles, *YABBY* 34 cycles) of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C; 72°C 5 min.

Data analysis

One-way analysis of variance was performed to determine the correlation of markers and fruit weight from plants in the $HGBC_1F_5$ population grown in 2008. Significant difference at $P < 0.01$ was used as threshold for estimating the linkage of *fw11.3* and the marker. To fine map *fw11.3*, the mean fruit weight of homozygous recombinants and homozygous non-recombinants from each progeny family were analyzed using Student's *t* test. A probability of $P < 0.01$ was used as threshold for determining that the locus was segregating in the parent. The gene action of *fw11.3* was estimated as the difference of mean fruit weight between the homozygous plants carrying the Howard German allele (EE), the LA1589 allele (PP), and heterozygous plants EP using the formula: $d/a = [2EP - (EE + PP)]/(EE - PP)$. To fine map *fas* in Orange Strawberry, the mean locule number of homozygous recombinants and homozygous non-recombinants from each progeny family were analyzed using Student's *t* test. A probability of $P < 0.01$ was used as threshold for determining that the locus was segregating in the parent.

Predicted genes in 149 kb *fw11.3* region

The predicted genes in the region encompassing *fw11.3* were identified by searching the available tomato genome annotation database, ITAG version 1 (SGN: http://solgenomics.net/genomes/Solanum_lycopersicum/genome_data.pl#annotation). Information about the expression of the predicted genes was found by BLASTN searching of the tomato full-length cDNA, Unigene, and ESTs databases, including Sol Genomics Network (SGN http://solgenomics.net) and The National Center for Biotechnology Information (NCBI http://www.ncbi.nlm.nih.gov).

Genome structure analysis at the *fas* locus in tomato

To determine the location of the breakpoint of the rearrangement in the first intron of the *YABBY* gene, fragments derived from the intron sequence were used as probe in Southern blot analysis according to standard protocols (Sambrook and Russell 2001) (Supplementary Table 1). To identify the sequence of the rearranged segment, inverse PCR was conducted as described previously (Rodriguez et al. 2011) and a fragment of approximately 4 kb was obtained. The sequence from the rearranged region was used as query to the tomato Heinz1706 genome sequence database using BLASTN at SGN (http://solgenomics.net/).

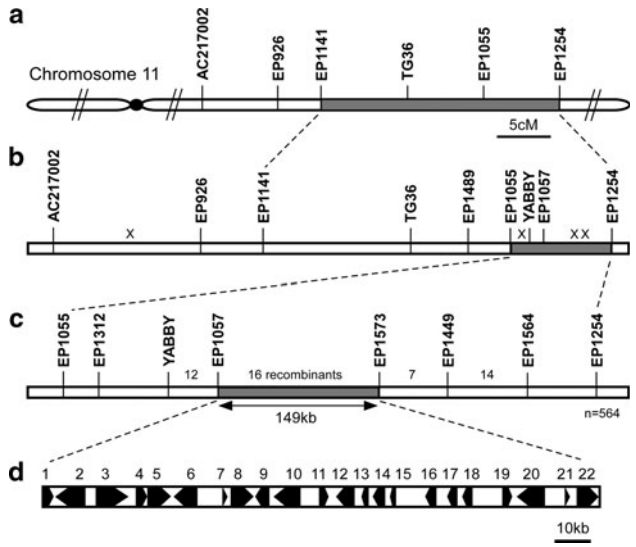

Fig. 1 Fine-mapping of the *fw11.3* locus **a** *fw11.3* locus was identified within EP1141-EP1254 interval on tomato chromosome 11 in the Howard German BC_1F_5 population. **b** *fw11.3* locus was confirmed and narrowed down to EP1055- EP1254 interval. **c** *fw11.3* was narrowed down to EP1057-EP1573 interval. **d** Annotated scaffold SL1.00sc06004 encompassing the *fw11.3* region. *Numbers* indicate predicted genes (Table 3) and the *arrows* indicate the direction of transcription

To check the validity of the genome rearrangement, sets of primers were developed spanning the breakpoints on both ends of the inversion (Supplementary Table 1).

Results

Identification of the *fw11.3* locus in tomato cultivar Howard German

To confirm the presence of the large-fruited allele of *fw11.3* in the variety Howard German, new markers were developed spanning approximately 33 cM and covering the bottom half of chromosome 11. These markers were used to genotype $HGBC_1F_5$ population derived from a cross between Howard German and LA1589 that would segregate for *fw11.3* if Howard German carried the large-fruited allele (Fig. 1a). The analysis of the fruit weight for each plant in the population showed that markers TG36 ($P < 0.001$), EP1055 ($P < 0.0005$) and EP1254 ($P < 0.01$) were significantly associated with the trait. This result indicated that the *fw11.3* locus was segregating in the Howard German-derived population and located in the EP1141-EP1254 interval on chromosome 11 (Fig. 1a), as reported previously (Grandillo et al. 1999; Van der Knaap and Tanksley 2003).

To better define the *fw11.3* region, a progeny test was performed on two plants that were recombinants between AC217002 and EP1254 (Table 1; Fig. 1b). The EP1055-EP1254 interval of 09S11-1 and the AC217002-EP1254 interval of 09S12-12 were heterozygous while many other regions were homozygous for the Howard German alleles, such as *fw1.1*, *fw2.2*, *fw3.1*, *fw3.2,* and *fw4.1* loci (Grandillo et al. 1999). When comparing the fruit weight of the non-recombinants with the recombinants in each family, a significant ($P < 0.00001$) increase in mean fruit weight for 09S11-1 (37.7 g) and 09S12-12 (21.4 g) was observed. This represented an increase of 57.6 and 50.1% in fruit mass, respectively (Table 1). These results indicated that the *fw11.3* locus was located in the EP1055-EP1254 interval on chromosome 11 (Fig. 1b).

Fine mapping of *fw11.3* locus

Based on the genomic sequence of tomato cv. Heinz 1706 (SL2.30ch11), the length of EP1055-EP1254 interval is 487 kb. To further delineate the position of *fw11.3*, five markers were developed to divide this interval in smaller segments (Fig. 1c). From the 564 seedlings that were genotyped with markers YABBY and EP1654, 49 recombinants were identified corresponding to an interval of

Table 1 Progeny test to confirm the *fw11.3* locus

BC1F3 Plant	Parental Pedigree	Filial Pedigree	Markers[a]								N[b]	AFW(g)[c]	P-value[d]	FW△(g)[e]	FW△(%)[f]
			AC217002	EP926	EP1141	TG36	EP1055	YABBY	EP1057	EP1254					
09S11-1	05S113	09S106	1	1	1	1	1	1	1	1	12	103.2 ± 13.2	2.97E-7	37.7	57.6
			1	1	1	1	1	3	3	1	12	65.5 ± 12.3			
09S12-12	05S120	09S107	1	1	1	1	1	1	1	1	11	64.1 ± 8.6	3.09E-6	21.4	50.1
			1	3	3	3	3	3	3	1	11	42.7 ± 6.5			

[a] Marker score: 1 homozygous for S. *lycopersicum* alleles (E); 3 homozygous for S. *pimpinellifolium* alleles (P)

[b] The number of tomato plants

[c] AFW: average fruit weight

[d] *P* values of t-tests for comparisons within each BC_1F_4 family

[e] The mean fruit weight in grams (g) for the EE class subtracted by the mean fruit weight (g) for the PP class

[f] Fruit weight △ (g) divided by the mean fruit weight (g) of the PP class

4.3 cM and a ratio of 76.4 kb/cM. Among the 49 recombinants, 12 recombinants located within YABBY-EP1057 interval; 16 recombinants located within EP1057-EP1573 interval; 7 recombinants located within EP1573-EP1449 interval; and 14 recombinants located within EP1449-EP1564 interval (Fig. 1c). From the total number of recombinant plants, 20 were selected for progeny testing based on the location of the crossover, sufficient number of seeds, maximum fruit weight, and regular fruit shape of the parental accession. The progeny test showed that *fw11.3* was located between markers EP1057 and EP1573, a 149-kb region on chromosome 11 (Fig. 1c, Supplementary Table 2).

Fine mapping and genome structure analysis of the *fas* locus

In an effort to develop near isogenic lines (NIL) for *fas*, we screened for recombinant plants in which the crossover occurred within 50 kb from *YABBY* gene. The source of the *fas* allele for NIL development was Orange Strawberry, a tomato cultivar bearing multilocular fruit that carries the mutant allele of *fas* (Rodriguez et al. 2011). From the recombinant screen using markers on BAC clone HBa0323E19 carrying the *YABBY* gene, 11 recombinants were identified that all fell within the 45-kb interval YABBY-EP1057 (for position of the markers, see Fig. 1b, c). Progeny testing of three recombinants, 08S610-18, 08S610-227, and 09S86-274, between YABBY and EP1057 showed that marker EP1057 is outside the *fas* locus (Supplementary Table 3). Progeny testing of an additional recombinant plant, with a crossover between EP1489 (derived from SGN marker T0302, approximately 375 kb upstream of YABBY) and YABBY, showed that *fas* is located in EP1489-EP1057 interval including YABBY (Supplementary Table 3), similar to what is published previously (Cong et al. 2008).

The dramatic change in recombination frequency on one compared with the other side of the *YABBY* gene (from 11 recombinants between YABBY and EP1057 to 0 recombinants between YABBY and EP1312) suggested that the *fas* mutation was due to an inversion and not to an insertion. To investigate the nature of the rearrangement, we sought to identify the sequence at the breakpoint of the rearrangement. As a first step, we conducted Southern blot analyses with DNA from tomato accessions that carried many loculed fruit and probes derived from the first intron where the rearrangement was located. These analyses suggested that the many loculed varieties exhibited a different genome structure compared with control accessions carrying 2–3 locules (Supplementary Fig. 2a and 2b). Moreover, only one band was observed in both many- and low-loculed varieties. Using inverse PCR, we obtained an approximately 4 kb fragment that covered the breakpoint of the rearrangement (Supplementary Fig. 3). Southern blot analysis using as probe a DNA fragment from the rearrangement, EP1067, showed also only one band in high- as well as low-loculed varieties (Supplementary Fig. 2c). These findings suggested that the rearrangement was not due to a duplication or deletion but instead might be the result of a translocation or an inversion. We genetically mapped EP1067 in an F_2 derived from a cross between Yellow Stuffer and LA1589 (Van der Knaap and Tanksley 2003). Yellow Stuffer does not carry the *fas* mutation and therefore represented wild-type gene structure at the locus. The mapping showed that the rearrangement originated 3 cM below TG36 and 2 cM above YABBY (data not shown). Therefore, the rearrangement appeared to be small and intrachromosomal. After the public release of the tomato genome sequence, we used the 4-kb rearranged fragment sequence as query against the tomato Heinz1706 genome sequence (http://solgenomics.net). The first part of the sequence (1–3,561 bp) corresponding to the rearranged part matched the SL2.30ch11 (51,956,539–51,960,099, +strand), and the last part of the sequence (3,560–4,137 bp) corresponding to the intron of *YABBY* gene matched the SL2.30ch11 (52,253,523–52,254,100, −strand) which is approximately 294 kb apart in wild-type tomato (Supplementary Fig. 3). To confirm the presence of the rearrangement in additional high-locule-number varieties, mainly those that were studied previously (Barrero and Tanksley 2004; Cong et al. 2008), we designed four sets of primer pairs that were predicted to span the breakpoints of the rearrangement (Supplementary Table 1). Amplification results indeed confirmed the presence of a ~294-kb inversion in the *fas* mutants (Fig. 2b, c). This inversion clearly affected the expression of *YABBY* as indicated by RT-PCR of RNA isolated from young flower buds (< 2 mm) of plants carrying the mutant allele of *fas* compared with wild type (Fig. 2). These results indicated that *YABBY* is knocked out as a result of the ~294-kb inversion.

Gene action of *fw11.3*

To determine gene action of *fw11.3* locus, fruit weight was measured in the homozygous and heterozygous plants in two families that were segregating for the EP1057-EP1573 interval (Table 2). Based on the mean fruit weights of EE, PP, and EP plants of the progeny of 09S225-49 and 09S225-185, the degree of dominance of Howard German allele was found to be 0.46 and 0.75, respectively. These results suggested that the *fw11.3* allele from Howard German was partially dominant over the allele from *S. pimpinellifolium* supporting earlier findings (Van der Knaap and Tanksley 2003).

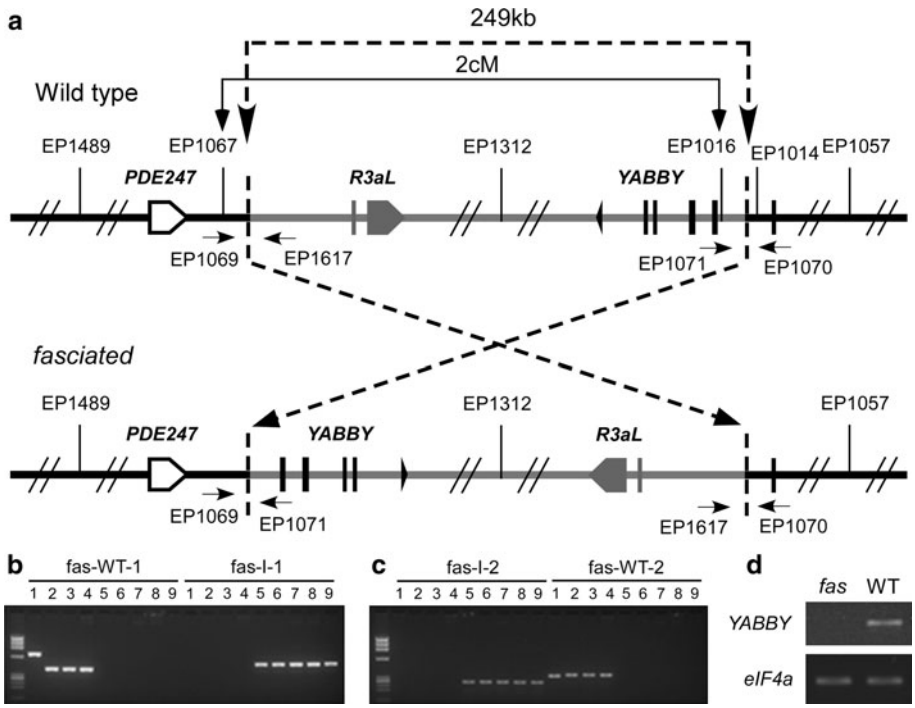

Fig. 2 The inversion of the *fas* locus in tomato **a** The *fas* locus harbored a 294-kb inversion. The left breakpoint of the inversion was between predicted gene *PDE247* (pigment defective 247, SL1.00sc06004_200.1) and *R3aL* (Disease resistance protein R3a-like protein, SL1.00sc06004_201.1), the right breakpoint of the inversion was within the first intron of *YABBY* gene (YABBY2-like transcription factor YAB2, SL1.00sc06004_243.1). fas-WT-1 (primer pair EP1069 and EP1617) and fas-WT-2 (primer pair EP1070 and EP1071) were used to check the wild type genome structure; fas-I-1 (primer pair EP1069 and EP1071) and fas-I-2 (primer pair EP1070 and EP1617) were used to check inversion at *fas*. **b** PCR amplification using the primers mentioned in (**a**) resulted in the detection of the inversion on the left. **c** PCR amplification using the primers mentioned in (**a**) resulted in the detection of the inversion on the right. *1* LA1589, *2* Heinz 1706, *3* Howard German, *4* Yellow Stuffer, *5* LA0767, *6* LA0925, *7* LA1786, *8* LA2452, *9* Orange Strawberry. *1*, *2*, *3*, *4* wild tomato or cultivars with low locule numbers; *5*, *6*, *7*, *8*, *9* tomato cultivars with high locule numbers (Barrero and Tanksley 2004; Cong et al. 2008; Rodriguez et al. 2011). **d** The expression of *YABBY* in wild-type and *fas* tomato. DNA marker, ΦX174 DNA-HaeIII Digest (NEB)

Table 2 Gene action of *fw11.3*

BC1F5 plant	Parental pedigree	Filial pedigree	Markers[a]									N^b	AFW(g)[c]	d/a[d]
			TG36	EP1055	YABBY	EP1057	EP1573	EP1449	EP1564	EP1254	EP1550			
09S225-49	09S106-2	10S93	1	1	1	1	1	1	1	1	1	12	83.5 ± 8.7	0.46
			1	1	2	2	2	2	2	1	1	11	78.4 ± 7.0	
			1	1	3	3	3	3	3	1	1	12	64.5 ± 4.4	
09S225-185	09S106-2	10S83	1	1	1	1	1	1	1	1	1	12	72.9 ± 7.7	0.75
			1	1	1	2	2	2	2	1	1	11	71.0 ± 9.5	
			1	1	1	3	3	3	3	1	1	12	57.5 ± 5.9	

[a] Marker score: 1 homozygous for S. *lycopersicum* alleles (E); 2 heterozygous (EP); 3 homozygous for S. *pimpinellifolium* alleles (P)

[b] The number of tomato plants

[c] AFW: average fruit weight

[d] d/a = degree of dominance of the E allele [(2EP-EE-PP)/(EE-PP)]

Gene annotation of the 149-kb *fw11.3* region

Twenty-two predicted genes were found in the 149-kb region corresponding to *fw11.3* by searching the tomato genome annotation database (Table 3). With the exception of SL1.00sc06004_256.1 and SL1.00sc06004_257.1, all are predicted to encode functional proteins. Most of the predicted genes, with the exception of SL1.00sc06004_250.1, SL1.00sc06004_262.1, and SL1.00sc06004_264.1, corresponded to a full-length cDNA or a unigene. Twelve

Table 3 Predicted genes in the *fw11.3* region

ID	Predicted CDS[a]	Putative protein function	Full-length cDNA	Unigene[b]	ESTs source tissues
1	SL1.00sc06004_250.1	CLAVATA 1, Serine/threonine kinase			
2	SL1.00sc06004_251.1	Outer envelope protein of 80 kDa		SGN-U588538	Leaf
3	SL1.00sc06004_252.1	Large subunit GTPase 1 homolog	AK319274	SGN-U568726, SGN-U569667	Crown gall, flower, fruit, leaf, root, seedlings, shoot
4	SL1.00sc06004_253.1	Serine/threonine protein phosphatase		SGN-U572554	Flower
5	SL1.00sc06004_254.1	Serine/threonine protein phosphatase		SGN-U572555	Fruit, leaf
6	SL1.00sc06004_255.1	DnaJ homolog subfamily C member 8	AK328152, BT013084	SGN-U569691, SGN-U589611	Crown gall, flower, fruit, leaf, root, seed, suspension cultures
7	SL1.00sc06004_256.1	Unknown protein	AK247611	SGN-U584550	Leaf
8	SL1.00sc06004_257.1	Unknown protein	AK328791	SGN-U586284	Fruit, leaf, root, seedling
9	SL1.00sc06004_258.1	FAD-linked sulfhydryl oxidase ALR		SGN-U598120	Leaf
10	SL1.00sc06004_259.1	Serine/threonine-protein kinase Nek5		SGN-U599760	Callus
11	SL1.00sc06004_260.1	Pyridine nucleotide-disulphide oxidoreductase		SGN-U571091, SGN-U596521	Root
12	SL1.00sc06004_261.1	ACTIN 4 structural constituent of cytoskeleton		SGN-U576083	Fruit, ovary
13	SL1.00sc06004_262.1	Homoserine dehydrogenase family protein			
14	SL1.00sc06004_263.1	Homoserine dehydrogenase family protein		SGN-U601085	Fruit
15	SL1.00sc06004_264.1	Polyubiquitin 9			
16	SL1.00sc06004_265.1	LTP family protein		SGN-U579533	Flower, leaf, trichomes
17	SL1.00sc06004_266.1	GDSL esterase/lipase	AK325531	SGN-U571716	Flower, fruit, leaf, root
18	SL1.00sc06004_267.1	F-box/WD-40 repeat-containing protein	AK320336	SGN-U569064, SGN-U579177	Flower, leaf, seed, trichomes
19	SL1.00sc06004_268.1	MYB family transcription factor-like		SGN-U584149	Trichomes
20	SL1.00sc06004_269.1	Speckle-type POZ protein	AK320634	SGN-U582672, SGN-U587095	Fruit, leaf, root
21	SL1.00sc06004_270.1	Kelch repeat-containing F-box family protein		SGN-U597645	Leaf, root
22	SL1.00sc06004_271.1	Translation initiation factor eIF-2B subunit gamma		SGN-U562684, SGN-U598746	Callus, crown gall, flower, fruit, leaf, root, shoot/meristem

[a] Data from http://solgenomics.net/genomes/Solanum_lycopersicum/genome_data.pl#annotation

[b] Data from http://solgenomics.net/search/direct_search.pl?search=unigene

genes corresponded to ESTs expressed in tomato reproductive tissues such as flower, ovary and/or fruit (Table 3).

Discussion

The *fw11.3* is not an allele of *fasciated*

The locus *fw11.3* is an important fruit weight locus explaining from 8 to 13% of the variation in F_2 and BC_1/BC_2 populations derived from crosses with wild relatives (Grandillo et al. 1999; Van der Knaap and Tanksley 2003). *fas* is also a major locus resulting in larger fruit weight by up to 37% through increasing the number of locules (Lippman and Tanksley 2001). *fw11.3* is found in a similar region on chromosome 11 as *fas* in low-loculed tomato cultivars (Grandillo et al. 1999; Van der Knaap and Tanksley 2003). Therefore, we hypothesized that *fw11.3* and *fas* were allelic and that *fw11.3* was the weaker allele of *fas*. The *FAS* gene has been cloned and has been found to encode a YABBY-like transcription factor (Cong et al. 2008). Our results show that the *fas* mutant allele is present in Orange Strawberry, a tomato cultivar bearing multilocular fruit. We confirmed the map location of *fas* in the

EP1489-EP1057 interval including *YABBY* (Supplementary Table 3) similar to what was found previously (Cong et al. 2008). However, the *fw11.3* locus in Howard German was fine-mapped to EP1057-EP1573 interval located 45 kb downstream of *YABBY* gene (Fig. 1c; Supplementary Table 2). Therefore, the fine mapping result of this study indicates that *FW11.3* and *YABBY* are two genes that map close to each other and are not alleles of the same gene.

Chromosomal rearrangements in tomato evolution

Chromosome rearrangements are mutations that alter the structure of chromosomes. Duplications, deletions, inversions, and translocations are the basic types of rearrangements (Pierce 2002). Chromosome rearrangements play important role in the plant evolution (Levin 2002). In tomato, 24.7 kb DNA fragment duplication mediated by retrotransposon *Rider* results in the increase of *SUN* gene expression and the elongation of fruit in several tomato cultivars (Xiao et al. 2008; Rodriguez et al. 2011). A 2.6-kb DNA fragment deletion in *Ripening-Inhibitor* (*Rin*) gene leads to tomato fruit that fails to ripen (Vrebalov et al. 2002). The emergence and selection of *fas* is a very important step in the tomato domestication (Lippman and Tanksley 2001; Cong et al. 2008). In this study, we found that the disruption of *FAS* resulted from a 294-kb DNA fragment inversion and its breakpoint was within the first intron of the gene (Fig. 2). Therefore, chromosome rearrangements might play more important roles in the evolution of phenotypic novelty within tomato than previously thought.

The large fruit *fw11.3* allele is partially dominant

Most QTL alleles are not completely dominant or recessive (Tanksley 1993). The genetic analyses of *fw2.2* and *fas* have shown that the large fruit alleles are partially recessive (Alpert et al. 1995; Barrero and Tanksley 2004). Molecular studies have shown that they are negative regulators of cell division and increase in carpel number, respectively. Moreover, in the case of *fas*, the large fruit phenotype resulted from a loss-of-function mutation (Cong et al. 2008). Unlike *fw2.2* and *fas*, the large fruit allele of *fw11.3* is partially dominant (Table 2). Therefore, the future cloning of *FW11.3* would shed novel insights into the molecular mechanism of increased fruit weight mediated by a partially dominant instead of a partially recessive allele.

Analysis of possible candidate genes

By searching the tomato genome annotation database we found twenty-two predicted genes in the 149-kb region corresponding to *fw11.3*. Nine of them are considered likely candidate genes of *FW11.3* based on their predicted role in cell division and/or cell expansion. SL1.00sc06004_250.1 and SL1.00sc06004_259.1 are putative serine/threonine kinase genes, SL1.00sc06004_253.1 and SL1.00sc06004_254.1 are putative serine/threonine phosphatase genes (Table 3). Serine/threonine kinases and serine/threonine phosphatases regulate many biological processes, such as cell cycle progression, growth factor response and hormone stimuli (Luan 2003; Inze and De Veylder 2006; Farkas et al. 2007; Krizek 2009). SL1.00sc06004_250.1 is a homolog of Arabidopsis *CLAVATA 1* gene. The loss of function of *CLAVATA 1* gene enlarges shoot and floral meristem size and increases floral organ number, mainly of the carpel (Clark et al. 1997). SL1.00sc06004_259.1 encodes a putative serine/threonine-protein kinase Nek5. The members of Nek family are involved in cell cycle control (O'Regan et al. 2007).

SL1.00sc06004_267.1 and SL1.00sc06004_270.1 are putative F-box genes. F-box protein is a component of Skp1/Cullin/F-box (SCF) complex, which is the largest and best characterized type of E3 enzyme. E3 enzyme is a very important component of ubiquitin–proteasome system (Smalle and Vierstra 2004). The ubiquitin–proteasome pathway plays an important role in the regulation of plant organ size (Disch et al. 2006; Song et al. 2007; Li et al. 2008; Kurepa et al. 2009; Sonoda et al. 2009).

SL1.00sc06004_252.1 is a putative large subunit GTPase 1 homolog gene. GTPases are involved in the regulation of many signaling pathways, including cell cycle procession, ubiquitin–proteasome pathway, and the auxin signaling pathway. The gene affects cell expansion and cell division (Nibau et al. 2006; Fu et al. 2009; Chen 2010).

SL1.00sc06004_255.1 is a putative DnaJ homolog subfamily C member gene. DnaJ/Hsp40 (heat shock protein 40) is a family of proteins containing a J-domain. They mostly play a role as chaperone and interact with Hsp70 to regulate protein translocation, assembly, and disassembly (Qiu et al. 2006; Kampinga and Craig 2010). Human Mrj protein is involved in the regulation of cell cycle (Dey et al. 2009). In addition to their function as chaperon proteins interacting with Hsp70, several plant DnaJ proteins also interact with other proteins and regulate abiotic stress responses (Ham et al. 2006; Yang et al. 2010), carotenoid accumulation (Lu et al. 2006), and formation of endosomes (Tamura et al. 2007).

SL1.00sc06004_268.1 encodes a putative MYB transcription factor-like protein. The MYB proteins are a large transcription factor family and have diverse function in all eukaryotes. Some MYB proteins are involved in the regulatory networks controlling cell cycle and cell differentiation (Berckmans and De Veylder 2009; Dubos et al. 2010).

In addition to the aforementioned candidate genes of *FW11.3*, SL1.00sc06004_257.1, SL1.00sc06004_261.1, SL1.00sc06004_263.1, SL1.00sc06004_265.1, SL1.00sc06004_266.1, and SL1.00sc06004_271.1 are expressed in tomato reproductive tissues (flower, ovary, and/or fruit) (Table 3). Therefore, these genes might also play a role in tomato fruit size.

To conclude, our results show that *fw11.3* is not an allele of *fas*. The *fw11.3* is found to be a major QTL controlling fruit weight in tomato. We fine-mapped the locus to a 149-kb region located 45 kb downstream of the *FAS-YABBY* gene. Unlike *fw2.2* and *fas*, the large fruit *fw11.3* allele is partially dominant over the wild-type allele from *S. pimpinellifolium* LA1589. Moreover, the mutation at *fas* was the result of a 294-kb inversion event that created a null allele of *YABBY* underlying the locus. The results from this study will not only contribute to the cloning of *FW11.3* gene, but also to further our understanding of the molecular mechanism of increased fruit weight in tomato and other crops.

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