High-Resolution Mapping of a Fruit Firmness-Related Quantitative Trait Locus in Tomato Reveals Epistatic Interactions Associated with a Complex Combinatorial Locus*1[W][OA]

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Fruit firmness in tomato (Solanum lycopersicum) is determined by a number of factors including cell wall structure, turgor, and cuticle properties. Firmness is a complex polygenic trait involving the coregulation of many genes and has proved especially challenging to unravel. In this study, a quantitative trait locus (QTL) for fruit firmness was mapped to tomato chromosome 2 using the Zamir Solanum pennelli interspecific introgression lines (ILs) and fine-mapped in a population consisting of 7,500 F2 and F3 lines from IL 2-3 and IL 2-4. This firmness QTL contained five distinct subpeaks, Firs.p.QTL2.1 to Firs.p.QTL2.5, and an effect on a distal region of IL 2-4 that was nonoverlapping with IL 2-3. All these effects were located within an 8.6-Mb region. Using genetic markers, each subpeak within this combinatorial locus was mapped to a physical location within the genome, and an ethylene response factor (ERF) underlying Firs.p.QTL2.2 and a region containing three pectin methylesterase (PME) genes underlying Firs.p.QTL2.5 were nominated as QTL candidate genes. Statistical models used to explain the observed variability between lines indicated that these candidates and the nonoverlapping portion of IL 2-4 were sufficient to account for the majority of the fruit firmness effects. Quantitative reverse transcription-polymerase chain reaction was used to quantify the expression of each candidate gene. ERF showed increased expression associated with soft fruit texture in the mapping population. In contrast, PME expression was tightly linked with firm fruit texture. Analysis of a range of recombinant lines revealed evidence for an epistatic interaction that was associated with this combinatorial locus.

Fruits such as tomato (Solanum lycopersicum) are important dietary sources for vitamins, minerals, and antioxidants, which make up the essential components of a balanced healthy diet. However, fruit quality attributes such as color, flavor, and firm texture strongly influence consumer choice in the purchase of these expensive and readily perishable crop products. Fruit texture not only affects consumer acceptance but influences transportability, resistance to disease, and shelf-life. Improving the texture quality of fruit will encourage a healthier diet, simplify logistics in the food chain, and reduce postharvest waste (Barrett et al., 2010).

Fruit firmness is determined by a number of factors including cell wall structure, turgor (Saladié et al., 2007), and cuticle properties (Chaïb et al., 2007) and is therefore likely to be a highly complex trait, involving numerous genes and pathways (Brummell and Harpster, 2001). Extensive research has focused on the biochemical and molecular aspects of fruit ripening using tomato as a model system. During tomato ripening, remodeling and degradation of the cell wall is intimately involved in softening (Crookes...
and Grierson, 1983; Seymour et al., 1990; Matas et al., 2009). Several single gene mutants in tomato have been identified that have a global effect on ripening and texture. These include ripening inhibitor and colorless nonripening, where the underlying genes have been identified by positional cloning (Thompson et al., 1999; Vrebalov et al., 2002; Eriksson et al., 2004; Manning et al., 2006). However, the links between these high-level regulators and the downstream effectors that modulate fruit firmness are still poorly understood. In order to unravel the molecular circuits controlling texture changes, efforts have concentrated on silencing cell wall-related genes in transgenic tomatoes (Brummell and Harpster, 2001). Targets have included polygalacturonase, pectin methylesterase, expansins, and galactanase. Reducing the expression of these genes individually usually has a comparatively small effect on fruit texture, although combining knockout lines can result in somewhat firmer fruits (Alexander and Grierson, 2002; Powell et al., 2003). These studies suggest that many cell wall activities are involved in texture changes, including multiple members of the same gene family. Additionally, it is now apparent that other factors, such as cuticle properties, play a much more important role than previously thought (Matas et al., 2009).

Recent advances in quantitative genetics, especially the development of tomato interspecific introgression lines (ILs), make possible the identification of genes underlying complex traits (Tanksley and Nelson, 1996; Lippman et al., 2007). Wild tomato species offer a rich and largely unexplored source of new genetic variation for breeders. A number of studies of tomato traits including fruit size (Frary et al., 2000; Cong et al., 2008), Brix (Fridman et al., 2004), parthenocarpic (Gorguet et al., 2008), and the evolutionary transition from allogamous flowers to autogamous flowers (Chen and Tanksley, 2004) have utilized wild tomato species ILs to identify genes involved in these processes. In this study, the 76 Solanum pennellii introgression lines in a S. lycopersicum background (cv M82; Eshed and Zamir, 1994) were used to identify QTLs for the textural properties. The S. pennellii ILs provide coverage of the entire genome and partition the genetic map into 107 bins that are defined by single or overlapping segments. This resource allows the "Mendelization" of QTLs and permits both the mapping and resolution of traits, while minimizing any artifacts due to environmental effects.

The aim of our study was to identify fruit firmness-associated QTLs in the S. pennellii introgression lines and reveal their underlying molecular basis. A robust firmness QTL was identified on chromosome 2. Further analysis revealed this to be a combinatorial locus in an 8.6-Mb region composed of several major effects. An ethylene response factor and a tandem triplication of pectin methylesterases were identified as QTL candidate genes based on linkage mapping and expression profiles. Analysis of a range of recombinant lines provided evidence for epistatic interactions associated with the combinatorial QTL region. Our work reveals new insights into the control of firmness in tomato and potentially other fleshy fruits.

RESULTS

Identification and Interval Mapping of a Chromosome 2 Firmness QTL

The S. pennellii IL population was screened for firmness-associated QTLs in ripe fruit, and robust effects were identified on chromosome 2 under both field and glasshouse conditions (Table I). The chromosome 2 QTL was associated with IL 2-3 and, in the field, with the overlapping introgression on IL 2-4. Therefore, we chose this chromosome 2 firmness-associated QTL for further study.

The aim of this work was to resolve the chromosome 2 firmness QTL and nominate candidate genes underlying the effect. A composite M82 × IL 2-3 F2 and M82 × IL 2-4 F3 mapping population (IL 2-4 having an overlapping region with IL 2-3) consisting of 7,500 lines was screened for recombinants with single nucleotide polymorphism TaqMan probes designed to RFLP marker sequences (Supplemental Text S1). A total of 124 informative recombinant individuals were identified: 72 M82 × IL 2-3 F2 lines and 52 M82 × IL 2-4 F3 lines. Mechanical measurements based on maximum load (force required to penetrate the

| Firmness Test | Growth Environment, Location, and Date | IL Containing Pericarp Firmness-Associated QTLs | P |
|---------------|----------------------------------------|-----------------------------------------------|---|
| Penetrometera | Field, France, 2000                    | IL 2-3                                        | <0.01|
|               |                                        | IL 2-4                                        | <0.001|
|               |                                        | IL 2-3                                        | <0.01|
| Maximum load, outer pericarpb | Glasshouse, United Kingdom, 2006 | IL 2-3                                        | <0.01|
|               |                                        | IL 3-4                                        | <0.001|
| Maximum load, inner pericarpb | Glasshouse, United Kingdom, 2006 | IL 2-3                                        | <0.001|
|               |                                        | IL 3-4                                        | <0.001|

aMeasured with a Durofel texture analyzer. A 3-mm probe was applied at two points on the fruit equator, the movement of the probe was recorded, and the average of the two measures (on 30 fruits) was used.  

bMaximum load was the force required to penetrate the pericarp tissue at 10 mm min−1 (see “Materials and Methods”).
pericarp tissue at 10 mm min\(^{-1}\) were obtained for the outer and inner pericarp of red ripe (breaker + 7 d) fruits. A total of 10 fruit were tested for each recombinant and parental line by probe penetration analysis, and substantial variation in fruit firmness was apparent (Fig. 1). For example, based on the 2007/2008 experiments, we were able to identify a number of recombinant individuals that showed the same or lower maximum load as the M82 parent (soft-textured fruit; e.g. line 654, which had mean maximum load values in both the outer and inner pericarp of 0.57 ± 0.14 N compared with M82 at 0.73 ± 0.16 N and 0.69 ± 0.13 N; Fig. 1). Other recombinants showed similar or higher maximum loads than the IL 2-3 and IL 2-4 parents (firm-textured fruit; e.g. for line 301, the mean maximum loads were 1.28 ± 0.30 N in the outer pericarp and 1.11 ± 0.27 N in the inner pericarp, which compared with values from IL 2-3 fruit of 1.04 ± 0.24 N for the outer pericarp and 0.98 ± 0.22 N for the inner pericarp; Fig. 1). We consistently observed that IL 2-4 had a softer outer pericarp than IL 2-3. Firmness differences between the outer and inner pericarp were apparent in both the IL 2-3 and M82 parental and recombinant lines (Fig. 1). The inner pericarp was generally softer than the outer pericarp in most recombinant lines (Fig. 1).

In order to establish the precise genomic location of the fruit firmness effect, a high-resolution genetic map of the IL 2-3 and IL 2-4 interval was constructed from 114 individuals with 50 PCR-based markers (Fig. 2; only informative markers are shown) using JoinMap 3.0. Interval mapping was used to generate QTL log of the odds (LOD) profiles for both the outer and inner pericarp (Fig. 2). The entire IL 2-3/IL 2-4 mapping interval delineated by markers CT255 and TG527 spanned a region of approximately 8.6 Mb.
according to the genomic sequence of Heinz 1706 (SL2.40ch02; http://solgenomics.net/). Within this 8.6-Mb region, the QTL map (Fig. 2) indicated that the major effect was located on the overlapping region of IL 2-3 and IL 2-4, with an additional effect on the remainder of the IL 2-4 introgressed segment. The main peak in the LOD trace contained five distinct subpeaks, which exceeded the significance threshold ($P < 0.05$) for either inner or outer pericarp tissue. These data suggested that the fruit firmness effect in this region of chromosome 2 was part of a combinatorial locus. For the purpose of the analysis, and in order to nominate candidate genes, we identified five subpeaks as $Fir^{p}\cdot QTL2.1$ to $Fir^{p}\cdot QTL2.5$. To enable an association between QTL intervals and the tomato physical map, genetic marker sequences were linked to the genomic sequence of Heinz 1706 scaffold SL2.40ch02. $Fir^{p}\cdot QTL2.1$, delineated by PCR-based markers TG453 and Le01498, encompassed a 667.3-kb region. $Fir^{p}\cdot QTL2.2$, flanked by PCR-based markers PH3700 and cLET-1-49, had a physical mapping interval of 222.2 kb. $Fir^{p}\cdot QTL2.3$, delineated by PCR-based markers LM0127 and LM1650, spanned a 168.3-kb region. $Fir^{p}\cdot QTL2.4$ had the smallest mapping interval of 110.1 kb and was delineated by PCR-based markers LE5100 and LE5200. $Fir^{p}\cdot QTL2.5$ had the largest mapping interval of 1.3 Mb defined by PCR-based markers HB5350 and TG567 (Fig. 2). The nonoverlapping S. pennelli segment in IL 2-4 extended beyond the TG567 marker to encompass a further 1.5-Mb region. Multiple QTL mapping (MQM) analysis was also undertaken with cofactors underlying either $Fir^{p}\cdot QTL2.4$ or $Fir^{p}\cdot QTL2.5$ and reduced the LOD score to below 1 at all other $Fir^{p}\cdot QTL$ regions, although small peaks remained. In all cases, a peak in the LOD trace of between 1 and 2 remained in the nonoverlapping part of IL 2-4, although the location of the peak varied depending on the cofactor selected. Both interval mapping and MQM analysis indicated that there was more than one effect contributing to the chromosome firmness QTL in the overlapping region, together with a further effect in the nonoverlapping component of IL 2-4.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{A genetic map of the chromosome 2 fruit firmness QTL mapping interval. The genetic map shows the M82 × IL 2-3 F2 and M82 × IL 2-4 F3 mapping population IL 2-3 and IL 2-4 chromosome 2 region. Distances are measured in Kosambi centimorgan units calculated from only those lines that recombined within the region of interest, and QTL regions are shaded in black. Physical marker distances in bp were obtained from the Heinz 1706 scaffold SL2.40ch02 and are marked by the bar on the far left. The outer pericarp QTL LOD profile is represented by the dark gray line, and the inner pericarp QTL LOD profile is represented by the light gray line. Vertical dashed lines represent the significance threshold $P$ value of 0.05.}
\end{figure}
Identification of Candidate Genes under \( \text{Fir}^{\text{pp}} \text{QTL2.5} \) and \( \text{Fir}^{\text{pp}} \text{QTL2.2} \)

A promising candidate gene mapping under the \( \text{Fir}^{\text{pp}} \text{QTL2.5} \) was identified from a GeneChip experiment (Supplemental Data Set S1). The expression of this gene, a pectin methylesterase (PME2.5), was approximately 101-fold higher \((P < 0.0001)\) in mature green fruits at 40 d post anthesis (DPA) in IL 2-3 fruit when compared with M82. The PME2.5 expression in IL 2-3 was higher than M82 at 15 and 25 DPA (15 d, 50-fold; 25 d, 89-fold) but returned to the levels seen in M82 in red ripe fruits (Supplemental Fig. S1). The GeneChip data were validated by quantitative PCR (Fig. 3, A and B). The genomic sequence of Heinz 1706 was used to identify a triplicated region containing three closely related PME gene models (Solyc02g080200.2, Solyc02g080210.2, and Solyc02g080220.2) and lying under \( \text{Fir}^{\text{pp}} \text{QTL2.5} \). All three PME genes matched the probe sequences on the array, and they could not be readily discriminated by quantitative PCR due to extremely high levels of sequence homology.

The \( \text{Fir}^{\text{pp}} \text{QTL2.2} \) mapping interval also contained a promising candidate gene showing differential expression between the parental lines (Fig. 3, C and D). The expression of 29 genes within \( \text{Fir}^{\text{pp}} \text{QTL2.2} \) was analyzed using semiquantitative reverse transcription (RT)-PCR (Table II), and candidate genes were selected on the criteria that they were expressed in fruit and showed marked differences in expression between the parental lines M82 and IL 2-3. From these data, three differentially expressed genes were identified. Their putative functions were an ethylene response factor (\( \text{ERF2.2} \); Solyc02g077840.1), an E6-2 protein kinase (Solyc02g077710.1), and an unknown protein (Solyc02g077690.2; Supplemental Fig. S2).

As ERFs are known to have a role in fruit ripening (Alexander and Grierson, 2002; Chung et al., 2010), we hypothesized that \( \text{ERF2.2} \) was a good candidate. Therefore, we chose to investigate this gene further, and using quantitative RT-PCR, the expression of \( \text{ERF2.2} \) was assayed at two developmental stages, mature green and breaker, in outer and inner pericarp tissue in lines M82 (soft-textured fruit) and IL 2-3 (firm-textured fruit). Expression of this gene at the breaker stage was substantially higher in M82 outer pericarp (approximately 12-fold) and inner pericarp (approximately 9-fold) compared with IL 2-3. However, we could find little difference in expression between either of the parents at the mature green stage (Fig. 3, C and D).

Modeling the Location of a Major Fruit Firmness QTL

We investigated the ability of simple statistical models based on a small number of genes, possibly interacting...
in an epistatic manner, to explain the observed variability between the firmness of the fruits from the populations. In the simplest model, containing only a single effect at \textit{PME2.5}, the deviance change from line-to-line variability unexplained by the model was 10.7 for the outer pericarp and 6.1 for the inner pericarp (both on 1 degree of freedom) and indicated a significant lack of fit \((P < 0.01\) and \(P < 0.05\), respectively). Therefore \textit{PME2.5} alone fails to explain the variability in fruit firmness observed between lines, which is consistent with the hypothesis that the QTL effect involves more than this one locus. When the model was extended to include both \textit{PME2.5} and the region on the nonoverlapping portion of IL 2-4, the deviance change from unexplained line-to-line variability was reduced to 9.1 for the outer pericarp and to 4.9 for the inner pericarp, which again indicated a significant lack of fit \((P < 0.01\) and \(P < 0.05\)) to the model. Extending the model further to include the additional \textit{ERF2.2} locus reduced the deviance change from unexplained line-to-line variability to 3.9 for the outer pericarp and to 0.8 for the inner pericarp, which is a marginally significant \((P < 0.05)\) lack of fit in the outer pericarp and is not significant in the inner pericarp. This additional analysis supports the observations of the interval mapping that our region of interest contains a combinatorial firmness QTL. The mean maximum load values for the different combinations of alleles at \textit{Fir}\textsuperscript{p} QTL2.2 and \textit{Fir}\textsuperscript{p} QTL2.5 and the nonoverlapping region of IL 2-4 were included in the model and are summarized in Figure 4 and Supplemental Table S3. When assessing the trends in fruit firmness across both the outer and inner pericarp with the various QTL combinations, the recombinant lines with the highest maximum load generally possessed two \textit{S. pennellii} alleles at either \textit{ERF2.2} or \textit{PME2.5} or at both these loci (e.g. lines homozygous for \textit{S. pennellii} alleles at \textit{ERF2.2} and heterozygous for \textit{PME2.5} and homozygous for \textit{M82} in the nonoverlapping IL 2-4 region were significantly \([P < 0.05]\) firmer than \textit{M82} in the outer pericarp; Fig. 4A, BHA). One \textit{S. pennellii} allele at either of these loci was insufficient to enhance fruit firmness (Fig. 4). The nonoverlapping IL 2-4 region appears to have an epistatic effect when \textit{S. pennellii} alleles are present within this region (Fig. 4). This trend occurs in both the outer and inner pericarp, although the effects are more apparent in the inner pericarp (e.g. lines heterozygous at \textit{ERF2.2}, with two

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**Table II. Expression of putative gene models underlying a 222.4-kb region of \textit{Fir}\textsuperscript{p} QTL2.2**

The expression of 29 genes within \textit{Fir}\textsuperscript{p} QTL2.2 was analyzed on the criteria that they were expressed in fruit and showed marked differences in expression between the parental lines \textit{M82} and IL 2-3.

| No. | Gene Identifier* | Gene Annotation* | Expressed in Fruit | Expression Difference, M82 Versus IL 2-3 |
|-----|------------------|------------------|--------------------|----------------------------------------|
| 1   | Solyc02g077600.2 | Guanylate-binding protein | Yes                | No                                     |
| 2   | Solyc02g077610.2 | NAC domain protein    | Yes                | No                                     |
| 3   | Solyc02g077620.1 | Sulfotransferase3a    | Yes                | No                                     |
| 4   | Solyc02g077630.2 | Receptor-like kinase, RLK | Yes          | No                                     |
| 5   | Solyc02g077640.1 | Unknown protein       | Yes                | No                                     |
| 6   | Solyc02g077650.1 | Pentatricopeptide repeat | Yes         | No                                     |
| 7   | Solyc02g077660.2 | Homeobox protein      | Yes                | No                                     |
| 8   | Solyc02g077670.2 | Chaperone protein dnaI | Yes                | No                                     |
| 9   | Solyc02g077680.2 | Phosphorylase         | Yes                | No                                     |
| 10  | Solyc02g077690.2 | Unknown protein       | No                 | No                                     |
| 11  | Solyc02g077700.1 | Unknown protein       | Yes                | Yes                                    |
| 12  | Solyc02g077710.1 | E6-2 protein kinase   | Yes                | Yes                                    |
| 13  | Solyc02g077720.2 | Unknown protein       | Yes                | No                                     |
| 14  | Solyc02g077730.2 | Unknown protein       | Yes                | No                                     |
| 15  | Solyc02g077740.2 | Nucleic acid-binding, OB-fold | Yes     | No                                     |
| 16  | Solyc02g077750.2 | Fiber protein Fb2     | Yes                | No                                     |
| 17  | Solyc02g077760.1 | Unknown protein       | _b_                | _b_                                    |
| 18  | Solyc02g077770.2 | Unknown protein       | Yes                | No                                     |
| 19  | Solyc02g077780.2 | Necrotic spotted lesions1 | Yes          | No                                     |
| 20  | Solyc02g077790.1 | Unknown protein       | No                 | No                                     |
| 21  | Solyc02g077800.1 | Unknown protein       | Yes                | No                                     |
| 22  | Solyc02g077810.1 | Ethylene-responsive transcription factor4 | No       | No                                     |
| 23  | Solyc02g077820.1 | Unknown protein       | Yes                | No                                     |
| 24  | Solyc02g077830.1 | Unknown protein       | No                 | No                                     |
| 25  | Solyc02g077840.1 | Ethylene response factor12 | Yes        | Yes                                    |
| 26  | Solyc02g077850.2 | Leu-rich repeat receptor-like Ser/Thr protein kinase | Yes    | No                                     |
| 27  | Solyc02g077860.1 | Ribulose bisphosphate carboxylase | Yes | No                                     |
| 28  | Solyc02g077870.1 | Unknown protein       | No                 | No                                     |
| 29  | Solyc02g077880.2 | Auxin-repressed protein | Yes               | No                                     |

*Data are from tomato sequence build SL2.40.ch02 (http://solgenomics.net/). _b_Missing data, failed amplification.
S. pennellii alleles at PME2.5 and homozygous for M82 in the nonoverlapping IL 2-4 region, were significantly [P < 0.05] firmer than similar lines homozygous for S. pennellii across the nonoverlapping IL 2-4 region; Fig. 4B, HBA compared with HBB).

Expression of QTL Candidate Genes in Recombinant Lines

The output from the statistical modeling is consistent with genes under Fir^{P} QTL2.2 and Fir^{P} QTL2.5 contributing to the chromosome 2 fruit firmness combinatorial QTL. The ERF2.2 and PME2.5 QTL candidate genes showed marked expression differences between the parental lines M82 and IL 2-3. We predicted that recombinant lines with M82 alleles at these loci would have, respectively, high ERF2.2 and low PME2.5 expression. The reverse patterns of expression should be observed where S. pennellii alleles were present at these loci. These predictions were tested by analysis of the expression of these genes in a range of different genotypes. Recombinant line 910 contained M82 alleles at both the ERF2.2 and PME2.5 loci. Expression of ERF2.2 was relatively high for both the outer and inner pericarp (Fig. 5) and had a similar profile to that for M82 (Fig. 3, C and D). However, in line 910, PME2.5 expression was undetectable (Fig. 5) and the fruit had a soft texture. In line 301, where the ERF2.2 and PME2.5 alleles were both from S. pennellii, the ERF expression was low and PME expression was high (Fig. 5). These profiles were similar to the IL 2-3 parent (Fig. 3), and line 301 had fruit with a firm texture. Line 2619 had S. pennellii alleles at the ERF2.2 locus and was heterozygous at the PME2.5 locus. This line had low expression of ERF2.2 and intermediate expression of PME2.5 and produced firm fruit. Line 1088 was heterozygous at both the ERF2.2 and PME2.5 loci, having an M82 and an S. pennellii allele at each of
these loci, but also heterozygous within the nonoverlapping IL 2-4 region. ERF2.2 expression in this line was lower than in line 910 (approximately 0.9-fold in the outer pericarp and approximately 5.2-fold in the inner pericarp) but higher than in line 301 (approximately 1.6-fold in the outer pericarp and approximately 0.1-fold in the inner pericarp). PME2.5 had lower levels of expression in line 1088 than might be expected from this genotype (Fig. 5) and a soft-fruit phenotype, which was consistent with an effect from S. pennellii alleles in the nonoverlapping IL 2-4 region. These results, and those in Figure 4 with respect to genotypes bearing the nonoverlapping IL 2-4 region, are suggestive of an epistatic interaction affecting fruit firmness and the expression of texture-related genes.

Analysis of Selected QTL-Near Isogenic Lines

A subset of recombinant lines were used to develop QTL-Near Isogenic Lines (NILs), and the firmness of fruits from selected QTL-NILs was measured (Fig. 6). A QTL-NIL, Q301, containing Fir\textsuperscript{p} QTL2.1-2.5 (delineated by markers T385 and TG567), had fruit with a similar outer and inner pericarp firmness to IL 2-3 but contained a smaller introgressed fragment than this parental line. Two additional lines were selected that together spanned the region covered by Q301 in an overlapping manner. Q678 containing Fir\textsuperscript{p} QTL2.1-2.3 (delineated by markers T385 and LM4500) was significantly (P < 0.01) softer in both outer and inner pericarp than Q301. The other QTL-NIL, Q5481, spanned Fir\textsuperscript{p} QTL2.3-2.5 (delineated by markers CT277 and TG567) and was significantly (P < 0.05) softer than Q301 in the outer pericarp (Fig. 6). We also selected a QTL-NIL, Q926, that harbored an introgression in the IL 2-4 nonoverlapping region (delineated by markers TG353 and TG527). In comparison with Q301, Q926 was significantly softer than Q301 in both outer (P < 0.01) and inner (P < 0.001) pericarp. Additionally, Q926 was also significantly (P < 0.001) softer in the inner pericarp than M82. These data provide further compelling evidence that the firmness QTL from S. pennellii on chromosome 2 is a combinatorial
locus that likely includes an enhanced softening effect on the IL 2-4 nonoverlapping region.

**DISCUSSION**

ILs and advanced backcross QTL approaches are now well-established tools for dissecting the genetic basis of complex polygenic traits in plant populations. In this study, *S. pennellii* ILs were used to identify a fruit firmness QTL located to an 8.6-Mb region on tomato chromosome 2. A prior study had identified a firmness QTL, *fir2.1* in *Solanum pimpinellifolium* (Doganlar et al., 2002), mapping within this region. Conservation of QTLs within the Solanaceae family has been demonstrated for a number of traits including fruit size, shape, and weight (Saliba-Colombani et al., 2001; Frary et al., 2004). However, QTL *fir2.1* in *S. pimpinellifolium* has not been further resolved by fine-mapping. In this work, we have used a composite mapping population of M82 × IL 2-3 F2 and M82 × IL 2-4 F3 to resolve the *S. pennellii* firmness QTL on chromosome 2 and report that at least several major firmness effects occur within a mapping interval of approximately 8.6 Mb, delineated by markers CT255 and TG527. Other studies in tomato have reported that multiple loci within a small genomic region can contribute to quantitative traits including fruit weight (Goldman et al., 1995), leaf dissection (Holtan and Hake, 2003), and stigma exsertion (Chen and Tanksley, 2004). Fine-mapping resolves multiple-loci QTLs that were previously masked as a single locus, due to the availability both of additional markers within the region and lines with smaller introgressed segments (Doganlar et al., 2002; Holtan and Hake, 2003; Lecomte et al., 2004). Our F2 data provide strong evidence that the firmness QTL on tomato chromosome 2, delineated by markers CT255 and TG527, is a combinatorial locus. Analysis of a small number of QTL-NIL lines demonstrated that those harboring only a subset of the *S. pennellii* firmness-associated QTL effects always had softer fruit than a line that contained all the *S. pennellii* QTLs. This provides further compelling evidence that the *S. pennellii* introgression on chromosome 2 harbors a combinatorial QTL for fruit firmness. The significance levels for the QTL effects differed between the outer and inner pericarp tissues (Figs. 1 and 2). A possible explanation for this disparity is that gene expression is not uniform across the different fruit tissues during ripening. This hypothesis is supported by a study of tissue-specific metabolites during tomato development, where differences in metabolite profiles were detected between specific tissues and at different ripening stages (Moco et al., 2007).

Recombination suppression hampered progress in linkage mapping within our combinatorial QTL interval, reducing substantially the number of available recombinant individuals, so that after screening a total of 7,500 F2 individuals, only 124 recombinants were identified. This level of suppression is relatively high in comparison with other equivalent fine-mapping studies. For example, Chen and Tanksley (2004) identified 123 recombinants from 1,535 F2 individuals within the *S. pennellii* IL 2-5 region for the fine-mapping of *se2.1* on chromosome 2. Recombination suppression varies across Solanaceae genomes and chromosomal locations (Alpert et al., 1995; Ku et al., 1999; Monforte and Tanksley, 2000; Gorguet et al., 2008), and in some regions, recombination hotspots have been identified, including on *S. pennellii* chromosome 9 (Fridman et al., 2000).

We identified several major fruit firmness effects in our study, and using the new tomato genome sequence, we were able to nominate candidate genes mapping within two of the subpeaks. A likely candidate gene under *Fir*-*p* QTL2.5 was identified from a tomato microarray experiment. A pectin methylesterase (PME2.5) was approximately 100-fold more highly expressed in IL 2-3 in comparison with M82 during most stages of fruit development and ripening. Previous studies have indicated the importance of PME during ripening in tomato and other fruits due to its function as a cell wall-modifying enzyme (Harriman et al., 1991; Hall et al., 1993; Goulao and Oliveira, 2008). PME acts to modify the structure of the cell wall.
by the deesterification of GalUA residues in high-M, pectin (Harriman et al., 1991; Hall et al., 1993; Phan et al., 2007). This can facilitate interactions between adjacent pectin molecules through calcium cross-linking. This is thought to increase cell-to-cell adhesion and tissue strength (Jolie et al., 2010). Studies with transgenic tomato lines containing reduced levels of a PME isoform (Pmeu1) showed enhanced softening (Phan et al., 2007), indicating that the expression of this PME gene is positively related to increased fruit firmness. Furthermore, elevated expression of the PME gene PEST1 was associated with improved texture in potato (Solanum tuberosum; Ross et al., 2011). These results indicate that PME may play an important role in enhancing fruit texture when expressed at high levels. The PME PEST1 also shares sequence homology with the three PME gene models underlying Fir<sup>fp</sup> QTL2.5.

ERF2.2 (Solyc02g077840.1) was identified under Fir<sup>fp</sup> QTL2.2. Ethylene plays an important role in tomato ripening and in controlling traits such as aroma, flavor, color, and texture (Alexander and Grierson, 2002). In other fleshy fruits, genes involved in ethylene biosynthesis and regulation have been associated with fruit firmness, including in melon (Cucumis melo), where a decrease in fruit firmness was correlated with an ethylene climacteric-ripening phenotype (Moreno et al., 2008). In kiwifruit (Actinidia delicosa), expression patterns of an ethylene receptor exhibited a strong association with fruit softening (Yin et al., 2008). ERFs are known to regulate ripening-related genes in tomato (Cara and Giovannoni, 2008), and overexpression of LeERF1 heightened both ripening and softening (Li et al., 2007). Expression of ERF2.2 was significantly (√<sub>P</sub> = 0.02) higher in the soft M82 parent in comparison with the firm IL 2-3 line. It is well established that ripening in tomato is coordinated by ethylene; therefore, it might be expected that elevated ERF2.2 levels would be associated with more rapid ripening and softening.

Genes associated with cell wall softening such as polygalacturonase and PME are regulated by ethylene (Alexander and Grierson, 2002), and in the latter case, PME2.2 is down-regulated at the onset of ripening. Therefore, ERF2.2 and PME2.5 may play major roles in our firmness QTL and have antagonistic effects. The higher ERF2.2 expression in M82 may have been preferentially selected for during domestication as a ripening trait. The QTL effects are summarized in a model that we believe encapsulates the data and is presented in Figure 7. When the ERF2.2 and PME2.5 alleles are both from <i>S. lycopersicum</i>, as for example in line 910 or M82, then at early stages of ripening, ERF2.2 expression is high and PME2.5 expression is low or undetectable and fruit softening proceeds rapidly. In line 301 or the IL 2-3 parent, ERF2.2 expression is low and PME2.5 is high during the early ripening phase. In comparison with M82, this results in slower ripening due to reduced ERF2.2 levels and higher firmness due to elevated PME2.5. In line 2619, which has homozygous <i>S. pennellii</i> ERF2.2 alleles but is heterozygous between <i>S. pennellii</i> and <i>S. lycopersicum</i> for the PME2.5 allele, ERF2.2 has low expression and PME expression is approximately 50% of that for the <i>S. pennellii</i> IL 2-3 parent. These results were consistent with those that could be predicted from the genotypes.

In all the above examples shown in Figure 7, the nonoverlapping IL 2-4 distal region was homozygous for M82 alleles. However, in line 1088 (Fig. 7), alleles at all loci are heterozygous and the fruit have a soft phenotype and lower levels of PME2.5 expression than would be predicted when compared with line 2619, which has the same genotype as line 1088 at PME2.5. A possible explanation for the phenotype of line 1088 and other lines containing <i>S. pennellii</i> alleles in the nonoverlapping IL 2-4 region is that the presence of <i>S. pennellii</i> alleles in this region results in an epistatic effect leading to softer fruit. This is also supported by the QTL-NIL analysis on line Q926 (Fig. 6). However, as yet, we have not been able to identify the

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**Figure 7.** Schematic model of the chromosome 2 mapping interval showing antagonistic effects between candidate genes ERF2.2 and PME2.5. The orange regions represent M82 DNA, and the green regions represent <i>S. pennellii</i> DNA. White boxes represent QTL regions containing candidate genes ERF2.2, PME2.5, and the distal region (DR) of IL 2-4, which does not overlap with IL 2-3. Red arrows show expression levels of candidate gene ERF2.2, and green arrows show expression levels of candidate gene PME2.5. The thickness of the arrow indicates high or low expression level. The boxes at bottom show line, ERF2.2 and PME2.5 genotypes as follows (A = M82, B = <i>S. pennellii</i>, and firmness phenotype.)
mechanism, because of the large mapping interval that harbors more than 200 gene models, and nominating candidate genes requires further investigation. Additionally, the statistical model we have used (Fig. 4) indicates that our two candidate genes and the IL 2-4 nonoverlapping region cannot fully account for all the line-to-line firmness variation. This indicates that there may be other genes within the 8.6-Mb region that are involved in this complex combinatorial locus, or that we have yet to select the best candidates. When recombinant individuals are created, with new combinations of S. pennellii and S. lycopersicum alleles within the chromosome 2 combinatorial fruit firmness locus, epistatic interactions occur (e.g. softer fruit phenotypes in lines containing S. pennellii alleles in the overlapping IL 2-3 and IL 2-4 region and S. pennellii alleles in the nonoverlapping IL 2-4 region). We speculate that there could be a direct or indirect epistatic interaction between the nonoverlapping IL 2-4 region and PME2.5. However, there are many factors that could be involved after the hybridization of genomes, including regulatory divergence and the epigenetic state of the loci of interest (Udall and Wendel, 2006; Shivaprasad et al., 2012), and these factors may also account for the transgressive firmness phenotypes observed in this study. For example, the prominent firm F2 line 301 was consistently firmer than the parental line IL 2-3. Also, among the recombinant individuals, some lines displayed softer phenotypes than the M82 parent (Fig. 1). The firmness trait is highly variable and affected by the environment, and this could be mediated through the ERF. Indeed, a recent report indicates that these genes can act as environmentally responsive (Gibbs et al., 2011). Our candidate firmness-related genes will now be validated in transgenic plants. We hypothesize that up-regulating the ERF2.2 gene in a firm-fruited background, containing S. pennellii alleles for the combinatorial firmness QTL on chromosome 2, will lead to rapid softening. It will be especially interesting to determine if it also leads to greatly reduced PME2.5 expression.

There are few published accounts of the dissection of combinatorial loci for crop traits. This study demonstrates that important agronomic traits such as fruit texture can be highly complex, involving combinatorial loci with multiple interactions. It seems likely that high-resolution studies on other traits of economic importance will reveal that this situation is much more common than would be supposed from the limited literature currently available after successful cloning of genes underlying QTLs. In summary, several major fruit firmness QTLs were identified and located to an 8.6-Mb region on chromosome 2. Candidate genes identified and profiled included an ERF and cell wall-modifying PMEs, which mapped under Fir2.2 QTL2.2 and Fir4.5 QTL2.5, respectively. This study provides evidence to construct a model to explain the associations between genotype and phenotype within the combinatorial QTL region and suggests that epistatic interactions are occurring in the hybrid backgrounds.

It provides important new insights into the complexity and gene interactions involved in fruit firmness that may be transferable to other fleshy fruit crops. Future experiments will involve testing our fruit firmness candidate genes in transgenic plants and further investigating possible epistatic interactions.

**MATERIALS AND METHODS**

**Plant Material and Mapping Population**

*Solanum pennellii* ILs and the tomato (*Solanum lycopersicum* ‘M82’) recurrent parent were grown in the United Kingdom during 2006 for a preliminary fruit firmness QTL screen under standard glasshouse conditions (16-h daylength, day temperature of 20°C, and night temperature of 18°C). Plants were grown in 7.5-L pots of Levington M2 pot/bedding compost. Irrigation was supplemented with Vitax 214. The population was grown in a randomized design planted in four blocks each containing one plant of the 76 S. pennellii lines. Four fruits from each line were tagged at the breaker stage and harvested 7 d later at the red ripe stage. The environmental conditions within the glasshouse were recorded throughout the experiment and included in the statistical analysis.

*S. pennellii* ILs and the *S. lycopersicum* ‘M82’ recurrent parent were grown under field conditions in France during 2000. Plant growth conditions were as described by Cause et al. (2004).

Parental lines used throughout the main study were *S. lycopersicum* ‘M82’ and *S. pennellii* IL 2-3 and IL 2-4. Seed for the IL 2-3 × M82 and IL 2-4 × M82 mapping population was provided by Dani Zamir (Hebrew University of Jerusalem). IL 2-3 × M82 F1 and IL 2-4 × M82 F2 plants that were heterozygous were selfed to produce IL 2-3 × M82 F2 and IL 2-4 × M82 F3 mapping populations. A total of 7,300 F2/F3 seedlings were screened using TaqMan probes designed to RFLP marker sequences CT255 and TG353 for M82 × IL 2-3 F2 and TG451 and TG583 for M82 × IL 2-4 F3 for the identification of recombinants (Supplemental Text S1). A total of 124 informative recombinant individuals were identified: 72 M82 × IL 2-3 F2 and 52 M82 × IL 2-4 F3 lines. Recombinants were grown in summer 2007 and 2008 under standard glasshouse conditions (16-h daylength, day temperature of 22°C, and night temperature of 20°C). Plants were grown in 7.5-L pots of Pro C2 coarse potting compost (Levington). Irrigation was supplemented with Vitax 214. Ten fruits per line were tagged at the breaker stage and harvested 7 d later. Replicates of 10 recombinant lines from 2007 were re-screened for firmness in 2008.

QTL-NILs were derived from IL 2-3 × M82 F2 and IL 2-4 × M82 F3 recombinant lines. Plants were grown during 2009 under standard glasshouse conditions (16-h daylength, day temperature of 22°C, and night temperature of 20°C). Plants were grown in 7.5-L pots of Pro C2 coarse potting compost (Levington). Irrigation was supplemented with Vitax 214. Ten fruits per line were tagged at the breaker stage and harvested 7 d later.

**Phenotypic Analysis**

Phenotypic analysis in the United Kingdom was carried out on both parental lines throughout the study and recombinant F2 and F3 individuals (124). A 6-mm transverse section was cut from each fruit, and the maximum load (the force required to penetrate the pericarp tissue at 10 mm min⁻¹) was measured using a Lloyd Instrument LF plus machine equipped with a 10-N load cell and 1.6-mm flat-head cylindrical probe. Measurements were taken separately from the outer and inner pericarp in duplicate. For the purposes of this study, outer pericarp was defined as below the skin but before the vascular boundary. Inner pericarp was defined as the cells between the vascular boundary and the endodermis. Fruit weight and color were also recorded. Color was measured using a Minolta Chroma Meter. Under field conditions in France, fruit firmness was measured using a Durafel texture analyzer: a 3-mm probe was applied at two points on the fruit equator, the movement of the probe was recorded, and the average of the two measures (on 30 fruits) was used.

**Statistical Analysis**

Maximum load data from the 2007 and 2008 recombinant experiments were analyzed separately for the inner and outer pericarp using restricted maximum likelihood (REML; Patterson and Thompson, 1971) after log transformation to
stabilize the residual variance. All analyses were carried out using the statistical analysis package GenStat (VSN International). The data from both years were analyzed jointly. Plant line was taken as the only fixed factor, while year, the number of the truss from which the fruit was harvested, the position in the glasshouse of the plant from which the fruit was harvested, the remaining variability between plants, and the remaining variability between fruit and between the two measurements on each fruit were all taken as random factors.

For the analysis of inner pericarp data, the variance component of the plant-to-plant variability was allowed to vary between years. For the analysis of outer pericarp data, the variance component of the truss was allowed to vary between years. The line means from this analysis were used in QTL mapping.

The maximum load data from the 2009 experiment on QTL-NILs were analyzed similarly to the recombinant experiments. The analysis was performed separately for the inner and outer pericarp, by REML, and on log-transformed data. Plant line was taken as the only fixed factor, while the number of the truss from which the fruit was harvested, the position in the glasshouse of the plant from which the fruit was harvested, the remaining variability between plants, and the remaining variability between fruit and between the two measurements on each fruit were all taken as random factors. After the analysis, t-tests were performed between informative pairs of treatments. A two-sided test was used to compare IL 2-3 and line Q301, since our model predicted that these lines would be similar. Other tests, where our model predicted one line as being firmer than the other, were one-sided.

Genotypic Analysis

Plant DNA was extracted from leaf material using the DNasey Plant Mini Kit (Qiagen) according to the manufacturer’s instructions. Primers were designed to chromosome 2 RFLP marker sequences and bacterial artificial chromosome end sequences obtained from the Sol Genomics Network Web site (http://www.sgn.cornell.edu/) using Primer3 (http://frodo.wi.mit.edu/; Supplemental Table S1). Markers were tested on parental varieties S. lycopersicum ‘M82’ and S. pennelli introgression lines IL 2-3 and IL 2-4. Polymorphic markers (Supplemental Table S1) were mapped using 119 recombinants. The PCR program consisted of a denaturing step of 95°C for 3 min, 35 cycles of 94°C for 30 s, 59°C for 45 s, and 72°C for 1 min, and a final extension step of 10 min at 72°C. PCR products were either sequenced or examined by single-strand conformational polymorphism assay using Sequa Gel MD (National Diagnostics) and visualized by silver staining (Bassam et al., 1991).

Map Construction and QTL Analysis

Linkage maps were calculated from recombination frequencies (0.4) and a LOD of 3.0 in JoinMap 3.0 (van Ooijen and Voorrips, 2001). QTL analysis was carried out using MapQTL version 5.0 (van Ooijen, 2004). For IL 2-3 × M82 F2 and IL 2-4 × M82 F3 mapping populations, predicted means generated from the REML analysis for each recombinant line were used in the analysis. Interval mapping was carried out on one linkage group created following the map construction of chromosome 2 using JoinMap 3.0 (van Ooijen and Voorrips, 2001). LOD scores were generated by a permutation test (1,000 cycles) to determine the genomic location of QTLs with a confidence interval of 95%. MQM analysis was carried out in MapQTL version 5.0 (van Ooijen, 2004) using a variety of different single markers as cofactors.

Model for the Location of Major Fruit Firmness Effects

The ability of models for the location of QTLs to explain the line data was assessed with further ML analyses of the original “maximum load” data. The fixed model in the REML analyses described earlier was replaced with a factor that had different levels for each combination of homozygotes and heterozygotes at all of the putative QTL loci included in the model, as well as a level for each of the three parental lines and any individual progeny line whose genotype could not be unambiguously categorized at any of the putative QTL loci. The random model in the previous REML analyses was supplemented with a term for the remaining variability between lines that were classified as the same by the putative QTL. If the putative QTL included in the model explains all the genetic variability among the lines, then the variance component of this factor will be zero, whereas if it does not, then this variance component will be positive. The significance of this variance component, and hence of the lack of fit of the putative QTL model, was assessed by comparing the deviance of the REML with and without this term with a χ² distribution on 1 degree of freedom.

RT-PCR

Expression levels of 29 putative genes were analyzed in lines M82 and IL 2-3. Total RNA was isolated from outer and inner pericarp fruit tissue collected 40 DPA (mature green) and at breaker using the RNeasy Plant Mini Kit (Qiagen) and treated with RNase-free DNase (Qiagen) according to the manufacturer’s instructions. The concentration of RNA was determined using an Agilent Bioanalyzer 2100 (Agilent Technologies). First-strand complementary DNA (cDNA) was synthesized from 0.5 μg of total RNA using 0.5 μg of random hexamers (Promega) in a 15-μL volume and incubated at 70°C for 5 min, followed by the addition of 0.5 μl deoxynucleoside triphosphates (Promega), 25 units of RNase inhibitor (Promega), 5 μL of Moloney murine leukemia virus reverse transcriptase buffer XS (Promega), 1 μL of Moloney murine leukemia virus reverse transcriptase (Promega), and made up to 25 μL with distilled water. The mixture was incubated at 25°C for 10 min, followed by 42°C for 1 h. After RT, the cDNA samples were diluted 4-fold. Primers were designed to the 29 annotated putative gene model sequences obtained from tomato sequence build SL2.4b (http://solgenomics.net/) using Primer3 (http://frodo.wi.mit.edu/; Supplemental Table S2). The PCR program consisted of a denaturing step of 95°C for 3 min, 35 cycles of 94°C for 30 s, 59°C for 45 s, and 72°C for 1 min, and a final extension step of 10 min at 72°C. PCR products were visualized.

Microarray

Lines M82 and IL 2-3 were used for the microarray expression studies to identify candidate genes underlying the firmness QTL on chromosome 2. Fruit were collected, consisting of three independent biological replicates per line, at 15 (immature green), 25 (rapid expansion stage), and 40 (mature green) DPA and immediately frozen in liquid nitrogen. Total RNA was isolated according to methods described by Manning et al. (2006). The concentration of RNA was determined using an Agilent Bioanalyzer 2100 (Agilent Technologies). Total RNA was treated with DNA-free (Ambion) as per the manufacturer's instructions. The .cel files were loaded into GeneSpring GX (Agilent Technologies) and normalized using the robust multichip average prenormalization algorithm, and subsequently each probe set signal was normalized to the median signal of that probe set across all hybridizations. The data were analyzed by two-way ANOVA of genotype differences.

Quantitative RT-PCR

Expression levels of ERF2.2 and PME2.5 in lines M82, IL 2-3, and recombinant individuals (lines 301, 2019, 910, and 1088) were validated using TaqMan quantitative RT-PCR. Samples consisting of three independent biological replicates were taken at the mature green stage for line M82 and for all lines and at the breaker stage. Primers and dual-labeled fluorescent probes (5'FAM and 3'TAMRA) were designed using Primer3 (http://frodo.wi.mit.edu/). The following primers and probes were used: EF2M forward (5'-CCGCTCTCTTACCTACGF3'), EF2M reverse (5'-ATACCCACCTGCGCTC3'), ERF TagMan probe (5'-TCCGGCCGGCCGACCTACT3'), PME forward (5'-TGCAGCAGCAAGAGTCTC3'), PME reverse (5'-GGCAGAACCTGAGATGTCG3'), PME TagMan probe (5'-TTCCCCAGTCGCCGACCTGAC3'), Elongation Factor1α (EF1α) forward (5'-ACCTTCTGATGACCTGAC3'), EF1α reverse (5'-TGGTTCAGCACAAGGCTC3'), EF1α TagMan probe (5'-TGGTTCAGCACAAGGCTC3'). The PCR contained a 5-μL cDNA pool, 7.5 μL of 2× LightCycler® 480 Probe Master (Roche Applied Science), 10 μM forward primer, 10 μM reverse primer, and 10 μM probe in a final volume of 15 μL. Three replicates per cDNA pool were used for EF1M, PME, and an internal standard, EF1α. Standard curves for each gene were run concurrently. TaqMan quantitative RT-PCR was run on a LightCycler® 480 System (Roche Applied Science), and PCR conditions consisted of an initial denaturation step at 95°C for 10 min, followed by 45 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 1 s, and a final cooling step of 40°C for 10 min. Standard curves were used to calculate relative mRNA concentrations from Crossing point values using absolute quantification with LightCycler® 480 software release 1.0 (Roche Applied Science) and normalized to the reference gene EF1α.

Combinatorial QTL Related to Tomato Fruit Firmness

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Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Expression of DME2.5 in lines M82 and IL 2-3 whole fruit tissue, over a developmental series of 15, 25, 40, and 54 DPA, using the Tomato Affymetrix GeneChip 20K genome array.

**Supplemental Figure S2.** RT-PCR expression differences of Solyc02g077860.1, Solyc02g077690.2, and Solyc02g077710.1 in lines M82 and IL 2-3 outer and inner pericarp tissue at developmental stages mature green and breaker.

**Supplemental Table S1.** Marker and primer information for *S. lycopersicum* cv M82 and *S. pennelli*.

**Supplemental Table S2.** Putative gene models underlying *Fir*'^*QTL*2.2', and primer sequences.

**Supplemental Table S3.** Recombinant and parental line raw data for Figure 4.

**Supplemental Text S1.** RFLP single nucleotide polymorphism sequences used to design TaqMan probes.

**Supplemental Data Set S1.** Syngenta Tomato GeneChip data set, top 20 genes.

**LITERATURE CITED**

Alexander L, Grierson D (2002) Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot 53: 2039–2055

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

Barrett DM, Beaulieu JC, Shewfelt R (2004) High-resolution mapping and functional analysis of se2.1: a major stigma exsertion quantitative trait locus ascribed to an invertase gene. Proc Natl Acad Sci USA 97: 4718–4722

Biswas DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW, Correa CS, Corbineau F, Theodoulou FL, Bailey-Serres J, et al (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. Nature 479: 415–418

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

Gorguet B, Eggink PM, Ocaña J, Tiwari A, Schipper D, Finkers R, Visser RGF, van Heusden AW (2008) Mapping and characterization of novel pathenorphylic *QTLs* in *Solanum*. Theor Appl Genet 116: 755–767

Hall LN, Tucker GA, Smith CJS, Watson CF, Seymour GB, Bundick Y, Bonwell JM, Fletcher JD, Ray JA, Schuch W, et al (1993) Antisense inhibition of pectin esterase gene-expression in transgenic tomatoes. Plant J 1: 121–129

Harriman RW, Tieman DM, Handa AK (1991) Molecular cloning of tomato pectin methylsterase gene and its expression in *Rutgers*, ripening inhibitor, nonripening and never ripe tomato fruits. Plant Physiol 97: 80–87

Holden HEE, Hake S (2003) Quantitative trait locus analysis of leaf dissection in tomato using *Lycopersicon pennelli* segmental introgression lines. Genetics 165: 1541–1550

Jolie RP, Duvetter T, Van Loey AM, Hendrickx ME (2010) Pectin methylesterase and its proteinaceous inhibitor: a review. Carbohydr Res 345: 2583–2595

Kim HM, Doganlar S, Chen KY, Tanksley SD (1999) The genetic basis of pear-shaped tomato fruit. Theor Appl Genet 99: 844–850

Lecomte I, Saliba-Coloto N, Gauthier A, Gomez-Jimenez MC, Dufpe P, Buret M, Causse M (2004) Fine mapping of *QTLs* of chromosome 2 affecting the fruit architecture and composition of tomato. Mol Breed 13: 1–14

Li YC, Zhu BZ, Xu WT, Zhu HL, Chen AJ, Xie YH, Shao Y, Luo YB (2007) LeERF1 positively modulated ethylene triple response on etiolated seedling, plant development and fruit ripening and softening in tomato. Plant Cell Rep 26: 1999–2008

Lippman ZH, Semel Y, Zamir D (2007) An integrated view of quantitative trait variation using tomato interspecific introgression lines. Curr Opin Genet Dev 17: 545–552

Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB (2006) A naturally occurring epigenetic mutation in a gene encoding an SBF-box transcription factor inhibits tomato fruit ripening. Nat Genet 38: 948–952

Matas AJ, Gapper NE, Chung MY, Giovannoni JJ, Rose JKC (2009) Bio- logic and genetic engineering of fruit maturation for enhanced quality and shelf-life. Curr Opin Biotechnol 20: 197–203

Moco S, Capanoglu E, Tikanov Y, Bino RJ, Boyacioglu D, Hall RD, Vervoort J, De Vos RCH (2007) Tissue specialization at the metabolite
level is perceived during the development of tomato fruit. J Exp Bot 58: 4131–4146

Monforte AJ, Tanksley SD (2000) Fine mapping of a quantitative trait locus (QTL) from Lycopersicon hirsutum chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor Appl Genet 100: 471–479

Moreno E, Obando JM, Dos-Santos N, Fernández-Trujillo JP, Monforte AJ, Garcia-Mas J (2008) Candidate genes and QTLs for fruit ripening and softening in melon. Theor Appl Genet 116: 589–602

Patterson HD, Thompson R (1971) Recovery of inter-block information when block sizes are unequal. Biometrika 58: 545–554

Phan TD, Bo W, West G, Lyceit GW, Tucker GA (2007) Silencing of the major salt-dependent isoform of pectinesterase in tomato alters fruit softening. Plant Physiol 144: 1960–1967

Powell ALT, Kalamaki MS, Kurien PA, Gurrieri S, Bennett AB (2003) Simultaneous transgenic suppression of LePG and LeExp1 influences fruit texture and juice viscosity in a fresh market tomato variety. J Agric Food Chem 51: 7450–7455

Ross HA, Wright KM, McDougall GJ, Roberts AG, Chapman SN, Morris WL, Hancock RD, Stewart D, Tucker GA, James EK, et al (2011) Potato tuber pectin structure is influenced by pectin methyl esterase activity and impacts on cooked potato texture. J Exp Bot 62: 371–381

Saladie M, Matas AJ, Isaacoa T, Jenks MA, Goodwin SM, Niklas KJ, Xiaolin R, Labavitch JM, Shackel KA, Fernie AR, et al (2007) A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiol 144: 1012–1028

Saliba-Colombani V, Causse M, Langlois D, Philouze J, Buret M (2001) Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. Theor Appl Genet 102: 259–272

Seymour GB, Colquhoun JJ, Dupont MS, Parsley KR, Selvendran RR (1990) Composition and structural features of cell-wall polysaccharides from tomato fruits. Phytochemistry 29: 725–731

Shivaprasad PV, Dunn RM, Santos BACM, Bassett A, Baulcombe DC (2012) Extraordinary transgressive phenotypes of hybrid tomato are influenced by epigenetics and small silencing RNAs. EMBO J 31: 257–266

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191–203

Thompson AJ, Tor M, Barry CS, Vrebalov J, Orfila C, Jarvis MC, Giovannoni JJ, Greer D, Seymour GB (1999) Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. Plant Physiol 120: 383–390

Udall JA, Wendel JF (2006) Polyploidy and crop improvement. Crop Sci 46: S3–S14

van Ooijen JW (2004) MapQTL Version 5.0: Software for the Mapping of Quantitative Trait Loci in Experimental Populations. Kyazma, Wagening, The Netherlands

van Ooijen JW, Voorips RE (2001) JoinMap 3.0: Software for the Calculation of Genetic Linkage Maps. Plant Research International, Wageningen, The Netherlands

Vrebalov J, Ruezinsky D, Padmanabhan V, White R, Medrano D, Drake R, Schuch W, Giovannoni J (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. Science 296: 343–346

Yin XR, Chen KS, Allan AC, Wu RM, Zhang B, Lalr N, Ferguson IB (2008) Ethylene-induced modulation of genes associated with the ethylene signalling pathway in ripening kiwifruit. J Exp Bot 59: 2097–2108