Journal of Experimental Botany, Vol. 56, No. 416, pp. 1591–1604, June 2005
doi:10.1093/jxb/eri154 Advance Access publication 25 April, 2005
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Journal of Experimental Botany

RESEARCH PAPER

Comparison of changes in fruit gene expression in tomato introgression lines provides evidence of genome-wide transcriptional changes and reveals links to mapped QTLs and described traits

Charles J. Baxter[1,*], Mohammed Sabar[1,*,†], W. Paul Quick[2] and Lee J. Sweetlove[1,‡]

[1] *Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK*

[2] *Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK*

Received 19 November 2004; Accepted 4 March 2005

Abstract

Total soluble solids content is a key determinant of tomato fruit quality for processing. Several tomato lines carrying defined introgressions from *S. pennellii* in a *S. lycopersicum* background produce fruit with elevated Brix, a refractive index measure of soluble solids. The genetic basis for this trait can be determined by fine-mapping each QTL to a single gene, but this is time-consuming and technically demanding. As an alternative, high-throughput analytical technologies can be used to provide useful information that helps characterize molecular changes in the introgression lines. This paper presents a study of transcriptomic changes in six introgression lines with increased fruit Brix. Each line also showed altered patterns of fruit carbohydrate accumulation. Transcriptomic changes in fruit at 20 d after anthesis (DAA) were assessed using a 12 000-element EST microarray and significant changes analysed by SAM (significance analysis of microarrays). Each non-overlapping introgression resulted in a unique set of transcriptomic changes with 78% of significant changes being unique to a single line. Principal components analysis allowed a clear separation of the six lines, but also revealed evidence of common changes; lines with quantitatively similar increases in Brix clustered together. A detailed examination of genes encoding enzymes of primary carbon metabolism demonstrated that few of the known introgressed alleles were altered in expression at the 20 DAA time point. However, the expression of other metabolic genes did change. Particularly striking was the co-ordinated up-regulation of enzymes of sucrose mobilization and respiration that occurred only in the two lines with the highest Brix increase. These common downstream changes suggest a similar mechanism is responsible for large Brix increases.

Key words: Brix, carbohydrate metabolism, introgression, tomato microarray, yield.

Introduction

Tomato fruit quality and yield are governed by a range of genetic and environmental factors that result in quantitative variation across varieties. The commercial value of processing tomato varieties is in part determined by a combination of total fruit yield and fruit soluble solids content (Brix). Ripe fruit with high soluble solids require the removal of less water to produce tomato-based food products of the appropriate consistency and taste. As such, the manipulation of fruit quality and yield are key targets of current tomato breeding programmes.

Different tomato varieties vary greatly in the form and abundance of the metabolites that determine Brix and the relationship between the concentrations of these metabolites and yield. Cultivated varieties such as *Solanum*

* These authors contributed equally to this work.
† Present address: Département de Biochimie, Faculté de Médecine, Université de Montréal, CP 6128, Succ. Centre-ville, Montréal, Qc H3C 3J7, Canada.
‡ To whom correspondence should be addressed. Fax: +44 (0)1865 275074. E-mail: lee.sweetlove@plants.ox.ac.uk

Abbreviations: DAA, days after anthesis; IL, introgression line; PCA, principal components analysis; SAM, significance analysis of microarrays; QTL, quantitative trait loci; EST, expressed sequence tag.

lycopersicum are generally high yielding and develop ripe fruit with low soluble solids contents (Brix 3–4%) made up predominantly by the accumulation of glucose and fructose (Yelle *et al*., 1988). By contrast, wild tomato progenitors, such as the green fruited *Solanum pennellii*, produce small fruit that store sucrose and have high (up to 15%) Brix contents (Stommel, 1992; Fridman *et al*., 2000). Although the biochemical pathways that are involved in the synthesis and storage of these metabolites are relatively well characterized in tomato fruit (Robinson *et al*., 1988; Yelle *et al*., 1991; N'tchobo *et al*., 1999) and differences between varieties have been described based on the measured activity of a small number of enzymes (Yelle *et al*., 1988; Miron and Schaffer, 1991; Sun *et al*., 1992; Stommel, 1992), a more complete analysis of regulatory and metabolic networks is required to understand the genetic basis of tomato fruit quality further.

In an attempt to gain an insight into the underlying genetic factors that govern differences between the cultivated and wild varieties, Zamir and colleagues generated a series of introgression lines in which defined genomic segments of the *S. pennellii* genome replaced homologous regions in a *S. lycopersicum* background (Eshed *et al*., 1992; Eshed and Zamir, 1995). A total of 76 lines were produced that collectively contained introgressions that covered the entire tomato genome. In a series of field studies, a number of phenotypic traits in these lines were quantified and QTLs identified (Eshed and Zamir, 1995; Gur *et al*., 2004). One such QTL for elevated Brix has been mapped to a single nucleotide substitution in a gene encoding an invertase enzyme (Fridman *et al*., 2000, 2004).

Although an extremely powerful and unbiased approach, delimiting a QTL to a single gene using genetic approaches is a time-consuming and technically demanding process (Fridman *et al*., 2000, 2004). Any additional information that could be linked to the observed traits in the introgression lines would therefore be beneficial in that it may provide clues as to the identity of the allele(s) responsible for a particular trait. Thus, metabolomic profiling of the introgression lines has been embarked upon to provide additional definition of the biochemical traits that are altered in each line (Overy *et al*., 2005). In addition, Causse and colleagues have taken a 'candidate gene approach' (Causse *et al*., 2004). They reasoned that many of the observed traits such as altered fruit soluble solids and yield are likely to be the result of alterations in fruit primary carbon metabolism. Therefore, they mapped a range of genes encoding enzymes of primary metabolism and were able to establish which genes lay in each introgression. In some cases, there were obvious links between the presence of *S. pennelli* alleles of these genes and the observed trait. Recent development of genomic resources for tomato has opened up an additional source of information: changes in the transcriptome of each introgression line. Given that one consequence of the presence of the introgression may be an altered expression pattern of the genes contained within it, a transcriptomic analysis could provide a route by which genetic changes can be linked to phenotype. This type of analysis will also reveal downstream changes as a result of the introgression that will provide important insight into the regulation of metabolic pathways that are related to the trait (Ruuska *et al*., 2002; Thimm *et al*., 2004; Sreenivasulu *et al*., 2004; Price *et al*., 2004).

There are currently available 180 000 tomato ESTs that have allowed the identification of approximately 30 000 unigenes across a range of tissues and developmental stages (Van der Hoeven *et al*., 2002; Fei *et al*., 2004). This has enabled the production of a tomato cDNA microarray containing 12 000 unique elements encoding 8500 genes covering a range of metabolic and developmental processes (http://bti.cornell.edu/CGEP/CGEP.html) (Alba *et al*., 2004). In this paper, this microarray resource is exploited in order to analyse transcriptomic changes in fruit of selected introgression lines. The focus is on a small group of lines that share a common phenotype of increased fruit Brix. These lines contain non-overlapping introgressions that would therefore be expected to lead to distinct primary transcriptomic changes. However, given the common phenotype, secondary transcriptomic changes (i.e. changes downstream of genes contained within the introgression) that share similar elements may also occur. The identification of such changes would reveal underlying regulatory mechanisms of fruit metabolism that lead to high Brix. To investigate the pattern of gene expression changes in the selected introgression lines, a replicated microarray analysis of fruit at 20 d after anthesis (DAA) from the *S. lycopersicum* parent and the introgression lines was performed. Multivariate statistics were used to analyse the pattern of changes between the lines as well as a directed analysis of statistically significant changes in expression of genes encoding enzymes of primary carbon metabolism. Wherever possible, these changes are related to the presence of known genes within each introgressed segment (Causse *et al*., 2004). The full dataset has been deposited in the public tomato expression database http://ted.bti.cornell.edu/cgi-bin/miame/home.cgi.

Materials and methods

Plant growth conditions and fruit sampling

Solanum lycopersicum (cv. M82 accession LA3475) tomato plants and plants from introgression lines IL1-4, IL2-6, IL7-3, IL7-5, IL4-4, and IL12-4 were grown in a greenhouse with supplementary lighting providing an irradiance of 250–400 μmol m^{-2} s^{-1}. Individual tomato plants were grown in pots (20 cm diameter) containing compost (Levingtons M3; 6 kg per pot) supplemented with Osmocote slow release fertilizer (30 g per pot). Plants were watered daily and prior to flowering given liquid fertilizer (Phostrogen plant food) on a weekly basis. Fruit were tagged at anthesis and harvested when they had reached the appropriate developmental stage. Individual fruit were removed from the plant, weighed, snap frozen in liquid nitrogen, and stored at −80 °C until required.

Measurement of fruit brix

Ripe fruit tissue was homogenized with a razor blade and the soluble solids (Brix) content of the resulting juice measured on a portable refractometer (Bellingham and Stanley Ltd, Kent, UK)

Carbohydrate assays

Frozen fruit powder was extracted with trichloroacetic acid (Sweetlove *et al*., 1996). Carbohydrates were assayed spectrophotometrically using the methods described in Baxter *et al*. (2003).

RNA isolation

Total RNA was isolated from homogenized, powdered tomato fruit tissue using a CTAB (hexadecyltrimethylammonium bromide) method (Chang *et al*., 1993).

Glass slide microarray

Glass slides containing arrayed tomato ESTs were obtained directly from The Centre for Gene Expression Profiling (CGEP) at the Boyce Thompson Institute (BTI), Cornell University. The tomato array contains approximately 12 000 unique elements randomly selected from cDNA libraries isolated from a range of tissues including leaf, root, fruit, and flowers and representing a broad range of metabolic and developmental processes. Technical details of the spotting and annotation of this file are provided on the BTI (http://bti.cornell.edu/CGEP/CGEP.html) website.

Fluorescent probe preparation and microarray hybridization

Microarray experiments were designed and conducted according to the MIAME guidelines (www.mged.org/miame) and all information relevant to this standard is presented in the Materials and methods and appropriate figure legends.

50 μg of total RNA was reverse transcribed to synthesize either Cy3 or Cy5 labelled cDNA probes. Total RNA was mixed with 1.25 μg Oligo d(T) primer (Invitrogen) and denatured at 65 °C for 5 min. A master mix containing 8 μl 5× first strand buffer, 4 μl low C dNTP mix (25 mmol each of dGTP, dATP, dTTP, and 10 mmol dCTP), 25 μmol Cy5 or Cy3 dCTP (Amersham), 4 μl 0.1 M DTT, and 40 U RNase out (Invitrogen) was added. Each sample was heated to 42 °C and then 2 μl (400 U) superscript II reverse transcriptase (Invitrogen, Karlsruhe) was added. The reaction was incubated for 2 h at 42 °C and stopped by the addition of 5 μl of 0.5 M EDTA. Template RNA was hydrolysed by the addition of 10 μl of 1 M NaOH and incubating at 65 °C for 30 min. The reaction was neutralized by the addition of 25 μl of 1 M TRIS (pH 8.0). Labelled cDNA was then precipitated by the addition of 8 μl of 3 M NaAc (pH 5.2) and 200 μl ethanol. Following incubation at −20 °C for 2 h, cDNA was pelleted by centrifugation at 12 000 *g* for 30 min at 4 °C. Pellets were allowed to dry, resuspended in 15 μl hybridization solution (0.1% SDS, 25% formamide, 5× SSC) and the Cy5 and Cy3 labelled probes were combined to a final volume of 30 μl. To account for dye bias, replicate experiments were done in which the Cy dyes used to label the samples to be compared were swapped.

Microarrays were prehybridized by immersion in prehybridization solution (0.1% (w/v) SDS, 25% (v/v) formamide, 5× SSC, 1% (w/v) BSA) for 90 min at 42 °C. Slides were rinsed in ddH_2O and air-dried. For hybridization, 2 μl of liquid block solution (Amersham) was added to the purified combined Cy3 and Cy5 labelled probes and these were denatured at 95 °C for 5 min. Probe solution was added to the spotted surface of the slide and hybridization carried out under a hybri-slip (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) in a humidified hybridization cassette (Telechem International, USA) for 16 h at 42 °C. Following hybridization, slides were washed for 5 min at 42 °C in 2× SSC, 0.1% (w/v) SDS, 10 min at room temperature in 0.1× SSC, 0.1% (w/v) SDS, and 4 min at room temperature in 0.1× SSC.

Microarray scanning and data analysis

Microarrays were scanned using an Affymetrix 428 Array scanner (Affymetrix, Inc, Santa Clara, Ca, USA) and acquisition software according to the manufacturer's instructions. After scanning images were analysed in Genepix Pro v. 4.1 software (Axon Instruments, Inc., USA) and the raw data collected and imported into Microsoft Excel for further analysis. To identify genes of interest whose expression levels change, the ratio of each feature (635 nm/532 nm) was calculated. Background fluorescence values were automatically calculated by the Genepix program and subtracted from all feature intensities prior to ratio calculation. It was determined that the array data should exclude all features that did not show a median pixel intensity of 2.5-fold greater than the overall mean slide background intensity. In order to account for differences in array-specific effects and to be able to average the results from replicate arrays, normalization between the Cy3 and Cy5 channels was further achieved by calculating the ratio for each spot based on the Cy3 and Cy5 fluorescence of the spot in relation to the total Cy3 and Cy5 fluorescence of the whole slide. Following data normalization and quality control all values were log transformed (log base 2) prior to further analysis.

Statistical analysis

Principal components analysis was performed on a complete microarray dataset consisting of log transformed expression values for each individual replicate. PCA was carried out using the R platform and relevant algorithms (http://www.r-project.org/). Briefly, the dimensionality of the dataset was reduced and described in a series of axes that defines the variance within the data. In this analysis 20.4% and 13.2% of the variance within the data was accounted for by the first two axes, respectively. These were used to plot the data and give an impression of the variation between the individual datasets; distances between points on the graph are indicative of the differences between replicates.

Microarray elements with significant changes in expression were identified using the significance analysis of microarrays protocol contained within the TIGR multiple experiment viewer (http://www.tigr.org/software/tm4/) (Tusher *et al*., 2001). For each introgression line (and wild-type *S. lycopersicum*) a dataset consisting of only those clones with expression values across each of the three replicated arrays was produced. SAM was performed on this dataset using the algorithms contained within the TIGR mev application. Lists of clones with significant changes in expression in comparison to wild-type *S. lycopersicum* were identified at delta values that gave a FDR of less than 10% (Tusher *et al*., 2001).

All other data generated in this study was analysed by *t*-test using the algorithm contained within Microsoft Excel software. Unless otherwise stated, all instances of the word 'significant' in the text denote a statistical significance of $P < 0.05$ as determined by the *t*-test.

Results

Selection of tomato introgression lines

Introgression lines with altered ripe fruit Brix were selected on the basis of previously published data from field-grown plants (Table 1) (Eshed and Zamir, 1995). To minimize environmental variation that could complicate the subsequent interpretation of experimental results, plants were grown in controlled environment greenhouses and supplied with supplementary lighting. Under these growth conditions, changes in Brix were observed that were consistent

with the traits recorded in field studies (Fig. 1; Table 1). On the basis of Brix and yield data the phenotypic variation within the selected lines could be grouped into three different categories; IL4-4 and IL7-3 were characterized by a large increase in Brix and a decrease in fruit weight and overall yield. IL2-6 and IL7-5 had a small increase in Brix, an increase in yield and a significant increase in Brix×yield (economic yield). Fruit from IL1-4 and IL12-4 displayed a small increase in Brix, with no significant effect on overall yield or Brix×yield (Fig. 1; Table 2).

Table 1. *Summary of plant weight, yield, and Brix×yield (economic yield) changes for selected introgression lines grown in the field (from Eshed and Zamir, 1995)*

Introgression line	Plant weight[a]	Yield[a]	Brix×yield[a]
IL4-4	Increase*	Decrease	No change
IL7-3	Increase*	Decrease	No change
IL7-5	Increase*	Increase*	Increase*
IL2-6	Increase*	Increase	Increase*
IL12-4	Decrease	Decrease	Decrease
IL1-4	Increase*	Increase	Increase

[a] An asterisk denotes a significant changes in comparison to *S. lycopersicum* (cv. M82).

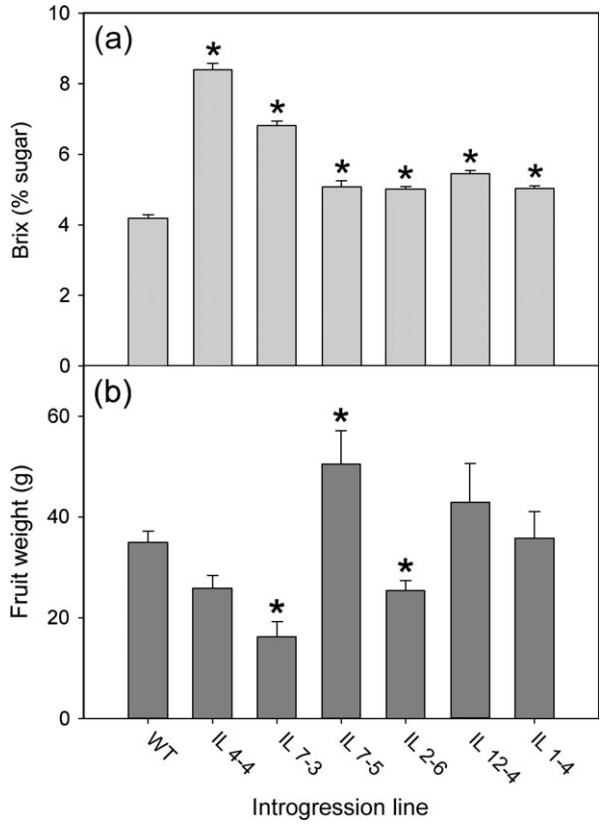

Fig. 1. Growth and fruit Brix content of *L. lycopersicum* wild-type (Wt) and introgression line plants. Wild-type (*S. lycopersicum* parent) and plants from ILs 1-4, 2-6, 4-4, 7-3, 7-5, and 12-4 were grown as described in the Materials and methods. (a) Brix value of ripe fruit. (b) Mean fruit weight of ripe fruit. Results presented are the mean of measurements from a minimum of five plants per line from two harvests. Asterisk indicates values in each introgression line that are significantly different from the *S. lycopersicum* parent ($P < 0.05$).

To define the biochemical phenotype imposed by each introgression further, fruit carbohydrate content was measured at three different stages of fruit development; 20 DAA, breaker (the stage of development when the fruit first begins to change from green to red) and ripe (Fig. 2). Generally, increased fruit Brix (Fig. 1) correlated with measured changes in the abundance of soluble carbohydrate (glucose, fructose, and sucrose) in ripe fruit (Fig. 2c). Specifically, in the high Brix, low yield lines IL4-4 and IL7-3, large increases (70–100%) in soluble carbohydrate were observed in the ripe fruit (Fig. 2c). This was due to increases in the abundance of glucose, fructose, and sucrose (Fig. 2c). In fruit from IL1-4 and IL12-4 (small Brix increase, no yield change), there were small changes in the abundance of soluble carbohydrate. Ripe fruit from IL2-6 and IL7-5, the lines with largest changes in Brix×yield, had similar soluble carbohydrate contents to the wild-type parent.

Differences in the abundance of soluble carbohydrate between the introgression lines and the wild type were also evident at two earlier stages of fruit development (20 DAA and breaker stage) (Fig. 2a, b). With the exception of IL4-4, increases in the abundance of soluble carbohydrate at earlier stages of development were related to changes in the abundance of hexose sugars rather than sucrose.

There was no significant difference in the starch content of ripe fruit from any of the introgression lines (Fig. 2f); however, in a number of lines there were significant increases in fruit starch at earlier stages of development (Fig. 2d, e). Starch abundance was significantly higher (2–3-fold) than in the wild type in fruit at 20 DAA from IL4-4, IL7-3, IL7-5, and IL1-4 (Fig. 2d). By contrast, at the breaker stage of development, increases in starch content were most apparent in fruit from IL4-4 (30-fold increase), IL12-4 (2-fold) and IL2-6 (2-fold) (Fig. 2e).

Principle and design of experiments profiling tomato fruit transcript abundance

The choice was made to profile transcript abundance in fruit at 20 DAA as this represents the peak of starch accumulation (Robinson *et al*., 1988) and a developmental stage at which a number of the important enzymes of fruit carbohydrate metabolism are active (Yelle *et al*., 1988, 1991; Robinson *et al*., 1988). Measuring the variation in gene expression at this stage of fruit development will allow the differences between lines to be described and to focus on processes that play an important role in determining fruit Brix and yield.

To obtain consistent and statistically valid comparisons of gene expression in each of the lines, triplicated samples from individual plants were hybridized against a pooled

Table 2. *Summary of gene expression data*

In section (a) the numbers of clones from each line that passed quality control criteria are listed. In section (b) the total numbers of clones that were deemed to change significantly in expression (SAM) are presented.

	Introgression line					
	IL4-4	IL7-3	IL7-5	IL2-6	IL12-4	IL1-4
(a) Clones present in triplicate arrays:						
Total	3305	5010	2723	6079	7664	5486
Up-regulated in introgression line	2476	1968	1231	3304	3827	2366
Up-regulated in wild type	829	3042	1492	2775	3837	3120
(b) Total clones changing significantly in expression (SAM):						
Percentage of total clones	40.3	30.9	1.25	8.6	0.5	1.4
Up-regulated in introgression line	1188	926	23	56	5	46
Up-regulated in wild type	144	623	11	469	35	30

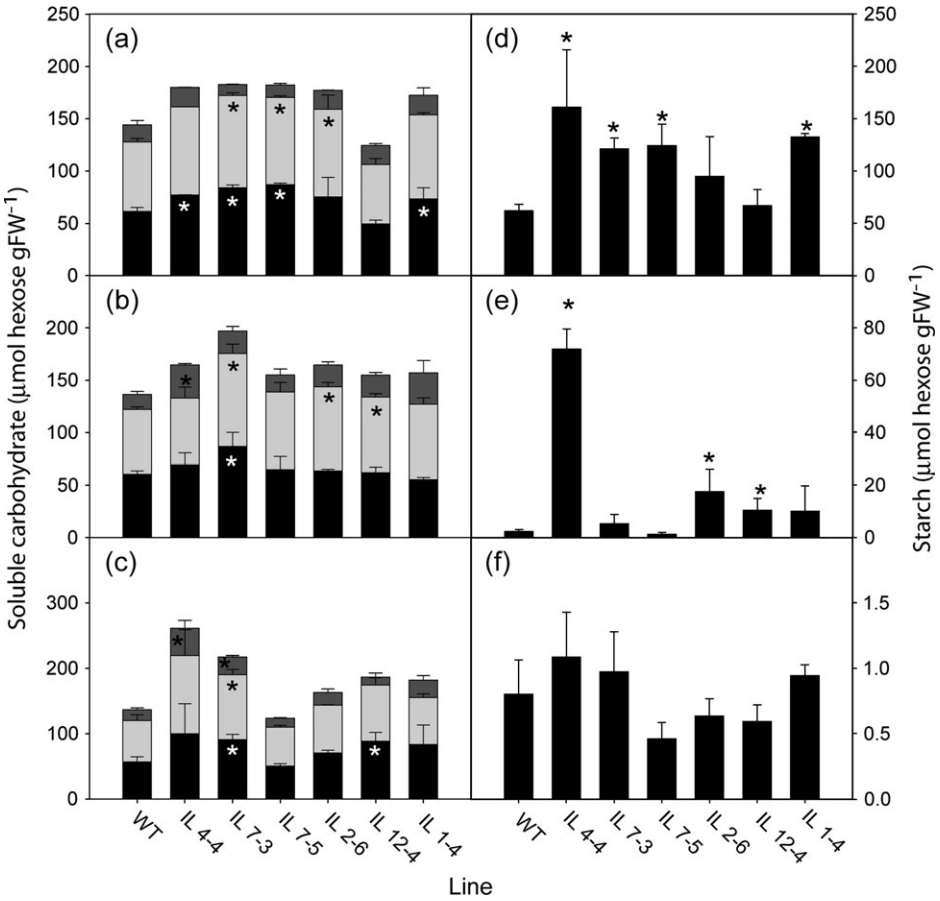

Fig. 2. The abundance of soluble carbohydrate and starch in fruit from tomato introgression lines. Soluble carbohydrate (glucose, black bar; fructose, grey bar; sucrose, dark grey bar) (a, b, c) and starch (d, e, f) was measured in fruit at 20 DAA (a, d), breaker stage (b, e), and ripe (c, f) from six selected introgression lines and wild-type plants. Values represent the mean ±SE; n=3–5 fruit per time point, harvested from different plants. Asterisk indicates values in each IL that are significantly different from the *S. lycopersicum* parent (P <0.05).

RNA sample obtained from wild-type *S. lycopersicum* (Fig. 3). The variation inherent in this pooled wild-type sample was itself accounted for by triplicated arrays comparing the pooled sample to individual wild-type plants (Fig. 3). This allowed the ratio of expression generated for each line to be reliably compared across the population of plants and significant changes in gene expression to be identified using the significance analysis of microarrays (SAM) method described by Tusher *et al*. (2001). SAM has a lower false discovery rate than conventional tests and can be used to identify statistically significant changes in gene expression by assimilating a set of gene-specific *t*-tests (Tusher *et al*., 2001). SAM was performed on a set of data for each line, containing expression values with an average

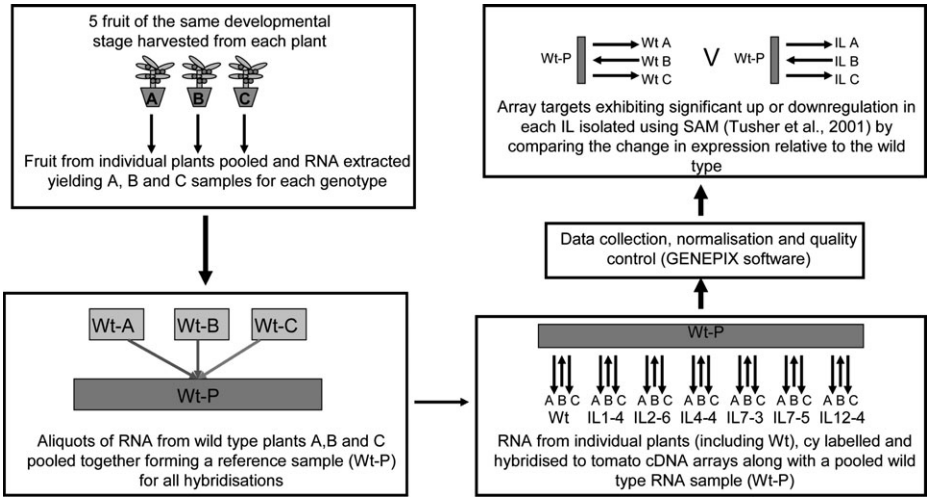

Fig. 3. Design of microarray experiment to compare transcript abundance across introgression lines. Fruit were sampled from individual plants and pooled, yielding replicated RNA samples for each line. A common reference sample was created for the wild type (*S. lycopersicum* parent) plants and hybridizations were carried out to compare transcript abundance to this reference. The degree of variation in the arrays was accounted for by including replicated wild-type arrays in which gene expression in individual wild-type plants was calculated by comparison to the pooled wild-type sample.

signal intensity of 2.5-times background in all three replicated arrays (Table 2). Data identified from triplicated arrays represent reliable and accurate measurements of gene expression: individual clones were represented in all replicates with a hybridization signal of 2.5-fold background. Clones identified in this way accounted for between 25% and 65% of the targets present on the array for individual lines (Table 2).

Comparative analysis of fruit gene expression across introgression lines

To demonstrate the degree of diversity between selected introgression lines at the level of the transcriptome, the array dataset was analysed using principal components analysis (PCA) (Fig. 4). PCA revealed that each line clustered independently and there was a large degree of separation between lines. The overall clustering pattern formed from PCA additionally discriminated between lines with different fruit phenotypes (Figs 1, 4); the high Brix, low yield IL4-4 and IL7-3 clustered at the upper end of both components, whilst the moderate Brix, high yield IL7-5 and IL2-6 clustered at the lower end (Fig. 4).

In a complementary approach to PCA, the number of lines in which individual transcripts change significantly in expression in comparison to the *S. lycopersicum* parent were identified and analysed (Fig. 5a). Of the genes displaying significant changes in expression the majority (78%) are unique to a single introgression line (Fig. 5a). A smaller proportion of significantly altered transcripts (12.6%) changed in expression in two of the six lines and only 1.5% were common to three or more lines. Transcripts common to more than one introgression line were evenly distributed across functional classes (Fig. 5a). A number of genes (8.1%) whose expression changed in more than one line appeared to be oppositely regulated in different lines (present as both black and grey bars in Fig. 5a).

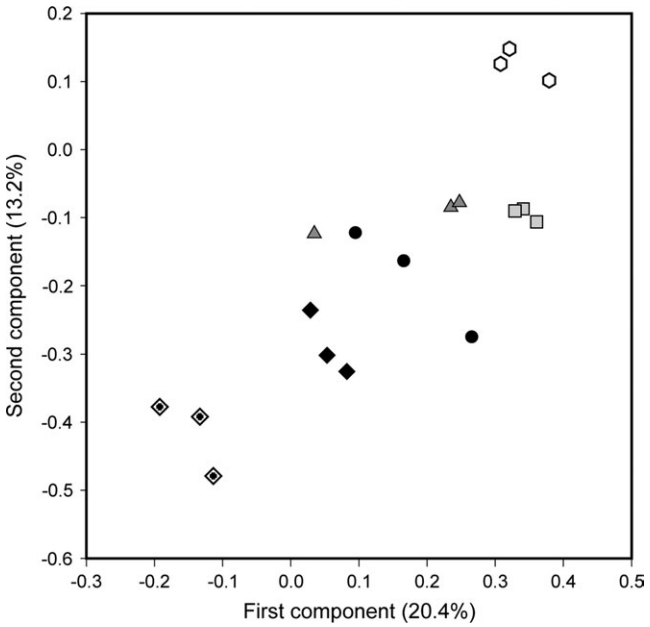

Fig. 4. Principal components analysis of the transcript profiles of 20 DAA fruit from tomato introgression lines. Data presented represent individual microarray hybridizations of RNA samples from tomato fruit of introgression lines 4-4 (grey-filled squares), 7-3 (open hexagons), 7-5 (black-filled diamonds), 2-6 (black circle in diamonds), 12-4 (grey-filled triangles), and 1-4 (black-filled circles). PCA vectors 1 and 2 were chosen to provide the best visualization of differences between lines and include 34% of the information derived from differences in the transcriptome.

The classification of significantly up-regulated or down-regulated genes from individual introgression lines spanned

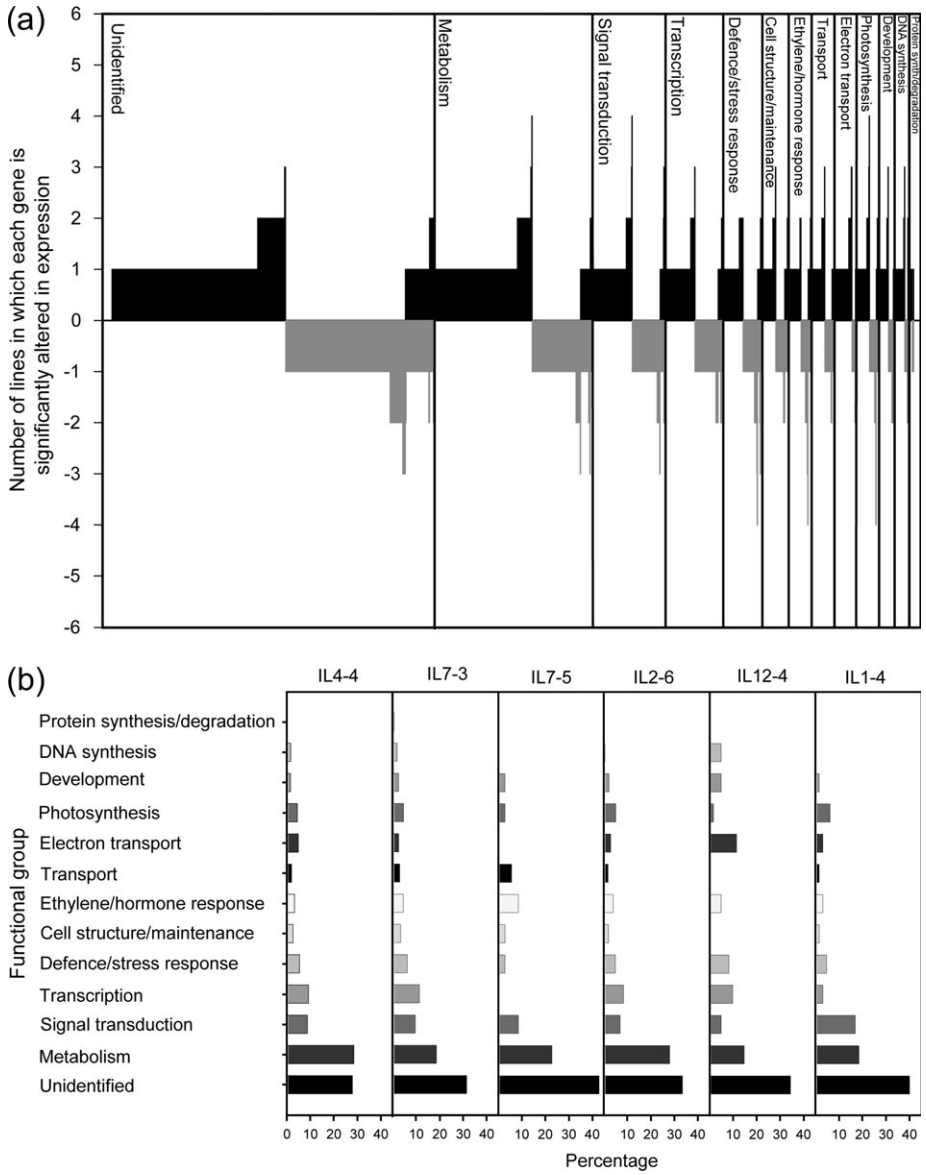

Fig. 5. (a) Occurrence of significant changes in gene expression in tomato fruit from introgression lines. The number of introgression lines in which significantly up-regulated (black bars) and down-regulated (grey bars) transcripts were detected is presented according to functional category. Genes with significant changes in expression were identified using SAM with delta values yielding FDRs <10%. (b) Distribution of significantly up-regulated and down-regulated genes in individual introgression lines by functional categories. Individual clones present on the BTI microarray were classified according to spot annotations (http://bti.cornell.edu/CGEP/CGEP.html), Tomato EST annotations (http://www.tigr.org/), pathway maps and literature reviews.

a range of functional categories (Fig. 5b); however, the number of genes in each functional category differed between lines. Although the unidentified group was the largest category in all lines, the metabolism subset of genes formed a proportionally greater part of the classification in IL2-6 (increased Brix×yield) and IL4-4 (large increase in Brix, decreased yield) than other lines (Fig. 5b). By contrast, a greater percentage of the transcripts changing in expression in IL12-4 (altered Brix, no change in yield) were in the electron transport (i.e. energy production), transcription, and DNA synthesis categories, whilst a larger number of the genes identified in IL1-4 (altered Brix, no change in yield) were classified as signal transduction or photosynthetic genes (Fig. 5).

Changes in the expression of genes encoding enzymes of fruit primary metabolism

Common changes in fruit carbohydrate accumulation in the six introgression lines (Fig. 2) suggests that similar metabolic pathways are affected (albeit to different degrees) by the different introgressions. Although the primary transcriptomic changes in each introgression line

must clearly be different (as each introgression is in a different, non-overlapping part of the genome) there may be common downstream transcriptomic changes that reflect common regulatory mechanisms of carbohydrate metabolism. Therefore the abundance of transcripts encoding enzymes of known metabolic function across the selected introgression lines was compared, in order to focus on the changing expression of genes related directly to carbohydrate metabolism (Table 3). Analysis was focused on the pathways that are known to play a direct role in the uptake, storage, and metabolism of carbohydrate transported to the fruit (Fig. 6) and was broken down into six processes (Table 3). Changes in transcripts relating to proteins involved in amino acid biosynthesis have also been included.

In general, what emerges from this analysis is a clear dichotomy in the transcriptomic response amongst the introgression lines (Table 3). In the high Brix, low yield lines, IL4-4 and IL7-3, there are striking co-ordinated increases in expression of genes encoding enzymes of sucrose metabolism, glycolysis, and the TCA cycle. By contrast, in the other four lines with small increases in Brix, relatively few significant changes in expression of metabolic genes were observed. Most changes in these lines were decreases in expression and there was little evidence of co-ordinated changes within metabolic pathways.

In fruit from IL4-4 there was evidence for synchronized changes in gene expression in a number of pathways. These changes included the significant down-regulation of an apoplastic invertase and up-regulation of a sucrose transporter in the pathway of phloem unloading, the up-regulation of sucrose synthase and fructokinase in cellular sucrose breakdown and an ADPGpyrophosphorylase involved in starch synthesis (Table 3; Fig. 6). In addition, there was significant up-regulation of most of the genes encoding enzymes involved in glycolysis (significant up-regulation of all genes detected with the exception of aldolase) and the TCA cycle (pyruvate dehydrogenase, citrate synthase, aconitase, succinate dehydrogenase, malate dehydrogenase, and malic enzyme) (Table 3).

Patterns of metabolic gene expression in IL7-3 were similar to IL4-4, particularly in terms of co-ordinated pathway-wide up-regulation of transcripts involved in glycolysis, and the TCA cycle (Table 3). However, in this line, notable differences were observed in phloem unloading (up-regulation of apoplastic invertase), cellular sucrose breakdown (down-regulation of acid invertase), starch metabolism (down-regulation of starch synthase, starch branching enzyme, and β-amylase) and in the decrease in the abundance of transcripts encoding cytosolic malate dehydrogenase (Table 3).

In IL2-6 significant down-regulation of expression was observed for genes encoding enzymes of glycolysis (aldolase, GapDH, enolase) and starch metabolism (ADPGpyrophosphorylase), whilst transcripts encoding pyruvate decarboxylase and glucose-6-phosphate dehydrogenase were up-regulated. A number of non-significant changes in gene expression in fruit from IL7-5 were similar to the significant changes measured in IL2-6, particularly for transcripts encoding glycolytic enzymes (Table 3). In addition, significant up-regulation of phosphoglucomutase and citrate synthase and down-regulation of aldolase were detected in IL7-5.

In IL12-4 and IL1-4 there were very few significant changes in gene expression in fruit at 20 DAA. In these lines measured changes in gene expression were confined to the down-regulation of transcripts encoding vacuolar acid invertase (IL12-4), PEPcarboxylase (IL1-4), pyruvate dehydrogenase (IL1-4), and up-regulation of a hexose transporter (IL1-4) (Table 3).

Discussion

Fruit from introgression lines with increased Brix have altered patterns of carbohydrate accumulation

The introgression line population of tomato plants produced from a cross between *S. pennellii* and *S. lycopersicum* (cv. M82) (Eshed *et al*., 1992) provides a powerful source of phenotypic variation in a defined genetic background and has facilitated the identification of a number of QTLs related to plant yield and fruit quality (Eshed and Zamir, 1995; Eshed *et al*., 1996; Liu *et al*., 2003; Causse *et al*., 2004). In this study, six introgression lines were selected to form the basis of an investigation into the genome-wide changes in the transcriptome of tomato fruit that underpin altered fruit Brix.

The growth of selected lines in the greenhouse resulted in a similar growth habit to that described previously (Eshed and Zamir, 1995; Causse *et al*., 2004) suggesting that the selected ripe fruit characteristics were robust in differing growth environments (Fig. 1; Table 2). Previously, QTL effects in tomato have been shown to be consistent across different environments (Monforte *et al.*, 2001). In all of the lines a significant increase in ripe fruit Brix was measured in comparison to the wild-type parent *S. lycopersicum* (Fig. 1). Although significant increases in Brix were recorded for all lines, they were largest in IL4-4 and IL7-3.

In tomato fruit, sugars and organic acids are the major metabolites contributing towards Brix (Grierson and Kader, 1986; Roessner-Tunali *et al*., 2003) and in many varieties soluble carbohydrate forms the most significant component of this value (Yelle *et al*., 1988; Stommel, 1992). In this study, increases in fruit Brix in three of the lines correlated with significant increases in the abundance of soluble carbohydrate in the ripe fruit (Fig. 2c).

In all of the selected introgression lines increases in the abundance of soluble carbohydrate and starch earlier in fruit development (Fig. 2) appear to underpin the significant increases in Brix observed in ripe fruit (Fig. 1) and suggest

Table 3. *Measurement of changes in the expression of transcripts encoding enzymes involved in fruit primary carbon metabolism*

Transcripts with significant changes in expression are marked with the following superscripts: a (up-regulated in introgression line) and b (down-regulated in introgression line). Genes with significant changes in expression were identified using SAM with delta values yielding FDRs <10%. Values presented are mean, log transformed ratios of expression (introgression line/*S. lycopersicum*). Genes mapped to the selected introgression lines (see Table 4) are marked with boxes. The numbers in the left hand column refer to steps described in the pathway in Fig. 6.

	Metabolic step	Introgression line					
		IL4-4	IL7-3	IL7-5	IL2-6	IL12-4	IL1-4
	Phloem unloading						
1	Apoplastic invertase-LIN6		0.316[a]		−0.409	0.167	
1	Apoplastic invertase-LIN5	−0.307[b]	0.375	0.616	−0.071	0.184	−0.273
1	Apoplastic invertase				0.329	0.066	
2	Sucrose transporter-SUT4	0.3394					0.103
2	Sucrose transporter-SUT1	0.7357[a]		−0.054	1.163	−0.366	0.169
3	Hexose transporter-HT1				0.096		
3	Hexose transporter-PR	1.4384[a]	2.263[a]	0.370	0.008	0.754	1.460[a]
	Soluble carbohydrate metabolism						
4	Sucrose phosphate synthase						
5	Sucrose synthase-Susy 2	1.187[a]	0.406	−1.253	0.222	0.337	−0.300
5	Sucrose synthase-Susy 3	0.911[a]	0.431[a]	−0.858	0.161	0.005	−0.146
5	Sucrose synthase-Susy 4	0.423			−0.274	0.247	−0.078
6	Acid invertase-vacuolar	−0.138	−0.129[b]		−0.201	−1.305[b]	−0.321
6	Acid invertase-vacuolar			−0.221	0.594	−0.001	0.187
6	Acid invertase-TAI		−0.299[b]	−0.521	−0.163	−0.252	−0.160
7	UDPGpyrophosphorylase	0.184	0.117	−0.281	−0.063	0.116	0.119
8	Fructokinase 2	0.806[a]	0.049	−0.322	−0.384	0.206	−0.405
8	Fructokinase 1		−0.018		−0.261	0.019	
9	Hexokinase						
	Glycolysis						
10	Phosphoglucomutase-chloroplastic	0.576[a]		0.921[a]		0.050	0.135
10	Phosphoglucomutase-cytoplasmic	0.707[a]	0.005	0.086	−0.248	0.250	−0.078
11	Phosphofructokinase		0.028		−0.105	0.248	
12	PFP α-subunit	0.336[a]	0.266		−0.163	−0.201	0.039
12	PFP β-subunit		0.132		−0.325	−0.057	0.210
13	Phosphofructokinase		−0.077		−0.347	−0.241	0.042
14	Aldolase	−0.518[b]	−0.388		−0.142	−0.076	−0.293
14	Fructose-bisphosphate aldolase	0.326[a]	0.134	−0.117	−0.251	−0.055	0.004
14	Plastidic aldolase	0.057[a]	0.076[a]	−0.720[b]	−0.267[b]	−0.009	−0.258
15	Triose phosphate isomerase	0.383[a]	0.078	−0.121	−0.076	0.273	−0.072
16	Glyceraldehyde-3-phosphate dehydrogenase	0.566[a]	0.319[a]	−0.372	−0.054	−0.041	
16	Glyceraldehyde-3-phosphate dehydrogenase-A chloroplast precursor	0.690[a]	0.423[a]	−1.223	−0.679[b]	−0.012	−0.118
16	Glyceraldehyde-3-phosphate dehydrogenase-B chloroplast precursor	0.785[a]	0.513[a]	−0.881	−0.384[b]	−0.174	0.100
16	Glyceraldehyde 3-phosphate dehydrogenase, cytosolic	0.457	0.520[a]	−0.155	−0.533[b]	−0.014	−0.045
17	Phosphoglycerate kinase, cytosolic	0.659[a]	0.388[a]	−0.148	−0.295	−0.033	0.047
18	Phosphoglycerate mutase						
19	Ethylene-responsive enolase	0.877[a]	0.115	−0.230	−0.153	0.117	0.233
19	Enolase	0.629[a]	0.251[a]	−0.252	−0.265[b]	0.175	0.076
20	Pyruvate kinase-cytosolic	0.369[a]	0.084	0.146	0.205	0.135	0.234
20	Pyruvate kinase, cytosolic isozyme		−0.310	−0.890	−0.255	−0.065	−0.265
20	Pyruvate kinase isozyme A, chloroplast precursor	0.143	−0.072		0.113	−0.193	−0.209
20	Pyruvate,phosphate dikinase, chloroplast precursor	−0.277		−0.534	0.269	−0.053	−0.237
21	PEPcarboxylase 1	0.306[a]	−0.052	−0.128	0.087	−0.008	−0.126[b]
21	PEPcarboxylase 2	0.085[a]	0.405[a]	−0.079	0.025	−0.156	−0.139
22	Pyruvate decarboxylase	0.762[a]	0.487[a]	0.462	0.564[a]	0.101	0.065
	Starch metabolism						
23	ADPGpyrophosphorylase LSU	0.034[a]	0.367[a]		−0.554[b]	−0.086	−0.308
24	ADPGpyrophosphorylase SSU	−0.347	−0.086		−0.527	−0.069	−0.669
25	Glucosyl transferase, jasmonate-induced		0.374[a]		−0.157	0.095	0.228
25	Glucosyltransferase IS5a (EC 2.4.1.-), salicylate-induced	0.282	0.227		0.062	−0.128	−0.003
26	Starch phosphorylase						
27	Starch synthase-isoform 3		−0.698[b]		−0.240	−0.036	
27	Starch synthase-isoform 4				0.035	0.159	
27	Starch synthase-isoform 5					−0.863	
28	Starch-branching enzyme		−0.492[b]		−0.005	−0.019	
29	β-amylase	−0.094	−0.425[b]		0.123	−0.211	0.034
	Oxidative pentose phosphate pathway						
30	Glucose 6 phosphate dehydrogenase-cytoplasmic isoform	0.451	−0.254		0.076[a]	−0.089	0.164
31	Lactonase		0.074	0.817	0.110	−0.123	
32	6-phosphogluconate dehydrogenase	0.100	0.479[a]	−0.151	0.169	0.049	0.005

Table 3. *Continued*

	Metabolic step	Introgression line					
		IL4-4	IL7-3	IL7-5	IL2-6	IL12-4	IL1-4
33	Pentose phosphate epimerase						
34	Transketolase 1	0.691	0.407		−0.226	0.010	−0.209
35	Transaldolase	0.378	−0.002	−0.094	−0.124	−0.119	0.028
35	Transaldolase 2						0.002
	Tricarboxylic acid cycle						
36	Pyruvate dehydrogenase E1 α subunit	0.341[a]	0.448[a]	−0.241	−0.088	−0.096	−0.142
36	Pyruvate dehydrogenase E1 β subunit isoform 1	0.186[a]	0.229[a]	0.679	−0.151	−0.040	−0.332[b]
36	Pyruvate dehydrogenase E1 β subunit isoform 2		0.146		−0.207	0.037	
37	Citrate synthase	0.328[a]	0.026[a]	0.850[a]	−0.138	−0.009	0.169
38	Aconitase	0.404[a]	−0.040	0.113	0.338	−0.196	0.071
39	NAD-dependent isocitrate dehydrogenase		0.462[a]		−0.009	0.029	0.220
40	2-oxoglutarate dehydrogenase					−0.321	0.055
41	Succinyl coA-ligase		0.488		−0.153	0.208	0.259
42	Succinate dehydrogenase, iron–sulphur subunit	0.487[a]	0.195	−0.161	−0.159	0.110	−0.018
42	Succinate dehydrogenase flavoprotein α subunit	0.235[a]	−0.064	−0.015	0.065	0.057	−0.055
43	Fumarase						0.388
44	Malate dehydrogenase 2, cytosolic	0.631[a]	0.437[a]	−0.209	−0.056	0.031	−0.234
44	Malate dehydrogenase mitochondrial	0.554[a]	0.064		−0.182	−0.161	0.129
44	Malate dehydrogenase cytosolic	0.557[a]	−0.824[b]	−0.680	0.044	−0.108	0.186
45	Cytosolic NADP-malic enzyme	0.620[a]	0.071	−0.487	0.146	−0.086	−0.062
	Amino acid metabolism						
	Glutamate synthase (ferredoxin)	0.505[a]				0.348	−0.033
	Glutamate synthase (NADH-dependent)	0.240	0.154	0.119	0.250	0.128	
	Glutamine synthase	−0.239	0.475[a]	−0.624		−0.440	−0.011
	Glutamate dehydrogenase A		0.032		0.024	−0.271	
	Aspartate kinase						−0.029
	Aspartate aminotransferase	−0.769[b]	−0.501[b]	−0.530	0.200	−0.134	−0.063
	Glycerate dehydrogenase	−0.464[b]	−0.185	−0.216	0.159	−0.350	−0.153
	Serine acetyltransferase	0.722[a]	0.243		−0.334	−0.034	
	3-deoxy-D-arabino-heptulosonate 7-phosphate synthase		0.153		−0.337[b]	−0.093	0.241
	Shikimate kinase	0.012	−0.044	0.213	−0.188	−0.133	0.025

that all lines show an altered pattern of carbohydrate metabolism in comparison with the *S. lycopersicum* parent.

Overall pattern of transcriptomic changes in the selected introgression lines

Each line used in this study contains a unique introgressed segment of the *S. pennellii* genome that results in a number of significant changes in fruit gene expression for each line. As one might predict, given that each of the introgressions are non-overlapping, the majority of changes in transcript abundance are unique to each line (Fig. 5a). Indeed, of those genes exhibiting significant changes in expression compared with the *S. lycopersicum* parent, 78% were unique to a single introgression line. This analysis reveals that the lines selected in this study each have a transcriptome that is largely specific for a particular introgression.

This is confirmed by PCA in which replicates from individual lines clearly clustered together (Fig. 4). At first glance, this observation would imply that despite the common biochemical characteristics of ripe fruit from the selected lines, there are no shared transcriptional changes that explain the increased fruit Brix phenotype. However, on closer examination, it is clear that PCA reveals apparent similarities in the overall transcriptome of fruit with similar phenotypes. Specifically, the high Brix, low yield lines (IL4-4 and IL7-3) cluster more closely to each other and at the opposite end of each component to the increased Brix and yield lines (IL2-6 and IL7-5).

Common changes in gene expression as a downstream consequence of the different introgressed genes

The effect of the introgression on fruit carbohydrate metabolism could either be a result of genes present on the introgressed section having altered expression patterns compared with the parent *S. lycopersicum* alleles or encoding proteins with altered properties. Indeed, recent characterization of the *S. pennellii* allele of an apoplastic invertase gene responsible for high fruit Brix identified an invertase protein with altered kinetic properties (Fridman *et al.*, 2004). Thus, transcriptomic changes in the introgression lines could either be the result of altered expression of the introgressed alleles or downstream gene expression changes resulting from the presence of proteins with altered properties (leading to, for example, altered metabolite signalling of gene expression). In an attempt to discriminate between these two possibilities, a detailed look

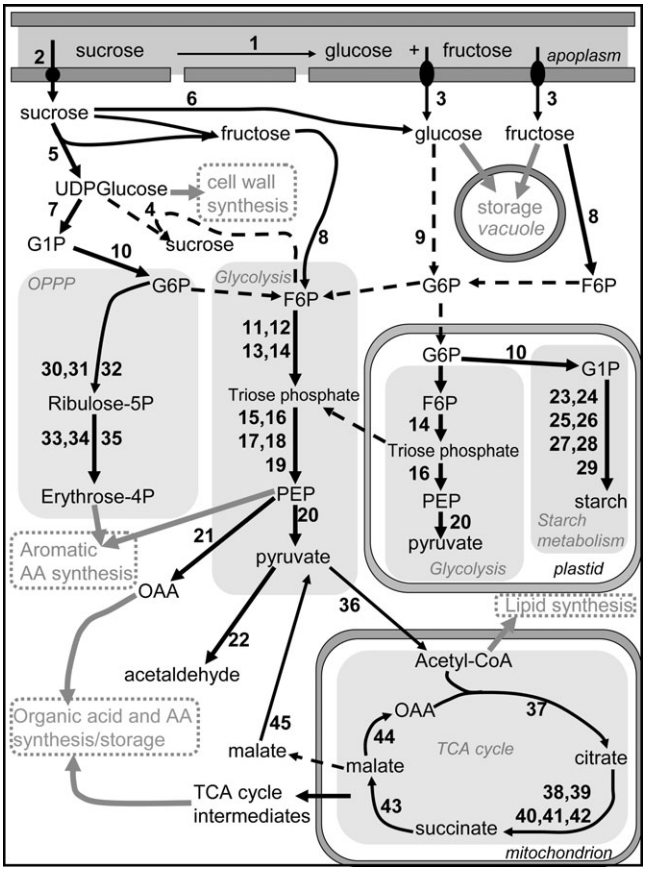

Fig. 6. Pathways of carbon uptake, storage, and energy production in tomato fruit. Black arrows represent pathways listed in table 3. Grey arrows refer to branches of metabolism that are not described in detail by selected genes. Numbers refer to enzymes listed in Table 3.

was taken at the expression of genes of carbon metabolism that have been previously mapped (Table 4).

For each introgression line, the expression of a range of genes encoding enzymes of carbohydrate metabolism, including those that have been mapped to locations within the introgressions (Table 4), was investigated (Causse *et al*., 2004). In general, genes known to occur within the introgression did not show significantly altered expression levels. Of the mapped genes described in Table 4, only fructokinase (IL4-4) and malate dehydrogenase (IL7-3) changed significantly in expression (Table 3). This suggests that, in general, the introgressed alleles conferred changes in fruit metabolism by encoding proteins with altered properties, rather than being expressed at different levels. However, some caution must be exercized in making this statement since this transcriptomic study was conducted at a single time point in fruit development (20 DAA). Allele-specific changes in gene expression at other stages of fruit development, or in tissues other than fruit cannot be ruled out. Furthermore, it is possible that the kinetics of carbohydrate accumulation and fruit development may be different between the lines (although fruit growth analysis suggests this is not the case; data not shown).

Table 4. *List of introgression lines and the identity of metabolism-related genes that have been mapped to the region of the introgression*

Introgression line	Mapped gene	Reference
IL4-4	Fructokinase	Causse *et al*., 2004
	Acyl transferase	Causse *et al*., 2004
	Phenylalanine ammonia lyase	Causse *et al*., 2004
IL7-3	Aconitase	Causse *et al*., 2004
	Malate dehydrogenase	Tanksley *et al*., 1992
	PEP carboxylase 1	Causse *et al*., 2004
	Phenylalanine ammonia lyase	Causse *et al*., 2004
	Fruit specific metallocarboxypeptidase	Causse *et al*., 2004
	ADPGpyrophosphorylase (SSU)	Schaffer *et al*., 2000
	Glutamate transaminase	Tanksley *et al*., 1992
IL7-5	Sucrose phosphate synthase	Fuglevand *et al*., 1998
	Sucrose synthase	Fuglevand *et al*., 1998
	ADPGpyrophosphorylase (LSU2)	Schaffer *et al*., 2000
	Pyrophosphatase	Causse *et al*., 2004
IL2-6	Succinate dehydrogenase	Causse *et al*., 2004
	ACC synthase	Tanksley *et al*., 1992
	Cyclin	Causse *et al*., 2004
	Ferritin	Causse *et al*., 2004
IL12-4	6 phosphofructokinase (α-subunit)	Causse *et al*., 2004
	Invertase inhibitor	Causse *et al*., 2004
	Cyclin dependent kinase	Causse *et al*., 2004
	β-Ureidohydrolase	Causse *et al*., 2004
IL1-4	Malate dehydrogenase	Causse *et al*., 2004
	Hexose transporter	Causse *et al*., 2004
	Triose phosphate isomerase	Causse *et al*., 2004
	Amidase	Causse *et al*., 2004
	Vacuolar ATPase	Causse *et al*., 2004
	Fructose bisphosphate aldolase	Causse *et al*., 2004
	ADPGpyrophosphorylase (LSU1)	Schaffer *et al*., 2000

Although few changes in the expression of introgressed alleles encoding enzymes of carbon metabolism were observed, there were significant changes in many other metabolic genes that are not contained within the introgressed regions (Tanksley *et al*., 1992; Fuglevand *et al*., 1998; Schaffer *et al*., 2000; Causse *et al*., 2004; Table 3). Such changes are presumably the result of perturbations in signalling and regulatory networks as a result of the presence of the introgressed alleles.

Common changes in the metabolic transcriptome in the two lines with the highest Brix increase

The observation that there are widespread downstream transcriptomic changes as a result of the introgressions raises the question as to whether the nature of these changes in the different introgression lines reveals common regulatory mechanisms that relate to fruit carbohydrate metabolism. The fact that PCA clustered lines with similar fruit Brix changes more closely together suggests that there are, in fact, some common elements of transcriptomic change (Fig. 4). Indeed, when the expression of genes encoding

enzymes of carbohydrate metabolism were analysed, a clear distinction could be made between the two lines with the largest increases in fruit Brix (IL4-4 and IL7-3) and the other lines with smaller increases in Brix. In the former, there is a pronounced and co-ordinated up-regulation of large sections of metabolism that does not occur in the latter. This suggests that a common mechanism for high Brix exists within these lines.

The significant changes in gene expression in IL4-4 and IL7-3 mainly related to pathways involved in sucrose mobilization and respiration (Table 3). The question then is what is the mechanism that links these changes in gene expression to increased fruit Brix? The identification of an apoplastic invertase as a QTL for increased Brix (Fridman *et al*., 2004) highlights one possibility, an increased supply of sucrose to the fruit tissue. The introgressed apoplastic invertase allele encodes a kinetically more efficient enzyme that is proposed to lead to an enhanced capacity for the movement of sucrose unloaded from phloem into the fruit tissues (Fridman *et al*., 2004; Baxter *et al*., 2005). There are, of course, many other possible routes to increasing the supply of sugar to the fruit (increased photosynthesis, increased partitioning of leaf photoassimilate to sucrose export, increased efficiency of phloem loading and unloading, as well as increased capacity for the uptake of sucrose into the fruit) and it is possible that this mechanism could be common amongst the high Brix introgression lines studied here. Thus, one explanation for the co-ordinated increase in expression of genes encoding enzymes involved in sucrose mobilization and respiration in IL4-4 and IL7-3 could be that these changes reflect an increased availability of sucrose in the fruit. Thus, the response is to increase the expression of enzymes of sugar mobilization and increase respiratory capacity. It is interesting that, in the opposite situation (sugar starvation), co-ordinated decreases in the same pathways occur (Thimm *et al*., 2004), suggesting that the transcriptional regulation of these pathways is tightly governed by sugar supply. In addition, an analysis of gene expression during *Arabidopsis* seed filling also highlighted synchronized induction of sucrose breakdown and glycolytic gene expression, although, in this study co-ordinated changes appeared to be specific to cytosolic or plastidial isoforms (Ruuska *et al*., 2002). Such specificity is not observed in the significant gene expression changes that were measured here, perhaps highlighting differences in sink metabolism between tomato and *Arabidopsis*. However, given that the array used in this study used EST as probes rather than gene-specific oligonucleotides, it is possible that cross-hybridization has limited the ability to distinguish closely related members of gene families. Apart from IL4-4 and IL7-3, there was no evidence for a co-ordinated up-regulation of genes encoding enzymes of sucrose mobilization and respiration in the other introgression lines in this study. Given that the changes in carbohydrate metabolism were much less pronounced in these lines compared with IL4-4 and IL7-3, it may be that there is a threshold of sugar-related changes required before re-programming of the metabolic transcriptome is observed.

The transcriptomic changes in fruit from IL4-4 and IL7-3 also provide insight into the regulation of starch metabolism in developing tomato fruit. Fruit from IL4-4 and IL7-3 at 20 DAA contained elevated levels of starch compared with fruit from the *S. lycopersicum* parent at the same stage (Fig. 2). In general, there didn't appear to be a co-ordinated change in the expression of genes encoding enzymes of starch metabolism that correlated with this change. In both lines, an increase in expression of the large subunit of ADPglucose pyrophosphorylase was observed. However, the increase was much smaller in IL4-4 despite the fact that fruit from both lines contained similar increases in starch (Fig. 2; Table 3). In IL4-4 no other significant changes in the expression of starch metabolism genes were observed. However, many of the genes were not detected on the arrays. In IL7-3, there were actually significant down-regulations of a starch synthase and a branching enzyme gene. These observations support previous work suggesting that the rate of starch synthesis in sink tissues is dependent on substrate supply rather than the amount of enzymes in the starch synthetic pathway (Schaffer and Petreikov, 1997; N'tchobo *et al*., 1999; Baxter *et al*., 2005). By contrast, in *Arabidopsis*, altered carbohydrate supply led to co-ordinated changes in the expression of a number of genes encoding enzymes of starch metabolism (Price *et al*., 2004). This highlights the different ways in which metabolism is regulated in storage organs such as fruit and in developing seedlings.

Summary

Transcriptomic changes have been described in a series of tomato introgression lines that share the common trait of increased ripe fruit soluble solids and increased accumulation of fruit carbohydrate. Each non-overlapping introgression leads to a distinct set of transcriptomic changes that are sufficient to be able to distinguish clearly between the lines using multivariate statistics. However, there are a small number of common changes that reveal mechanistic relationships between the lines. In particular, the two lines with the largest increases in fruit soluble solids both show a co-ordinated up-regulation of genes encoding enzymes of sucrose mobilization and respiration. By contrast, lines with smaller increases in Brix show relatively few changes in the expression of genes encoding enzymes of primary carbon metabolism. At the developmental stage analysed in this study, few genes that have been mapped within the introgressions changed in expression, raising the possibility that the majority of transcriptomic changes are a downstream consequence of the expression of the introgressed genes. This transcriptomic approach provides additional

information that characterizes the Zamir introgression lines and adds to the growing collection of data that includes metabolome profiles (Overy *et al*., 2005) and map positions of candidate genes (Causse *et al*., 2004). The complete transcriptome dataset from this study has been deposited in the tomato expression database http://ted.bti.cornell.edu/cgi-bin/miame/home.cgi.

Acknowledgement

Financial support to LJS and WPQ by the BBSRC is acknowledged.

References

Alba R, Fei Z, Paxton P, *et al*. 2004. ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. *The Plant Journal* **39,** 697–714.

Baxter CJ, Foyer CH, Turner J, Rolfe SA, Quick WP. 2003. Elevated sucrose-phosphate synthase activity in transgenic tobacco sustains photosynthesis in older leaves and alters development. *Journal of Experimental Botany* **54,** 1813–1820.

Baxter CB, Carrari F, Bauke A, Overy S, Hill SA, Quick P, Fernie A, Sweetlove LJ. 2005. Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. *Plant Cell Physiology* (in press).

Causse M, Duffe P, Gomez MC, Buret M, Damidaux R, Zamir D, Gur A, Chevalier C, Lemaire-Chamley M, Rothan C. 2004. A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. *Journal of Experimental Botany* **55,** 1671–1685.

Chang S, Puryear J, Cairney J. 1993. A simple and efficient method for isolating RNA from pine trees. *Plant Molecular Biology Reporter* **11,** 113–116.

Eshed Y, Abu-Abied M, Saranga Y, Zamir D. 1992. *Lycopersicum esculentum* lines containing small overlapping introgressions from L. pennellii. *Theoretical and Applied Genetics* **83,** 1027–1034.

Eshed Y, Gera G, Zamir D. 1996. A genome-wide search for wild-species alleles that increase horticultural yield of processing tomato. *Theoretical and Applied Genetics* **93,** 877–886.

Eshed Y, Zamir D. 1995. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. *Genetics* **141,** 1147–1162.

Fei Z, Tang X, Alba RM, White JA, Ronning CM, Martin GB, Tanksley SD, Giovannoni JJ. 2004. Comprehensive EST analysis of tomato and comparative genomics of fruit ripening. *The Plant Journal* **40,** 47–59.

Fuglevand G, Phillips W, Mozzanega P, Corley S, Chengappa S, Shields R. 1998. Mapping of tomato genes associated with sugar metabolism. *Tomato Genetics Co-operative Report* **48,** 22–23.

Fridman E, Pleban T, Zamir D. 2000. A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. *Proceedings of the National Academy of Sciences, USA* **97,** 4718–4723.

Fridman E, Carrari F, Liu YS, Fernie AR, Zamir D. 2004. Zooming in on a quantitative trait for tomato yield using interspecific introgressions. *Science* **305,** 1786–1789.

Grierson D, Kader AA. 1986. Fruit ripening and quality. In: Atherton JG, Rudich J, eds. *The tomato crop. A scientific basis for improvement*. Dordrecht: Kluwer Academic Publishers, 241–280.

Gur A, Semel Y, Cahaner A, Zamir D. 2004. Real time QTL of complex phenotypes in tomato interspecific introgression lines. *Trends in Plant Science* **9,** 107–109.

Liu YS, Gur A, Ronen G, Causse M, Damidaux R, Buret M, Hirschberg J, Zamir D. 2003. There is more to fruit colour than candidate carotenoid genes. *Plant Biotechnology Journal* **1,** 195–207.

Miron D, Schaffer AA. 1991. Sucrose phosphate synthase, sucrose synthase, and invertase activities in developing fruit of *Lycopersicon esculentum* Mill, and the sucrose accumulating *Lycopersicon hirsutum* Humb and Bonpl. *Plant Physiology* **95,** 623–627.

Monforte AJ, Fridman E, Zamir D, Tanksley SD. 2001. Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: Deductions about natural variation and implications for germplasm utilization. *Theoretical and Applied Genetics* **102,** 572–590.

N'tchobo H, Dali N, Nguyen-Quoc B, Foyer CH, Yelle S. 1999. Starch synthesis in tomato remains constant throughout fruit development and is dependent on sucrose supply and sucrose synthase activity. *Journal of Experimental Botany* **50,** 1457–1463.

Overy SA, Walker HJ, Malone S, Howard TP, Baxter CJ, Sweetlove LJ, Hill SA, Quick WP. 2005. Application of metabolite profiling to the identification of traits in a population of tomato introgression lines. *Journal of Experimental Botany* **56,** 287–296.

Price J, Laxmi A, St Martin SK, Jang JC. 2004. Global transcription profiling reveals multiple sugar signal transduction mechanisms in *Arabidopsis*. *The Plant Cell* **16,** 2128–2150.

Robinson NL, Hewitt JD, Bennett AB. 1988. Sink metabolism in tomato fruit. 1. Developmental changes in carbohydrate metabolizing enzymes. *Plant Physiology* **87,** 727–730.

Roessner-Tunali U, Hegemann B, Lytovchenko A, Carrari F, Bruedigam C, Granot D, Fernie A. 2003. Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. *Plant Physiology* **133,** 84–99.

Ruuska SA, Girke T, Benning C, Ohlrogge JB. 2002. Contrapuntal networks of gene expression during *Arabidopsis* seed filling. *The Plant Cell* **14,** 1191–1206.

Schaffer AA, Levin I, Oguz I, Petreikov M, Cincarevsky F, Yeselson Y, Shen S, Gilboa N, Bar M. 2000. ADPGlucose pyrophosphorylase activity and starch accumulation in immature tomato fruit: the effect of a *Lycopersicum hirsutum*-derived introgression encoding for the large subunit. *Plant Science* **152,** 135–144.

Schaffer AA, Petreikov M. 1997. Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. *Plant Physiology* **113,** 739–746.

Sreenivasulu N, Altschmied L, Radchuk V, Gubatz S, Wobus U, Weschke W. 2004. Transcript profiles and deduced changes of metabolic pathways in maternal and filial tissues of developing barley grains. *The Plant Journal* **37,** 539–553.

Stommel JR. 1992. Enzymic components of sucrose accumulation in the wild tomato species *Lycopersicum peruvianum*. *Plant Physiology* **99,** 324–328.

Sun J, Loboda T, Sung SJS, Black CC. 1992. Sucrose synthase in wild tomato, *Lycopersicon chmielewskii,* and tomato fruit sink strength. *Plant Physiology* **98,** 1163–1169.

Sweetlove LJ, Burrell MM, ap Rees T. 1996. Starch metabolism in tubers of transgenic potato (*Solanum tuberosum*) with increased ADPglucose pyrophosphorylase. *Biochemical Journal* **320,** 493–498.

Tanksley SD, Ganal MW, Prince JP, *et al*. 1992. High-density molecular-linkage maps of the tomato and potato genomes. *Genetics* **132,** 1141–1160.

Thimm O, Blasing O, Gibon Y, Nagel A, Meyer S, Kruger P, Selbig J, Muller LA, Rhee SY, Stitt M. 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. *The Plant Journal* **37,** 914–939.

Tusher VG, Tibshirani R, Chu G. 2001. Significance analysis of microarrays applied to the ionizing radiation response. *Proceedings of the National Academy of Sciences, USA* **98,** 5116–5121.

Van der Hoeven R, Ronning C, Giovannoni J, Martin G, Tanksley SD. 2002. Deductions about the number, organization and evolution of genes in the tomato genome based on analysis of a large expressed sequence tag collection and selective genome sequencing. *The Plant Cell* **14,** 1441–1456.

Yelle S, Chetelat RT, Dorias M, DeVerna JW, Bennett AB. 1991. Sink metabolism in tomato fruit. 4. Genetic and biochemical analysis of sucrose accumulation. *Plant Physiology* **95,** 1026–1035.

Yelle S, Hewitt JD, Robinson NL, Damon S, Bennett AB. 1988. Sink metabolism in tomato fruit. 3. Analysis of carbohydrate assimilation in a wild species. *Plant Physiology* **87,** 737–740.