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1 **Genotype-dependent response to carbon availability in growing tomato**

2 **fruit**

3 Short running title: Responses to carbon availability in tomato fruit

4

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1 **Abstract**

2 Tomato fruit growth and composition depend on both genotype and environment. This paper
3 aims (i) at studying how fruit phenotypic responses to changes in carbon availability can be
4 influenced by genotype and (ii) at identifying genotype-dependent and -independent changes in
5 gene expression underlying variations in fruit growth and composition. To this end, we grew a
6 parental line (*S. lycopersicum*) and an introgression line from *S. chmielewskii* harboring QTL for
7 fresh weight and sugar content under two fruit loads (FL). Lowering fruit load increased fruit cell
8 number and reduced fruit developmental period in both genotypes. In contrast, fruit cell size was
9 increased only in the parental line. Modifications in gene expression were monitored in expanding
10 fruits using microarrays and RT-qPCR for a subset of genes. FL changes induced more deployments
11 of regulation systems (transcriptional and post transcriptional) than massive adjustments of whole
12 primary metabolism. Interactions between genotype and FL were especially noticeable for 99
13 genes mainly linked to hormonal and stress responses, and on gene expression kinetics during
14 fruit development. Links between gene expression and fruit phenotype were found for aquaporin
15 expression levels and fruit water content, and invertase expression levels and sugar content during
16 fruit ripening phase. In summary, the present data emphasized age- and genotype-dependent
17 responses of tomato fruit to carbon availability, at phenotypic as well as at gene expression level.

18 **Keyword index**

19 Fruit growth, gene expression, genotype x environment interaction, hormone, metabolism,
20 regulations, *Solanum lycopersicum*, stress response, sugar, transcriptome

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1

2 **Introduction**

3 Carbohydrate availability is a major factor limiting plant growth, in particular for sink organs such
4 as fruits. In tomato, carbohydrates needed for fruit growth come from photosynthetic sources
5 such as mature leaves, fruits having a low photosynthetic activity (Farrar & Williams 1991).
6 Increasing carbohydrate availability to the reproductive organs by reducing fruit load on the plant
7 enhances cell division in the ovary and thereby the final fruit size (Baldet *et al.* 2006). Conversely, a
8 low carbohydrate supply during the phase of rapid cell expansion leads to a reduction of fruit size
9 (Bertin *et al.* 2003; Heuvelink 1997) and even of dry matter (Gautier, Guichard & Tchamitchian
10 2001). In the case of severe carbon stress, sugar, protein and amino-acid contents can all be
11 reduced (Baldet *et al.* 2002; Gary *et al.* 2003). Fruit sugar content is the consequence of sucrose
12 import, carbohydrate metabolism, and dilution by water (Ho 1996). Sucrose enters the cells either
13 via the apoplast after conversion to hexose by a parietal invertase, or via the symplast. In the
14 cytoplasm, sucrose may be converted into fructose and glucose by invertase, or into fructose and
15 UDP-glucose by sucrose synthase (Frommer & Sonnewald 1995; Yelle *et al.* 1988). Hexoses are
16 then transformed into starch by the successive actions of fructokinase, hexokinase,
17 phosphoglucoisomerase, phosphoglucomutase, ADP-glucose-pyrophosphorylase and starch
18 synthase (Schaffer & Petreikov 1997a; Schaffer & Petreikov 1997b; Damari-Weissler *et al.* 2006).
19 Starch is transiently stored in the amyloplasts, and constitutes a carbon reservoir for hexose
20 synthesis (Dinar & Stevens 1981). The accumulation of carbohydrates in the fruit leads to a
21 gradient of osmotic pressure, causing first a massive entrance of water, notably *via* aquaporins
22 (see Kaldenhoff *et al.* 2008 for review), and subsequently cell expansion (Ho, Grange & Picken
23 1987). This expansion relies on cell wall plasticity, determined partly by the activity of enzymes
24 related to the synthesis or degradation of cell wall components in the epidermis as well as in the

1 pericarp (Thompson, Davies & Ho 1998). Many of these processes are affected by hormones.
2 Cytokinins may affect fruit sugar content (Martineau *et al.* 1995), while auxins and gibberellins can
3 be involved in the regulation of cell enlargement, by controlling the expression of genes encoding
4 cell wall modifying proteins like expansins (Catala, Rose & Bennett 2000; Chen & Bradford 2000;
5 Chen, Nonogaki & Bradford 2002; Guillon *et al.* 2008).

6 Large-scale quantification of gene expression in tomato has been shown to be a powerful tool for
7 characterizing plant response to a variety of conditions, including salt stress (Ouyang *et al.* 2007),
8 light (Facella *et al.*, 2008), developmental changes (Alba *et al.* 2005; Vriezen *et al.* 2008),
9 differentiation of specialized tissues (Lemaire-Chamley *et al.* 2005), mutants (Kolotilin *et al.*, 2007)
10 or introgressions of genomic segments (Baxter *et al.* 2005). To our knowledge, the effect of
11 carbohydrate availability on tomato transcriptome has never been investigated, although many
12 genes are known to be sugar sensitive (Koch 1996). In fruit, the effect of carbohydrate availability
13 was only investigated for target enzymes and genes at few developmental stages. For example, it
14 was shown in peach fruit that high crop load leads to increased acid invertase activity at a final
15 stage of fruit growth (Morandi *et al.* 2008). In tomato, an obscurity-induced carbohydrate
16 limitation led to changes in the expression of some sugar transporters, and of enzymes involved in
17 sugar- or amino-acid metabolism at two stages of fruit development (cell division and cell
18 expansion) (Baldet *et al.* 2002). However, although recent studies emphasized that source-sink
19 relationships between vegetative and reproductive organs are genetically controlled and are a
20 central hub for controlling fruit metabolism in tomato (Schauer *et al.* 2006; Lippman, Semel &
21 Zamir 2007), comparative analysis of fruit response to carbohydrate availability in different
22 tomato genotypes has not been looked at in any depth.

23 This study aims at identifying genes and gene categories differentially regulated in growing fruit in
24 response to changes in carbon availability induced by alteration of fruit load on the plant.
25 Transcriptome analysis using tomato microarrays was carried out on fruit pericarps harvested 21

1 days after anthesis, a stage of rapid fruit growth, cell expansion and storage of major
2 carbohydrates such as starch. To get an insight into interactions between genotype and
3 environment on gene expression, two closely-related tomato genotypes were grown at two
4 contrasted fruit loads, one (high load, HL) inducing competition for carbon among fruits (trusses
5 were not pruned), and the other (low load, LL) inducing low or no competition (trusses pruned to
6 one fruit). The two accessions differed by an introgressed chromosome fragment on chromosome
7 9, carrying several quantitative trait loci whose expression is either independent of the fruit load
8 (for fruit developmental duration, fresh weight, seed number, dry matter and sugar content) or
9 specific to one fruit load (cell number, cell size or fruit cracking) (Prudent *et al.* 2009). In a last
10 step, the expression of a subset of genes related to carbon metabolism, cell wall modification, and
11 water fluxes were analyzed using RT-quantitative PCR along fruit expansion and ripening.

12 **Materials and methods**

13 ***Plant growth, fruit thinning and sampling***

14 Two tomato genotypes, *Solanum lycopersicum* cv. Moneyberg (hereafter called M) and an
15 introgression line (hereafter called C9d) carrying a fragment from the bottom of chromosome 9 of
16 *Solanum chmielewskii* in the M genetic background, were grown under controlled greenhouse
17 conditions at a day-night temperature set point of 25/15 °C during spring 2007 in Avignon, France.
18 The position of the introgression of genotype C9d as well as the quantitative trait loci (QTL)
19 previously identified, are described in Prudent *et al.* (2009). For both genotypes, all trusses were
20 pruned to one fruit when flower 2 was at anthesis (low fruit load condition or LL) on 40 randomly
21 selected plants, while trusses of 16 other plants were not pruned (high fruit load condition or HL).
22 Under HL condition, the average number of fruit sets per truss was similar in C9d and M (around
23 seven fruits). Anthesis was recorded three times a week, allowing the determination of fruit age
24 and fruit developmental duration. Fruits were harvested at five different developmental stages

1 from the cell expansion period until maturity (21, 28, 35, 42 days after anthesis (daa) and red ripe).
2 Fruits were weighed, and locular tissue and seeds were removed. The pericarp tissue was then
3 weighed, frozen in liquid nitrogen and stored at -80°C until nucleic acid manipulations or stored at
4 -20°C until phenotypic measurements.

5 ***Phenotypic measurements***

6 At each developmental stage, six fruits per genotype and per fruit load were randomly harvested
7 between the fourth and the ninth truss of the plants, at proximal positions: flowers 2, 3 or 4 under
8 HL and only flower 2 under LL. Pericarp powders were lyophilized and weighed and pericarp water
9 content was then deduced. Soluble sugars (glucose, fructose, sucrose) and starch were extracted,
10 quantified by an enzymatic method (Gomez *et al.* 2007; Gomez, Rubio & Auge 2002) and
11 expressed in g per 100 g of pericarp dry matter. Additional phenotypic measurements were
12 carried out on fruits harvested at the red ripe stage: seeds were counted, and the number and the
13 mean size of pericarp cells were assessed according to the method described in Bertin, Gautier &
14 Roche (2002). An analysis of variance was performed on phenotypic measurements in order to
15 study the effects of fruit age, fruit load, genotype and their two- or three-way interactions with R
16 Software (<http://www.r-project.org>).

17 ***RNA extractions***

18 All fruits used for RNA extraction were randomly harvested between the fourth and the ninth truss
19 of the plants, at proximal positions, similarly to phenotypic measurements. Fruit samples used for
20 microarray and quantitative real time PCR were different. Total RNA used for microarray
21 experiment was isolated from two biological pools of 25 fruits at 21 daa, following the procedure
22 described by Chang, Puryear & Cairney (1993). Total RNA used for quantitative real time PCR was
23 isolated from three biological pools of ten fruits (at 21, 28, 35, 42 daa and red ripe stage) with TRI

1 Reagent[®] Solution (Ambion) following the procedure described by the manufacturer, with minor
2 modifications.

3 ***Microarray experiments***

4 TOM2 cDNA glass slides were fabricated by arraying Array-Ready Oligo Set[™] for the Tomato
5 Genome (Operon) onto Corning[®] UltraGAPS[™] slides using a BioRobotics MicroGridII arrayer
6 (Genomic Solutions) in Instituto de Biología Molecular y Celular de Plantas (Valencia, Spain). Slides
7 contained 12,160 oligos corresponding to 11,862 unique and randomly selected transcripts of the
8 tomato genome. Information about the oligo set was described on the Operon website
9 (http://omad.operon.com/download/storage/lycopersicon_V1.0.2_datasheet.pdf). Fluorescent
10 probes were prepared from 50 µg of total RNA, using the Amino Allyl MessageAmp[™] II aRNA
11 Amplification Kit (Ambion), and following manufacturer's specifications. Purified Cy3- or Cy5-
12 labelled AA-aRNAs were dried in a speed-vac, resuspended in 9 µL nuclease free water and
13 fragmented using RNA Fragmentation Reagents (Ambion). Probes were mixed with 88 µL of the
14 hybridization solution consisting of 0.5 µg.mL⁻¹ denatured salmon sperm DNA (Stratagene), 5X SSC,
15 0.25% SDS and 5X Denhardt's solution and 50% formamide. The solution was denatured for 1
16 min at 100°C and cooled down to 37°C. Labelled AA-aRNAs were hybridized with slides for 16h at
17 37°C under agitation. After hybridization, slides were washed at 30°C with 2X SSC, 0.2% SDS for 5
18 min, with 0.2X SSC, 0.1% SDS for 5 min, with 0.2X SSC for 3 min, and with 0.02X SSC for 30 s.
19 Finally, slides were dried with nitrogen gas for 3 min at 30°C before scanning.

20 ***Data analysis of microarray experiments***

21 The microarray experimental design consisted of two biological replicates, and for each biological
22 replicate the dyes were reversed (dye swap) for a total of four slides per comparison (Fig. 3a).

23 The raw data corresponding to the median spot intensities, with no background subtraction were
24 analyzed using the Bioconductor (<http://www.bioconductor.org>) package R/Maanova v1.4.1 (Cui,

1 Kerr & Churchill 2003) in R-2.6 (<http://www.r-project.org>). Data visualization, normalization, and
2 statistical analysis, including multiple test adjustments (FDR) were performed as described by
3 Mounet *et al.* (2009). *F* statistics computed on the James-Stein shrinkage estimates of the error
4 variance (Wu *et al.* 2003) were calculated and genes with a *P* value $< 5 \cdot 10^{-2}$, FDR < 0.05 , fold
5 change ≥ 1.6 and average intensity $>$ background mean + 2 background s_D were selected.
6 In order to analyze specifically the interaction between the genotype and the fruit load, another
7 statistical study was performed on normalized data using Bioconductor LIMMA package v2.13.8
8 (Smyth 2005a). The data were normalized using the printtploess (within-array normalisation) and
9 scale (between-arrays normalisation) functions (default parameters). Flagged spots were given a
10 weight of 0.1 using the weight function. A factorial design analysis (Smyth 2005b) was performed
11 and a linear model with a coefficient for each of the four factor combinations (C9dLL, C9dHL, MLL
12 and MHL) was fit. The interaction term (C9dLL-C9dHL) - (MLL-MHL) was extracted. The *P* values
13 resulting from moderated *t* test were corrected for multiple testing using the Benjamini-Hochberg
14 FDR adjustment. As above, genes were considered to be significantly differentially expressed if
15 adjusted *P* values were $< 5 \cdot 10^{-2}$, fold change was ≥ 1.6 . For each of the four conditions, spots with
16 an average intensity higher than background mean + 2 background s_D were considered as
17 detected.

18 ***Quantitative real-time PCR***

19 The quantitative real-time PCR experiment followed the eleven golden rules proposed by Udvardi,
20 Czechowski & Scheible (2008). Reverse transcription was performed with 2 μ g of total RNA from
21 each sample treated with DNase in 50 μ L with oligo-dT (10 μ M) and AMV Reverse Transcriptase
22 (10 U/ μ L) (Promega), according to the manufacturer's instructions. The RT mix was diluted 5-fold
23 in water and 2 μ L aliquots were stored before use. Quantitative real-time PCR was carried out
24 using a Stratagene Mx3005P[®] thermocycler (Stratagene, Cedar Creek, TX) in a reaction volume of

1 20 μ L, in 96-well plates. PCR were conducted using 7.5 μ L SYBR Green mix (133 mM KCl, 27 mM
2 Tris HCl pH 9, 0.2 % Triton[®] X-100, 8 mM MgCl₂, 2 μ L of 1500-fold dilution SYBR[®] Green I Nucleic
3 Acid Gel Strain 10000X (Lonza), 0.5 mM each dNTP, 2 μ L of the 5-fold dilution of RT mix, 1 U Taq
4 DNA polymerase, 9.8 μ L H₂O, and 0.12 μ M of each primer. Primer sequences are detailed in
5 Supporting Information Table S1. PCR conditions were: 2 minutes at 95°C, followed by 40 cycles at
6 95°C for 20 sec, 20 sec at the primer specific temperature (~55°C) and at 72°C for 35 sec. A
7 thermal denaturation curve of the amplified DNA was carried out, in order to measure the melting
8 temperature of the PCR product. For each reaction, three technical replicates were run. Relative
9 gene expression was calculated by the $2^{-\Delta\Delta C_T}$ method (Livak & Schmittgen 2001), with the
10 eukaryotic translation initiation factor 4A-2 (eIF-4A-2) (U213502) as an internal control. The use of
11 this unigene as a reference was validated under our experimental conditions, as advised by
12 Gutierrez *et al.* (2008). For each genotype, and at each developmental stage, fruit load effect on
13 gene expressions was evaluated using a Student's t test with R software, and the significance of
14 the interaction between genotype and fruit load was tested via a two-way analysis of variance in
15 R. Normalized gene expression data were analyzed by principal component analysis (PCA) using
16 the "princomp" function in R. The principal component scores were then plotted for individual
17 observations.

18 ***Functional categorization***

19 Classifications of unigenes into functional groups were obtained from MapMan ontology
20 classifications (Thimm *et al.* 2004), gene family assignments from TAIR
21 (<http://www.arabidopsis.org>), and the literature when unigenes exhibited no homology with
22 *Arabidopsis thaliana* proteins. Functional categories were restricted to eleven: cell wall
23 modification, electron transport, hormonal responses, photosynthesis, primary metabolism,

1 protein metabolism, secondary metabolism, signalling, stress responses, transcription and
2 transport.

3 **Results**

4 ***Fruit load effect on fruit phenotype***

5 Tomato fruits of the two lines, Moneyberg (M) and the introgression line C9d, were harvested at
6 five developmental stages from 21 daa to the red ripe stage, and their phenotype was described
7 either throughout fruit development (Fig. 1), or only at maturity (Fig. 2). The analysis of variance
8 (not shown) underlined significant interactions between fruit age and fruit load for all traits
9 measured kinetically. At maturity, seed number was the only trait which was not affected by fruit
10 load. For the other traits, the significant fruit load effect was either genotype-independent or
11 associated to significant genotype x fruit load interactions. When fruit load was reduced to one
12 fruit (LL), fruit development duration decreased and cell number increased, without any
13 interaction with genotype (Fig. 2b). On the other hand, larger fruits (Fig. 1a), associated with
14 higher cuticular macrocracks (Fig 2a), lower pericarp water and sugar contents (Fig. 1b and 1d)
15 and higher starch content (Fig. 1c) were consistently observed during fruit development in fruits
16 grown under LL, when compared to HL fruits in both genotypes. However sugar contents were
17 similar at maturity. For these traits, the effect of fruit load interacted significantly with genotype,
18 for at least one fruit developmental stage. It was also the case of cell size, which was higher under
19 LL conditions for M, but not for C9d (Fig. 2b).

20 At 21 daa, which corresponds to the period of rapid fruit growth and to the developmental stage
21 chosen for microarray transcriptome analysis, modification of fruit composition in response to
22 changes in fruit load was significant in both genotypes for sugar content (Fig. 1d) and in M for
23 water and starch content, indicating a strong significant interaction between genotype and fruit
24 load (G x FL) for these two traits.

1

2 ***Fruit load effect on the transcriptome from 21 daa fruits and genotype-fruit load***
3 ***interactions***

4 **Microarray experimental design and data analysis**

5 In the tomato genotypes studied, the 21 daa stage of fruit development corresponds to the cell
6 expansion phase during which rapid fruit growth occurs and starch accumulation peaks (Dinar &
7 Stevens 1981). At 21 daa, fruit load effect was obvious for starch and soluble sugar contents; no
8 significant effect on fruit weight and water contents could be observed (Fig. 1a and 1b). This stage
9 of fruit development appears therefore critical for the control of fruit growth and of the
10 concomitant changes in fruit composition.

11 In order to reduce the biological variability between samples, we used 40 randomly
12 selected plants for the low load (LL) condition and 16 plants for the high load (HL) condition. For a
13 given genotype and developmental stage, up to 50 fruits were randomly picked from the plants.
14 From these, large pools of 25 fruits each were constituted at random, as previously described
15 (Buret, Duby & Flanzky 1980). This sampling and pooling strategy effectively reduces the variability
16 between biological replicates e.g. by excluding the environmental effects linked to plant location
17 in the field, and allows thus to focus only on the effects of genotype and fruit load.

18 In addition, the microarray experiment was designed as a loop where C9dLL was compared
19 to C9dHL itself compared to MHL itself compared to MLL itself compared to C9dLL (Fig.3a).
20 Compared to single comparisons, this design decreases considerably the variance of estimated
21 effects (Churchill 2002; Yang & Speed 2002), allowing much more confidence in the estimation of
22 the differentially expressed genes. Four technical replicates (including 2 dye swaps) were done per
23 comparison *i.e.* 8 hybridizations per comparison (2 biological repeats X 4 technical repeats). To
24 further increase the significance of the results from statistical analyses, the threshold for log₂ fold-

1 change was set-up to 0.68 (1.6 fold change) *i.e.* a value higher than that commonly used in similar
2 studies (e.g. Wang *et al.* 2009). As a result of this combination of pooling strategy, experimental
3 design and statistical analyses, a smaller number of differentially expressed genes identified by
4 transcriptome analysis can be anticipated but more robust results are expected. Indeed, in a
5 previous study done in tomato using a similar design with fewer technical replicates, most of the
6 results from microarray analysis were further validated by qRT-PCR (Mounet *et al.*, 2009).

7 8 **Impact of fruit load on fruit transcriptome**

9 Because fruit load effect on fruit development and composition is the main focus of our study, we
10 excluded from the statistical analyses the comparison of genotypes for the same load. The effect
11 of fruit load on tomato fruit transcriptome in each genotype was analyzed by comparing gene
12 expressions under HL and LL in M and in C9d (Fig. 3a). In total, 103 genes out of 11,862 (0.9 %)
13 were differentially expressed in M, versus 147 in C9d (1.2 %) (Fig. 3b). Among them, 56
14 differentially expressed genes were common to C9d and M with similar responses whatever the
15 genotype, except for one unigene showing an opposite response depending on the genotype.

16 To identify which biological processes are modified by a fruit load change, unigenes were classified
17 into functional categories. Figure 4a displays the distribution of the differentially expressed
18 unigenes into functional categories (Supporting Information Table S2 and Table S3). According to
19 the Mapman classification, all biological processes were affected by fruit load but to a variable
20 extent. As expected from the modification of the source/sink relationships under LL, the
21 expression of genes implicated in primary and secondary metabolism was indeed affected.
22 Accordingly, LL fruits also displayed changes in cell wall related genes (mostly up-regulated) and in
23 electron transport (mostly down-regulated). A large proportion of the differentially expressed
24 genes, mostly down-regulated, have no function attributed. However, the most striking
25 differences between HL and LL –in terms of expression level-, were observed for genes involved in

1 signalling, in regulation of gene expression (hormonal responses, transcription) and in protein
2 metabolism (protein synthesis, post-translational modifications and degradation). Details of
3 several categories showing remarkable changes are displayed in Table 1. In primary metabolism,
4 the enzyme exhibiting the highest changes under LL conditions in both M and C9d genotypes (\log_2
5 fold-change = ~ -1.6 in M, ~ -1.5 in C9d) was the fructose-2,6-bisphosphatase (F26BPase). Several
6 differentially expressed genes with putative function in signalling exhibited high fold-changes
7 under low load conditions in both M and C9d ($1.4 < \log_2$ fold-changes < 4.8). While functions of
8 genes encoding proteins with calmodulin-binding motif and leucine rich repeats may remain
9 elusive, the COP9 signalosome is a key player of the machinery controlling protein degradation
10 that regulates a variety of processes in plants including light-regulated development and hormone
11 signalling (Chamovitz 2009). In addition, some ethylene, auxin and cytokinin-related genes with
12 roles in hormone biosynthesis, degradation or responses were also up- or down-regulated under
13 LL (Supporting Information Table S2 and Table S3). Among the 29 differentially expressed genes
14 encoding transcription factors and other proteins involved in gene regulation, 9 displayed very
15 consistent up-regulation (2 genes) or down-regulation (7 genes) in both M and C9d under LL.
16 Several transcription factor families were represented (HB, bHLH, AP2/EREBP, C2H2 zinc finger,
17 bZIP ...). Of these, the gene encoding a TAZ zinc finger protein exhibited very high and strikingly
18 similar changes in M and C9d under low load. The fold change \log_2 value was ~ -5.3 for both
19 genotypes, i.e. a ~ 40 -fold reduction in transcript abundance in the 21 daa fruits from M and C9d
20 cultivated under LL conditions. With the notable exception of the elongation factor 1-alpha
21 implicated in the protein synthesis machinery and of protein phosphatase 2C, most of the genes
22 classified into the protein metabolism category and differentially expressed under LL are involved
23 in protein degradation.

24

25 **Interactions between genotype and fruit load**

1 The previous study showed independently the effect of fruit load on each genotype. Several genes
2 displayed very similar fold changes in the two genotypes (e.g. the F26BPase and the TAZ
3 transcription factor) while others were mostly affected in one or the other genotype (e.g. the AS2
4 or the CCAAT box transcriptional regulators preferentially up-regulated in C9d) (Table 1). In order
5 to specifically identify the LL affected genes displaying a genotype-dependent change in gene
6 expression, we analyzed the genotype x fruit load (G x FL) interaction using LIMMA. Ninety-nine
7 genes showing significant G x FL interactions (Supporting Information Table S4) were identified
8 and further classified into functional categories (Fig. 4b). Among them, a high proportion belonged
9 to hormonal and stress response categories albeit all biological processes were targets of G x FL
10 interactions. For all functional categories, most of the genes up-or down-regulated under low load
11 conditions showed a greater variation of gene expression in one or the other genotype
12 (Supporting Information Table S4). In this table, positive values indicates that the corresponding
13 gene was more up-regulated or down-regulated under LL relative to HL conditions in C9d than in
14 M while negative values indicate the opposite. Their absolute value gives an indication of the
15 extent of the differences between the two genotypes. The largest category comprised genes
16 related to various hormone biosynthetic pathways including ethylene (ACC synthase and ACC
17 oxidase), auxin (IAA hydrolase), jasmonic acid (lipoxygenase, ent-kaurenoic acid hydroxylase) and
18 phytoalkylamines and salicylate (Table 2). All the hormone-related genes displaying G x FL
19 interactions were more expressed in C9d while cell wall related genes were more or less expressed
20 in one or the other genotype (Table 2).

21 **Validation of microarray data**

22 The abundance of transcripts from selected genes was monitored by qRT-PCR in order to validate
23 the microarray data, on independent fruit samples. Quantitative PCR was carried out on eleven
24 unigenes, and confirmed the changes in transcript abundance (Supporting Information Fig. S1).

1 Because of the difference in sensitivity of the two techniques (already observed in Mohammadi,
2 Kav & Deyholos 2007 and Fernandez *et al.* 2008), in a lot of cases the ratio values based on
3 quantitative PCR were higher than those based on microarray but the Pearson's correlation
4 coefficient between the two methods was high ($r = 0.92$, $p = 7.10^{-16}$).

5 ***Fruit load effect on expression of selected genes along fruit development***

6 To gain further insight into how genes reacted to the fruit load change in the two genotypes, we
7 performed qRT-PCR on five stages from the cell expansion phase to fruit maturity, in the two
8 genotypes and under the two fruit loads. The functions of the 15 selected genes were related to
9 processes potentially involved in fruit growth and carbohydrate accumulation. Some genes were
10 chosen based on microarray results while others were selected because of their importance in
11 metabolism (when absent from the DNA chip). The correspondence between gene codes, their
12 annotations and their sequence references are detailed in Table 3. As shown on Fig. 5, for each of
13 the 15 observed genes, we identified (i) a significant fruit load effect whatever the fruit
14 developmental stage, for M, for C9d or for both genotypes and (ii) a significant G x FL interaction
15 at least at two developmental stages.

16 **Water flux-related genes**

17 Two aquaporins: a delta-tonoplast integral protein (delta-TIP), and a plasma membrane intrinsic
18 protein (PIP1), involved in water flux across biological membranes, were particularly affected by a
19 change in fruit load. The down-regulation of delta-TIP under LL condition was confirmed for both
20 genotypes (except at 42 daa) and PIP1 was similarly affected by fruit load. Moreover, a shift in
21 expression with fruit load was observed for PIP1: its expression was down-regulated earlier under
22 LL than under HL.

1 **Cell wall- related genes**

2 Five cell wall (CW) related genes were selected, two involved in synthesis, UDP-glucose-4-
3 epimerase (UDP-G-4-epi) and UDP-glucose-pyrophosphorylase (UDP-G-PPase), and three involved
4 in CW degradation : polygalacturonase (PG), and two xyloglucan endotransglycosylases (XTH6 and
5 BR1). The expression of UDP-G-4-epi was lower under LL than under HL during the fruit growth
6 phase (the first three stages), while the opposite occurred during fruit ripening (the two last
7 stages). The expression patterns of UDP-G-PPase and XTH6 were similar and slightly affected by
8 fruit load, with a high G x FL interaction at 28 daa. BR1 and PG were highly affected by fruit load
9 (figures are drawn with logarithmic scales), but for BR1 the variations depended on the fruit
10 developmental stage. For M, a shift in expression was revealed as BR1 expression was up-
11 regulated earlier under LL than under HL. PG showed the highest fold-change expression values
12 between HL and LL, during the whole of fruit development and its expression level was higher
13 under LL than under HL during the fruit ripening for both genotypes.

14 **Metabolism- related genes**

15 Some genes involved in key processes of starch metabolism, sugar metabolism, organic-acid and
16 amino-acid metabolism were analysed. Starch metabolism was represented by two genes involved
17 in its synthesis, the ADP-glucose-pyrophosphorylase large sub-unit (ADP-G-PPase) and a starch
18 branching enzyme (SBE), and one gene involved in its degradation: a beta-amylase (β -AM). LL
19 conditions mainly reduced the ADP-G-PPase expression during fruit ripening, whereas SBE
20 expression was mainly reduced during the fruit growth phase. The expression of β -AM was
21 dependent on fruit load in both genotypes, but it was more affected during the fruit growth phase
22 in C9d, and during fruit ripening in M.

23 Three genes involved in sugar metabolism were studied: two acid invertases (TIV1 and β -FR) and a
24 sucrose synthase (SUS2). Sucrose synthase is involved in phloem unloading and is mostly

1 expressed at early stages of fruit development while vacuolar invertases cleave sucrose into
2 glucose and fructose that are further accumulated into the vacuole of fruit cells. Sucrose synthase
3 SUS2 exhibited a very strong interaction effect with fruit age since the highest expression level was
4 observed at 28 daa in C9d, whatever the fruit load. In contrast, the transcript abundance of
5 vacuolar invertase (TIV1 and β -FR) was highly dependent on fruit load conditions and displayed a
6 strong increase in both genotypes during fruit ripening.

7 A gene coding for a phosphoenolpyruvate carboxylase (PEPC) was studied because it is a
8 multifaceted enzyme providing precursors for amino acid and organic-acid synthesis (Stitt 1999).
9 Whatever the genotype, LL led to a slight decrease in PEPC expression, similarly to SUS2. A gene
10 coding for glutamate decarboxylase (GAD1) was studied because this enzyme is involved in the
11 synthesis of GABA, the major amino-acid stored in tomato fruit (Akihiro *et al.* 2008). In C9d, the
12 level of GAD1 transcripts was lower under LL than under HL whatever the fruit developmental
13 stage, while in M no clear tendency was deduced.

14 **Principal component analysis (PCA)**

15 Gene expression patterns throughout fruit development were studied *via* PCA (Fig. 6) in order to
16 have a global view on the main changes that occurred at the two fruit loads. The first two principal
17 components (PC) accounted for 56 % of the total gene expression variations. The first PC
18 accounted for 33% of the variation and was mainly represented by expressions of PIP1, PEPC, SBE
19 and UDP-G-PPase, *ie* water fluxes, organic and amino-acid metabolism, starch and cell wall
20 synthesis while the second PC was mainly explained by the two studied invertases (β -FR and TIV1),
21 UDP-G-4-epi and PG *ie* soluble sugar metabolism and cell wall synthesis / degradation.

22 By plotting the PC scores during growth stages in relation to the first two PC axes, it appeared that
23 the developmental courses of the fruits mostly differed between HL and LL rather than between
24 the two genotypes. Under HL and for both genotypes, the beginning of the fruit growth phase was

1 characterized by high expressions in BR1, followed by high expressions in GAD1 and ADP-G-PPase
2 related to cell wall degradation, amino-acid metabolism, and starch synthesis, respectively, while
3 the ripening phase was mainly characterized by reduced expressions of UDP-G-4-epi, and β -AM,
4 related to cell wall synthesis, and starch degradation. Under LL the same two phases could be
5 distinguished: during the fruit growth phase, variations throughout fruit development were
6 related to a down-regulation of UDP-G-4-epi while during fruit ripening, they were related to high
7 expressions of the two invertases (TIV1, and β -FR) and PG and low expressions of SUS2.

8 **Discussion**

9 In tomato, as in other plant species, the development and composition of strong sink
10 organs such as seeds and fruits depend largely upon the size of the carbohydrate pool available
11 and on the sink strength (Baldet *et al.* 2006; Burstin *et al.* 2007). Carbohydrate pool size and
12 partitioning between the different plant tissues can be affected by a large variety of
13 environmental conditions and by genetic factors. Environmental conditions, including cultural
14 practises, are well-known determinants of fruit growth and quality (Heuvelink 1997; Gautier,
15 Guichard & Tchamitchian 2001; Bertin *et al.* 2003). Recent studies also highlighted the genetic
16 control of fruit growth and composition at both plant (Schauer *et al.* 2006) and fruit (Fridman,
17 Pleban & Zamir 2000; Fridman *et al.* 2004) levels. In addition, detailed transcriptome analysis of
18 tomato lines carrying introgressions from *S. pennellii* into *S. lycopersicum* genetic background
19 indicated the coordinated up-regulation of enzymes of sucrose mobilization and respiration in
20 early developing fruit in lines displaying high Brix values (Baxter *et al.* 2005). However, the
21 mechanisms by which environment and genotype interact and modulate fruit growth and quality
22 remain poorly known.

23 In order to get some new insights upon these mechanisms, we have altered the size of the
24 carbohydrate pool available to the fruit by reducing the fruit load (FL) of the plant and analyzed

1 the transcriptome of the developing fruit under these conditions. This was done for two closely
2 related genotypes differing for an introgression of 40 cM at the bottom of chromosome 9. The
3 introgression carries quantitative trait loci whose expression is either independent of fruit load
4 (fruit developmental duration, fresh weight, seed number, dry matter and sugar content) or
5 specific to fruit load (cell number, cell size or fruit cracking), already indicating the occurrence of G
6 x FL interactions (Prudent *et al.* 2009).

7

8 ***The C9d and the M tomato genotypes display both common and specific fruit***

9 ***phenotypic variations under low fruit load.*** In both genotypes, the increased carbohydrate

10 availability led to a spectacular increase in fruit size, with the appearance of large macro-cracks in

11 the cuticle (Fig. 2a). Increase in fruit fresh weight can be related to an increase in cell number

12 through the regulation of cell-proliferation genes at very early stages of flower and fruit

13 development (Baldet *et al.* 2006), or to enhanced cell enlargement (Bertin 2005). In the present

14 study, cell number was increased in both genotypes but cell expansion was affected only in the M

15 genotype (Fig. 2b). In both genotypes, this resulted in the acceleration of fruit growth, more

16 pronounced in the M genotype, and in the shortening of the duration of fruit development (Fig. 1a

17 and 2b). As expected from previous studies (Gautier, Guichard & Tchamitchian 2001), fruit

18 composition was also affected since the decrease in fruit load led to a strong increase in starch

19 content in M and C9d. Surprisingly, since starch degradation is the major source of soluble sugars

20 that are further accumulated in the vacuole of ripening fruit cells (Dinar & Stevens 1981), the

21 soluble sugar content of the ripe fruit was not different under LL (Fig. 1d). This result, obtained in

22 both genotypes, suggests that starch degradation products further enter fruit metabolism and/or

23 are respired. Linked with the increased carbohydrate accumulation in the fruit, the dry matter

24 content of the fruit was higher in the two genotypes, as indicated by the lower water

25 concentration under LL (Fig. 1b). However, when considering both fruit weight (Fig. 1a) and water

1 concentration in the fruit (Fig. 1b), the water flux towards the fruit was considerably enhanced
2 under LL, in particular in the M genotype. In addition, the presence of cuticle macro-cracks under
3 LL may have considerably increased the water flux in the fruit.

4

5 ***Fruit transcriptome analysis highlights regulatory processes as common targets of***

6 ***low fruit load conditions in both genotypes.*** Transcriptome analysis provides a systems-

7 level view of plant response to carbon availability, and allows a global approach of this response

8 (Smith & Stitt 2007). Transcript profiles under two conditions of fruit load in two genotypes were

9 analysed during cell expansion phase (21 daa) because (i) fruit load affected cell size in M but not

10 in C9d (Fig. 2b), (ii) fruit load affected starch storage (Fig. 1c) which is maximum during this period

11 (Dinar & Stevens 1981). Despite the large changes in starch accumulation in the fruit, indicating a

12 change of its carbohydrate status (Fig. 1c), main gene categories affected by fruit load and

13 common to both genotypes were mostly related to transcriptional and post-transcriptional

14 regulation processes, to stress and to unknown functions, but not to primary and secondary

15 metabolism (Fig. 4a). Several hypotheses can be considered to explain this result. First, microarray

16 transcriptome analyses were done at only one stage, 21 daa, which may not be representative for

17 the metabolic processes occurring during the whole fruit development. However, the

18 transcriptome analysis of introgression lines by Baxter *et al.* (2005), done at the same stage of

19 development, effectively highlighted sugar-metabolism related genes in lines differing in fruit

20 composition. Second, analyses of transcriptome data could have been too stringent, excluding a

21 large group of differentially expressed genes. This hypothesis is sustained by the comparatively

22 low number of differential genes identified. At last, fruit primary metabolism can be mainly

23 controlled at other levels than transcript abundance *via* for instance protein abundance, which can

24 be uncoupled from transcript level (Piques *et al.* 2009), and enzymatic regulation, as proposed by

25 Gibon *et al.* (2009).

1 The most striking and unexpected result was the strong up- or down-regulation in response
2 to changes in carbon availability of genes involved in signaling, in regulation of gene expression
3 (hormonal responses, transcription) and in protein metabolism (protein synthesis, post-
4 translational modifications and degradation). Carbohydrate status of the plant is known to affect a
5 wide range of processes in the fruit, such as fruit cell division (Bertin 2005), whole-plant
6 respiration rate (Gary *et al.* 2003), fruit cuticle elasticity (Gilbert *et al.* 2007), fruit transpiration
7 (Guichard *et al.* 2005), ethylene emission (Génard & Gouble 2005), and carbon- and nitrogen-
8 metabolism (Baldet *et al.* 2002). Accordingly, in our experiment, fruit growth and composition
9 were notably affected in both genotypes. According to the results presented here, the fruit may
10 cope with the new nutritional status of the plant under LL by setting up new regulations in order
11 to adjust its growth and its metabolism, whatever the genotype. It is noteworthy that several of
12 the most highly induced or repressed genes, which displayed high and almost identical variations
13 in expression under LL in both genotypes, may potentially regulate whole pathways or processes,
14 like the fructose-2,6-bisphosphatase (F26BPase) and the TAZ transcription factor. In plants, the
15 F26BPase enzyme is involved in both the synthesis and the degradation of the Fru-2,6-P₂, which is
16 believed to be involved in the regulation of sink metabolism (Nielsen, Rung & Villadsen 2004). The
17 *Arabidopsis* BT2 gene homologous to the tomato TAZ transcription factor described here encodes
18 a protein with BTB and TAZ domains which has recently been shown to be a central component of
19 an interconnected signalling network that detects and responds to multiple inputs, including
20 sugars and hormones (Mandadi *et al.* 2009). In addition, several other transcription factors such as
21 the b-ZIP, AP2 and C2H2 zinc finger protein, known to be involved in the mediation of diverse
22 hormone, light, circadian rhythm, stress and metabolic effects (Liu *et al.* 2001; McGrath *et al.*
23 2005; Nakano *et al.* 2006; Rushton *et al.* 2008; Robert *et al.* 2009; Yáñez *et al.* 2009), were also
24 regulated by fruit load. Among the possible functions of the other large “protein modification”
25 category, which includes proteins with diverse roles in protein synthesis and fate, there is the

1 post-transcriptional control of protein abundance and activity e.g. for enzymes from primary
2 metabolism.

3

4 ***Genotype x fruit load interactions are unravelled by fruit transcriptome analysis***
5 ***and by qRT-PCR analysis of specific target genes along fruit development.*** All

6 functional categories analyzed showed significant G x FL interactions (Supporting Information
7 Table S4). Genes showing G x FL interactions were (i) differentially expressed in LL conditions
8 relative to HL conditions in at least one genotype but (ii) more or less expressed in one or the
9 other genotype, if both genotypes were affected. As expected from the fruit growth phenotype
10 (Fig. 1a), the presence of cuticle macro-cracks which may be perceived as wounds (Fig. 2a) and the
11 large difference in seed numbers between the two lines (Fig. 2b and Prudent *et al.* 2009), the 21
12 daa fruits submitted to LL conditions displayed genotype-dependent variations of expression for
13 genes involved in hormone synthesis, hormone response, stress response, transcription and cell
14 wall modifications. Most of the hormones concerned are either involved in fruit growth control,
15 such as auxins, gibberellins, cytokinins, and brassinosteroid (Gillapsy, Ben-David & Gruissem 1993)
16 and/or in stress response such as ethylene, jasmonic acid and abscisic acid (Table 2). Accordingly,
17 several transcription factors showing differential expression in the two genotypes under LL such as
18 AP2/EREBP and WRKY are known as regulators of hormone mediated stress response (Birnbaum
19 *et al.* 2003; Ülker & Somssich 2004). Hormonal control may also play a role in the differential
20 expression in both genotypes of genes involved in cell wall modifications (Table 2) (Catala, Rose &
21 Bennett 2000; Chen & Bradford 2000; Chen, Nonogaki & Bradford 2002; Guillon *et al.* 2008) and in
22 the possibly linked fruit cell size (Fig. 2b). Interestingly, several metabolism genes (isocitrate lyase,
23 GABA transaminase, tryptophan synthase) displayed significant G x FL interactions (Supporting
24 Information Table S4), confirming the need for large scale metabolomic investigation of the LL
25 effect on fruit composition in both genotypes. Thus, the function of the genes showing significant

1 G x FL interactions could be to modulate the fruit response to the LL condition in accordance with
2 the specific plant and fruit characteristics of a given genotype.

3 As discussed above, the transcriptome study was done at only one stage of fruit
4 development. It may therefore have overlooked some fruit responses to LL conditions and G x FL
5 interactions. For that reason, we analyzed the expression patterns along fruit development of
6 selected genes implicated in various aspects of plant metabolism and growth (Fig. 5 and Fig. 6).
7 The strongest effect was observed for the vacuolar invertases (TIV1 and β -fructosidase), which
8 catalyze the cleavage of sucrose to glucose and fructose further stored in the vacuole (for a
9 review, see Sturm 1999) and for the polygalacturonase gene involved in cell wall degradation
10 (Hadfield & Bennett 1998). Both showed consistent changes of expression in both genotypes
11 during fruit ripening, suggesting a strong regulation by carbohydrate availability or by induced
12 changes in the fruit. In addition, increased invertase expression could be linked with the increased
13 starch degradation (Fig. 1c) and soluble sugar accumulation (Fig. 1d) under LL in both genotypes.
14 In the case of the aquaporins, which allow fluxes of water across biological membranes (Baiges *et al.*
15 *et al.* 2002; Tyerman, Niemietz & Bramley 2002; Maurel *et al.* 2008), decreased aquaporin expression
16 could be linked with decreased fruit water content (Fig. 1b) under LL in both genotypes. However,
17 though the expression patterns of many genes during fruit development were in agreement with
18 previous studies dealing with carbon-metabolism related genes (Alba *et al.* 2005; Kortstee *et al.*
19 2007), a consistency between gene expressions on the one hand and possible phenotypic changes
20 under LL on the other hand was not found for all the analyzed genes. It was for example the case
21 for starch synthesis and degradation related genes. Possible reasons are the limited number of
22 genes studied by qRT-PCR or the existence of other genes with similar functions in the fruit. For
23 example, β -amylase is not the only enzyme responsible for starch degradation as α -amylase and
24 starch phosphorylase both fulfil similar roles (Robinson, Hewitt & Bennett 1988).

1 Additional explanations are the existence of strong G x FL interactions. Indeed, significant
2 genotype effects and G x FL interactions were detected in this study for most genes studied, as
3 well as interactions between responses to carbon availability and fruit developmental stage. The
4 significant difference in fruit development between the two genotypes and the shift between the
5 two fruit loads (fruit developmental duration is shorter under LL than under HL), could possibly
6 explain the genotype effect and the G x FL interactions for some genes. At early stages of fruit
7 development, a clear effect of the M genotype is observed for the sucrose cleaving enzyme
8 sucrose synthase SUS2 which has a prominent role in sink tissues (Claussen, Lovey & Hawjer 1986;
9 Sung, Wu & Black 1989; Amor *et al.* 1995; Kleczkowski 1994), possibly reflecting the differences in
10 competition for assimilates between the two genotypes. Shift in fruit development between HL
11 and LL may also account for e.g. the differences in ADP-G-PPase expression patterns in M
12 compared to C9d. However, several other genes, including the cell wall degradation gene BR1, the
13 starch degradation gene β -amylase and the GABA biosynthesis gene GAD1, displayed clear G x FL
14 interactions independently from the fruit development shift under LL. Considering the phenotypic
15 data available, no link could be established between the G x FL interactions at gene expression
16 level and the G x FL interactions at fruit phenotypic level, again highlighting the need for more
17 global and comprehensive analysis of the mechanisms involved in fruit response to increased
18 carbon availability.

19 **Conclusion**

20 The present paper aimed at identifying processes that were influenced by a change in fruit carbon
21 availability in two tomato genotypes. Our results suggested that a change in carbon availability
22 affected very few genes but all biological processes and that primary metabolism was globally less
23 modified at the transcriptional level than regulation systems. To get a better insight on these
24 regulations, a transcriptome study could be envisaged similarly to the work of Mustroph *et al.*

1 (2009), as well as a metabolome study in order to identify the regulation networks between
2 metabolites and genes, similarly to the work of Mounet *et al.* (2009). This study also emphasized
3 the recurrent interactions between genotype and carbon availability, at the phenotypic level as
4 well as at the gene expression level throughout fruit development. All these interactions, arising at
5 different levels, thus raise the difficulty of a consistent characterization of responses to various
6 environments, if conducted on a single genotype at a single developmental stage. Therefore, once
7 enough data will be available on tomato, meta-analyses will offer the possibility to decipher those
8 interactions.

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1 Tables

2 **Table 1: Transcripts affected by fruit load** and belonging to (A) primary metabolism, (B)
 3 signaling, (C) transcription and (D) protein metabolism. Expression fold-change values
 4 (expressed in log₂-fold change) between the two fruit load conditions were indicated, as well
 5 as the sense of differential expression when significant at the 0.05 probability level, for
 6 Moneyberg (M) and C9d.

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Functional category	Gene ID	Annotation	Log ₂ -fold change M (LL/HL)	Sense in M	Log ₂ -fold change C9d (LL/HL)	Sense in C9d
A) Primary metabolism						
	sgn-U214573	delta-1-pyrroline-5-carboxylate dehydrogenase	0.72	+LL	0.38	ns
	sgn-U223594	formamidase	0.75	+LL	0.44	ns
	sgn-U214006	aldehyde dehydrogenase	0.98	+LL	0.78	+LL
	sgn-U213084	S-adenosylmethionine decarboxylase	1.07	+LL	0.5	ns
	sgn-U225810	adenine phosphoribosyltransferase	1.21	+LL	0.45	ns
	sgn-U233626	phospholipid hydroperoxide glutathione peroxidase	3.49	+LL	0.49	ns
	sgn-U219737	galactinol synthase	0.54	ns	0.76	+LL
	sgn-U212595	histidine decarboxylase	-0.12	ns	1.20	+LL
	sgn-U225897	aldo/keto reductase	0.37	ns	2.54	+LL
	sgn-U214272	succinate dehydrogenase	0.22	ns	2.90	+LL
	sgn-U221770	dihydroipoamide S-acetyltransferase	0.14	ns	3.73	+LL
	sgn-U234104	Fruc2,6BisPase	-1.83	-LL	-1.56	-LL
	sgn-U230970	glycosyltransferase	-1.64	-LL	-1.52	-LL
	sgn-U212765	alcohol dehydrogenases	-0.78	-LL	-0.85	-LL
	sgn-U217846	bis(5'-adenosyl)-triphosphatase	-0.12	ns	-0.99	-LL
B) Signalling						
	sgn-U222606	transducin	0.73	+LL	0.44	ns
	sgn-U220461	calreticulin 3	0.75	+LL	0.63	ns
	sgn-U238875	receptor kinase DUF 26	1.38	+LL	2.56	+LL
	sgn-U223661	COP9 signalosome	2.18	+LL	4.07	+LL
	sgn-U222229	calmodulin-binding motif	2.27	+LL	3.53	+LL
	sgn-U231007	leucine rich repeat VIII-2	3.56	+LL	4.78	+LL
	sgn-U213199	GCN5-related N-acetyltransferase	0.51	ns	0.76	+LL
	sgn-U219767	calmodulin-like MSS3	0.62	ns	0.78	+LL
	sgn-U218626	calmodulin-related protein	0.1	ns	0.87	+LL
	sgn-U218749	G-proteins	0.23	ns	2.29	+LL
	sgn-U219841	transducin	-0.05	ns	2.96	+LL
	sgn-U222057	ubiquitin E3 SCF FBOX	0.61	ns	3.24	+LL
	sgn-U231736	COP9	-0.09	ns	-2.31	-LL
	gi-AF130423	cryptochrome 1	-0.07	ns	-2.27	-LL
	sgn-U218306	phospholipase C	-0.47	ns	-0.78	-LL

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C) Transcription

sgn-U222912	unspecified	0.68	+LL	-0.21	ns
sgn-U213736	RNA helicase	0.77	+LL	0.23	ns
gi-BT012912	MYB-related transcription factor family	0.93	+LL	0.54	ns
sgn-U222648	GCN5-related N-acetyltransferase	1.36	+LL	0.63	ns
sgn-U223859	HB,Homeobox transcription factor family	2.66	+LL	4.52	+LL
sgn-U241461	bHLH	2.76	+LL	0.6	ns
sgn-U242104	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family	3.19	+LL	3.41	+LL
sgn-U217991	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family	-0.1	ns	0.80	+LL
sgn-U225111	WRKY domain transcription factor family	0.15	ns	0.85	+LL
sgn-U216769	zinc finger protein (PM2) -related	0.19	ns	0.85	+LL
sgn-U216297	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family	0.39	ns	0.87	+LL
sgn-U218605	WRKY domain transcription factor family	0.35	ns	0.90	+LL
sgn-U213139	C2H2 zinc finger family	0.25	ns	0.92	+LL
sgn-U214213	PHOR1	0.22	ns	0.96	+LL
sgn-U217394	C2H2 zinc finger family	0.65	ns	0.97	+LL
sgn-U218476	bHLH	0.02	ns	1.31	+LL
sgn-U224219	histone deacetylase	0.21	ns	2.43	+LL
sgn-U239113	AS2,Lateral Organ Boundaries Gene Family	-0.02	ns	2.84	+LL
sgn-U233205	ribonucleases	0.23	ns	3.78	+LL
sgn-U224917	CCAAT box binding factor family, HAP2	0.03	ns	4.02	+LL
sgn-U230225	TAZ zinc finger family protein / BTB/POZ domain-containing protein	-5.36	-LL	-5.28	-LL
sgn-U217339	putative transcription regulator	-3.49	-LL	-0.68	-LL
sgn-U218544	Ring3-related bromodomain protein	-2.30	-LL	-2.24	-LL
sgn-U236234	ELF3 /	-2.12	-LL	-1.75	-LL
sgn-U222954	C2H2 zinc finger family	-1.74	-LL	-1.78	-LL
sgn-U228377	chromatin remodeling factors	-1.74	-LL	-0.1	ns
sgn-U240661	transcriptional regulator	-1.62	-LL	-1.44	-LL
sgn-U216671	bZIP transcription factor family	-1.45	-LL	-1.17	-LL
sgn-U225321	DHHC-type zinc finger	0.03	ns	-2.95	-LL

D) Protein metabolism

sgn-U222514	AAA type protein	0.84	+LL	0.26	ns
sgn-U214901	26S proteasome regulatory subunit	1.03	+LL	0.62	ns
sgn-U220965	serine protease	1.08	+LL	2.16	+LL
sgn-U213363	proteinase inhibitor	1.23	+LL	1.59	+LL
sgn-U225461	ubiquitin E1	1.39	+LL	1.84	+LL
sgn-U215055	Rer1A	1.78	+LL	0.57	ns
sgn-U212847	elongation factor 1-alpha	2.09	+LL	0.93	+LL
sgn-U221937	phosphatase 2C	0.18	ns	0.71	+LL
sgn-U213770	cysteine protease XBCP3	0.11	ns	0.72	+LL
sgn-U215101	postranslational modification	0.34	ns	0.81	+LL
sgn-U213693	ubiquitin E3 RING	0.38	ns	0.90	+LL
sgn-U215602	AAA-type ATPase family	0.38	ns	0.92	+LL
sgn-U218603	30S ribosomal protein	0.18	ns	0.96	+LL
sgn-U214535	ubiquitin E3 RING	0.5	ns	1.12	+LL
sgn-U222008	ubiquitin E3 RING	0.08	ns	1.49	+LL
sgn-U234442	metalloprotease	0.03	ns	1.60	+LL
sgn-U217270	receptor like cytoplasmatic kinase VIII	-0.08	ns	2.78	+LL
sgn-U215048	protein kinase family	0.14	ns	4.39	+LL
sgn-U230025	peptidase	-3.70	-LL	-3.94	-LL
sgn-U236595	receptor like cytoplasmatic kinase VIII	-2.35	-LL	0.01	ns
sgn-U223430	degradation	-1.66	-LL	-1.71	-LL
sgn-U229834	receptor like cytoplasmatic kinase VII	-1.64	-LL	-0.56	ns
sgn-U221944	protein phosphatase 2C	-1.62	-LL	-1.43	-LL
sgn-U220298	peptidylprolyl isomerase	-1.07	-LL	-0.90	-LL

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3 +LL: significant up-regulation under LL

4 -LL: significant down-regulation under LL

5 ns: non-significant fruit load effect

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1 **Table 2: Transcripts showing significant genotype x fruit load interactions** at the 0.05
2 probability level and belonging to (a) hormonal responses and (b) cell wall modification.
3 Differences in fruit load fold change expression between Moneyberg (M) and C9d are
4 indicated and expressed in log₂ fold-change.

	Functional category	Gene ID	Annotation	Interaction = (Log ₂ -fold change in C9d) - (Log ₂ -fold change in M)
A)	Hormonal responses	sgn-U214292	GA2 oxidase	0.68
		sgn-U213942	jasmonate ZIM-domain protein 1	0.70
		sgn-U213522	1-aminocyclopropane-1-carboxylate synthase	0.71
		sgn-U227363	auxin induced protein	0.75
		sgn-U219331	jasmonic acid2	0.76
		sgn-U214505	putative phytoalkyl peptide precursor	0.78
		sgn-U226232	IAA-Ala hydrolase (IAR3)	0.79
		sgn-U214304	ethylene-responsive late embryogenesis-like protein (ER5)	0.79
		sgn-U214303	ethylene-responsive late embryogenesis-like protein (ER5)	0.80
		U77719	ethylene-responsive late embryogenesis-like protein (ER5)	0.82
		sgn-U215161	auxin induced protein	0.83
		sgn-U221333	steroid 5 alpha reductase DET2	0.90
		sgn-U232919	putative phytoalkyl peptide precursor	0.91
		sgn-U214504	putative phytoalkyl peptide precursor	0.91
		sgn-U214986	auxin-regulated protein	0.94
		sgn-U212798	2-oxoglutarate-dependent dioxygenase	0.99
		sgn-U212786	1-aminocyclopropane-1-carboxylate oxidase	1.08
		sgn-U213253	ent-kaurenoic acid hydroxylase/oxygenase	1.12
		sgn-U241804	auxin induced protein	1.13
		sgn-U215638	S-adenosyl-L-methionine:salicylic acid carboxyl methyltrans	1.43
sgn-U212784	lipoxygenase	1.70		
sgn-U214919	1-aminocyclopropane-1-carboxylate oxidase	2.13		
B)	Cell wall modification	sgn-U215495	extensin	-0.94
		sgn-U215382	xyloglucan endotransglycosylase	-0.80
		sgn-U215860	xyloglucan endotransglycosylase	-0.71
		sgn-U216615	arabinogalactan protein	-0.71
		sgn-U214352	UDP-glucose-4-epimerase	0.74
		Sgn-U222466	elastin	0.74
		sgn-U220299	elastin	1.06

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1 **Table 3: List of the 18 genes used for qRT-PCR analysis.** For each gene studied in qRT-PCR,
 2 the code used in the text, annotation and reference of the sequence are added. Asterisk
 3 means that the gene was chosen according to microarray results.

4

Code	Annotation	Accession number	References
* PIP1	plasma membrane intrinsic protein 1c (PIP1c)	U212567	(Johanson et al., 2001)
* delta-TIP	delta tonoplast integral protein 1 (delta-TIP)	U214295	(Bayer et al., 2006)
* UDP-G-4-epi	UDP-glucose-4-epimerase	U214352	(Oomen et al., 2004)
* UDP-G-PPase	UDP-glucose-4-pyrophosphorylase	U213088	(Borovkov et al., 1997)
* XTH6	xyloglucan-endotransglycosylase (XTH6)	U215860	(Saladie et al., 2006)
BR1	xyloglucan-endotransglycosylase (BR1)	TC 94351	(Koka et al., 2000)
* PG	polygalacturonase 2A	U213213	(Grierson et al., 1986)
ADP-G-PPase	ADP-glucose-pyrophosphorylase large sub-unit (AGP-S1)	U81033	(Chen et al., 1998)
* SBE	starch branching enzyme II (SBE II)	U221404	(Larsson et al., 1998)
* β -AM	beta-amylase 1	U220865	(Ren et al., 2007)
TIV1	acid-invertase TIV1	M81081	(Klann et al., 1992)
* β -FR	beta-fructosidase	U212903	(Ohyama et al., 1992)
SUS2	sucrose synthase 2 (sus2)	AJ011535	(Fu and Park, 1995)
PEPC	phosphoenolpyruvate carboxylase	U219207	(Koizumi et al., 1991)
GAD1	glutamate decarboxylase 1 (GAD1)	U314961	(Yun and Oh, 1998)
* ACC-ox	1-aminocyclopropane-1 carboxylate oxidase	U212787	(Kock et al., 1991)
* E8	2-oxoglutarate-dependent dioxygenase	U213299	(Trentmann and Kende, 1995)
* eIF-4A-2	eukaryotic initiation factor 4A-2	U213502	(Owtrim et al., 1991)

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1 **Figure legends**

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3 **Figure 1: Temporal trends of fruit physiological characteristics in two lines.** From 21 days
4 after anthesis to the red ripe stage: fruit fresh weight (a), pericarp water content (b), starch
5 content of the pericarp (c), and soluble sugar content of the pericarp (d) were measured on
6 M (triangles) and C9d (circles) under HL (black) and under LL (grey). Each point corresponds
7 to the mean of five points, and bars are standard deviations. For each genotype, from 21 daa
8 to red ripe stage, fruit load effect significance and genotypic effect significance were
9 evaluated by t-tests. Letters *a* and *b* indicate a significant effect of fruit load at the 0.05
10 probability level for M and C9d, respectively. Interactions between genotype and fruit load
11 were evaluated by a two-way analysis of variance. Significant interactions at the 0.05
12 probability level are indicated by letter *c*.

13

14 **Figure 2: Phenotypic characteristics of fruits at the red ripe stage.**

15 (a) Images of red ripe fruits from M and C9d grown under HL and LL conditions.

16 (b) Means and standard deviation (s.d) for the fruit developmental duration (Dura expressed
17 in days), seed number (SdN), pericarp cell number (CIN) and cell size (CIS expressed in
18 nanoliters (nL)) of five fruits in two genotypes (C9d and Moneyberg (M)) under high load (HL)
19 or low load (LL) conditions. For each trait, the fruit load effect and the genotype effect were
20 evaluated by t-tests and interactions between genotype and fruit load were evaluated via a
21 two-way analysis of variance.

22

23 **Figure 3: Microarray analysis of gene expression under HL and LL conditions, in M and C9d.**

24 (a) Microarray experimental design. Each arrow represents a hybridized microarray slide.

1 (b) Number of genes up-regulated (↑) or down-regulated (↓) under LL for Moneyberg (on
2 the left) and for C9d (on the right). The number of genes differentially expressed according
3 to fruit load that were common to both genotypes, is in parentheses.

4

5 **Figure 4: Distribution of differentially expressed genes into biological classes**, according to
6 the Mapman annotation.

7 (a) Percentage of genes differentially regulated with fruit load effect for C9d (black) and M
8 (grey).

9 (b) Percentage of genes showing significant genotype x fruit load interactions

10

11 **Figure 5: Gene expression patterns along fruit development.** Expression of 15 genes at 21,
12 28, 35, 42 daa and the red ripe stage (RR), under high load (HL, black) and low load
13 conditions (LL, grey) for Moneyberg (M, triangles) and C9d (circles). Each value is the mean
14 of 3 biological and 3 technical replicates and was normalized either using M under HL at 21
15 daa as a reference for expression data. Bars are standard deviations.

16 These 15 genes encode a plasma membrane aquaporin (PIP1), a delta-tonoplast intrinsic
17 protein (delta-TIP), a UDP-glucose-4-epimerase (UDP-G-4-epi), a UDP-glucose-
18 pyrophosphorylase (UDP-G-PPase), two xyloglucan endotransglycosylases (XTH6 and BR1), a
19 polygalacturonase (PG), a ADP-glucose-pyrophosphorylase (ADP-G-PPase), a starch-
20 branching enzyme (SBE), a b-amylase (β -AM), a vacuolar invertase (TIV1), a β -fructosidase
21 (b-FR), a sucrose synthase (SUS2), a phosphoenolpyruvate carboxylase (PEPC), and a
22 glutamate decarboxylase (GAD1).

23 For each genotype, from 21 daa to red ripe stage, fruit load effect significance was evaluated
24 by t-tests. Letters *a* and *b* indicate a significant effect of fruit load at the 0.05 probability
25 level for M and C9d, respectively. Interactions between genotype and fruit load were

1 evaluated by a two-way ANOVA. Significant interactions at the 0.05 probability level are
2 indicated by letter c.

3

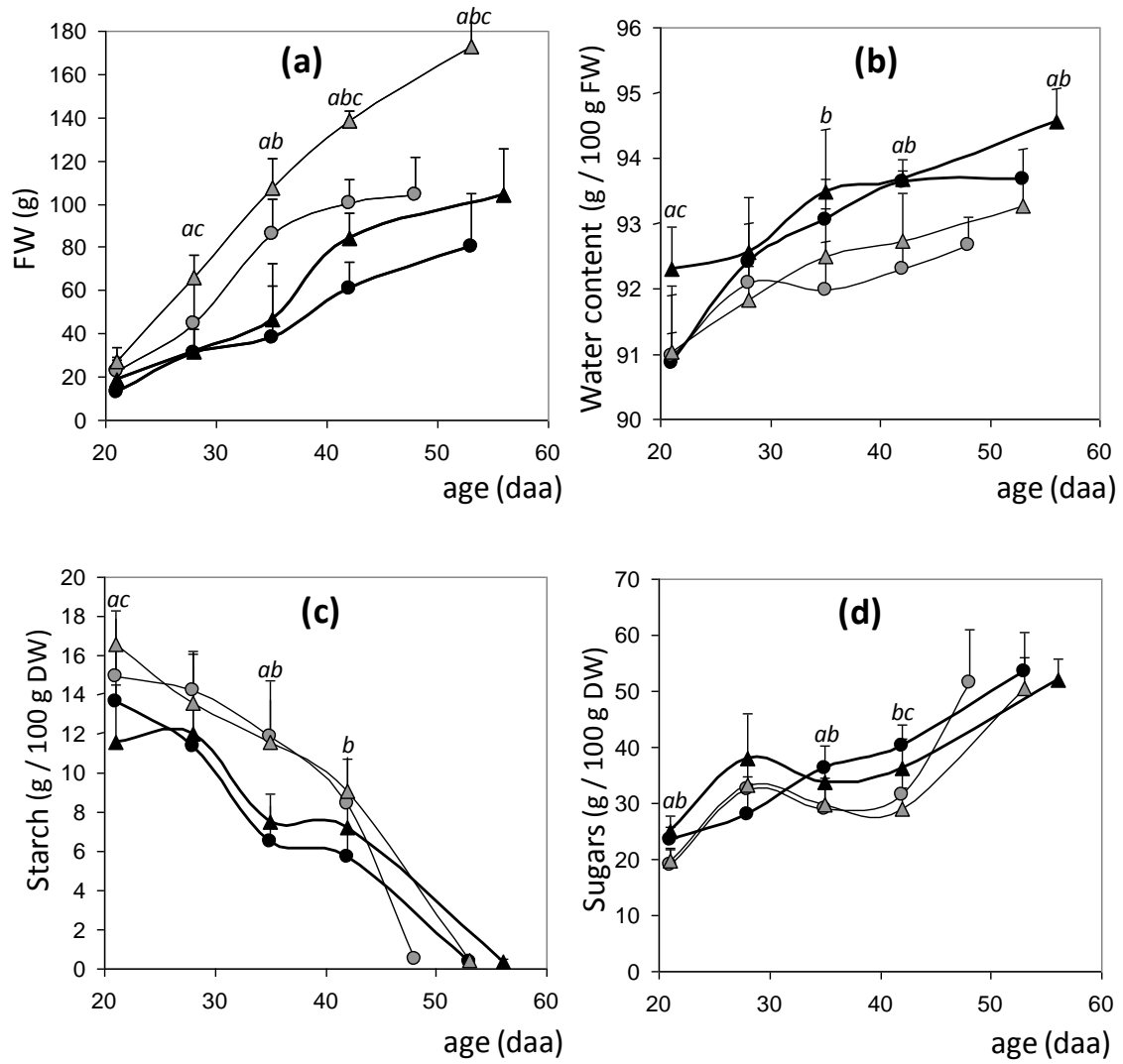
4 **Figure 6: Principal component analysis (PCA) of gene expression during fruit development**
5 **for M and C9d under high load (HL) and low load conditions (LL).**

6 (a) First plan of the PCA constructed with 15 gene expression patterns for the two first
7 principal components. Percentage in brackets represents the variance explained by each
8 component. The 15 genes encode a plasma membrane aquaporin (PIP1), a delta-tonoplast
9 intrinsic protein (delta-TIP), a UDP-glucose-4-epimerase (UDP-G-4-epi), a UDP-glucose-
10 pyrophosphorylase (UDP-G-PPase), two xyloglucan endotransglycosylases (XTH6 and BR1), a
11 polygalacturonase (PG), a ADP-glucose-pyrophosphorylase (ADP-G-PPase), a starch-
12 branching enzyme (SBE), a β -amylase (b-AM), a vacuolar invertase (TIV1), a β -fructosidase
13 (b-FR), a sucrose synthase (SUS2), a phosphoenolpyruvate carboxylase (PEPC), and a
14 glutamate decarboxylase (GAD1).

15 (b) Principal component scores for M (triangles) and C9d (circles) under HL (black) and LL
16 (grey) for each of the five developmental stages (21, 28, 35, 42 daa and red ripe (RR)).

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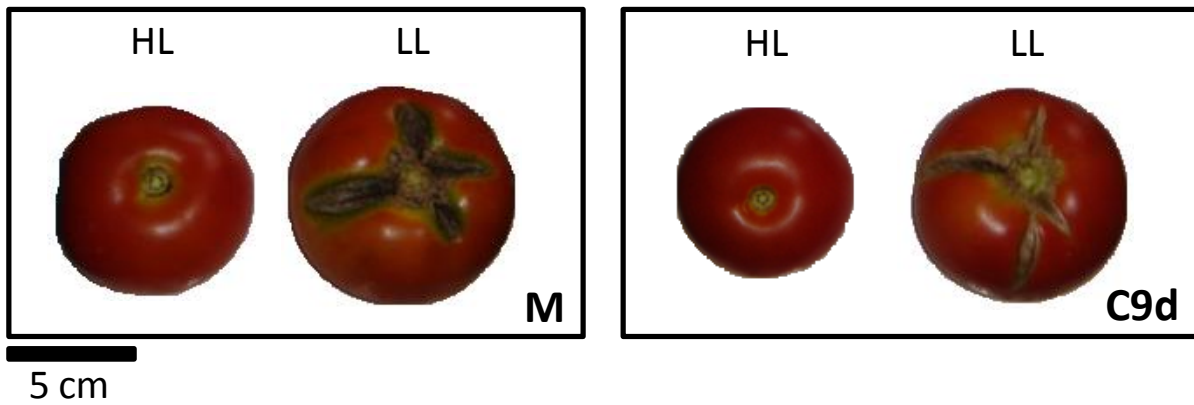
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Figure 1.

(a)



(b)

Genotype	Fruit load	Dura (days)		SdN		CIN		CIS (nL)	
		Mean	s.d	Mean	s.d	Mean	s.d	Mean	s.d
		<i>ab</i>				<i>ab</i>		<i>ac</i>	
C9d	HL	53.50	2.6	117.24	12.4	7.08	0.36	9.05	0.66
C9d	LL	46.80	2.3	84.41	24.7	8.63	0.60	9.03	0.82
M	HL	57.32	2.0	185.60	21.7	9.28	0.98	7.63	0.39
M	LL	50.47	2.1	166.46	21.5	13.49	0.84	8.68	0.42

a : significant fruit load effect at $p < 0.05$ in M

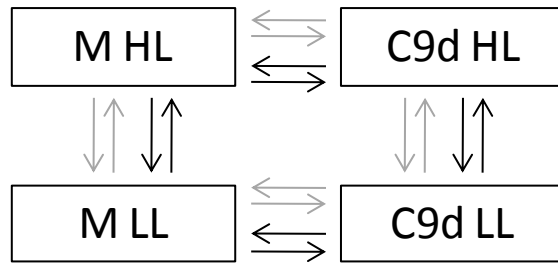
b : significant fruit load effect at $p < 0.05$ in C9d

c : significant genotype x fruit load interaction at $p < 0.05$

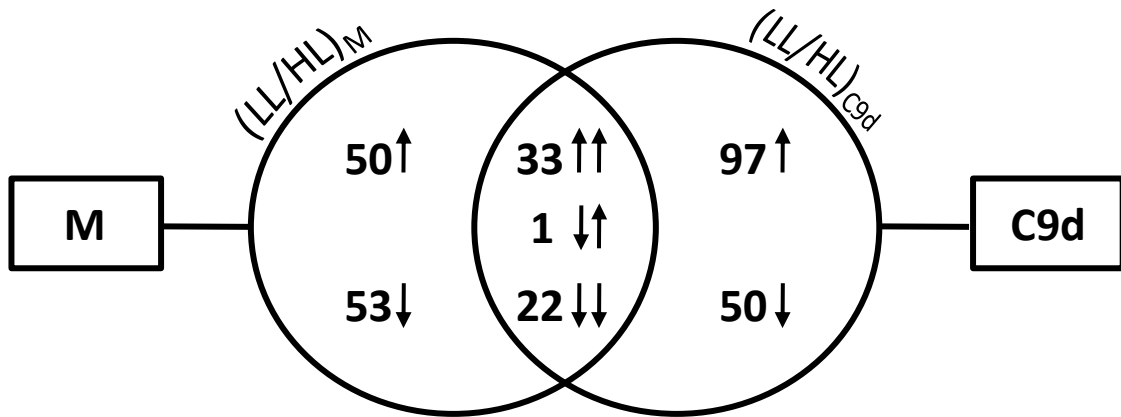
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Figure 2.

(a)



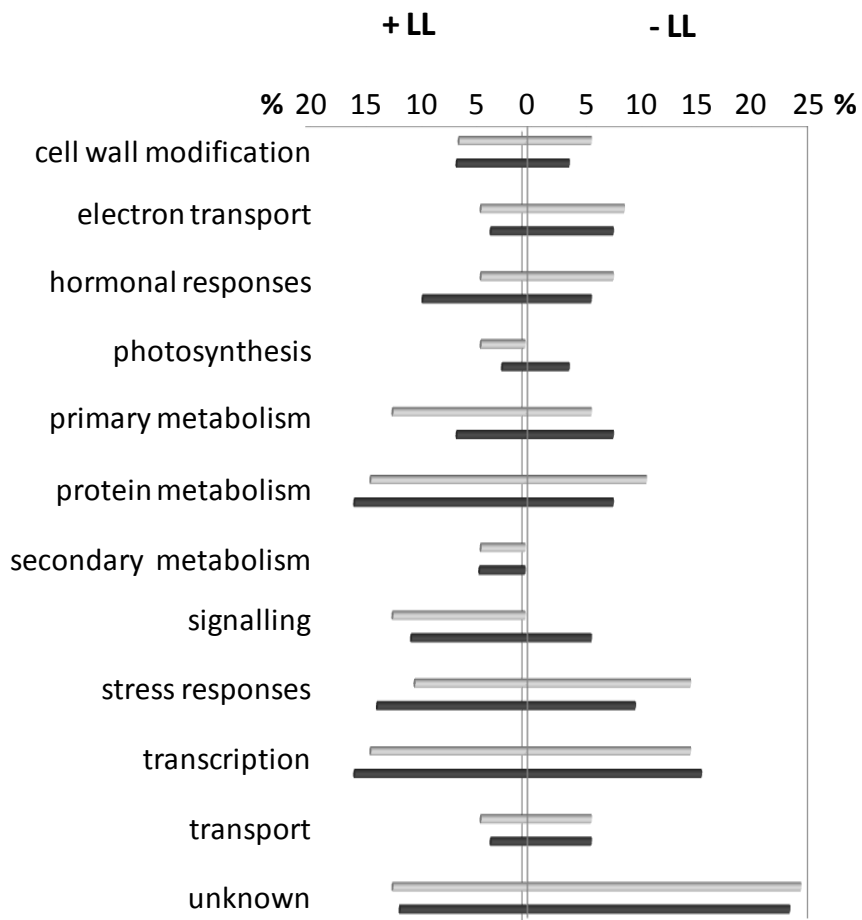
(b)



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Figure 3.

(a)



(b)

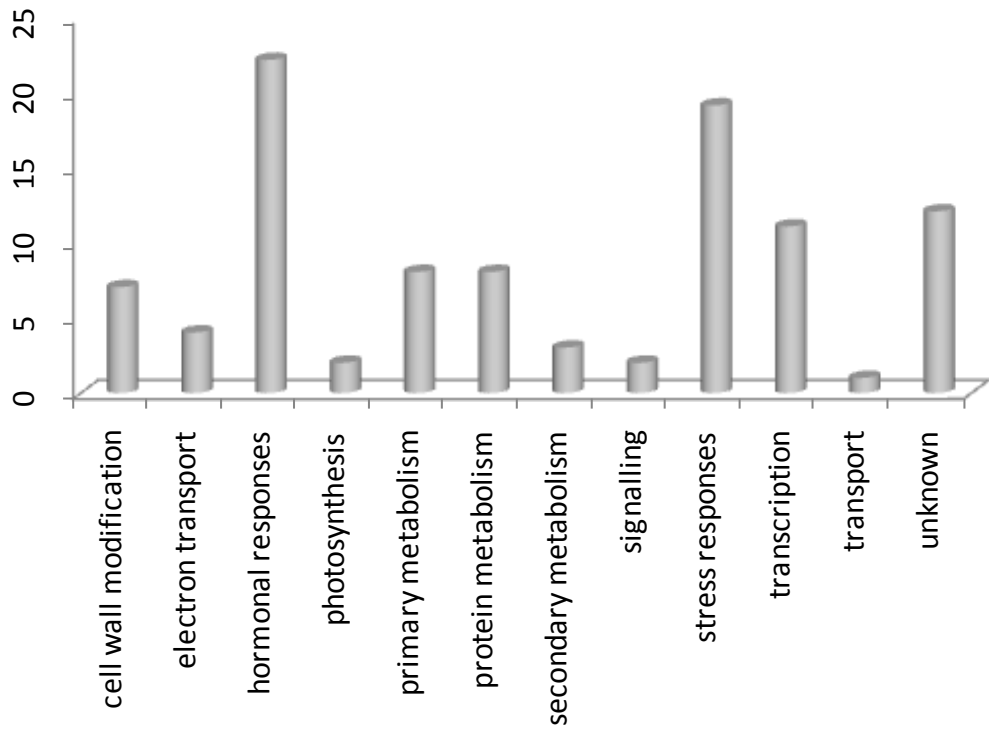
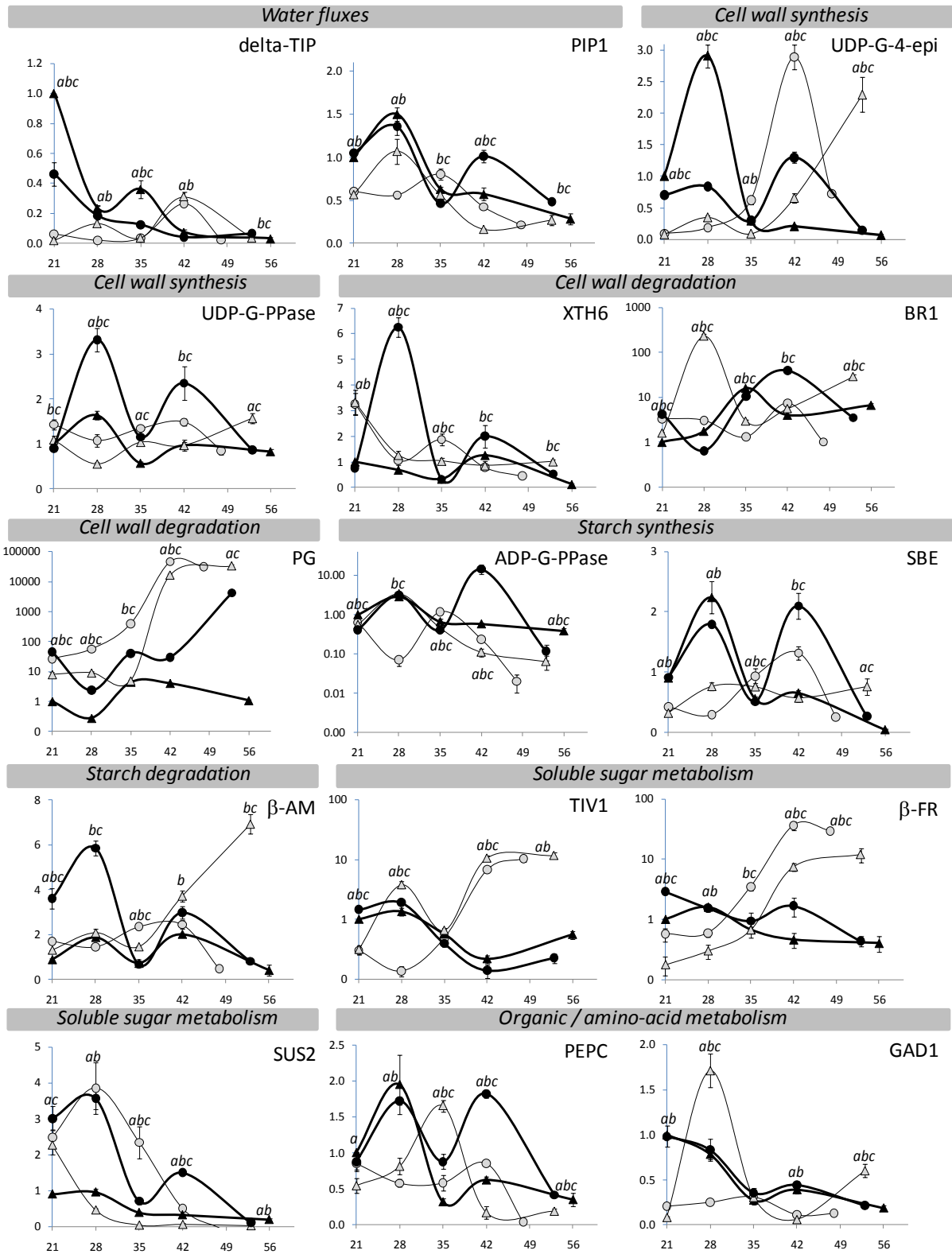


Figure 4.

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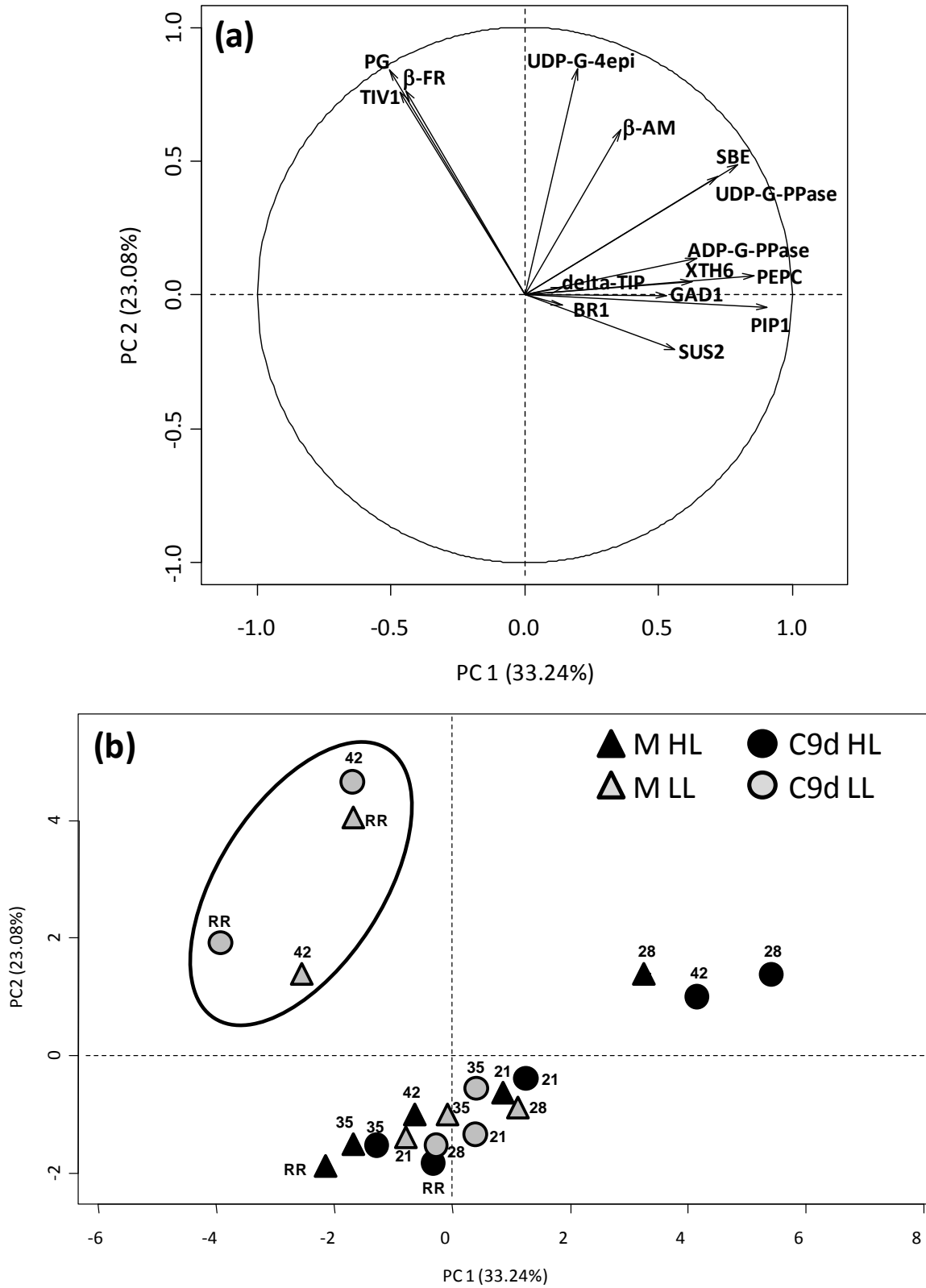
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Figure 5.

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Figure 6.

1 Supporting information

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4 **Table S1: List of the 18 genes and primers used for qRT-PCR analysis.** For each gene studied
5 in qRT-PCR, the code used in the text and annotation of the sequence are added.

6

Code	Annotation	Accession number	Forward primer 5'--> 3'	Reverse primer 5'--> 3'
PIP1	plasma membrane intrinsic protein 1c (PIP1c)	U212567	GTCTTGGTGCTATCTGTGG	GTCCATCACCTTTGGTGTAAC
delta-TIP	delta tonoplast integral protein 1 (delta-TIP)	U214295	GTGGGTTAGCTGGTCTTATC	CTGCAAACCAAAAACCTAAC
UDP-G-4-epi	UDP-glucose-4-epimerase	U214352	GTATATGGCAACGACTACCC	GAAGTCTCTGAAGTGCAAC
UDP-G-PPase	UDP-glucose-4-pyrophosphorylase	U213088	CTGAAAAGCTCAACAACCTC	CTTAGATAGCGACCGACAAG
XTH6	xyloglucan-endotransglycosylase (XTH6)	U215860	GAGGGACCATACTCACACAC	CTCCCATGAAACTTAAATC
BR1	xyloglucan-endotransglycosylase (BR1)	TC 94351	CTCGTTGCTGGAAACTCTG	GTAGGGTCAAACCAAGG
PG	polygalacturonase 2A	U213213	GAGAATATCAAGGGCACAAG	TCTAGTGAAGTGCAAGTGTGG
ADP-G-PPase	ADP-glucose-pyrophosphorylase large sub-unit (AGP-S1)	U81033	CATATTAGGAGGAGGCGAAG	GAGCAATGTGACGATTCAG
SBE	starch branching enzyme II (SBE II)	U221404	GAGCTTCTTGACGTTATCTG	CACCTCATCTCTGCGTATG
b-AM	beta-amylase 1	U220865	CCTGACTTATCCATCCTG	GCTTCCCTCTCTACCTGTTT
TIV1	acid-invertase TIV1	M81081	GACAGTGAATCTGCTGACC	GGCCACTGAAGTAGATGTG
b-FR	beta-fructosidase	U212903	CATGTAGGTTTCAGTTGCTC	GTTAGCTCAGATAGCGTTTG
SUS2	sucrose synthase 2 (sus2)	AJ011535	TATTGATCCATATCACGGTGAGCAAG	ATAAACAGCAGCCAGCGTCAGTAGTC
PEPC	phosphoenolpyruvate carboxylase	U219207	TGGCCATTCTTTAGGGTAAC	CTCTTCGTAAGGACCTC
GAD1	glutamate decarboxylase 1 (GAD1)	U314961	GCAAGGGTACTAAGAGAAGG	GAGCATCTGGTGGCATAGTG
ACC-ox	1-aminocyclopropane-1 carboxylate oxidase	U212787	GGCAGAGGAAAGTACACAAG	TTGAATTGGGATCTAAGCAC
E8	2-oxoglutarate-dependent dioxygenase	U213299	GTCGATTCAGGGATAGTTC	CTACGTTGAAAATCGTCTCC
eIF-4A-2	eukaryotic initiation factor 4A-2	U213502	GATCAGCGTATCCTTCAGAG	GGCATTGTAGCAGAGAAAAC

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1 **Table S2: List of transcripts down-regulated under LL conditions.** Genes were classified
2 according to their belonging to a biological process. Expression fold-change values
3 (expressed in log₂-fold change) between the two fruit load conditions were indicated, as well
4 as the sense of differential expression when significant at the 0.05 probability level, for
5 Moneyberg (M) and C9d.

Functional category	Gene ID	Log ₂ -fold change M (LL/HL)	Sense in M	Log ₂ -fold change C9d (LL/HL)	Sense in C9d	Annotation
Cell wall modification	sgn-U215187	-1.70	-LL	-1.18	-LL	keratin
	sgn-U216459	-1.07	-LL	-0.62	ns	elastin
	sgn-U215382	-0.88	-LL	-1.47	-LL	xyloglucan endotransglycosylase
Electron transport	sgn-U225019	-5.30	-LL	-5.13	-LL	4Fe-4S ferredoxin iron-sulfur binding
	sgn-U228865	-1.76	-LL	-1.52	-LL	cytochrome P450
	sgn-U230985	-1.54	-LL	-0.08	ns	glutaredoxins
	sgn-U220890	-1.23	-LL	-0.65	ns	glutaredoxins
	sgn-U221959	-0.83	-LL	-0.37	ns	glutaredoxins
	sgn-U232104	0.07	ns	-4.00	-LL	cytochrome c reductase
	sgn-U215744	0.51	ns	-0.83	-LL	F1-ATPase
Hormonal responses	sgn-U212706	-1.76	-LL	-0.86	-LL	cytokinin repressed protein
	sgn-U214919	-2.54	-LL	-0.77	-LL	1-aminocyclopropane-1-carboxylate oxidase
	sgn-U212787	-1.93	-LL	-0.64	ns	1-aminocyclopropane-1-carboxylate oxidase
	sgn-U217786	-0.95	-LL	-0.84	-LL	auxin induced protein
Photosynthesis	sgn-U230677	-0.12	ns	-4.03	-LL	PSII polypeptide subunits
	sgn-U234083	-0.48	ns	-0.71	-LL	PSII LHC-II
Primary metabolism	sgn-U234104	-1.83	-LL	-1.56	-LL	Fruc2,6BisPase
	sgn-U230970	-1.64	-LL	-1.52	-LL	glycosyltransferase
	sgn-U212765	-0.78	-LL	-0.85	-LL	alcohol dehydrogenases
	sgn-U217846	-0.12	ns	-0.99	-LL	bis(5'-adenosyl)-triphosphatase
Protein metabolism	sgn-U230025	-3.70	-LL	-3.94	-LL	peptidase
	sgn-U236595	-2.35	-LL	0.01	ns	receptor like cytoplasmatic kinase VIII
	sgn-U223430	-1.66	-LL	-1.71	-LL	degradation
	sgn-U229834	-1.64	-LL	-0.56	ns	receptor like cytoplasmatic kinase VII
	sgn-U221944	-1.62	-LL	-1.43	-LL	protein phosphatase 2C
	sgn-U220298	-1.07	-LL	-0.90	-LL	peptidylprolyl isomerase
Signalling	sgn-U231736	-0.09	ns	-2.31	-LL	COP9
	gi-AF130423	-0.07	ns	-2.27	-LL	cryptochrome 1
	sgn-U218306	-0.47	ns	-0.78	-LL	phospholipase C
Stress responses	sgn-U221778	-2.62	-LL	-2.97	-LL	disease resistance protein
	sgn-U216469	-1.61	-LL	-0.29	ns	heat shock protein
	gi-U72396	-1.50	-LL	-0.43	ns	heat shock protein
	sgn-U216965	-1.32	-LL	0	ns	stress induces protein
	gi-AJ225049	-1.07	-LL	-0.78	-LL	heat shock protein
	sgn-U216888	-1.07	-LL	-0.68	-LL	DNAJ
	sgn-U212696	-0.99	-LL	-0.87	-LL	heat shock protein
	sgn-U215426	-0.98	-LL	-0.53	ns	SENU1
	sgn-U217418	-0.44	ns	-0.96	-LL	heat shock protein HSP81-1
	Transcription	sgn-U230225	-5.36	-LL	-5.28	-LL
sgn-U217339		-3.49	-LL	-0.68	-LL	putative transcription regulator
sgn-U218544		-2.30	-LL	-2.24	-LL	Ring3-related bromodomain protein
sgn-U236234		-2.12	-LL	-1.75	-LL	ELF3 /
sgn-U222954		-1.74	-LL	-1.78	-LL	C2H2 zinc finger family
sgn-U228377		-1.74	-LL	-0.1	ns	chromatin remodeling factors
sgn-U240661		-1.62	-LL	-1.44	-LL	transcriptional regulator
sgn-U216671		-1.45	-LL	-1.17	-LL	bZIP transcription factor family
sgn-U225321		0.03	ns	-2.95	-LL	DHHC-type zinc finger

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Transport	sgn-U230991	-4.01	-LL	0.08	ns	metabolite transporters at the mitochondrial membrane
	sgn-U214295	-1.23	-LL	-1.01	-LL	major intrinsic protein TIP
	sgn-U218010	-0.09	ns	-3.89	-LL	mannitol transporter
	sgn-U216406	-0.46	ns	-0.73	-LL	amino acids transport
Unknown	sgn-U222947	-5.52	-LL	-0.08	ns	unknown
	sgn-U239621	-4.88	-LL	-1.56	-LL	unknown
	sgn-U240531	-2.49	-LL	-0.45	ns	unknown
	sgn-U220450	-2.24	-LL	0.05	ns	unknown
	sgn-U242297	-1.97	-LL	-1.75	-LL	unknown
	sgn-U225758	-1.95	-LL	0.09	ns	unknown
	sgn-U224962	-1.77	-LL	-2.88	-LL	unknown
	sgn-U228268	-1.75	-LL	-1.66	-LL	unknown
	sgn-U239771	-1.67	-LL	-0.58	ns	unknown
	sgn-U220856	-1.41	-LL	-0.86	-LL	unknown
	sgn-U225490	-1.38	-LL	-0.17	ns	unknown
	sgn-U222595	-1.37	-LL	-1.01	-LL	unknown
	sgn-U229617	-1.25	-LL	-0.79	-LL	unknown
	sgn-U231794	-0.07	ns	-3.76	-LL	unknown
	sgn-U229103	-0.01	ns	-1.91	-LL	unknown
	sgn-U239488	0.03	ns	-1.50	-LL	unknown
	sgn-U217425	-0.45	ns	-1.33	-LL	unknown
	sgn-U218174	-0.39	ns	-0.72	-LL	unknown

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5 ns: non-significant fruit load effect

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1 **Table S3: List of transcripts up-regulated under LL conditions.** Genes were classified
2 according to their belonging to a biological process. Expression fold-change values
3 (expressed in log₂-fold change) between the two fruit load conditions were indicated, as well
4 as the sense of differential expression when significant at the 0.05 probability level, for
5 Moneyberg (M) and C9d.

Functional category	Gene ID	Log ₂ -fold change M	Sense in M	Log ₂ -fold change C9d	Sense in C9d	Annotation
Cell wall modification	sgn-U217045	0.69	+LL	0.49	ns	pectate lyases and polygalacturonases
	sgn-U215860	1.38	+LL	0.83	+LL	xyloglucan endotransglycosylase
	sgn-U213213	1.79	+LL	1.90	+LL	polygalacturonase
	sgn-U220299	-0.16	ns	0.76	+LL	elastin
	sgn-U222710	0.31	ns	0.85	+LL	beta-1,4-xylosidase
	sgn-U215711	-0.08	ns	2.92	+LL	expansin
	sgn-U213668	0	ns	3.58	+LL	pectate lyases and polygalacturonases
Electron transport	sgn-U217593	0.72	+LL	0.42	ns	thioredoxin
	sgn-U214691	1.13	+LL	0.86	+LL	alternative oxidase
	sgn-U225537	0.15	ns	1.73	+LL	5'-adenylylsulfate reductase
	sgn-U220136	0.35	ns	2.20	+LL	mitochondrial carrier protein family
Hormonal responses	sgn-U213299	1.01	+LL	0.59	ns	2-oxoglutarate-dependent dioxygenase
	sgn-U216133	1.35	+LL	1.26	+LL	cytokinin oxidase
	sgn-U227363	0.04	ns	0.70	+LL	auxin induced protein
	sgn-U218918	0.47	ns	0.72	+LL	2-oxoglutarate-dependent dioxygenase
	sgn-U212798	-0.05	ns	0.73	+LL	2-oxoglutarate-dependent dioxygenase
	sgn-U241804	-0.07	ns	0.79	+LL	auxin induced protein
	sgn-U215161	0.12	ns	0.83	+LL	auxin induced protein
	sgn-U212800	0.34	ns	0.97	+LL	2-oxoglutarate-dependent dioxygenase
	sgn-U212799	0.47	ns	1.10	+LL	2-oxoglutarate-dependent dioxygenase
sgn-U236633	-0.06	ns	1.67	+LL	auxin induced protein	
Photosynthesis	sgn-U213011	0.86	+LL	0.53	ns	rubisco subunit2A
	sgn-U212665	0.92	+LL	0.83	+LL	PSII polypeptide subunits
	sgn-U223602	-0.07	ns	2.77	+LL	chlorophyll synthetase
Primary metabolism	sgn-U214573	0.72	+LL	0.38	ns	delta-1-pyrroline-5-carboxylate dehydrogenase
	sgn-U223594	0.75	+LL	0.44	ns	formamidase
	sgn-U214006	0.98	+LL	0.78	+LL	aldehyde dehydrogenase
	sgn-U213084	1.07	+LL	0.5	ns	S-adenosylmethionine decarboxylase
	sgn-U225810	1.21	+LL	0.45	ns	adenine phosphoribosyltransferase
	sgn-U233626	3.49	+LL	0.49	ns	phospholipid hydroperoxide glutathione peroxidase
	sgn-U219737	0.54	ns	0.76	+LL	galactinol synthase
	sgn-U212595	-0.12	ns	1.20	+LL	histidine decarboxylase
	sgn-U225897	0.37	ns	2.54	+LL	aldo/keto reductase
	sgn-U214272	0.22	ns	2.90	+LL	succinate dehydrogenase
	sgn-U221770	0.14	ns	3.73	+LL	dihydrolipoamide S-acetyltransferase
Protein metabolism	sgn-U222514	0.84	+LL	0.26	ns	AAA type protein
	sgn-U214901	1.03	+LL	0.62	ns	26S proteasome regulatory subunit
	sgn-U220965	1.08	+LL	2.16	+LL	serine protease
	sgn-U213363	1.23	+LL	1.59	+LL	proteinase inhibitor
	sgn-U225461	1.39	+LL	1.84	+LL	ubiquitin E1
	sgn-U215055	1.78	+LL	0.57	ns	Rer1A
	sgn-U212847	2.09	+LL	0.93	+LL	elongation factor 1-alpha
	sgn-U221937	0.18	ns	0.71	+LL	phosphatase 2C
	sgn-U213770	0.11	ns	0.72	+LL	cysteine protease XBPC3
	sgn-U215101	0.34	ns	0.81	+LL	postranslational modification
	sgn-U213693	0.38	ns	0.90	+LL	ubiquitin E3 RING
	sgn-U215602	0.38	ns	0.92	+LL	AAA-type ATPase family
	sgn-U218603	0.18	ns	0.96	+LL	30S ribosomal protein
	sgn-U214535	0.5	ns	1.12	+LL	ubiquitin E3 RING
	sgn-U222008	0.08	ns	1.49	+LL	ubiquitin E3 RING
	sgn-U234442	0.03	ns	1.60	+LL	metalloprotease
	sgn-U217270	-0.08	ns	2.78	+LL	receptor like cytoplasmatic kinase VIII
	sgn-U215048	0.14	ns	4.39	+LL	protein kinase family

Secondary metabolism	sgn-U213010	1.05	+LL	0.38	ns	phenazine biosynthesis PhzC/PhzF family protein	
	sgn-U216112	1.58	+LL	1.81	+LL	ascorbate oxidase SRG1	
	sgn-U218068	0.19	ns	0.68	+LL	cinnamoyl-CoA reductase	
	sgn-U217320	0.35	ns	0.79	+LL	redox.glutaredoxins	
	sgn-U221863	-0.15	ns	2.29	+LL	secondary metabolism.flavonoids.flavonols.oxidoreductase	
Signalling	sgn-U222606	0.73	+LL	0.44	ns	transducin	
	sgn-U220461	0.75	+LL	0.63	ns	calreticulin 3	
	sgn-U238875	1.38	+LL	2.56	+LL	receptor kinase DUF 26	
	sgn-U223661	2.18	+LL	4.07	+LL	COP9 signalosome	
	sgn-U222229	2.27	+LL	3.53	+LL	calmodulin-binding motif	
	sgn-U231007	3.56	+LL	4.78	+LL	leucine rich repeat VIII-2	
	sgn-U213199	0.51	ns	0.76	+LL	GCN5-related N-acetyltransferase	
	sgn-U219767	0.62	ns	0.78	+LL	calmodulin-like MSS3	
	sgn-U218626	0.1	ns	0.87	+LL	calmodulin-related protein	
	sgn-U218749	0.23	ns	2.29	+LL	G-proteins	
	sgn-U219841	-0.05	ns	2.96	+LL	transducin	
	sgn-U222057	0.61	ns	3.24	+LL	ubiquitin E3 SCF FBOX	
	Stress responses	sgn-U215393	0.72	+LL	0.69	+LL	peroxidases
		gi-Y12640	0.76	+LL	0.48	ns	Cf-4A gene
sgn-U218488		0.97	+LL	0.6	ns	disease resistance protein-related	
sgn-U213790		1.99	+LL	0.99	+LL	glycosyl hydrolase	
sgn-U212922		2.58	+LL	1.23	+LL	PR protein	
sgn-U213021		-0.58	ns	0.70	+LL	protease inhibitor	
sgn-U225818		0.48	ns	0.71	+LL	Avr 09	
sgn-U218566		0.14	ns	0.71	+LL	heat shock protein	
sgn-U214985		0.55	ns	0.76	+LL	hevein-related protein precursor (PR-4)	
gi-M13938		-0.08	ns	0.77	+LL	proteinase inhibitor I gene	
sgn-U215231		0.5	ns	0.80	+LL	peroxidases	
sgn-U213023		-0.3	ns	0.97	+LL	protease inhibitor	
sgn-U213338		-0.31	ns	1.12	+LL	trypsin inhibitor	
sgn-U212578		-0.27	ns	1.42	+LL	transferase	
sgn-U227798		0.43	ns	3.02	+LL	peroxidases	
Transcription		sgn-U222912	0.68	+LL	-0.21	ns	unspecified
	sgn-U213736	0.77	+LL	0.23	ns	RNA helicase	
	gi-BT012912	-0.93	+LL	0.54	ns	MYB-related transcription factor family	
	sgn-U222648	1.36	+LL	0.63	ns	GCN5-related N-acetyltransferase	
	sgn-U223859	2.66	+LL	4.52	+LL	HB,Homeobox transcription factor family	
	sgn-U241461	2.76	+LL	0.6	ns	bHLH	
	sgn-U242104	3.19	+LL	3.41	+LL	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family	
	sgn-U217991	-0.1	ns	0.80	+LL	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family	
	sgn-U225111	0.15	ns	0.85	+LL	WRKY domain transcription factor family	
	sgn-U216769	0.19	ns	0.85	+LL	zinc finger protein (PMZ) -related	
	sgn-U216297	0.39	ns	0.87	+LL	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family	
	sgn-U218605	0.35	ns	0.90	+LL	WRKY domain transcription factor family	
	sgn-U213139	0.25	ns	0.92	+LL	C2H2 zinc finger family	
	sgn-U214213	0.22	ns	0.96	+LL	PHOR1	
	sgn-U217394	0.65	ns	0.97	+LL	C2H2 zinc finger family	
	sgn-U218476	0.02	ns	1.31	+LL	bHLH	
	sgn-U224219	0.21	ns	2.43	+LL	histone deacetylase	
	sgn-U239113	-0.02	ns	2.84	+LL	AS2,Lateral Organ Boundaries Gene Family	
	sgn-U233205	0.23	ns	3.78	+LL	ribonucleases	
	sgn-U224917	0.03	ns	4.02	+LL	CCAAT box binding factor family, HAP2	
	Transport	sgn-U212831	0.71	+LL	0.28	ns	ABC transporters and multidrug resistance systems
sgn-U225466		0.79	+LL	0.29	ns	cyclic nucleotide or calcium regulated channels	
sgn-U217738		0.59	ns	1.08	+LL	heavy-metal-associated domain-containing protein	
sgn-U219528		0.16	ns	3.31	+LL	inorganic phosphate transporter	
Unknown	sgn-U222203	0.68	+LL	0.68	+LL	unknown	
	sgn-U228082	0.70	+LL	0.52	ns	unknown	
	sgn-U220025	0.74	+LL	0.46	ns	unknown	
	sgn-U227036	0.95	+LL	0.51	ns	unknown	
	sgn-U217364	1.07	+LL	0.88	+LL	unknown	
	sgn-U226746	2.31	+LL	0.6	ns	unknown	
	sgn-U218518	-0.11	ns	0.68	+LL	unknown	
	sgn-U216027	0.23	ns	0.75	+LL	unknown	
	sgn-U215236	0.37	ns	0.78	+LL	unknown	
	sgn-U216791	0.15	ns	0.90	+LL	unknown	
	sgn-U223726	0.11	ns	0.95	+LL	unknown	
	sgn-U242049	0.08	ns	2.37	+LL	unknown	
	sgn-U238051	-0.08	ns	2.76	+LL	unknown	
	sgn-U223823	-0.01	ns	3.39	+LL	unknown	
	sgn-U221474	0.05	ns	4.02	+LL	unknown	

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ns: non-significant fruit load effect

1 **Table S4: List of transcripts showing significant genotype x fruit load interactions** at the
 2 0.05 probability level. Genes were classified according to their belonging to a biological
 3 process. Differences in fruit load fold change expression between Moneyberg (M) and C9d
 4 are indicated and expressed in log₂ fold-change.

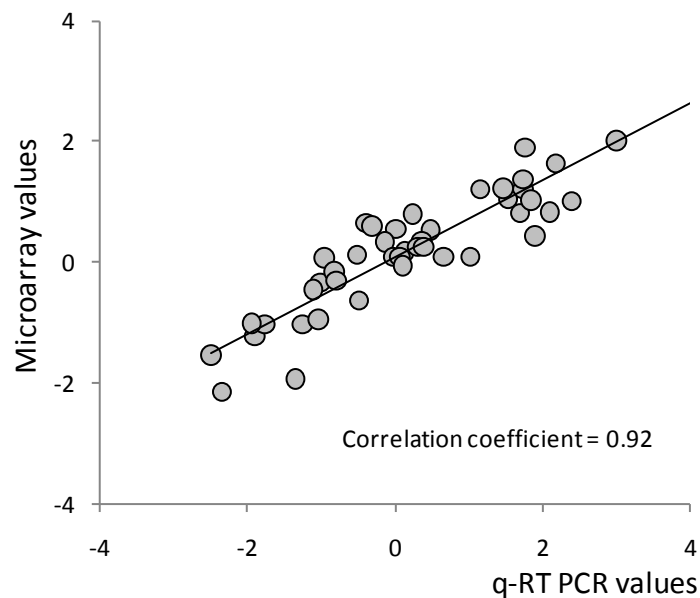
Functional category	Gene ID	Interaction = (Log ₂ -fold change in C9d) - (Log ₂ -fold change in M)	Annotation
Cell wall modification	sgn-U215495	-0.94	extensin
	sgn-U215382	-0.80	xyloglucan endotransglycosylase
	sgn-U215860	-0.71	xyloglucan endotransglycosylase
	sgn-U216615	-0.71	arabinogalactan protein
	sgn-U214352	0.74	UDP-glucose-4-epimerase
	sgn-U222466	0.74	elastin
	sgn-U220299	1.06	elastin
Electron transport	sgn-U220817	-0.71	adrenodoxin
	sgn-U213859	0.72	cytochrome P450
	sgn-U217384	0.93	cytochrome P450
	sgn-U230985	1.57	glutaredoxin
Hormonal responses	sgn-U214292	0.68	GA2 oxidase
	sgn-U213942	0.70	jasmonate ZIM-domain protein 1
	sgn-U213522	0.71	1-aminocyclopropane-1-carboxylate synthase
	sgn-U227363	0.75	auxin induced protein
	sgn-U219331	0.76	jasmonic acid2
	sgn-U214505	0.78	putative phytosulfokine peptide precursor
	sgn-U226232	0.79	IAA-Ala hydrolase (IAR3)
	sgn-U214304	0.79	ethylene-responsive late embryogenesis-like protein (ER5)
	sgn-U214303	0.80	ethylene-responsive late embryogenesis-like protein (ER5)
	U77719	0.82	ethylene-responsive late embryogenesis-like protein (ER5)
	sgn-U215161	0.83	auxin induced protein
	sgn-U221333	0.90	steroid 5 alpha reductase DET2
	sgn-U232919	0.91	putative phytosulfokine peptide precursor
	sgn-U214504	0.91	putative phytosulfokine peptide precursor
	sgn-U214986	0.94	auxin-regulated protein
	sgn-U212798	0.99	2-oxoglutarate-dependent dioxygenase
	sgn-U212786	1.08	1-aminocyclopropane-1-carboxylate oxidase
	sgn-U213253	1.12	ent-kaurenoic acid hydroxylase/oxygenase
	sgn-U241804	1.13	auxin induced protein
	sgn-U215638	1.43	S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase
sgn-U212784	1.70	lipoxygenase	
sgn-U214919	2.13	1-aminocyclopropane-1-carboxylate oxidase	
Photosynthesis	sgn-U212734	0.70	chlorophyll a/b binding protein
	sgn-U218909	0.71	photosystem II LHC-II
Primary metabolism	sgn-U222648	-1.06	GCN5-related N-acetyltransferase
	sgn-U225810	-0.80	adenine phosphoribosyltransferase
	sgn-U213496	0.73	isocitrate lyase
	sgn-U214061	0.75	xanthine dehydrogenase
	sgn-U213220	0.78	GABA transaminase
	sgn-U215314	0.90	triacylglycerol lipase
	sgn-U217603	1.01	acetyl-CoA acetyltransferase
	sgn-U218246	1.08	tryptophan synthase
Protein metabolism	sgn-U212847	-1.29	elongation factor 1-alpha
	M13938	0.72	protease inhibitor I
	sgn-U213020	0.82	protease inhibitor I
	sgn-U213022	0.88	protease inhibitor I
	sgn-U212706	0.90	cytokinin repressed protein
	sgn-U213019	0.95	protease inhibitor I
	AY129402	1.06	proteinase inhibitor II
sgn-U213023	1.25	protease inhibitor	

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Secondary metabolism	sgn-U213120	0.75	oxidase like protein
	sgn-U213739	0.81	aldehyde decarboxylase, CER1
	sgn-U227503	0.83	reticuline oxidase
Signalling	sgn-U214886	0.78	calcium/calmodulin-dependent protein kinase CaMK2
	sgn-U218626	0.85	calmodulin-related protein
Stress responses	sgn-U212922	-1.50	PR protein
	sgn-U213790	-1.31	glycosyl hydrolase
	sgn-U227867	-0.73	disease resistance response protein-related
	sgn-U214145	0.69	annexin
	sgn-U222728	0.69	senescence-associated protein
	sgn-U213993	0.72	peptide methionine sulfoxide reductase
	sgn-U217299	0.73	sec14 cytosolic factor family
	sgn-U218566	0.75	heat shock protein
	AJ225046	0.75	heat shock protein Hsp20.1
	sgn-U225027	0.92	heat shock protein family
	sgn-U212932	0.99	heat shock protein HSP101
	sgn-U216468	1.04	heat shock protein, class II, HCT2
	U72396	1.23	class II small heat shock protein Le-HSP17.6
	sgn-U213338	1.33	trypsin inhibitor
	sgn-U213021	1.35	protease inhibitor
	sgn-U216469	1.39	heat shock protein
	sgn-U214580	1.45	Pto-responsive gene
	sgn-U214579	1.48	Pto-responsive gene
	sgn-U223591	1.19	glutathione S transferases
	Transcription	sgn-U222912	-0.83
sgn-U213736		-0.68	RNA helicase
sgn-U223608		0.77	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family
sgn-U222261		0.78	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family
sgn-U213139		0.79	C2H2 zinc finger family
sgn-U213772		0.81	ribonucleases
sgn-U225111		0.83	WRKY domain transcription factor family
sgn-U214213		0.86	PHOR1
sgn-U215259		0.96	Heat-shock transcription factor family
sgn-U217991		1.03	AP2/EREBP, APETALA2/Ethylene-responsive element binding protein family
sgn-U224122		1.10	glutamate permease
Transport	sgn U220945	6.11	shrunken seed protein
Unknown	sgn-U217425	-0.71	unknown
	sgn-U220121	0.69	unknown
	sgn-U217695	0.73	unknown
	sgn-U215211	0.75	unknown
	sgn-U221141	0.76	unknown
	sgn-U215210	0.77	unknown
	sgn-U214265	0.79	unknown
	sgn-U218259	0.80	unknown
	sgn-U217613	0.82	unknown
	sgn-U232416	0.88	unknown
	sgn-U223726	0.91	unknown
	Sgn-U213285	0.93	unknown

1 **Figure S1: Verification of the microarray data.** Eleven different unigenes (PIP1, delta-TIP,
2 UDP-G-4-epi, UDP-G-Ppase, XTH6, PG, SBE, β -AM, β -FR, ACC-ox and E8) were used to
3 compare the two techniques. qRT-PCR expression analyses (X-axis) normalized to the inner
4 control (eukaryotic translation initiation factor 4A-2 (eIF-4A-2)) were compared to microarray
5 expression analysis (Y-axis) for the four comparisons: LL versus HL on genotype C9d, C9d
6 versus M under HL conditions, C9d versus M under LL conditions and LL versus HL on
7 genotype M. Data are indicated in the \log_2 base, and each quantitative PCR value is the mean
8 of three biological and three technical replicates.

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