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RESEARCH PAPER

# Combining ecophysiological modelling and quantitative trait locus analysis to identify key elementary processes underlying tomato fruit sugar concentration

**Marion Prudent[1,2,*], Alain Lecomte[1], Jean-Paul Bouchet[2], Nadia Bertin[1], Mathilde Causse[2] and Michel Génard[1]**

[1] INRA, UR1115 Plantes et Systèmes de culture Horticoles, F-84000 Avignon, France
[2] INRA, UR1052 Génétique et Amélioration des Fruits et Légumes, F-84000 Avignon, France

* To whom correspondence should be addressed. UMR Génétique et Ecophysiologie des Légumineuses à Graines, 17 rue de Sully, 21 000 Dijon, France. E-mail: marion.prudent@dijon.inra.fr

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## Abstract

**A mechanistic model predicting the accumulation of tomato fruit sugars was developed in order (i) to dissect the relative influence of three underlying processes: assimilate supply (S), metabolic transformation of sugars into other compounds (M), and dilution by water uptake (D); and (ii) to estimate the genetic variability of S, M, and D. The latter was estimated in a population of 20 introgression lines derived from the introgression of a wild tomato species (*Solanum chmielewskii*) into *S. lycopersicum*, grown under two contrasted fruit load conditions. Low load systematically decreased D in the whole population, while S and M were targets of genotype×fruit load interactions. The sugar concentration positively correlated to S and D when the variation was due to genetic introgressions, while it positively correlated to S and M when the variation was due to changes in fruit load. Co-localizations between quantitative trait loci (QTLs) for sugar concentration and QTLs for S, M, and D allowed hypotheses to be proposed on the processes putatively involved at the QTLs. Among the five QTLs for sugar concentration, four co-localized with QTLs for S, M, and D with similar allele effects. Moreover, the processes underlying QTLs for sugar accumulation changed according to the fruit load condition. Finally, for some genotypes, the processes underlying sugar concentration compensated in such a way that they did not modify the sugar concentration. By uncoupling genetic from physiological relationships between processes, these results provide new insights into further understanding of tomato fruit sugar accumulation.**

**Key words:** Assimilate supply, dilution, fruit load, fruit quality, genetic variation, metabolism, model, *Solanum chmielewskii*, *Solanum lycopersicum*, sugar and starch accumulation.

## Introduction

Tomato organoleptic quality is highly linked to the balance between the concentrations of acids and sugars in the fruit (Stevens *et al.*, 1977; Bucheli *et al.*, 1999). Sweetness particularly depends on sugar concentration, which is synthesized and accumulated during fruit growth. Fruit growth follows a sigmoid curve, which can be subdivided into three main steps (Ho and Hewitt, 1986). The first period is a division phase with an intense mitotic activity, leading to an increase in cell number which determines the potential size of the fruit (Ho, 1996*a*). The second phase corresponds to cell enlargement. During this period, the degradation of starch (considered to be a transient storage form of sugars) into soluble sugars leads to a maximal accumulation of sugars (essentially glucose and fructose), acids, and parietal components accompanied by a high water accumulation (Davies and Cocking, 1965; Dinar and Stevens, 1981; Schaffer and Petreikov, 1997). Finally, during the last slow growth period, intensive metabolic changes occur concomitantly with fruit ripening, while glucose and fructose continue to accumulate (Carrari *et al.*,

2006). The two last growth periods are thus essential in the final sugar accumulation in the fruit. From a physiological point of view, tomato sugar concentration is the consequence of various linked physiological processes such as carbon and water fluxes entering the fruit (Guichard *et al.*, 2001), carbon metabolism for the synthesis of sugars and starch, and the synthesis of other compounds such as organic acids or cell wall constituents (Ho, 1996*b*). These processes are influenced by environmental conditions, linked either to climate (temperature, humidity, and irradiance), to cultural practices, or to internal plant conditions (such as the sink:source ratio). The present study takes place in this context as little is currently known about the effect of sink:source ratio variations on processes underlying tomato fruit sugar concentration.

From a genetic point of view, many chromosome regions carrying quantitative trait loci (QTLs) for sugar content have been identified in various tomato populations carrying fragments from wild species (*Solanum habrochaites*, *S. peruvianum*, *S. neoricki*, *S. pimpinellifolium*, *S. pennellii*, and *S. lycopersicum* cv cerasiforme) (Saliba-Colombani *et al.*, 2001; Causse *et al.*, 2002, 2004; Fulton *et al.*, 2002). Moreover, the stability of QTLs involved in sugar concentration partly depends on the environment considered as the year of growing periods (Chaib *et al.*, 2006). The importance of the environment in the stability of QTLs for sugar concentration has also been reported in a QTL analysis performed under two fruit load conditions, in which Prudent *et al.* (2009) have identified chromosome regions involved in the control of sugar content which are either susceptible to or independent from changes in carbon availability within the plant. If these studies allow the suggestion of clues as to the instability of some chromosome regions towards environmental variations, the physiological processes underlying QTLs for sugar concentration which could be responsible for this susceptibility to the environment remain to be elucidated.

During the last 10 years, approaches combining ecophysiological modelling and QTL analyses have been developed to understand the key processes involved in the control of complex traits. Such an approach has been applied to study specific leaf area in barley (Yin *et al.*, 1999), leaf elongation in maize (Reymond *et al.*, 2003), and fruit quality in peach (Quilot *et al.*, 2005). The method consists of simultaneously studying the genotypic variation of a given complex trait, and the genotypic variation of ecophysiological model parameters linked to key processes involved in the development of this trait. Then, co-localizations of QTLs for the trait and QTLs for parameters give new insights into the processes involved in the trait at the QTL level, and then may help in the choice of candidate genes to characterize it, or may give clues as to the regions to be combined in an ideotype. This approach is particularly well adapted to the study of interrelated processes linked to complex traits, and appeared to be an essential tool in the context of sugar accumulation in fruits. However, in tomato, current available ecophysiological models mainly concern fruit development, with descriptions of cell division (Bertin *et al.*, 2003), DNA endoreduplication (Bertin *et al.*, 2007), or fruit growth (Bussieres, 2002; Liu *et al.*, 2007), but none of these models concerns the organoleptic quality of the tomato fruit. It thus emerged that an ecophysiological model describing sugar accumulation in tomato fruit is necessary in order to make some advances in the understanding of the physiological processes underlying sugar concentration.

In order to assess the key processes underlying sugar concentration, an approach combining ecophysiological modelling and QTL analysis was applied. For this purpose, a first model predicting tomato fruit sugar concentration was adapted from a previous model built on peach fruit (Quilot *et al.*, 2004), allowing the dissection of three interrelated elementary processes: the assimilate supply provided to the fruit (hereafter referred to as S), the metabolic transformation of sugars into other compounds (hereafter referred to as M), and the dilution of sugars by water uptake (hereafter referred to as D). Two sources of variation were used to modulate the sugar concentration: a genetic method, by working on a population of introgression lines (ILs), and a physiological method, by modifying the sink:source ratio leading to increased carbon availability to the fruit. This approach allowed: (i) observation of, under contrasting conditions of carbon availability, the intergenotypic relationships first among elementary processes and then between elementary processes and sugar concentration; (ii) estimation of whether two different sources of variation for sugar concentration (i.e. genotypic and physiological) lead to similar changes in the underlying processes; and (iii) identification at each QTL for sugar concentration those processes which were supposedly involved.

## Materials and methods

### *Model of sugar accumulation*

The model was adapted from the one-parameter model of Quilot *et al.* (2004), which is a simplified form of the SUGAR model for peach (Génard and Souty, 1996) and predicts the sugar concentration in peach flesh. The present model simulates, at a daily time step, the total sugar concentration of tomato pericarp (including soluble sugars and starch) during the last two developmental phases: cell enlargement and ripening. The variation rate of carbon in the form of sugars (*Csug*, in g fruit pericarp$^{-1}$) is expressed as:

$$\frac{\mathrm{d}Csug}{\mathrm{d}t} = \gamma_{\mathrm{DW}} \cdot \frac{\mathrm{d}DW}{\mathrm{d}t} - k(t) \cdot Csug \qquad (1)$$

where $t$ [in days after anthesis (daa)] is the time, $\gamma_{\mathrm{DW}}$ is the carbon concentration of dry pericarp [$\gamma_{\mathrm{DW}}$=0.44 gC gDW$^{-1}$; Génard and Souty (1996)], d$DW$/d$t$ (in gC d$^{-1}$) is the growth rate of pericarp dry weight, and $k(t)$ (in d$^{-1}$) is a function of time reflecting the rate of consumption of sugars contained in tomato pericarp for synthesis of other compounds. Changes in the model were added for the variable $k$, which depends on the relative growth rate (Génard *et al.*, 2003):

$$k(t) = k_0 \cdot \left( \frac{\mathrm{d}DW}{\mathrm{d}t} \cdot \frac{1}{DW(t)} \right)^{\varepsilon} \qquad (2)$$

where $k_0$ (in d$^{-1}$) is a genotype-dependent parameter, reflecting the value of $k$ when the relative growth rate is equal to 1, while $\epsilon$ is

a dimensionless parameter assumed to be constant whatever the genotype and the fruit load condition, which was estimated and set to 1.36.

As the total sugar concentration relative to pericarp fresh weight SUG (in gC. 100gFW$^{-1}$) is calculated as:

$$SUG = \frac{100.Csug}{\gamma_{sug}.FW} \quad (3)$$

where FW (in g) is the pericarp fresh weight, and $\gamma_{sug}$ (in gC g$^{-1}$) is the carbon concentration in sugars ($\gamma_{sug}$ =0.42 gC g$^{-1}$), a calculation of the variation rate of carbon in the form of sugars was obtained from Equations 2 and 3:

$$\frac{dSUG}{dt} = \frac{100}{\gamma_{sug}}\left(\frac{\gamma_{DW}}{FW}\frac{dDW}{dt} - \frac{k(t).C_{sug}}{FW} - \frac{C_{sug}}{FW^2}\frac{dFW}{dt}\right) \quad (4)$$

At time τ corresponding to fruit maturity, the sugar concentration SUG relative to pericarp fresh weight is then calculated by integrating Equation 4:

$$SUG(\tau) - SUG(t_0) = S - M - D \quad (5)$$

where S is the sugar import to the fruit, expressed as $S = \int_{t_0}^{\tau} \frac{100}{\gamma_{sug}} \frac{\gamma_{DW}}{FW}\left(\frac{dDW}{dt}\right).dt$ (in g 100gFW$^{-1}$), M is the metabolic transformation of sugars into others compounds, expressed as $M = \int_{t_0}^{\tau} k(t).SUG(t).dt$ (in g 100gFW$^{-1}$), D is the dilution of sugars by water uptake expressed as $D = \int_{t_0}^{\tau} \frac{SUG(t)}{FW(t)}\left(\frac{dFW}{dt}\right).dt$ (in g 100gFW$^{-1}$), and $t_0$ is the time when the first sugar data were recorded (at 21 daa).

At maturity, the starch concentration of tomato fruit is negligible (Robinson *et al.*, 1988). Sugar concentration is thus assimilated into the concentration of soluble sugars (glucose, fructose, and sucrose).

### Plant material

The study was performed using the *S. lycopersicum* line 'Moneyberg' and 20 ILs carrying single or multiple introgressions of the *S. chmielewskii* LA1840 in the background of Moneyberg. The locations of the introgressions for the 20 ILs as well as the description of the QTLs they harbour have been previously detailed in Prudent *et al.* (2009).

### Growth conditions, experimental treatment, and sampling

Experiments were conducted over 2 years in 3.6 plants m$^{-2}$ density greenhouses in Avignon (Southern France). Seeds were sown at the end of February; 400 plants were grown at day–night temperature set points of 24/16 °C during spring 2006 (March–July) and 25/15 °C during spring 2007 (March–July). Plants were randomly distributed in two blocks, each containing 200 plants and facing North and South, respectively. Plant nutrition and chemical pest and disease control followed commercial practices. Starting from anthesis of the first truss, flowers were pollinated with an electrical shaker every 2–3 d.

Two fruit loads were applied: a high fruit load (HL) with competition for assimilates among fruits, and a low fruit load (LL) in order to place all fruits in non-limiting growth conditions. On 12 plants per genotype, all trusses were pruned to one fruit (LL) while on seven other plants trusses were not pruned (HL). Under HL conditions, the average number of fruits per truss within the population was 5.3. On each inflorescence of the LL plants, all the flowers except the second one were removed just after fruit set. All the plants were stopped two leaves above the ninth truss.

In both years, six fruits per genotype and per fruit load were randomly harvested between the fourth and the ninth truss of the plants, at proximal positions: flowers 2, 3, or 4 under HL and only flower 2 under LL at the red ripe stage. For three contrasting genotypes (Moneyberg, C9d, and C12d), under both fruit loads, and only in 2007, six fruits were also harvested at four other developmental stages: 21, 28, 35, and 42 daa.

### Fruit and sugar measurements

Each fruit harvested was weighed, seeds were removed, and pericarp was weighed (FW) and ground in liquid nitrogen before lyophilization and storage at –20 °C. Pericarp dry weight (DW) and dry matter concentration (DMC) were then measured. Sugars and starch were extracted from the powders with a methanol–chloroform mix (Gomez *et al.*, 2002), and quantified by enzymatic assay in 96-well microplates (Gomez *et al.*, 2007).

### Model inputs

On genotypes Moneyberg, C9d, and C12d for which growth data were measured in kinetics, FW and DW were fitted to three-parameter Gompertz curves, separately under each fruit load (Fig. 1). For the other 18 genotypes and under each fruit load condition, FW and DW data were recorded only at maturity. Their FW and DW growth curves were thus reconstituted using the Gompertz parameters estimated on Moneyberg, C9d, and C12d, assuming that two out of the three Gompertz parameters do not vary with genotypes (Supplementary Appendix 1 available at *JXB* online). On the basis of the calculated values of FW and DW from the fitted Gompertz equation, it was then possible to assess the values of S, M, and D indirectly, according to Equation 5 for each genotype, and under each fruit load condition.

### Statistical analysis

Parameters estimation was carried out using the 'lsqnonlin' function of Matlab-version 7.3.0.267 (http://www.mathworks.com/). The goodness of fit of the model was evaluated through the relative root mean square error (RRMSE) (Kobayashi and Salam, 2000), which is a common criterion to quantify the mean difference between simulation and measurement:

$$RRMSE = \frac{1}{y}\sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y_i})^2} \quad (6)$$

where $y_i$ is the observed value, $\hat{y_i}$ is the corresponding predicted value, $N$ is the number of observed data, and $\bar{y}$ is the mean of all measured values. The smaller the value of RRMSE, the better the goodness of fit. The RRMSE value of the model is presented in Fig. 2.

QTL analysis was performed either under each fruit load condition for fruit load-dependent variables (S, M, or D) or by pooling fruits grown under HL and LL for parameter $k_0$, which is a genetic parameter, common to both fruit loads. QTL detection in a population of ILs relies on comparing each IL with the parent conferring the genetic background (Moneyberg).

For this purpose, the bootstrap method was performed considering 200 successive random drawings from the original data set, and permitted to re-estimate S, M, D, and $k_0$. It consisted of 'resampling' from the original sample with replacement, with the same size as the original.

Then, in order to compare S, M, D, and $k_0$ from each genotype with Moneyberg, the variable $z$ was calculated:

$$z_i = \frac{\left|\bar{y}_{i,b} - \left|\bar{y}_{i,b} - \bar{y}_{i,d}\right|\right| - \left|\bar{y}_{M,b} - \left|\bar{y}_{M,b} - \bar{y}_{M,d}\right|\right|}{\sqrt{\frac{\sigma^2_{i,b} + \sigma^2_{M,b}}{n}}} \quad (7)$$

where $\bar{y}_{i,b}$ and $\bar{y}_{M,b}$ are the means of the 200 variable $y$ (S,M, D, or $k_0$) estimations calculated from bootstrap drawings for genotype $i$ and Moneyberg, respectively; $\bar{y}_{i,d}$ and $\bar{y}_{M,d}$ are the estimations of the same variable $y$ calculated from the observed data set for genotype $i$ and Moneyberg, respectively; $\sigma^2_{i,b}$ and $\sigma^2_{M,b}$ are the variance of the 200 variable $y$ estimations calculated from bootstrap drawings for genotype $i$ and Moneyberg,

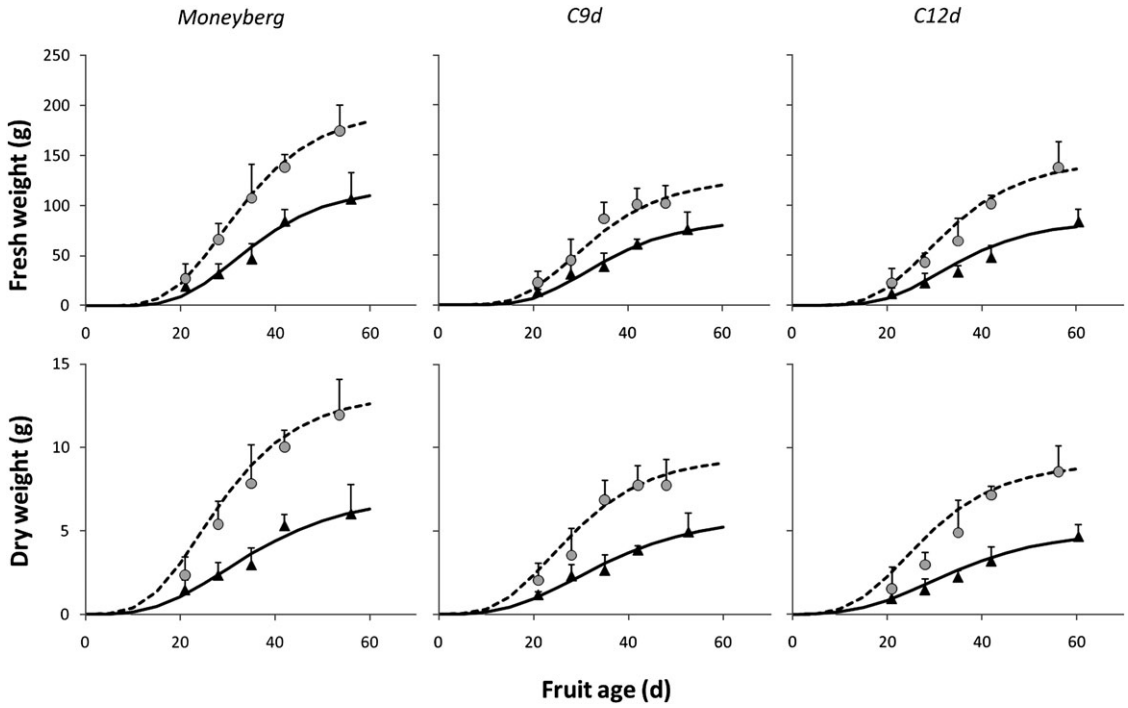



**Fig. 1.** Measured (points) and simulated (lines) fresh and dry weights according to the Gompertz equation fitting for genotypes Moneyberg, C9d, and C12d. Data are means ±SD ($n$=6). Filled triangles and solid lines indicate high load (HL) conditions, while shaded circles and dashed lines indicate low load (LL) conditions.

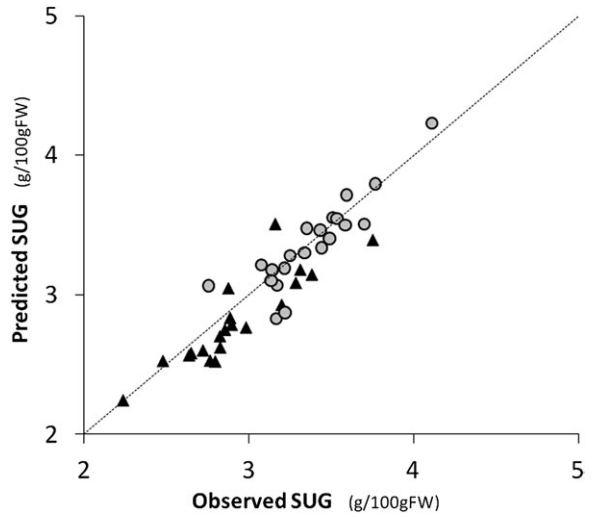



**Fig. 2.** Predicted versus observed sugar concentration relative to pericarp fresh weight (SUG). Each point represents the mean of the observed/predicted values of a genotype under a given fruit load (21 genotypes×2 fruit loads=42 points). Filled triangles indicate high load (HL) conditions, while shaded circles indicate low load (LL) conditions. The dotted line indicates the bisecting line. The value of global RRMSE is given.

respectively, and $n$ is the number of bootstrap drawings (in this case $n$=200). Then, a Z-test was applied as $z$ followed the reduced centred normal law (Sprinthall, 2002): for each genotype $i$, $|z_i|$ was compared with the threshold $z$ value corresponding to a 99% significance level ($z_{th}$=2.58). If $|z_i|>z_{th}$, genotype $i$ was significantly different from Moneyberg, meaning that it carried a QTL.

The genotypic effect ($\Delta_G$) was presented as a percentage of difference from Moneyberg:

$$(\Delta_G) = \frac{\bar{y}_{i,b} - \bar{y}_{M,b}}{\bar{y}_{M,b}} \times 100 \quad (8)$$

To evaluate fruit load effect, the same Z-test as the one used for QTL analysis was performed: for each genotype, the mean of the variable being calculated under HL was compared with the mean of the variable under LL. For each trait, the percentage of fruit load variation ($\Delta_{FL}$) from HL to LL was calculated following:

$$(\Delta_{FL}) = \frac{\bar{y}_{LL} - \bar{y}_{HL}}{\bar{y}_{HL}} \times 100 \quad (9)$$

where $\bar{y}_{HL}$ and $\bar{y}_{LL}$ are the general means of the variable $y$ under HL and LL, respectively.

### *Estimation of sugar content from QTLs*

For each genotype carrying a single introgression, an estimation of sugar content was obtained from QTLs for sugar content. When the genotype $i$ carried a QTL for variable $y$ (SUG), the estimated value of variable $y$ was equal to its observed value in genotype $i$: $y_{i,\,pred}=y_{i,\,obs}$. When the genotype $i$ did not carry any QTL for variable $y$, then the estimated value of variable $y$ was equal to the observed value of variable $y$ in Moneyberg: $y_{i,\,pred}=y_{Moneyberg,\,obs}$.

Then, estimations of SUG components were obtained from QTLs for S, M, and D, with the same method as for sugar content. It was thus possible to calculate for each genotype the estimated sugar content from QTLs for S, M, and D, by replacing the predicted values of S, M, and D in Equation 5.

### *Identification of candidate genes*

Some gene families annotated as corresponding to aquaporins, glucose, sucrose, or sugar transporters in the Sol Genomics

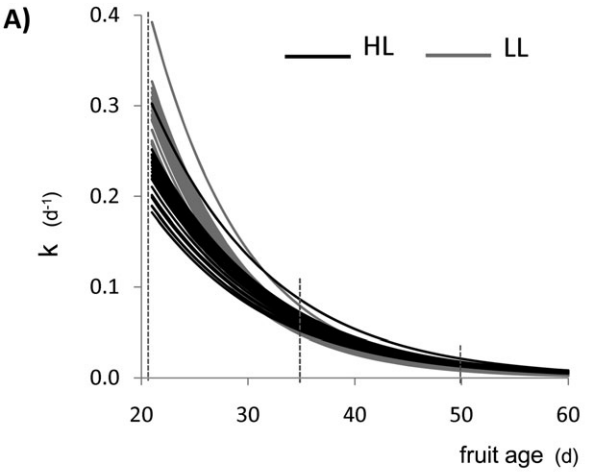
Network web site (Mueller *et al.*, 2005) were selected for the analysis. These gene families were selected as they are complete with respect to the study of Bermudez *et al.* (2008) concerning candidate genes related to metabolism. These gene families were built in 2007 with SGN unigene builds (version Tomato 200607 #1) and the *Arabidopsis* proteome (version 2004) with three values of stringency in grouping genes together (http://solgenomics.net). Among these gene families, 94 tomato unigenes were identified as gene candidates that could be involved in the QTLs detected in the present study. To assess co-localization between these tomato unigenes and QTLs for S, M, and D, the 2.10 version of the tomato genome assembly delivered by the International Tomato Genome Sequencing Consortium (Mueller *et al.*, 2009) which is distributed on the Sol Genomics Network web site was used. This version includes the first release of tomato chromosome pseudomolecule sequences assembled from 3433 scaffolds placed and oriented using multiple physical maps. Homologies between both the set of unigenes and the 13 pairs of markers flanking QTLs on chromosomes 1, 3, 4, 6, 7, 8, 9, 10, and 12 were searched with the NCBI blastn program (version 2.2.19-blastall-p blastn) (Altschul *et al.*, 1997). All high-scoring segment pairs selected with an e-value $<10^{-25}$ were sorted according to marker positions to verify consistency with positions on pseudochromosomes. Thus all unigenes and all markers were localized with high confidence on the pseudochromosomes. The co-localization between candidate genes and QTLs for S, M, and D are presented in Supplementary Table S1 at *JXB* online.

## Results

### *The model allowed the estimation of metabolism intensity*

The generic one-parameter model of Quilot *et al.* (2004), originally built for peach fruit, was adapted to tomato fruit in order to predict its sugar concentration at maturity. Comparisons between observed and simulated sugar concentration data are shown in Fig. 2. This model allowed (i) the dissection of sugar accumulation into three physiological processes: the assimilate supply to the fruit (S), the metabolic transformation of sugars into other compounds (M), and the dilution of sugars by water uptake (D); and (ii) the estimation of a genetic parameter $k_0$ relating the rate of sugar depletion to the relative growth rate in dry mass (Equation 2). Two different sources of variation were used to modulate the sugar concentration in tomato fruit. The first source of variation was genetic via the use of a population consisting of 20 ILs, and the line conferring the genetic background. The second source of variation was physiological via the modulation of the carbon availability to the fruit by pruning trusses to a single fruit.

The metabolic transformation of sugars into other compounds (M) depends on the rate ($k$) of consumption of sugars for their transformation, the latter decreasing during fruit development for all genotypes (Fig. 3A). However, during the first days of cell expansion (at ~21 daa), the rate of consumption of sugars for their transformation was significantly higher under high carbon availability (LL conditions) than under low carbon availability (HL conditions), while the opposite occurred during fruit maturation (at ~50 daa) (Fig. 3B).




B)

| fruit age (d) | $k_{HL}$ ($d^{-1}$) mean | s.d | $k_{LL}$ ($d^{-1}$) mean | s.d | FL effect on k p-value |
|---|---|---|---|---|---|
| 21 | 0.224 | 0.027 | 0.291 | 0.035 | 3.33E-08 |
| 35 | 0.063 | 0.008 | 0.058 | 0.007 | 2.00E-02 |
| 50 | 0.016 | 0.002 | 0.010 | 0.001 | 2.20E-16 |

**Fig. 3.** Effect of fruit load on $k$ values, reflecting the rate of consumption of sugars for the synthesis of other compounds. (A) $k$ values (in $d^{-1}$) calculated from Equation 2 plotted against time (in days). Each line refers to a genotype grown under high load (HL) conditions (black) or low load (LL) conditions (grey). (B) At three fruit developmental stages (21, 35, and 50 daa), the mean and SD of $k$ values under HL and LL were calculated, and the effect of the fruit load was tested using a Student's test.

### *Low fruit load conditions, in interaction with the genotype, modulated tomato fruit sugar concentration, the underlying processes, and their relationships*

For each genotype, the significance of the fruit load effect was tested at the red ripe stage fruit for pericarp sugar concentration (SUG), the assimilate supply (S), the metabolic transformation of sugars (M), and the dilution of sugars by water uptake (D). Genotypes were then grouped into five groups according to their responses to the fruit load change (Table 1). Decreasing fruit load resulted in significantly increased sugar concentration for most of the genotypes (15 out of 21) and in a systematic decreased dilution effect (D). The percentage of variation due to fruit load ($\Delta_{FL}$, Equation 9) was the highest for the dilution effect, as its mean (calculated on the 21 genotypes) was ~3-fold higher than those of S and M. Indeed, low load conditions did not significantly affect either the assimilate supply (S), except for seven genotypes belonging to groups 1, 2, and 4 for which S increased, or the metabolic transformation of sugars into other compounds (M), except for six genotypes belonging to groups 1 and 4, for which M increased.

Relationships and correlations between the three variables estimated by the model (S, M, and D) were studied separately under the two fruit load conditions (Fig. 4A–C). Assimilate supply was significantly positively correlated to dilution of sugars by water uptake and to metabolic transformation of sugars regardless of the fruit load.

**Table 1.** Effect of fruit load on sugar concentration relative to pericarp fresh weight (SUG), assimilate supply (S), metabolic transformation of sugars into other compounds (M), and dilution attributable to water uptake (D)

Genotypes were ordered into five groups according to their responses to the fruit load modification. Arrows showed the group trend by indicating if the variable significantly increased (↑), decreased (↓), or remained stable (–) at the 0.05 probability level from high load (HL) to low load (LL) conditions. When fruit load effect was significant, the percentage variation ($\Delta_{FL}$) from HL to LL was calculated (Equation 9). For each variable, the mean $\Delta_{FL}$ calculated on the 21 genotypes is indicated at the end of the table.

| Group | Genotype | SUG $\Delta_{FL}$ | S $\Delta_{FL}$ | M $\Delta_{FL}$ | D $\Delta_{FL}$ |
|---|---|---|---|---|---|
| **1** | | ↑ | ↑ | ↑ | ↓ |
| | C10b | 41 | 12 | 10 | –16 |
| | C4c | 15 | 12 | 10 | –13 |
| | C5b | 17 | 15 | 13 | –12 |
| | C8a | 28 | 12 | 10 | –14 |
| | C8e | 23 | 13 | 11 | –13 |
| **2** | | ↑ | ↑ | – | ↑ |
| | C1a | 22 | 11 | NS | –14 |
| **3** | | ↑ | – | – | ↓ |
| | C11b | 30 | NS | NS | –25 |
| | C3c | 11 | NS | NS | –21 |
| | C6e | 11 | NS | NS | –19 |
| | C7b | 21 | NS | NS | –18 |
| | C8c | 20 | NS | NS | –19 |
| | C9a | 15 | NS | NS | –17 |
| | C9c | 21 | NS | NS | –15 |
| | C9d | 11 | NS | NS | –23 |
| | Moneyberg | 16 | NS | NS | –18 |
| **4** | | – | ↑ | ↑ | ↓ |
| | C7a | NS | 12 | 9 | –14 |
| **5** | | – | – | – | ↓ |
| | C3a | NS | NS | NS | –24 |
| | C3d | NS | NS | S | –19 |
| | C4d | NS | NS | NS | –15 |
| | C7d | NS | NS | NS | –19 |
| | C12d | NS | NS | NS | –25 |
| | **Mean** | **16** | **7** | **5** | **–18** |

Similar positive correlations were found for the genotypic [$\Delta_G$ (S, M, D)] and fruit load [$\Delta_{FL}$ (S, M, D)] effects (Fig. 4D, E, G, H). On the other hand, a significant correlation between metabolic transformation of sugars and their dilution by water uptake was only observed for the fruit load effect (Fig. 4C, F, I).

Relationships and correlations linking sugar concentration (SUG) to variables estimated by the model (S, M, D) and the model parameter ($k_0$) were drawn (Fig. 5). Tomato sugar concentration was highly positively correlated to assimilate supply, whatever its source of variation (genetic or physiological). On the other hand, the sugar concentration was positively correlated to the metabolic transformation of sugars only if these two variables were expressed as a percentage of variation due to the fruit load change ($\Delta_{FL}$). The opposite occurred for the correlation between the sugar concentration and the dilution of sugars by water uptake, which was significantly positive when variables were not expressed as a percentage of variation due to the fruit load change. Finally, intergenotypic relationships between the sugar concentration and the rate oftransformation of sugars into other compounds ($k_0$) were fruit load dependent as a significant negative correlation between them was found only under HL conditions.

### *QTL detection*

QTLs were detected (i) for the model parameter $k_0$ reflecting the rate of consumption of sugars for their transformation, independently from fruit load; and (ii) for each variable estimated by the model (S, M, D), separately under each fruit load condition (Table 2). Positive or negative QTLs corresponded to a region where the alleles of *S. chmielewskii* increased or decreased the trait, respectively, compared with Moneyberg. Fourteen QTLs were detected for $k_0$, almost all with positive allele effects (except one negative on C3a) and with $\Delta_G$ effects (Equation 8) comprised between 9% (on C3a) and 51% (on C11b) compared with Moneyberg. Eleven QTLs were identified for sugar supply (S); among them, eight were positive, with half carrying similar allele effects under HL and LL conditions, and three were negative, with only one carrying similar effects under both fruit loads. Their $\Delta_G$ effects were consistently lower than the effects detected for QTLs for $k_0$, and the highest value was carried by C4d (~20%). Fifteen QTLs were identified for the metabolic transformation of sugars into other compounds (M): among them, 12 were positive and nine were common to both fruit loads. Their $\Delta_G$ effects ranged from 9% (on C4c) to 26% (on C8a). Then, 12 QTLs were identified for the dilution of sugars by water uptake D, mostly with negative effects, except on C4d, C9d, and C3c, and seven carried similar effects under both fruit loads. Their $\Delta_G$ effects were of the same order of magnitude as for sugar supply, with the highest value carried by C4d (22%).

### *QTL co-localizations*

Based on QTL detection, it was possible to study the 14 genotypes carrying a single introgressed fragment for co-localizations among QTLs for variables estimated by the model (S, M, D), the model parameter $k_0$, and QTLs for sugar concentration (Fig. 6). Some links already emphasized in Fig. 5 were confirmed at the genetic level as co-localizations between QTLs for sugar supply and for metabolic transformation of sugars (on C3d, C4c, C4d, C8a, C9d, C10b, and C12d); also co-localizations between QTLs for sugar supply and for sugar dilution (on C4d, C9d, C10b, and C12d) systematically carried similar allele effects.

Among the five QTLs for sugar concentration detected within genotypes carrying a single introgression, four of them (on C4d, C9d, C10b, and C12d) also co-localized with QTLs for sugar supply, for metabolic transformation of sugars, and for sugar dilution, with similar allele effects, and the last one (on C3a) co-localized with a QTL for the rate of consumption of sugars for their transformation, with a negative allele effect. However, these co-localizations were mostly dependent on the fruit load (on C3a, C9d, C10b, and C12d),

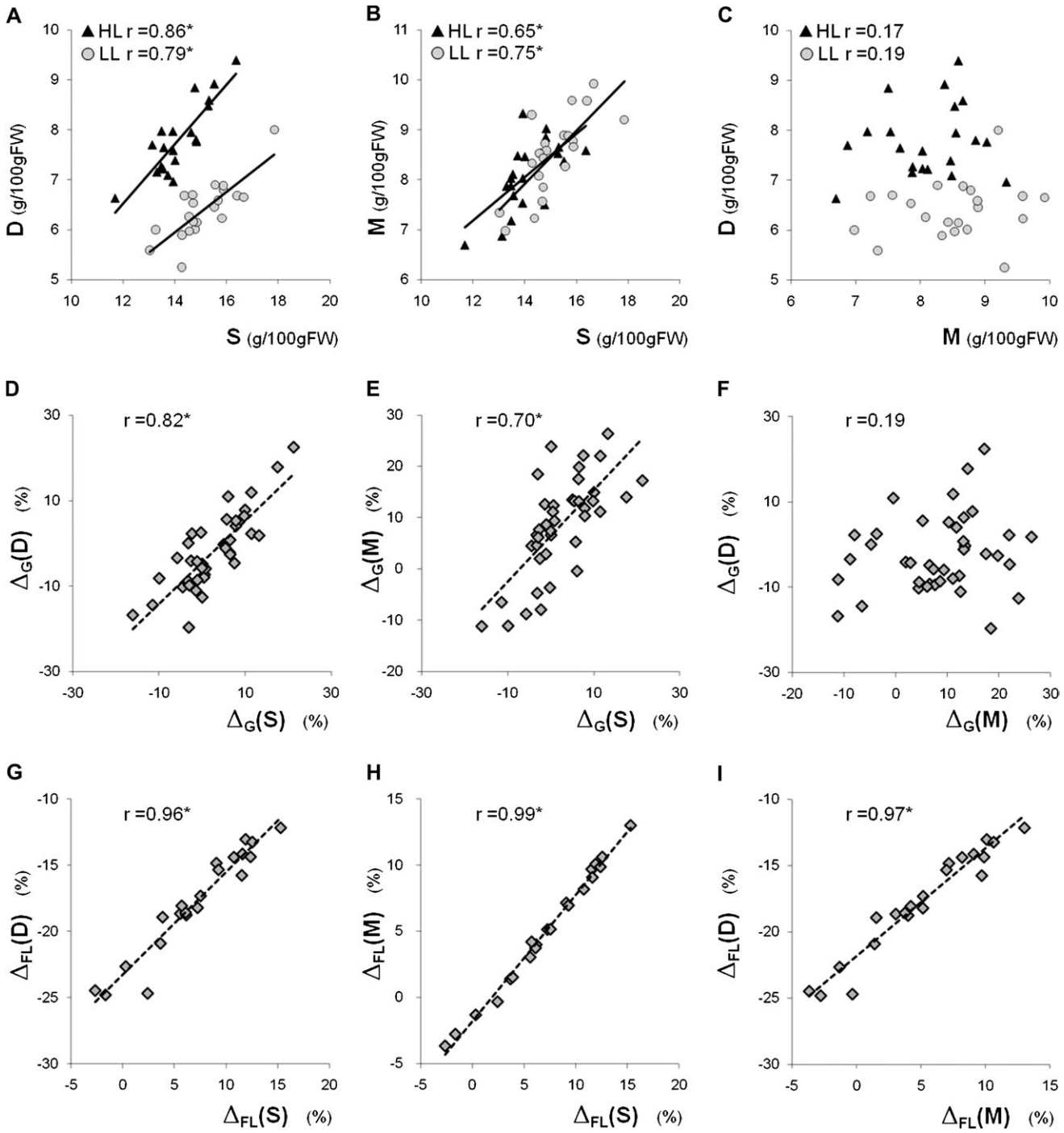



**Fig. 4.** Relationships between the three variables estimated by the model (sugar supply, S; metabolic transformation of sugars, M; dilution of sugars, D) (A–C), between their genotypic effects ($\Delta_G$, Equation 8) (D–F), and between their fruit load effects ($\Delta_{FL}$, Equation 9) (G–I). In A–C, each point corresponds to a genotype under high load (HL: filled triangles) and under low load (LL: shaded circles) conditions. In D–I, each point corresponds to a genotype under both fruit loads (shaded diamonds). Pearson correlations among variables were calculated either separately under each fruit load condition or under both fruit loads, and the correlation (*r*) is shown in the left corner of each graph. Asterisks indicate that correlations are significant at the 0.05 probability level.

indicating that depending on the carbon availability to the fruit, the physiological processes underlying QTLs for sugar concentration changed. For example, for genotype C9d, the QTL for sugar concentration could be related to a higher value of sugar supply under LL while it could be related to a combination of higher values of sugar supply, of metabolic transformation of sugars, and of sugar dilution by water uptake under HL.

In some chromosome regions, co-localizations among QTLs for the variables estimated by the model were not associated with QTLs for sugar concentration. This indicated that in these regions, the physiological processes

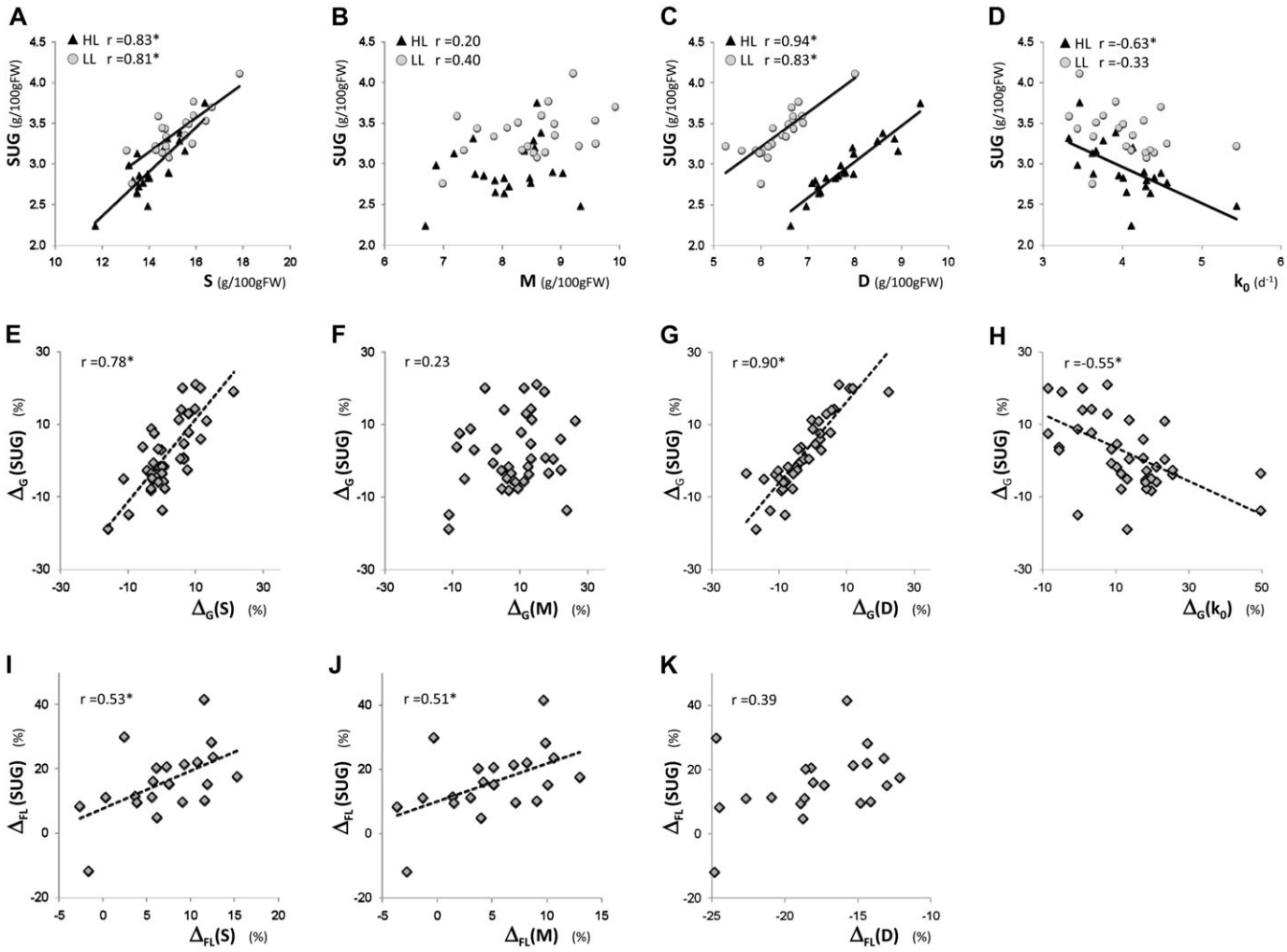



**Fig. 5.** Relationships between sugar concentration (SUG) and the three variables estimated by the model (sugar supply, S; metabolic transformation of sugars, M; dilution of sugars, D) and the model genetic parameter $k_0$ (reflecting the rate of consumption of sugars for the synthesis of other compounds). These variables were expressed either as their estimated/measured values (A–D), as a percentage of variation due to the introgression ($\Delta_G$, Equation 8) (E–H), or as a percentage of variation due to the fruit load ($\Delta_{FL}$, Equation 9) (I–K). Each point corresponds to a genotype under high load (HL: filled triangles) and under low load (LL: shaded circles) conditions, or under both fruit loads (shaded diamonds). Pearson correlations among variables were calculated either separately under each fruit load condition or under both fruit loads, and the correlation ($r$) is shown in the left corner of each graph.

underlying sugar concentration compensated in such a way that they did not modify the sugar concentration. For instance, for genotype C3d, QTLs for assimilate supply and sugar metabolic transformation were detected with positive allele effects, but the absence of a QTL for sugar concentration in this segment revealed that a higher assimilate supply was compensated for by a higher transformation of sugars into other compounds.

*In silico* analyses of four gene families (aquaporins, glucose transporters, sucrose transporters, and sugar transporters) allowed the identification of candidate genes which co-localized with QTLs for variables estimated by the model (S and D) (Supplementary Table S1 at *JXB* online). Over the seven QTLs detected for sugar dilution by water uptake, five co-localized with unigenes coding for aquaporins and, among the seven QTLs detected for sugar supply, three co-localized with unigenes coding for sugar transporters.

### *Estimation of sugar content from identified QTLs*

In order to assess if sugar content was better predicted by QTLs for its components (S, M, D) than predicted by QTLs for itself, the two methods were compared (Fig. 7). The prediction by QTLs identified for sugar content itself had an RRMSE lower than the prediction by QTLs identified for S, M, and D (RRMSE=0.07 and 0.17, respectively).

## Discussion

### *The model approach allows dissection of sugar concentration into interrelated elementary processes*

A modelling approach has been proposed to identify the processes involved in tomato fruit sugar concentration in a large set of genotypes without resorting to expensive and

**Table 2.** QTL detection for S, M and D under each fruit load condition (HL. high load condition; LL, low load condition), and for $k_0$ under both fruit loads

Genotypes are ordered according to the introgressions they carried (single or multiple) and chromosomes carrying introgressions are indicated for each genotype. QTL effects for sugar concentration (SUG) previously found (Prudent *et al.*, 2009) were added. NS indicates that the QTL was not significant at the 0.05 probability level. The effect of the QTL ($\Delta_G$) is expressed as a percentage of the difference from Moneyberg (Equation 8).

| Genotype | Chromosome | QTL effect for SUG | | QTL effect for $k_0$ | QTL effect for S | | QTL effect for M | | QTL effect for D | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | HL | LL | HL and LL | HL | LL | HL | LL | HL | LL |
| Genotypes carrying a single introgression | | | | | | | | | | |
| C1a | 1 | NS | NS | 18 | NS | 12 | 18 | 22 | NS | NS |
| C3a | 3 | 20 | NS | –9 | NS | NS | NS | NS | NS | NS |
| C3d | 3 | NS | NS | NS | 10 | 8 | 13 | 10 | NS | NS |
| C4c | 4 | NS | NS | NS | –6 | NS | –9 | NS | NS | NS |
| C4d | 4 | 35 | 19 | NS | 18 | 21 | 14 | 17 | 18 | 22 |
| C6e | 6 | NS | NS | 21 | NS | NS | 12 | 11 | –7 | –8 |
| C7a | 7 | NS | NS | 17 | NS | NS | NS | NS | –10 | –6 |
| C7b | 7 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| C7d | 7 | NS | NS | 15 | NS | NS | 13 | 13 | NS | NS |
| C8a | 8 | NS | NS | 23 | NS | 13 | 20 | 26 | NS | NS |
| C8c | 8 | NS | NS | 19 | NS | NS | NS | NS | –9 | –10 |
| C9d | 9 | 20 | 14 | NS | 11 | 6 | 11 | NS | 12 | NS |
| C10b | 10 | –19 | NS | 12 | –16 | –11 | –11 | NS | –17 | –14 |
| C12d | 12 | NS | –15 | NS | NS | –10 | NS | –11 | NS | –8 |
| Genotypes carrying multiple introgressions | | | | | | | | | | |
| C3c | 2;3 | 21 | NS | 10 | 10 | 8 | 15 | 12 | 8 | NS |
| C5b | 4;5;7;11 | NS | NS | 26 | NS | 8 | 13 | 22 | –11 | NS |
| C8e | 3;8 | NS | NS | 11 | NS | 7 | NS | 13 | NS | NS |
| C9a | 7;9;11 | NS | NS | 19 | NS | NS | NS | 9 | –9 | –9 |
| C9c | 1;7;9;11 | NS | NS | 14 | NS | NS | NS | NS | –9 | NS |
| C11b | 11;12 | NS | NS | 51 | NS | NS | 24 | 18 | –13 | –20 |

time-consuming methods. As this trait is influenced by carbon and water fluxes entering the fruit, and by the proportion of metabolic transformation of carbon into sugars, acids, and structural components occurring in the cells (Ho, 1996*b*; Guichard *et al.*, 2001), the present model allowed the assessment of the effects of three key physiological processes involved in sugar accumulation: sugar supply (S), metabolic transformation of sugars into other compounds (M), and dilution of sugars attributable to change in fruit volume by water uptake (D). In contrast to peach fruit, for which sugar supply and dilution of the sugars are the most active processes (Quilot *et al.*, 2004), in tomato fruit all three processes were of the same order of magnitude (Figs 4, 5).

Relationships between the different processes dissected by the model were emphasized, such as the positive correlation between the sugar supply to the fruit and sugar dilution by water uptake (Fig. 4A, D, G). This relationship has already been tackled by the use of a tomato fruit growth model: when a virtual carbon stress is applied (by a decrease in the phloem carbon concentration), then water influxes from xylem and phloem decrease (Liu *et al.*, 2007), thus leading to a reduction of carbohydrate dilution by water uptake. In this study, this relationship was also confirmed at the genetic level by co-localizations of QTLs for assimilate supply and sugar dilution with similar allele effects.

### *Fruit load change affects assimilate supply, metabolic transformation, and sugar dilution in interaction with the genotype*

In order to allow the fruit to reach its growth potential, a modification of competition for assimilates among fruits was applied by modulating fruit load (Ho, 1996*b*; Tanksley, 2004). As fruit load directly influences fruit growth (Bertin, 2005; Baldet *et al.*, 2006), this treatment was directly taken into account in the model through model inputs (pericarp fresh and dry weights). In grape berries, variations in sugar concentration due to a fruit load modification are mainly due to the variation of the assimilate supply (Dai *et al.*, 2009). In tomato, dilution was the process which was the most affected by fruit load, as it significantly increased with fruit load for all genotypes and by up to 20–25% in five genotypes (Table 1). It was expected that low fruit load led on one hand to an increase in assimilate supply as it increases phloem fluxes (Guichard *et al.*, 2005) and on the other hand to an increase in metabolic transformation as, under high carbon availability conditions, protein and amino acid concentrations are increased (Baldet *et al.*, 2002). Even if it was the case for seven and six genotypes, respectively, it appeared that for the majority of them, including Moneyberg, assimilate supply and metabolic transformation were not susceptible to the fruit load change, indicating the occurrence of interactions between

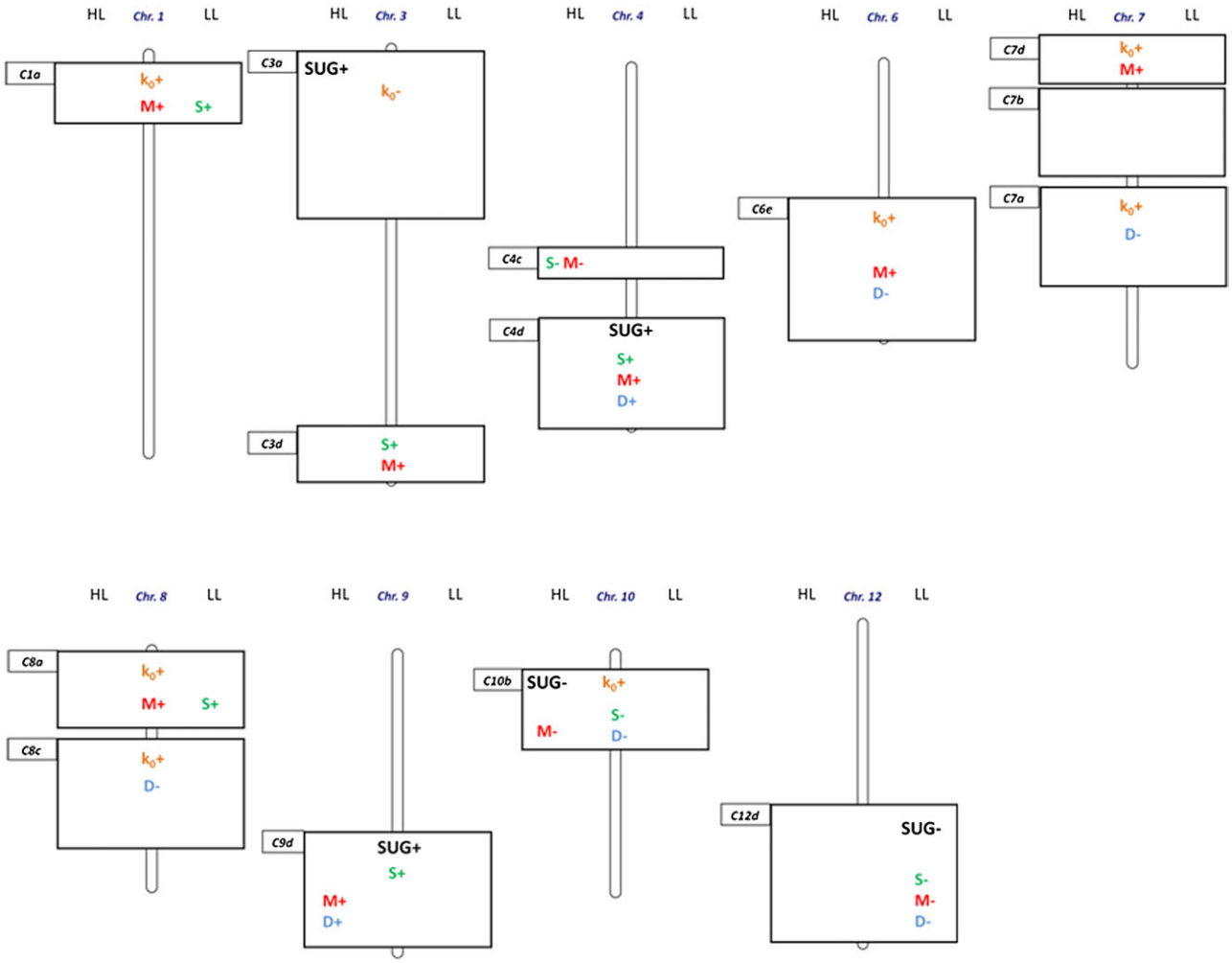



**Fig. 6.** Genetic map of QTLs for model parameters detected on genotypes carrying a single introgressed fragment. QTLs previously detected in Prudent *et al.* (2009) are indicated: they concerned sugar concentration relative to pericarp fresh weight (SUG). QTLs for sugar supply (S), for metabolic transformation of sugars into other compounds (M), for dilution of sugars by water uptake (D), and for the rate of consumption of sugars for synthesis of other compounds ($k_0$) were added. QTLs only detected under high fruit load (HL) are on the left of the chromosome; QTLs only detected under low fruit load (LL) are on the right of the chromosome; QTLs detected whatever the fruit load are at the middle of the chromosome. (–) and (+) indicate if the *S. chmielewskii* alleles had negative or positive effects on the trait, respectively. (This figure is available in colour at *JXB* online.)

fruit load and genotype at the ecophysiological process level, confirming what has already been observed at the molecular level (Prudent *et al.*, 2010). Moreover, analysis of relationships among the three trait components (Fig. 4) revealed that correlations were higher when the source of variation was related to the fruit load effect than when the source of variation was related to a genetic effect. This observation can be explained by the fact that the fruit load effect only modified fruit growth, while the genotypic effect not only modified fruit growth, but also the genetic parameter $k_0$ which makes the metabolic transformation of sugars (M) vary.

### *Relationships between fruit sugar concentration and the underlying processes depend on their source of variation*

The three main processes taken into account in the model were not linked in a similar way to the final sugar concentration, but depended on their source of variation. When the sugar concentration variation was due to a change in fruit load, then the sugar concentration positively correlated to assimilate supply and metabolic transformation of sugars. On the other hand, when applying a variation of sugar concentration via genetic introgression, the sugar concentration was still positively correlated to assimilate supply but also to sugar dilution. The relationships observed with a genetic variation were in accordance with previous molecular studies. One of them shows that the rapid hexose accumulation in developing tomato fruit is explained more by the expression level of a gene coding for a hexose transporter than by sugar metabolism (Dibley *et al.*, 2005). Another study highlights that inhibiting a TRAMP aquaporin leads to a decrease in sugar concentration and an increase in organic acid content (Chen *et al.*, 2001). Moreover, in this sense, enzymatic studies demonstrate that high fruit sugar concentrations depend on sugar

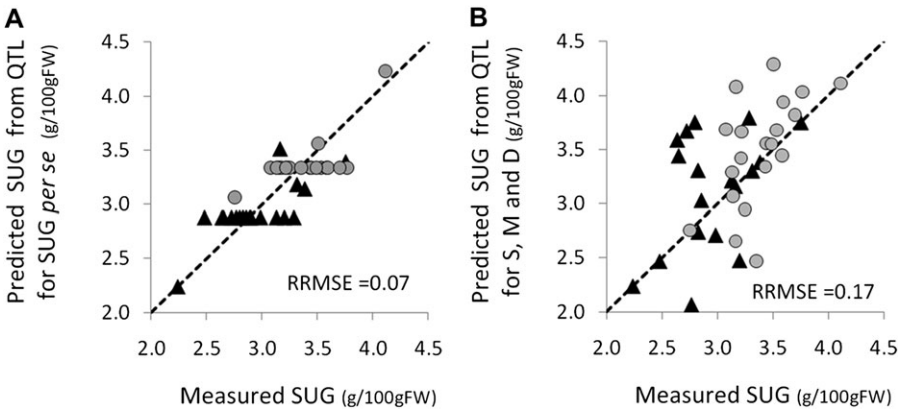



**Fig. 7.** Comparison between observed values of sugar concentration (SUG) and (A) those predicted from QTLs identified for SUG and (B) those predicted from QTLs for SUG component traits (supply, S; metabolism, M; and dilution, D). The dotted line indicates the bisecting line. The value of global RRMSE is given. Each point represents the mean of the observed/predicted values of a genotype under a given fruit load (20 introgression lines×2 fruit loads=40 points). Filled triangles indicate high load (HL) conditions, and shaded circles indicate low load (LL) conditions.

import rather than on sucrose metabolism (Balibrea *et al.*, 2006). This suggests that, in the context of a tomato breeding programme for enhanced sugar concentration, many efforts should be made to understand the mechanisms leading to sugar import and dilution process.

### *Physiological processes underlying QTLs for sugar concentration*

Co-localizations between QTLs for sugar concentration and QTLs for each of the three processes defined in the model were identified under the two fruit load conditions (Fig. 6). All the QTLs for sugar content co-localized with QTLs for trait components, giving clues as to which physiological processes could be involved in sugar content at each QTL. There were also a lot of QTLs for trait components which did not co-localize with QTLs for sugar content, indicating that for some genotypes, different processes can compensate. This situation has already been described for another complex trait (yield) in barley (Yin *et al.*, 2002) and, even if more QTLs have been detected for component traits than for grain yield, a poorer performance of the estimation of yield variation based on component trait QTLs has been observed (Yin *et al.*, 2002). In the present case, the same conclusions could be drawn, as the sugar concentration was better estimated from sugar concentration-based QTL analysis than from component trait QTL analysis. It could be explained both by the accumulation of errors in model parameterization for calculating component traits S, M, and D, and by the fact that the model did not account fully for the variation of SUG (Fig. 2). In order to improve the prediction of sugar concentration based on component trait QTL analysis, first the model should be tested using data independent from those for model parameterization, and, secondly, a model whose input components can be easily measured directly from an experiment could be developed.

Co-localizations between QTLs for sugar content and QTLs for its trait components could also facilitate bridging the gap between QTLs and genes by helping to choose candidate genes (Quarrie *et al.*, 2006). For example, albeit that a decrease in the confidence intervals of the QTLs is necessary, a path could be explored on genotype C4d as the QTLs for sugar concentration and for metabolic transformation of sugars into other compounds were identified, while in this same chromosome region the gene HXK4 coding for a hexokinase [an enzyme that phosphorylates hexoses (Kandel-Kfir *et al.*, 2006)], as well as a gene coding for a pectinesterase [an enzyme involved in cell wall modifications (Bermudez *et al.*, 2008)] have both been mapped. In other regions carrying co-localizations between QTLs for sugar concentration and QTLs for metabolic transformation, a coupling with metabolome analyses could be relevant in order to target the processes to be precisely dissected. On the other hand, in some regions, no QTL for M was identified, while genes involved in metabolism have already been mapped in previous studies. For instance, this is the case for the chromosome region carried by genotype C7a where a gene coding for a xyloglucan endotransglycosylase (an enzyme involved in cell wall modifications) is located (Bermudez *et al.*, 2008). This could be explained by compensatory effects of different genes located in this region, which could mask the effect of a single gene (Supplementary Table S1 at *JXB* online). Most of the QTLs detected for the dilution of sugar by water uptake co-localized with some members of the aquaporin gene family, indicating that these genes play a key role in the sugar content, as they modulate the water availability to the fruit and consequently the sugar dilution (Supplementary Table S1). Similarly, as expected, several genes coding for hexose transporters were located in chromosome regions carrying QTLs for assimilate supply, giving clues about candidate genes which could be studied in order to enhance tomato fruit sugar concentration.

### *Conclusion*

Using a model-based approach followed by genetic analyses, it was possible to dissect physiological processes

underlying QTLs for fruit sugar concentration such as the assimilate supply, the transformation of sugars into other compounds, and their dilution by water uptake. This work allowed uncoupling of genetic from physiological relationships among processes, as most of them acted in a fruit load-dependent manner and displayed compensatory effects. It is a first step in the construction of ideotypes for a sugar-enhanced tomato breeding programme, as it suggests a simulation model for the analysis of a genetic parameter. The next step will be to find the best associations between this parameter and other variables linked to fruit growth, for instance by coupling the sugar accumulation model with a fruit growth model.

## Supplementary data

Supplementary data are available at *JXB* online.

**Table S1.** List of candidate genes for each introgression carrying a QTL for component traits (S, M, and D).

**Supplementary Appendix 1.** Reconstitution of tomato fruit growth curves in dry weight and fresh weight of the 18 ILs different from Moneyberg, C9d, and C12d.

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