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A kinetic model of sugar metabolism in peach fruit allows the exploration of genetic variability

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Keywords: mathematical model, *P.persica*, sugar concentrations, enzyme capacities, compartmentation

Abstract

The organoleptic properties of peach, as fruit in general, largely depend on the accumulated sugars and acids. From sucrose, glucose and fructose, main sugars found in peach, fructose is the sweetest one. While commercial peach has equivalent fructose and glucose concentration, some wild or ornamental accessions display an imbalanced fructose-to-glucose ratio with a very low fructose concentration. In addition to genetic control, sugar metabolism is driven by fruit development and environment. The relative role of biochemical strengths and gene regulation in the elaboration of fruit sugar content at maturity is not well known. As the complex interplay between synthesis, degradation, transport and storage held in the cell is difficult to formulate, mathematical modelling appears to be an effective tool to challenge the issue. Thus, on the basis of profiling data, we developed a kinetic model of sugar metabolism in peach fruit. It simulates the evolution of sucrose, glucose, fructose and sorbitol concentrations during fruit development. A particular attention has been given to represent cellular compartmentation (cytosol and vacuole) in order to modulate the availability of the metabolites for the enzymatic reactions. The model was parameterized for different peach genotypes including a particular phenotype with low fructose-to-glucose ratio. It described well genetic variability. It was then used to further explore the system including the mechanisms driving genotypic differences.

Keywords: mathematical model, *P.persica*, sugar concentrations, enzyme capacities, compartmentation

INTRODUCTION

Sugars are the principal component of fruit controlling organoleptic quality. Nevertheless, the accumulation of sugars during fruit development is a complex mechanism not well understood yet. The sugar content in fruit evolves along fruit growth and depends on the environment and the genotype. At maturity, peach fruit (*Prunus persica* (L.) Batsch) are characterized by high amount of sucrose equal moderate amounts of glucose and fructose, and low amount of sorbitol (Moriguchi et al., 1990a). Despite this general trend, peach accessions display a large natural variability in sugar

concentrations (Cantín et al., 2009). The main point of variation in wild or ornamental peaches is a very low fructose concentration compared to glucose concentration (Moriguchi et al., 1990a; Kanayama et al., 2005). The mechanism involving in this particular feature is not yet known. Three hypotheses can be made to explain this low fructose-to-glucose ratio: I) the fructose may be less synthesized, four enzymes synthesize fructose in peach fruit namely acid invertase (AI), neutral invertase (NI), sucrose synthase (SuSy) and sorbitol dehydrogenase (SDH). Among these enzymes, only SuSy and SDH may lead to a modification of the fructose-to-glucose ratio II) fructose may be more phosphorylated by the fructokinase (FK) or III) a default in the fructose storage capacity into the vacuole may result in a higher amount of fructose in the cytosol where it can be phosphorylated by the FK.

Recent studies characterizing a large number of metabolites and enzyme capacities (maximal activity) related to sugar metabolism during fruit development showed that there is no clear link between the concentration of a given sugar and enzymatic maximal activities (Biais et al., 2014; Desnoues et al., 2014). This suggests that the regulation of sugar metabolism is system-driven rather than reaction-driven. Therefore kinetic models are especially useful to investigate functional hypotheses or perform *in silico* experiments, due to the mechanistic description of metabolic functions.

The present study is aiming at exploring the fruit sugar metabolism during peach fruit development. More precisely, it intends at investigating hypotheses to explain the major change in fructose concentration between genotypes. For this purpose, we developed a kinetic model of sugar metabolism based on profiling data (Desnoues et al., 2016).

MATERIALS AND METHODS

Model construction

We built a dynamical model of sugar metabolism in peach fruit mesocarp (Desnoues et al., in prep). From the measured flesh dry weights during fruit development, we estimated the carbon inflow as done by Génard et al. (2003). A series of ordinary differential equations describes the distribution of this carbon through the metabolites. We used irreversible Michaelis Menten (MM) equations to represent enzymatic reactions.

Sub Cellular Compartmentation

We performed a cytological analysis as described in Biais et al. (2014) to estimate the compartmentation between the cytosol and the vacuole during fruit development. This compartmentation was then represented explicitly in the model so as to calculate the appropriate concentration of metabolites available for the enzymatic reactions in each compartment.

We used a linear function of the cytosolic sucrose concentration to represent the import flow of sucrose in the vacuole (Preisser and Komor, 1991). Concerning the

hexoses, we represented the active and passive transporters separately since both active and passive transports occur in the vacuolar membrane in fruit (Martinoia et al., 2012). Concerning sorbitol transport in the fruit, little information is available in the literature (Wei et al., 2014). Since sorbitol has been observed in the vacuole in peach fruit (Jiang et al., 2013), we assumed the existence of a vacuolar transporter and chose a passive transport mechanism. Following Beauvoit et al. (2014), we supposed that the density of transporters per unit surface area was constant for both active and passive transports. Consequently, flows linked to transport augmented proportionally to the tonoplast surface during fruit development.

Model Parameterization and Simulation

Parameter values corresponding to respiration rate were taken from Génard et al. (2010). A polynomial curve fitted to the V_{max} measured in Desnoues et al. (2014) was used to parameterize enzyme kinetics. We used K_m values from literature, primarily those concerning peach or plants (Moriguchi et al., 1990b; Moriguchi et al., 1991; Vorster and Botha, 1998; Oura et al., 2000). In absence of information in the literature, we numerically estimated the six parameters linked to vacuolar transport kinetics that we assumed constant.

Matlab software (MATLAB R2014a, The MathWorks Inc., Natick, MA) was used for model integration and calibration. For each genotype we performed twenty estimations to ensure good exploration of the parameter space. Among the estimations, we selected for each genotype those with no more than 10% deviation from the lowest sum of squared difference between simulations and observations. Then, based on flow minimization criteria (Holzhutter, 2004), we kept only one for the subsequent result analyses.

RESULTS and DISCUSSION

The dynamic model we developed combines enzyme activities with an explicit description of subcellular compartmentation and their evolution over time. It describes well the accumulation of four sugars (sucrose, glucose, fructose and sorbitol) in peach fruit during its development. It can simulate the genetic diversity observed between the eight genotypes studied (Fig. 1), including a particular phenotype with a low fructose concentration. The application of our model to a panel of genotypes with different fructose-to-glucose ratio opens the way to an in-depth investigation of the molecular mechanisms underlying this variation. Three possible mechanisms were hypothesized for the modification of the fructose content, namely, fructose synthesis, degradation and/or storage.

Fructose synthesis: SuSy

Previous studies have shown the presence of different isoforms of SuSy (Tanase and Yamaki, 2000) which is why the K_M values related to this enzyme were estimated.

Moreover, the presence of two genes coding for SuSy have been reported in the QTL controlling the low fructose-to-glucose ratio (Desnoues et al., 2016), making Susy a good candidate to explain this feature. However, the estimated K_M for both fructose types are not significantly different (Fig. 2a) although we observe a trend of a higher K_M for the ‘low fructose-to-glucose ratio’ genotypes, which is consistent with the hypothesis of a lower synthesis of fructose. To explore this functional hypothesis we simulated the concentrations of sugars by exchanging the value of the K_M of SuSy parameter with the average estimated value of the opposite fructose type (Fig 2b, c). Changing this parameter alone has no effect on the simulation of sugar concentration. Thereby SuSy does not seem to be responsible of the ‘low fructose-to-glucose ratio’ phenotype.

Fructose degradation: FK

In the same way as the SuSy, Kanayama et al. (1998; 2005) showed the presence of isoforms of FK with different K_M . The estimated parameter representing the K_M of FK shows a significant difference between the two types of fructose (Fig 2d), with a lower K_M for the ‘low fructose-to-glucose ratio’ genotypes. This is in line with an increase of the degradation of fructose by FK and is therefore consistent with the hypothesis.

We then simulated the concentration of sugars by exchanging the value of the parameter K_M of FK with the average estimated values of the opposite fructose type (Fig 2e, f). While the concentration of the other sugars is not changed, the fructose concentration changes drastically. Indeed, the genotypes initially ‘standard fructose-to-glucose ratio’ display a strong fall of their fructose concentration to a concentration close to that observed in the ‘low fructose-to-glucose ratio’ genotypes and inversely.

Thus the presence of these isoforms in different proportions in the two fructose types may result in differential degradation of fructose and may lead to the emergence of two phenotypes.

Fructose storage: passive tonoplasmic transporter

A similar low-fructose phenotype in Arabidopsis leaves has been attributed to the action of the tonoplasmic transporter AtSWEET17, a fructose exporter from vacuoles to the cytosol (Chardon et al., 2013). Similarly, in apple, Wei et al. (2014) found higher expression of the MdSWEET4.1 transporter (phylogenetically similar to AtSWEET17) in the leaves than in the fruit, and the fructose concentration was lower in the leaves. The AtSWEET17 transporter is a bidirectional passive transporter (Guo et al., 2014), and its activity corresponds to the TpassifFru parameter of the model.

However, the estimated parameter values TpassifFru were not significantly different between the two fructose types (Fig 2g). The model estimated a higher value for the ‘low fructose-to-glucose ratio’ genotypes which cause a higher flow of fructose from the vacuole to the cytosol allowing its phosphorylation into the cytosol. The use of the parameter estimated from the other fructose type causes a modification of fructose concentration (without changing the concentrations of other sugars) but lower than the parameter of K_M of FK (Fig. 2h, i).

However, it has to be noted that TpassifFru is represented in the model as a passive transporter, whose action only depends on the fructose gradient across the tonoplastic membrane. Its effect should thus be strengthened if coupled to a “pump” mechanism, such as FK, which hydrolyzes fructose in the cytosol. The fructose vacuolar transport and the two isoforms of FK are therefore compatible mechanisms that might act together for the elaboration of the ‘low fructose-to-glucose ratio’ phenotype.

In conclusion, the model presented here proved to be a helpful tool for the investigation of sugar metabolism in peach and the identification of the mechanisms underlying phenotypic variability. Indeed, this study reveals that a difference in fructokinase affinity can be responsible for the ‘low-fructose-to glucose-ratio’ phenotype observed in the studied population. We do not exclude that other mechanisms, such as a modification of fructose storage in the vacuoles, may act with the fructokinase affinity to cause this particular phenotype.

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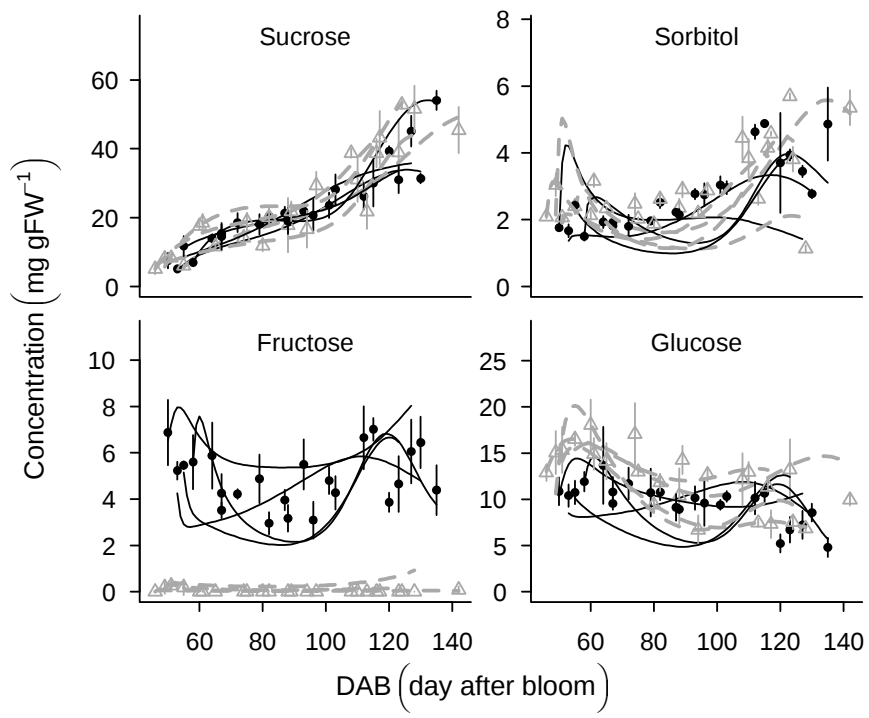
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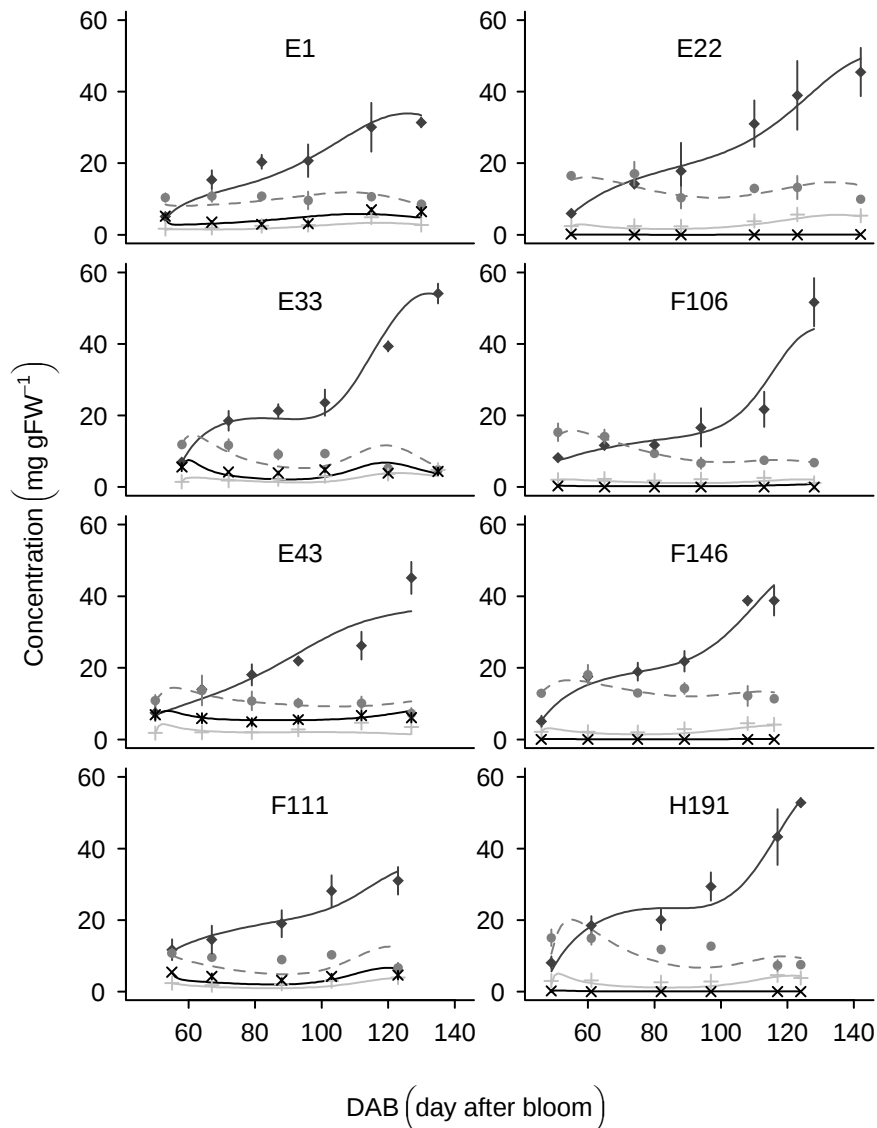
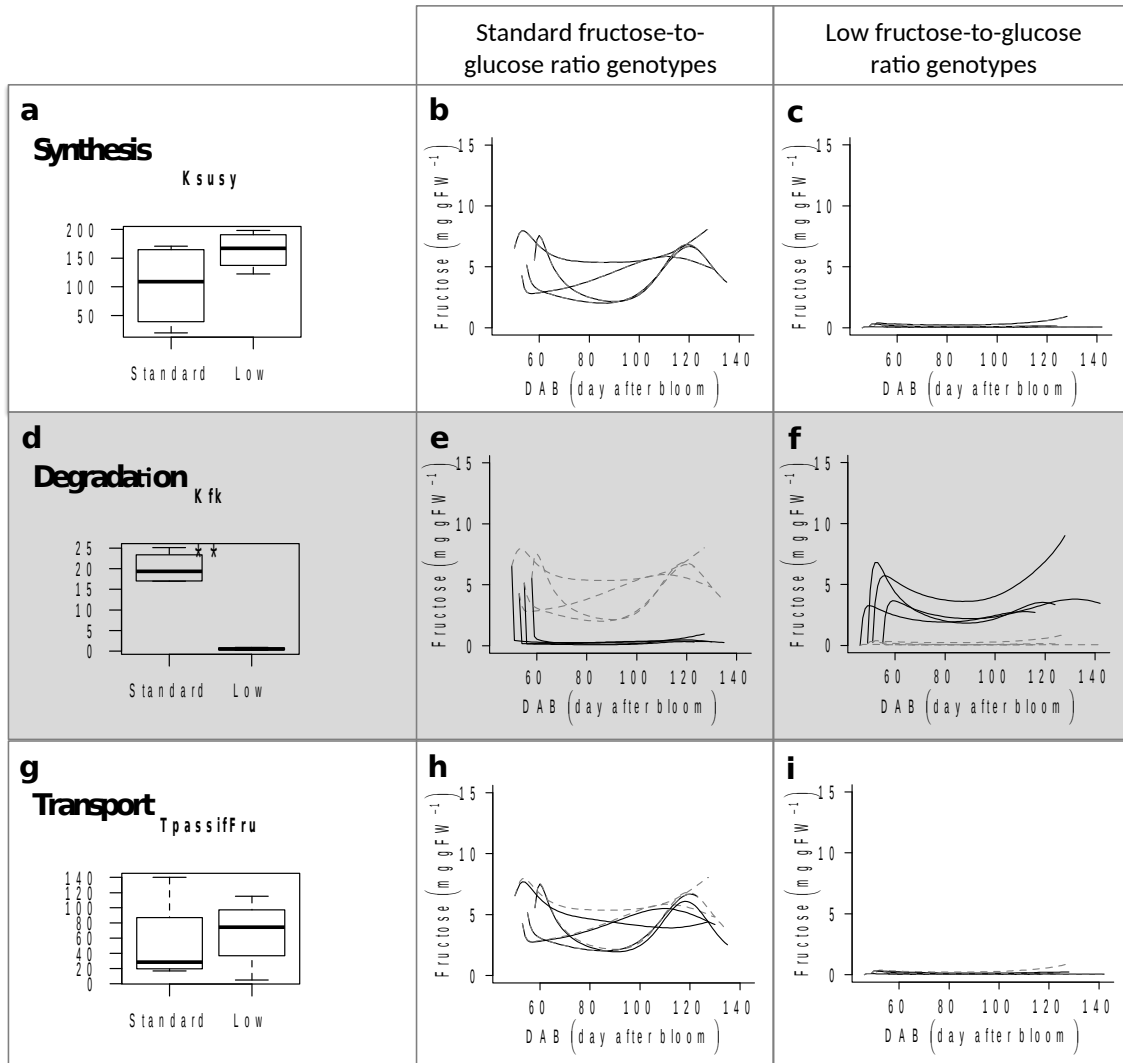


Fig. 1. Observed and simulated concentrations (mg gFW^{-1}) of sucrose, glucose, fructose and sorbitol during the fruit development (DAB, day after bloom) for four different genotypes with the ‘standard fructose-to-glucose ratio’ phenotype (left) and four different genotypes with the ‘low fructose-to-glucose ratio’ phenotype (right). Symbols and bars correspond to mean and standard deviation of experimental data from Desnoues et al. (2014). Lines correspond to the model simulation displaying the minimum cost function. Sucrose concentration is represented by diamonds and dark gray lines, glucose concentration is represented by points and gray dotted lines, fructose concentration is represented by cross and black lines and sorbitol concentration is represented by plus signs and light gray lines.



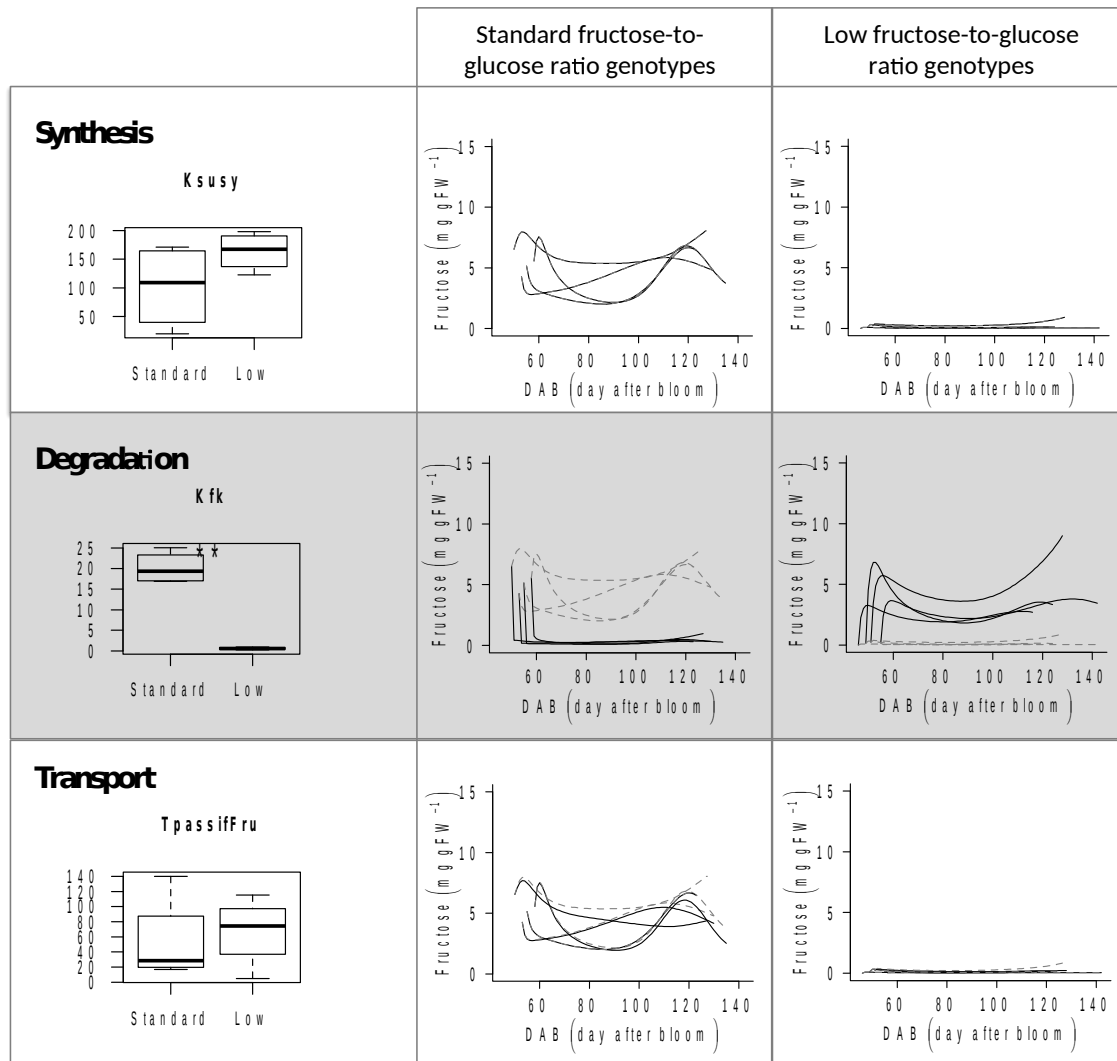


Fig. 2. The three functioning hypotheses to explain the low fructose-to-glucose ratio phenotype are a modification of the fructose synthesis, degradation or transport. a, d and g) For the three hypotheses, a box plot represents the variability of the parameter values relative to the hypotheses (K_{susy} , K_{fk} and $T_{passiFru}$ respectively). For each hypotheses, the parameter values estimated for the four genotypes with the ‘standard-fructose-to-glucose’ phenotype correspond to the box named Standard and the four genotypes with the ‘low fructose-to-glucose’ phenotype correspond to the box named Low. ** Significant differences at p -value < 0.01 (Student’s t-test between the two fructose types). b, e and h) Solid black lines correspond to fructose concentration (mg gFW^{-1}) during fruit development (DAB, days after bloom) corresponding to simulations of the four genotypes with the ‘standard-fructose-to-glucose ratio’ phenotypes with average values of the parameters corresponding to the hypothesis (K_{susy} , K_{fk} and $T_{passiFru}$ respectively) estimated from the genotypes with the ‘low fructose-to-glucose ratio’ phenotype. Gray

dotted lines correspond to the original fructose concentration simulations.). c, f and i) Solid black lines correspond to fructose concentration (mg gFW^{-1}) during fruit development (DAB, days after bloom) corresponding to simulations of the four genotypes with the 'low fructose-to-glucose ratio' phenotypes with average values of the parameters corresponding to the hypothesis (Ksusy, Kfk and TpassiFru respectively) estimated from the genotypes with the 'standard-fructose-to-glucose ratio' phenotype. Gray dotted lines correspond to the original fructose concentration simulations.