Supporting Information

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SI Text

Modeling. The intramolecular isotope composition ($\delta^{13}C$) was modeled using a steady-state approach ["forward modeling" of Tcherkez et al. (1)]. That is, the model assumes a steady state for both metabolite concentrations and isotope ratios [with the exception of (*i*) leaf metabolites in the dark and (*ii*) accumulated metabolites in sink organs (vacuolar sucrose, starch), see below]. The equations are similar to those in ref. 1, with steady-state isotopic ratios calculated from mass-balance equations. Quite generally, if a compound i is (*i*) consumed by n reactions associated with isotope effects (denoted as α_k, $k \in [1, \ldots, n]$) and (*ii*) comes from m reactions associated with isotope effects (denoted as β_j, $j \in [1, \ldots, m]$), we have (isotopic steady state)

$$R_i \sum_{k=1}^{n} \frac{F_k}{\alpha_k} = \sum_{j=1}^{m} \frac{G_j R_j}{\beta_j}, \qquad [S1]$$

where F_k and G_j are the fluxes associated with reactions consuming and producing i, respectively. R_js are the isotope ratios of substrates of reactions producing i. The steady state applied to concentrations is such that

$$\sum_{k=1}^{n} F_k = \sum_{j=1}^{m} G_j. \qquad [S2]$$

The model starts from the production of photosynthates by the Calvin cycle, which produces triose phosphates (glyceraldehyde-3-phosphate and dihydroxyacetone phosphate) and fructose-6-phosphate. In the present paper, new equations were added to the model of Tcherkez et al. (1) to account for (*i*) the isomerization of fructose to glucose to synthesize leaf transitory starch in chloroplasts, (*ii*) the isomerization between fructose and glucose to synthesize leaf sucrose in the cytoplasm, and (*iii*) the reactions that interconvert sugars in sink organs (Fig. 3, main text). For simplicity, the compounds are abbreviated as follows:

Compound	Abbreviation
Ribulose-1,5-bisphosphate	RuBP
Glyceraldehyde-3-phosphate	G3P
Dihydroxyacetone phosphate	DHAP
Fructose-6-phosphate	F
Fructose-1,6-bisphosphate	FBP
Glucose-6-phosphate	G
Sucrose (fructosyl and glucosyl moieties)	S (SF and SG)

Eqs. **S7**–**S9** below use isotope ratios ($^{13}C/^{12}C$) and reciprocal isotope effects ($1/\alpha$). Isotope ratios are denoted as [$X - Ck$], where X is the compound and k is the atom position of interest. Reciprocal isotope effects are simpler to use in numerical calculations because they simply multiply to isotope ratios. The main parameters used are described in the following table:

Parameter	Description
Φ	Glycine production flux in % of carboxylation ($\Phi = v_o/v_c$)
T	Flux of transitory starch synthesis in leaves
R^*	Isotope ratio of CO_2 fixed to RuBP by Rubisco
L	Proportion of sucrose produced in the light in source sucrose used for storage in sink organ
a_2	Reciprocal thermodynamic isotope effect of aldolase in C-2 of FBP
a_3	Reciprocal thermodynamic isotope effect of aldolase in C-3 of FBP
a_4	Reciprocal thermodynamic isotope effect of aldolase in C-4 of FBP
$i_1, \ldots, i_6$	Reciprocal thermodynamic isotope effect of glucose isomerase in C-1, … , C-6 during fructose conversion to glucose
$z_1, \ldots, z_6$	Reciprocal kinetic isotope effect of invertase in C-1, … , C-6 in the fructosyl moiety of sucrose during sucrose hydrolysis
g	Isotope kinetic fractionation of glycine decarboxylase (photorespiration)
s	Commitment of FBP to aldolase cleavage (glycolysis) in wheat grains (i.e., proportion of FBP molecules not converted back to glucose but consumed by glycolysis)
x	Fraction of source sucrose cleaved by invertase in beet root
ξ	Fraction of FBP cleaved by aldolase (glycolysis) in beet root
θ	Commitment of source sucrose to invertase-catalyzed cleavage in wheat grains

We further denote by μ_k (for $k = 1, \ldots, 6$) the quotient

$$\mu_k = \frac{1 + \Phi + 3T}{1 + \Phi + 3Ti_k} \qquad [S3]$$

and A, B, and C, given by the expressions

$$A = \left(1 + \frac{\Phi}{2} - \mathrm{T}\right) + a_4\left(T + \frac{1+\Phi}{3}(1-\mu_4)\right) - \frac{1}{3}\frac{\Phi}{1 + \frac{1}{1+g}}\left((1+\mu_2)a_2\tilde{a}_2B + a_3\tilde{a}_3\mu_3\right) \qquad [S4]$$

$$B = \frac{\frac{1}{3}a_3\tilde{a}_3\left(\mu_3 + \frac{\Phi}{2}\right) + (1+\mu_1)C\frac{\Phi}{6}}{2 + \frac{3}{2}\Phi - \frac{1}{3}a_2\tilde{a}_2(1+\mu_2) - \frac{1}{3}(1+\Phi)(2+\mu_5)} \qquad [S5]$$

$$C = \frac{\frac{1}{3}\left(1 + \frac{\Phi}{2}\right)a_3\tilde{a}_3}{2 + \frac{3}{2}\Phi - (1+\Phi)\frac{2+\mu_6}{3} - \frac{1}{3}\left(1 + \frac{\Phi}{2}\right)(1+\mu_1)}, \qquad [S6]$$

where $\tilde{a}_2$ and $\tilde{a}_3$ are given by $3(1 + \Phi/2)/[2a_2 + 1 + \Phi(2a_2 - 1/2)]$ and $3(1 + \Phi/2)/[2a_3 + 1 + \Phi(2a_3 - 1/2)]$, respectively.

Starch and Sucrose in Leaves. In the steady state, isotope ratios in chloroplastic G3P in leaves (in the light) are given by [G3P-C1] = R^*/A, [G3P-C2] = BR^*/A, and [G3P-C3] = CR^*/A, where A, B, and C are given by Eqs. **S4**–**S6** above. The isotope ratio in carbon positions of starch residues (glucose) and fructosyl and glucosyl moieties in sucrose is then calculated with a

substitution procedure, using the equations of Tcherkez et al. (1) that include the action of isomerization between glucose and fructose. Isotope ratios in source sucrose exported by leaves and imported by grains to synthesize starch are referred to by "source" below. They are calculated as a weighted average of sucrose produced by leaves in the dark (from transitory starch) and in the light (in the cytoplasm from triose phosphates): The proportion of sucrose synthesized in the light is denoted as L.

Starch in Wheat Grains. If s and θ denote the relative commitment of fructose-6-phosphate to glycolytic degradation and the relative commitment of sucrose to invertase-catalyzed cleavage, respectively, we have, for k = 1–6,

$$[G-Ck] = \frac{1}{2-s}\left([SG-Ck\ \ source] + [SF-Ck\ \ source]\frac{i_k(1-s)(\theta z_k+(1-\theta))}{\frac{s}{a_k}+(1-s)i_k}\right). \quad \textbf{[S7]}$$

In Eq. **S7**, the reciprocal isotope effects of aldolization a_1, a_5, and a_6 (in C-1, C-5, and C-6, respectively) are equal to unity (no recognized fractionation at these positions). It should be noted that introducing the equilibration between triose phosphates (G3P and DHAP) and possible subsequent resynthesis of fructose via aldolase does not change isotope ratios eventually calculated in grain starch, because only the net effect of metabolic reactions matters in the framework of this model, in the steady state.

Sucrose in Beet Root. The fraction (commitment) of sucrose cleaved by invertase is denoted as x, the fraction of fructose cleaved by aldolase (glycolysis) is denoted as ξ, and the fraction of fructose recycled to sucrose after invertase-catalyzed cleavage is denoted as b. In other words, if the input (import) of sucrose is denoted as F, the net flux of sucrose and fructose to vacuolar buildup is $(1-\xi)F$ and $b(x-\xi)F$, respectively. x and ξ are <1 and are expressed relative to F and therefore we have $\xi < x$. Eqs. **S8** and **S9** below are both simplified by F, which is an unnecessary variable:

$$[SG-Ck] = \frac{[SG-Ck\ \ source]}{1+(z_k-1)(xb+(1-b)\xi)} \quad \textbf{[S8]}$$

$$[SF-Ck] = \frac{[SF-Ck\ \ source]}{(1-xb)-(1-b)\xi+xz_k-(1-b)(x-\xi)\frac{xz_k}{x+\xi\left(\frac{1}{a_k}-1\right)}}. \quad \textbf{[S9]}$$

Assumptions for Numerical Applications. ***Isotope effects of isomerase and invertase.*** In the present calculations, we take into account the thermodynamic isotope effect of glucose isomerase and the kinetic isotope effect of invertase. The phosphoglucose isomerase reaction is generally believed to be very close to equilibrium although there is some evidence that the isomerization between glucose-6-phosphate and fructose-6-phosphate might not be exactly at equilibrium. In the illuminated chloroplast, there is a net flux to glucose-6-phosphate production to sustain transitory starch synthesis but a mass action ratio between 0.9 and 1.3 has been suggested from experiments, meaning that fructose-6-phosphate production might be favored (the equilibrium constant K_{eq} = [glucose-6-phosphate]/[fructose-6-phosphate] is about 2) (2, 3). Nevertheless, it should be noted that such an actual mass action ratio would have little effect on the effective isotope effect. Classical equations of reaction kinetics indicate that the isotope fractionation would change by only a few per mil at most and keep the same sign, simply because the reaction remains kinetically close to equilibrium (there is much less than one order of magnitude between the equilibrium constant and the actual mass action ratio). We therefore have used the thermodynamic isotope fractionation of glucose isomerase rather than the kinetic isotope fractionation.

Glucose-6-phosphate leaf pool in the night. In the dark, leaf starch is consumed to synthesize sucrose via maltose and glucose-1-phosphate. It is believed that phosphoglucose and phosphofructose accumulate somewhat in the dark so that their associated pools are not in the steady state (4) and glucose-6-phosphate tends to accumulate in the cytosol (2). Glucose-6-phosphate and fructose-6-phosphate are thus likely to freely equilibrate and, in fact, the glucose-6-phosphate to fructose-6-phosphate ratio is near 3. Therefore, calculations of isotope ratios in night sucrose were simply based on the thermodynamic isotope fractionation between glucose-6-phosphate and fructose-6-phosphate, with no steady-state constraint.

Isotope effects of aldolase and transketolase. In the present calculations, we took into account the thermodynamic isotope effect of (trans)aldolase. In fact, the reaction is believed to be close to equilibrium (ref. 2, $\Delta G \sim +1$ kcal/mol); thus, the effective isotope fractionation is thermodynamic (for a specific discussion, see ref. 1). There is presently some uncertainty about the numerical values of fractionation associated with the reaction. In vitro, the enzyme purified from rabbit muscle is associated with a thermodynamic isotope fractionation against ^{12}C of 3.6‰ and 4.9‰ in the C-3 and C-4 positions, respectively, during FBP production (5). However, these published values further indicate that the kinetic isotope effect associated with FBP cleavage is against ^{12}C in the C-4 of FBP (C-1 position of glyceraldehyde-3-phosphate). An inverse isotope effect is very unusual and its origin is still not explained. An artifact during isotopic measurements at this position remains possible. Calculation of isotope effects has further suggested larger values (up to 16‰) in C-4 during FBP production for any input parameters (photorespiration rate) chosen (1). In addition, the thermodynamic isotope effect during acetoin synthesis has been shown to be about 8‰ at the carbonyl position (6), which may correspond here to the C-4 of FBP. Thus, the calculations carried out here used an isotope fractionation against ^{12}C (thermodynamic effect) of 3.6‰ and 10‰ (which is roughly the average of 4.9‰, 8‰, and 16‰) in C-3 and C-4, respectively, during FBP production. To our knowledge, there are no data in the literature on isotope fractionation associated with transketolase. The equilibrium constants of the two transketolase-catalyzed reactions (G3P + F → erythrose-4-phosphate + ribulose-5-phosphate and G3P + sedoheptulose-7-phosphate → ribulose-5-phosphate + xylulose-5-phosphate) are near 0.8 and 0.08, respectively (7) whereas the mass action ratios are about 0.1 and >2, respectively (8). In other words, although the reaction that produces erythrose-4-phosphate is roughly close to equilibrium, this is not true for xylulose-5-phosphate production, which is very far from equilibrium. For the transketolase-catalyzed reaction, we thus believe that the effective isotope fractionation is not thermodynamic but influenced by the kinetic isotope effect. We thus chose an average value of 10‰ against ^{13}C at positions involved in the C–C bond cleaved by the reaction (the fractionation at the position inherited from C-1 of glyceraldehyde-3-phosphate does not appear in the final equations and thus has no importance). These values are within the range of rather similar reactions such as ribulose epimerase (9).

Numerical values of other parameters. The values used and the associated references are tabulated below:

Parameter	Value used here	Reference or source
g	0.020 (= 20‰)	(10)
L	From 0.1 to 0.5[†]	(11–15)
Φ	From 0.35 to 0.5[†]	(16)
T	0.055	(17–19)
θ	0.1	(20, 21)
R^*	0.01092256[‡]	Arbitrary (represents a source carbon 28‰ depleted compared with internal CO_2)
s	0.1	(22)
x	0.03	(23, 24)
b	0.35	(25)
ξ	0.05	(23)

[†]See main text and Fig. 1 legend.

[‡]Note that this value is unimportant because the results are expressed as $\delta^{13}C$ deviations relative to the molecular average.

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