



HAL
open science

Enzyme activity profiles during fruit development in tomato cultivars and *Solanum pennellii*

Marie-Caroline Steinhauser, Dirk Steinhauser, Karin Koehl, Fernando Carrari,
Yves Y. Gibon, Alisdair R. Fernie, Mark Stitt

► **To cite this version:**

Marie-Caroline Steinhauser, Dirk Steinhauser, Karin Koehl, Fernando Carrari, Yves Y. Gibon, et al..
Enzyme activity profiles during fruit development in tomato cultivars and *Solanum pennellii*. *Plant
Physiology*, 2010, 153 (1), pp.80-98. 10.1104/pp.110.154336 . hal-02666951

HAL Id: hal-02666951

<https://hal.inrae.fr/hal-02666951>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Enzyme Activity Profiles during Fruit Development in Tomato Cultivars and *Solanum pennellii*

Marie-Caroline Steinhauser^{*,1}, Dirk Steinhauser¹, Karin Koehl¹, Fernando Carrari², Yves Gibon^{1,3}, Alisdair R. Fernie¹ and Mark Stitt¹

¹Max Planck Institute of Molecular Plant Physiology, Am Muehlenberg 1, 14476 Potsdam-Golm, Germany

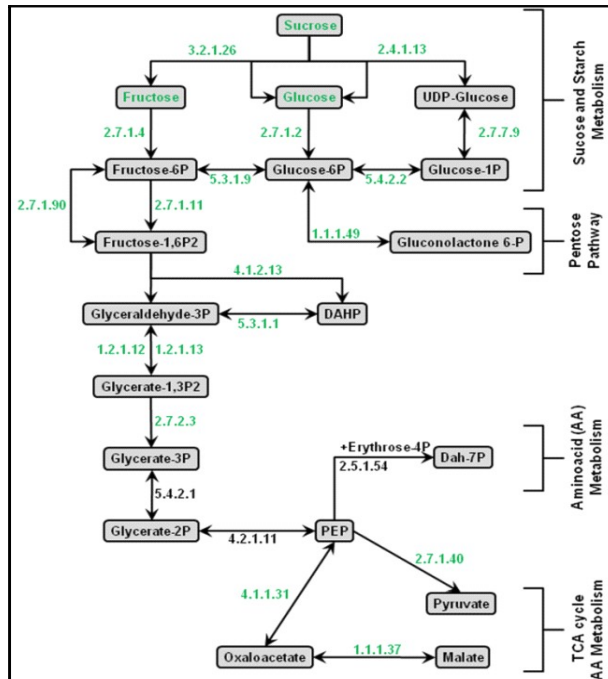
²Instituto de Biotecnología, CICVyA, Instituto Nacional de Tecnología Agropecuaria (IB-INTA), Argentina

³Present address: INRA Bordeaux, University of Bordeaux 1&2, UMR619 Fruit Biology, F-33883 Villenave d'Ornon, France

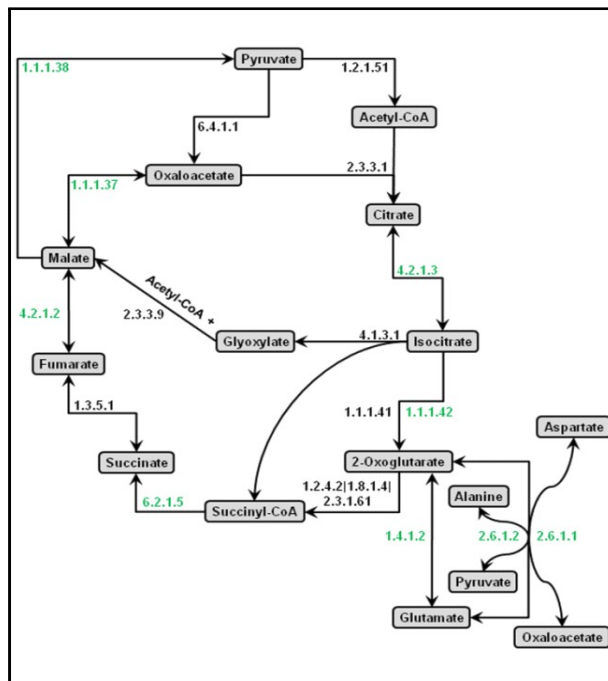
Supplemental Information

Supplementary figures

A, Sucrose and starch metabolism and glycolysis

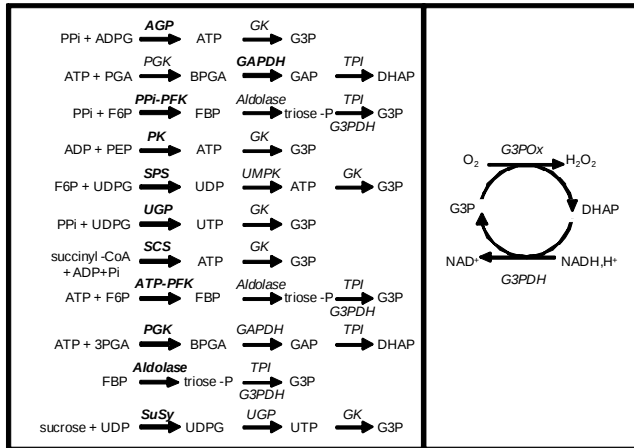


B, TCA cycle

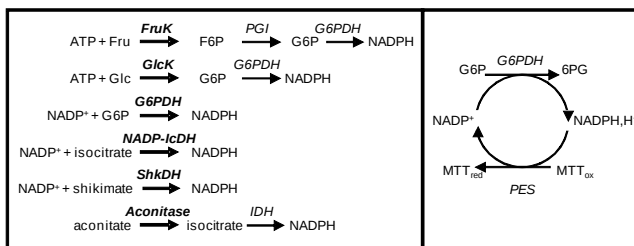


Supplemental Figure S1. Simplified scheme of the carbohydrate metabolism and associated reactions. (A) Starch and sucrose metabolism and glycolysis. (B) TCA cycle. The EC number of the enzymes that were optimized for measurement is depicted as bold text. Some associated pathways are illustrated on the right side of the graph. The sub-cellular compartmentation of certain reactions is ignored. For names of enzymes, see Supplemental Table S1.

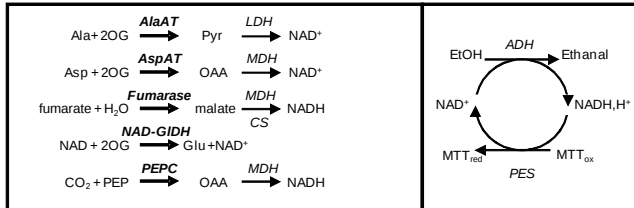
A, Stopped assay,
product determined via a
glycerol-3-P / DHAP
cycling reaction



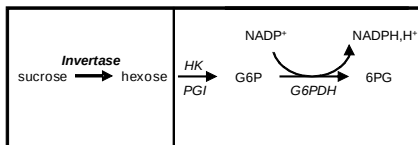
B, Stopped assay, product
determined via a NADP /
NADPH cycling reaction



C, Stopped assay,
product determined via a
NAD / NADH cycling
reaction



D, Stopped assay, product
determined via NADPH
determination



E, Continuous assays

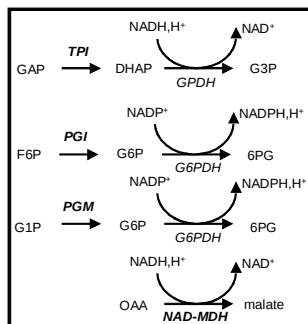


Figure S2. Scheme of the enzyme assays (A) Stopped assay, product determined via a glycerol-3-P / DHAP cycling reaction. (B) Stopped assay, product determined via a NADP / NADPH cycling reaction. (C) Stopped assay, product determined via a NAD / NADH cycling reaction. (D) Stopped assay, product determined via NADPH determination. (E) Continuous assays

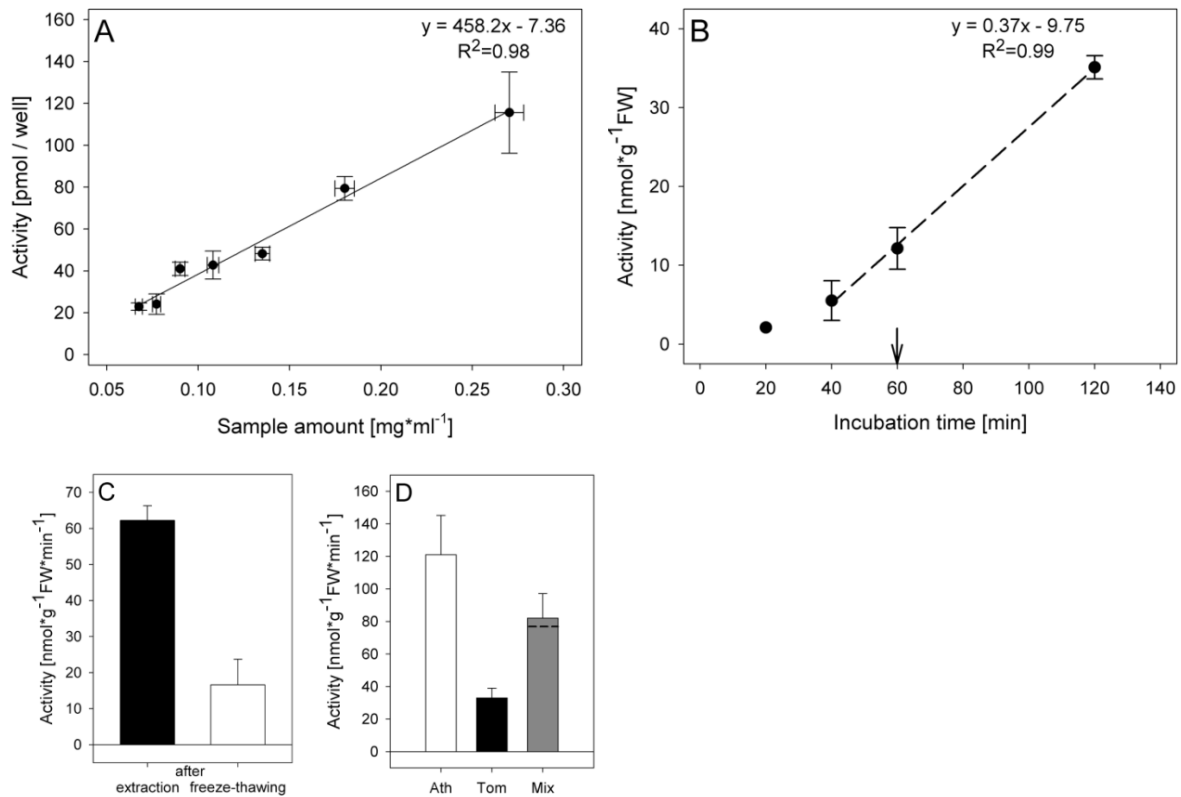
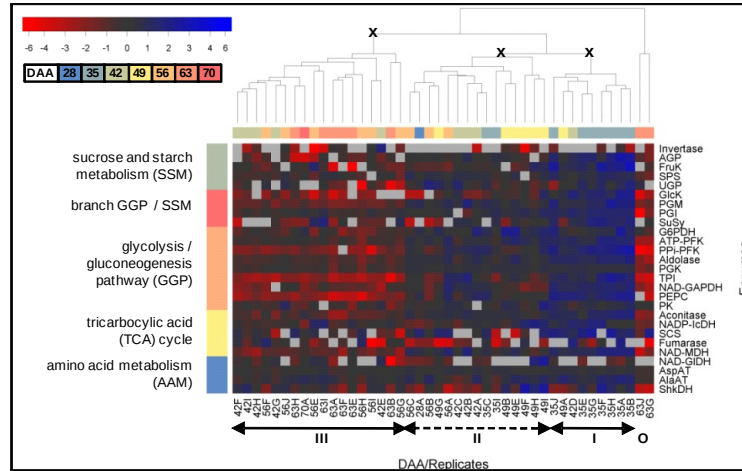
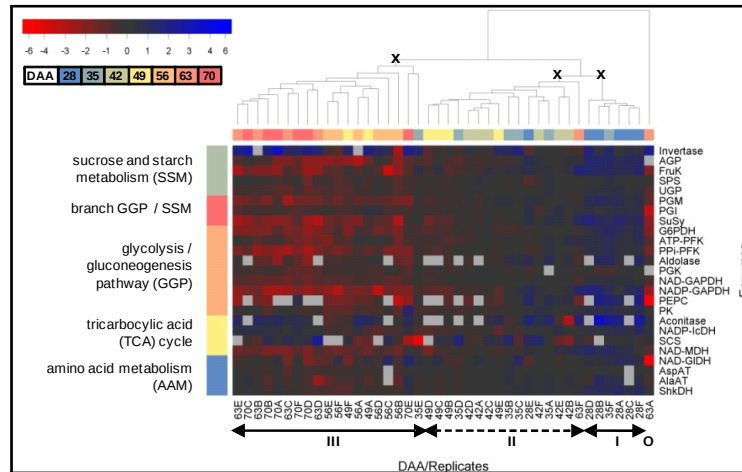


Figure S3. Optimization of the extraction and enzyme assay exemplarily depicted for fructokinase. All plots show fructokinase activity from fully ripe red *S. lycopersicum* 'M82' fruit pericarp (fleshy part) (A) Dependence of fructokinase activity on the amount of the extract in the assay. The regression line represents the range of dilution for which the determined activities are linearly dependent on extract dilution. The black arrow shows the value of the chosen final dilution factor used for all further analyses. (B) Incubation time. Fructokinase activity after an incubation time of 20, 40, 60 and 120 minutes. The blue regression line represents the incubation time range for which the determined activities are linear. The black arrow shows the value of the chosen final incubation time used for all further analyses. (C) Stability to freeze-thaw cycles. Comparison of the maximal enzyme activities of fully ripe red tomato fruit pericarps determined directly after extraction or after a freeze-thaw cycle. Values are given as normalized average values \pm standard error in nmol min⁻¹ g FW⁻¹. (D) Recovery. Activity measured in *A. thaliana* leaf extracts (light grey), tomato fruit extracts (black), and measured (grey) activity of a 1:1 mix. The theoretical value calculated as the average activity of the 1:1 mix is depicted as dotted line in the grey bar of the 1:1 mix.

A, *S. lycopersicum*
cv. M82



B, *S. lycopersicum*
cv. MM



C, *Solanum pennellii*

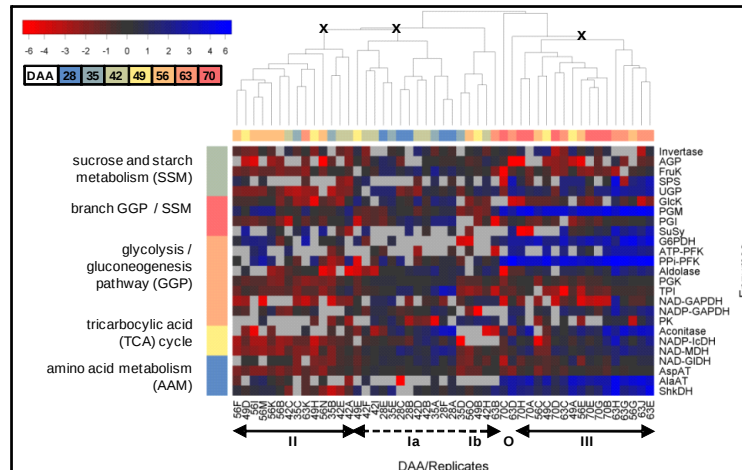


Figure S4. Heat map visualization and cluster tree representation based on enzyme activities, expressed on the fresh weight basis, during tomato fruit developmental from 28 to 70 DAA in a 7-day interval.

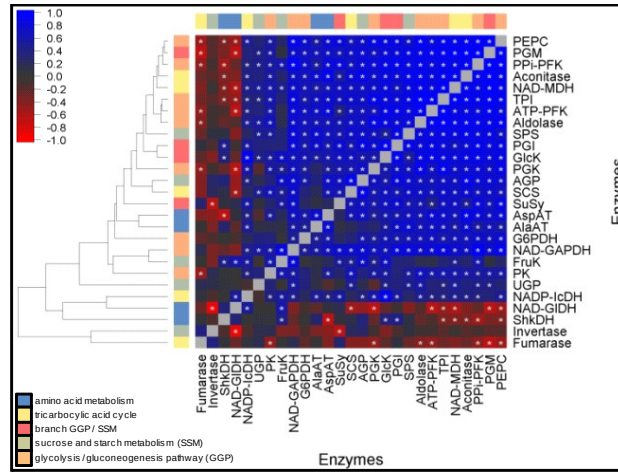
(A) *S. lycopersicum* `M82`,

(B) *S. lycopersicum* `MM`,

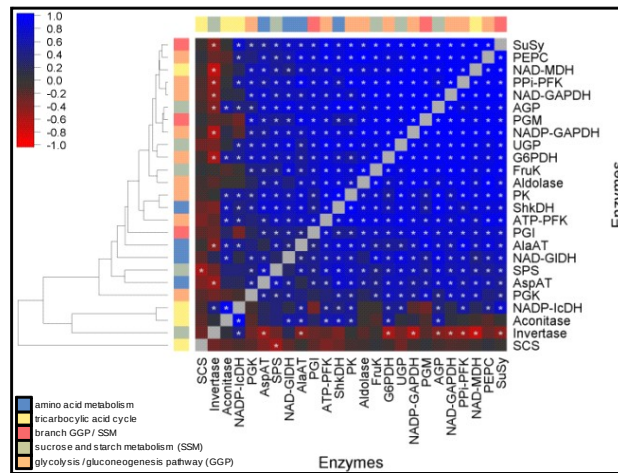
(C) *Solanum pennellii*.

This analysis underlies Table I in the hard copy. The cluster tree was drawn on the basis of the Euclidean distances between DAA samples by using the average linkage cluster algorithm. The heat map was drawn on the basis of the shrunk and outlier-removed normalized data sets for each genotype, comprising 27 enzymes for *S. lycopersicum* 'M82', for 25 for *S. lycopersicum* 'MM' and for 25 for *S. pennellii*. Enzymes are represented by rows and DAA samples by columns. Enzyme activities are shown relative to the average activity at 42 DAA of the respective genotype (cf. Material and Methods); lower activities are colored red, higher activities are blue (see legend in top left corner of each panel) and missing values are colored grey. The color-coded row on the left hand side of the display represents the functional assignment of enzymes to metabolic pathways. The time of harvest is indicated by a number (days DAA), and biological replicates for each DAA as an uppercase letter. The color-coded top column indicates the different DAA groups as drawn in the DAA legend. Cuts were made manually (indicated as 'x') to generate individual clusters, resulting in three main cluster assignments and outlier samples (I, II, III and O, respectively, shown at the bottom of each display).

A, *S. lycopersicum*
cv. M82



B, *S. lycopersicum*
cv. MM



C, *S. pennellii*

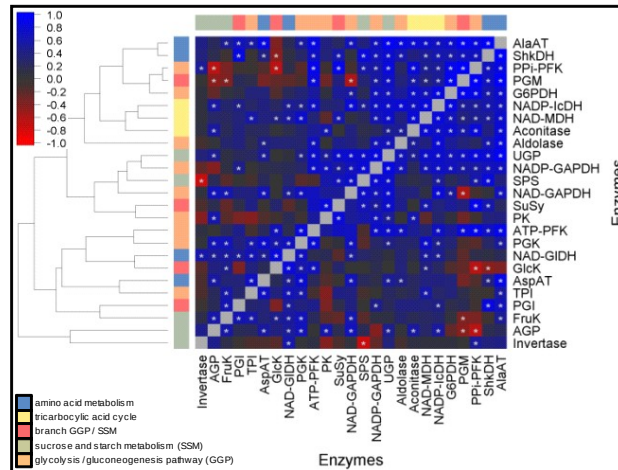


Figure S5. Heat map visualization and cluster tree representation of enzyme correlations according their activity changes during fruit development

(A) *S. lycopersicum* `M82`,

(B) *S. lycopersicum* `MM`,

(C) *Solanum pennellii*.

The heat map was drawn on the basis of obtained robust Pearson's (r) correlation for the shrunk and outlier-removed normalized data sets for each genotype, comprising 27 enzymes for *S. lycopersicum* 'M82', for 25 for *S. lycopersicum* 'MM' and for 25 for *S. pennellii*. The heat map colors indicating the strength of correlation from -1 to 1: negative and positive correlations are colored red and blue, respectively, according to the top left color panel. Significant correlations ($p < 0.05$ with Benjamini-Hochberg correction) are marked by a star in each cell. The cluster tree was drawn on the basis of the distances obtained by $1-r$ by using the average linkage cluster algorithm. The color-coded left-/top-sided row/column represents the functional assignment of enzymes as depicted in the graph.

Supplementary tables

Table S1: Overview of the optimized enzyme assays, their EC number, the abbreviations used in this work and the principle of the assay.

Enzyme	EC number	Abbreviation	Principle of the assay
NAD-malate dehydrogenase	1.1.1.37	NAD-MDH	Continuous
Aconitase	4.2.1.3	Aconitase	Coupled to a NADPH cycle
ADP-glucose pyrophosphorylase	2.7.7.27	AGP	Coupled to a Glycerol-3P cycle
Aspartate aminotransferase	2.6.1.1	AspAT	Coupled to a NAD ⁺ cycle
NAD-glyceraldehyde 3-P dehydrogenase	1.2.1.12	NAD-GAPDH	Coupled to a Glycerol-3P cycle
NADP-glyceraldehyde 3-P dehydrogenase	1.2.1.13	NADP-GAPDH	Coupled to a Glycerol-3P cycle
NAD-dependent glutamate dehydrogenase	1.4.1.3	NAD-GIDH	Coupled to a NAD ⁺ cycle
Acid invertase	3.2.1.26	Invertase	Coupled to sugar determination
Pyrophosphate: fructose 6-P 1-phosphotransferase	2.7.1.90	PPI-PFK	Coupled to a Glycerol-3P cycle
Phosphoglucomutase	5.4.2.2	PGM	Continuous
Pyruvate kinase	2.7.1.40	PK	Coupled to a Glycerol-3P cycle
Sucrose phosphate synthase	2.4.1.14	SPS	Coupled to a Glycerol-3P cycle
Sucrose synthase	2.4.1.13	SuSy	Coupled to a Glycerol-3P cycle
UDP-glucose pyrophosphorylase	2.7.7.9	UGP	Coupled to a Glycerol-3P cycle
Alanine aminotransferase	2.6.1.2	AlaAT	Coupled to a NAD ⁺ cycle
Fructose-1,6-bisphosphate aldolase	4.1.2.13	Aldolase	Coupled to a Glycerol-3P cycle
Fructokinase	2.7.1.4	FruK	Coupled to a NADPH cycle
Fumarate hydratase	4.2.1.2	Fumarase	Coupled to a NAD ⁺ cycle
Glucose 6-P 1-dehydrogenase	1.1.1.49	G6PDH	Coupled to a NADPH cycle
Glucokinase	2.7.1.2	GlcK	Coupled to a NADPH cycle
NADP-Isocitrate dehydrogenase	1.1.1.42	NADP-IcDH	Coupled to a NADPH cycle
Phosphoenol pyruvate carboxylase	4.1.1.31	PEPC	Coupled to a NAD ⁺ cycle
ATP-phosphofructokinase	2.7.1.11	ATP-PFK	Coupled to a Glycerol-3P cycle
Phosphoglucose isomerase	5.3.1.9	PGI	Continuous
Phosphoglycerate kinase	2.7.2.3	PGK	Coupled to a Glycerol-3P cycle
Shikimate dehydrogenase	1.1.1.25	ShkDH	Coupled to a NADPH cycle
Succinyl CoA ligase	6.2.1.5	SCS	Coupled to a Glycerol-3P cycle
Triose phosphate isomerase	5.3.1.1	TPI	Continuous

Table S2: Overview of the enzyme assays, their optimal range of dilution and the dilution factor chosen for this work.

Enzyme	Abbreviation	Optimal range of dilution in $\mu\text{L. mg}^{-1}$ FW	Dilution factor for the experiment in $\mu\text{L. mg}^{-1}$ FW
NAD-malate dehydrogenase	NAD-MDH	10-50	15
Aconitase	Aconitase	25-200	45
ADP-glucose pyrophosphorylase	AGP	25-150	45
Aspartate aminotransferase	AspAT	15-125	45
NAD-glyceraldehyde 3-P dehydrogenase	NAD-GAPDH	25-100	45
NADP-glyceraldehyde 3-P dehydrogenase	NADP-GAPDH	25-200	45
Glutamate dehydrogenase	NAD-GIDH	25-200	45
Acid invertase	Invertase	25-100	45
Pyrophosphate: fructose 6-P 1-phosphotransferase	PPI-PFK	10-80	45
Phosphoglucomutase	PGM	25-200	45
Pyruvate kinase	PK	15-125	45
Sucrose phosphate synthase	SPS	25-150	45
Sucrose synthase	SuSy	25-200	45
UDP-glucose pyrophosphorylase	UGP	25-150	45
Alanine aminotransferase	AlaAT	50-200	150
Fructose-1,6-bisphosphate aldolase	Aldolase	50-300	150
Fructokinase	FruK	100-160	150
Fumarate hydratase	Fumarase	100-400	150
Glucose 6-P 1-dehydrogenase	G6PDH	25-200	150
Glucokinase	GlcK	50-200	150
NADP-Isocitrate dehydrogenase	NADP-IcDH	100-160	150
Phosphoenol pyruvate carboxylase	PEPC	50-300	150
ATP-phosphofructokinase	ATP-PFK	100-400	150
Phosphoglucose isomerase	PGI	50-200	150
Phosphoglycerate kinase	PGK	25-200	150
Shikimate dehydrogenase	ShkDH	50-200	150
Succinyl CoA ligase	SCS	100-160	150
Triose phosphate isomerase	TPI	750-2,500	1500

Table S3: Comparison of the maximal enzyme activity of *S. lycopersicum* 'M82' tomato fruit pericarp harvested at different days after anthesis (DAA). Values are given as normalized average values \pm standard error in nmol min⁻¹ g⁻¹ FW. The colors in the table show the significant changes of enzyme activity compared to the activity at 63 DAA, where light-green colored cells represent significant changes of uncorrected $p < 0.05$ and dark-green colored cells those with Bonferroni corrected significant changes of $p_{adj} < 0.05$.

	35	42	49	56	63
Aconitase	154 \pm 18	71.81 \pm 14.77	94.13 \pm 10.13	66.18 \pm 10.59	30.65 \pm 5.72
AGP	20.76 \pm 3.58	8.22 \pm 2.08	8.06 \pm 0.87	6.14 \pm 0.93	4.53 \pm 0.89
AlaAT	89.2 \pm 12.92	35.77 \pm 2.73	87.06 \pm 10.57	67.68 \pm 0.89	58.35 \pm 7.36
Aldolase	57.42 \pm 6.73	25.62 \pm 4.35	43.7 \pm 4.68	25.96 \pm 4.49	15.13 \pm 2.2
AspAT	72.65 \pm 3.59	60.4 \pm 4.64	70.06 \pm 1.49	63.33 \pm 2.79	54.44 \pm 4.54
ATP-PFK	132 \pm 17	54.76 \pm 6.09	110 \pm 12	68.18 \pm 12.93	41.03 \pm 7.32
FruK	295 \pm 68	74.62 \pm 7.14	100 \pm 26	47.61 \pm 8.13	36.82 \pm 11.29
Fumarase	150 \pm 57	158 \pm 44	131 \pm 84	142 \pm 62	122 \pm 39
G6PDH	86.7 \pm 11.38	39.21 \pm 2.98	136 \pm 29	33.09 \pm 4.9	36.31 \pm 4.79
GlcK	167 \pm 34	46.09 \pm 15.13	76.42 \pm 17.72	14.31 \pm 2.18	19.25 \pm 6.54
Inv	1405 \pm 417	3152 \pm 2309	1344 \pm 443	1887 \pm 769	1416 \pm 550
NAD-GAPDH	130 \pm 3	52.93 \pm 14.27	29.25 \pm 3.97	33.62 \pm 5.97	32.01 \pm 6.04
NAD-GIDH	21.3 \pm 3.2	36.23 \pm 6.61	116 \pm 47	24.42 \pm 7.87	23.47 \pm 9.29
NAD-MDH	5691 \pm 697	1934 \pm 444	3151 \pm 435	1682 \pm 449	1138 \pm 238
NADP-IcDH	147 \pm 29	64.77 \pm 5.16	173 \pm 36	60.23 \pm 4.64	93.75 \pm 24.58
PEPC	435 \pm 54	155 \pm 54	153 \pm 10	37.66 \pm 5.94	20.98 \pm 3.13
PGI	1924 \pm 135	647 \pm 72	1340 \pm 135	912 \pm 66	606 \pm 126
PGK	180 \pm 1	129 \pm 13	164 \pm 6	124 \pm 13	78.55 \pm 10.42
PGM	5085 \pm 687	1448 \pm 312	2235 \pm 256	1535 \pm 344	816 \pm 72
PK	91.01 \pm 10.41	63.77 \pm 7.49	83.27 \pm 13.12	61.69 \pm 7.97	40.69 \pm 6.73
PPi-PFK	335 \pm 42	106 \pm 29	94.92 \pm 13.32	33.45 \pm 5.73	26.76 \pm 6.94
SCS	396 \pm 73	129 \pm 31	209 \pm 66	172 \pm 42	194 \pm 83
ShkDH	50.93 \pm 11.88	45.51 \pm 10.27	118 \pm 43	35.48 \pm 8.36	23.09 \pm 7.06
SPS	249 \pm 24	145 \pm 16	186 \pm 18	143 \pm 28	126 \pm 19
SuSy	86.8 \pm 8.52	35.35 \pm 8.03	26.78 \pm 5.6	30.06 \pm 12.14	24.15 \pm 8.75
TPI	34160 \pm 7725	15073 \pm 5710	15691 \pm 3744	3638 \pm 478	2676 \pm 589
UGP	23308 \pm 4737	11779 \pm 1292	12372 \pm 1416	17154 \pm 4982	11076 \pm 3244

Table S4: Comparison of the maximal enzyme activity of *S. lycopersicum* ‘MoneyMaker’ tomato fruit pericarp harvested at different days after anthesis (DAA). Values are given as normalized average values \pm standard error in $\text{nmol min}^{-1} \text{g}^{-1} \text{FW}$. The colors in the table show the significant changes of enzyme activity compared to the activity at 63 DAA, where light-green colored cells represent significant changes of uncorrected $p < 0.05$ and dark-green colored cells those with Bonferroni corrected significant changes of $p_{\text{adj}} < 0.05$.

	35	42	49	56	63
Aconitase	18.25 \pm 6.4	14.92 \pm 4.18	21.37 \pm 5.24	19.49 \pm 5.9	32.3 \pm 3.94
AGP	12.38 \pm 1.69	14.98 \pm 1.92	8.64 \pm 1.48	5.96 \pm 1.82	6.38 \pm 1.53
AlaAT	54.51 \pm 4.72	53.56 \pm 2.81	46 \pm 5.44	32.88 \pm 3.25	42.92 \pm 7.9
Aldolase	51.1 \pm 5.42	33.3 \pm 4.02	26.72 \pm 2.15	20.57 \pm 1.93	22.7 \pm 1.99
AspAT	73.22 \pm 5.09	73.66 \pm 1.65	64.58 \pm 2.74	57.4 \pm 3.59	63.62 \pm 1.44
ATP-PFK	86.02 \pm 13.1	75.2 \pm 4.7	61.48 \pm 5.6	59.04 \pm 12	62.02 \pm 14.13
FruK	131 \pm 6	97.61 \pm 9.29	82.29 \pm 6.92	40.52 \pm 8.25	31.6 \pm 7.88
G6PDH	45.18 \pm 5.18	54.02 \pm 3.05	34.36 \pm 1.14	26.69 \pm 2.98	15.82 \pm 0.46
Invertase	1037 \pm 214	506 \pm 43	900 \pm 256	565 \pm 207	1357 \pm 317
NAD-GAPDH	74.72 \pm 3.05	73.45 \pm 1.84	52.83 \pm 3.73	40.95 \pm 3.14	38.62 \pm 3.47
NAD-GIDH	14.16 \pm 2.15	12.09 \pm 0.62	10.52 \pm 2.02	8.76 \pm 1.28	6.59 \pm 1.89
NAD-MDH	2738 \pm 160	3193 \pm 360	1717 \pm 235	1646 \pm 171	1528 \pm 186
NADP-GAPDH	45.15 \pm 7.78	42.32 \pm 4.39	19.94 \pm 1.18	10.23 \pm 2.05	10.71 \pm 1.42
NADP-IcDH	42.14 \pm 6.51	43.88 \pm 4.85	26.81 \pm 0.81	29.24 \pm 0.8	54.06 \pm 4.75
PEPC	288 \pm 48	242 \pm 48	121 \pm 10	97.21 \pm 7.8	57.75 \pm 21.3
PGI	1014 \pm 49	872 \pm 62	890 \pm 81	730 \pm 52	641 \pm 49
PGK	154 \pm 6	192 \pm 7	155 \pm 9	149 \pm 4	134 \pm 14
PGM	4753 \pm 652	4099 \pm 358	2783 \pm 171	1937 \pm 129	1751 \pm 312
PK	79.87 \pm 12.1	66.04 \pm 4.2	49.25 \pm 3.63	48.69 \pm 5.56	65.7 \pm 6.18
PPi-PFK	137 \pm 11	145 \pm 7	89.18 \pm 8.72	64.63 \pm 8.02	38.5 \pm 5.46
SCS	317 \pm 116	228 \pm 74	300 \pm 4	227 \pm 95	297 \pm 65
ShkDH	27.62 \pm 3.76	23.99 \pm 1.02	19.37 \pm 1.31	17.45 \pm 1.15	21.39 \pm 1.9
SPS	140 \pm 17	121 \pm 6	101 \pm 6	99.43 \pm 10.63	113 \pm 4
SuSy	108 \pm 15	90.99 \pm 6.59	50.21 \pm 5.11	35.06 \pm 5.67	22.6 \pm 4.54
UGP	14623 \pm 1327	14370 \pm 587	11127 \pm 885	10311 \pm 864	8143 \pm 409

Table S5: Comparison of the maximal enzyme activity of *S. pennellii* tomato fruit pericarp harvested at different days after flowering (DAF). Values are given as normalized average values \pm standard error in nmol min⁻¹ g⁻¹ FW. The colors in the table show the significant changes of enzyme activity compared to the activity at 63 DAF, where light-green colored cells represent significant changes of uncorrected $p < 0.05$ and dark-green colored cells those with Bonferroni corrected significant changes of $p_{adj} < 0.05$.

	35	42	49	56	63
Aconitase	44.07±20.46	17.42±6.13	5.67±1.19	41.56±20.44	82.6±31.32
AGP	24.02±6.69	24.4±7.15	11.43±3.77	2.87±0.94	8.68±1.38
AlaAT	6.48	5.49±1.52	28.27±15.06	17.18±5.6	38.7±10.72
Aldolase	16.51±5.98	12.06±4.46	17.66±1.19	12.22±3.01	19.74±4.12
AspAT	41.69±11.29	57.4±5.55	38.63±5.24	28.81±3.92	39.51±6.05
ATP-PFK	4.3	12.01±1.37	25.66±20.4	33.77±11.61	64.15±6.75
FruK	56.24±16.43	35.89±4.18	31.45±4.61	23.49±3.75	23.32±6.78
G6PDH	2.73±1.32	3.29±0.75	8.38±0.92	6.3±1.54	19.44±4.88
GlcK	57.17±27.06	33.31±7.33	24.24±8.17	26.44±4.15	14.64±3.91
Invertase	322±50	568±190	878±271	435±61	544±147
NAD-GAPDH	30.79±12.08	76.16±28.12	58.34±18.1	29.68±8.93	55.01±14.45
NAD-GIDH	32.19±14.93	27.46±4.33	20.04±3.36	27.42±4.4	36.28±4.43
NAD-MDH	2333±1100	2077±272	1149±191	535±50	3874±971
NADP-GAPDH	21.36±5.72	15.73±2.73	15.08±3.51	18.59±3.02	39.21±9.44
NADP-IcDH	18.18±1.72	36.26±9.71	14.86±3.7	7.28±1.25	61.05±16.58
PGI	1561±594	1073±252	441±110	506±29	915±226
PGK	98.28±31.72	121±13	92.86±13.85	56.32±7.12	103±16
PGM	106±7	98.57±16.46	212±110	479±124	1412±363
PK	68.14±34.54	56.6±21.88	74.55±26.53	45.46±16.53	101±16
PPi-PFK	1.33±0.3	1.62±0.28	15.42±7.83	10.95±2.6	53.95±12.37
ShikDH	3.73±1.53	2.52±0.6	3.94±0.9	2.35±0.46	19.8±6.78
SPS	40.2	20.55±8.99	18.64±4.42	43.38±8.14	106±36
SuSy	10.95	7.7±2.74	18.74±8.46	15.35±5.92	34.71±5.75
TPI	4814±1739	9070±1548	5051±734	3498±585	4579±1051
UGP	1209±646	1270±491	1647±434	1302±478	5118±974

Table S6: Overview of complete (cpl) and shrunk (shr) enzyme activity datasets for the different cultivars and species under investigation. The number of samples (n_{DAP}) and enzymes (n_{Enzymes}) as well as the number of measured ($n_{\text{Measurements}}$) and missing (n_{Missing}) values are displayed. The number of samples for each DAA (n/DAA) is described as list corresponding to 28, 35, 42, 49, 56, 63, and 70 DAA, respectively. Moreover, not determined (n.d.) or fully excluded enzymes (removed) are listed at the table bottom. All enzyme activities in the complete data sets are expressed on the basis of the fresh weight. The shrunk data sets were normalized by the average activity of 42 DAA and the corresponding ratios were log base 2 transformed.

Species	<i>S. lycopersicum</i> cv. M82		<i>S. lycopersicum</i> cv. MM		<i>S. pennellii</i>	
Dataset	M82cpl	M82shr	MMcpl	MMshr	PENcpl	PENshr
n_{DAP}	44	44	42	42	64	49
n_{Enzyme}	28	27	26	25	26	25
$n_{\text{Measurements}}$	1105 (89.7%)	1090 (91.8%)	1026 (94%)	1005 (95.7%)	1227 (73.7%)	1012 (82.6%)
n_{Missing}	127 (10.3%)	98 (8.2%)	66 (6%)	45 (4.3%)	437 (26.3%)	213 (17.4%)
n/DAA	1,9,9,7,9,8,1	1,9,9,7,9,8,1	6,6,6,6,6,6,6	6,6,6,6,6,6,6	6,5,9,8,16,11,9	5,5,8,6,10,8,7
n.d.			TPI, GlcK		Fumarase, PEPC	
removed		NADP-GAPDH		Fumarase		SCS

Table S7. Correlation matrix, depicted as signed probabilities, between enzymes and metabolites during fruit development in *S. lycopersicum* 'MM'. All pairwise Pearson correlations were calculated between enzyme activities (see Supplemental Table S4) and metabolites Carrari et al. (2006). The values depicted represent the signed p-values, i.e. probabilities of negative correlations are given as negative p-values, whereas the probabilities of positive correlations are depicted as positive (without sign) p-values. The parameters are grouped with enzyme activities on the left/upper section and metabolites in the right/lower section. Sectors corresponding to enzyme-enzyme, metabolite-metabolite and enzyme-metabolite pairs are indicated. Color code: for negative correlation: red if $p < -0.01$ and orange if $-0.01 < p < -0.05$, for positive correlation: light blue if $0.01 < p < 0.05$ and dark blue if $p < 0.01$. This is an expanded version of Figure 7 in the hard copy.

See the attached excel file (TableS7)

Table S8. Correlation matrix, depicted as signed probabilities, between enzymes and transcripts during fruit development in *S. lycopersicum* 'MM'. Pairwise correlations were calculated between enzyme activities (see Supplemental Table S3) and transcripts annotated as encoding the enzymes (Carrari *et al.*, 2006). The values depicted represent the signed p-values, i.e. probabilities of negative correlations are given as negative p-values, whereas the probabilities of positive correlations are depicted as positive (without sign) p-values. Color code: for negative correlation: red if $p < -0.01$ and orange if $-0.01 < p < -0.05$, for positive correlation: light blue if $0.01 < p < 0.05$ and dark blue if $p < 0.01$. Sectors corresponding to parameter pairs in which the transcript is annotated as encoding the enzyme are highlighted in grey. Significant correlations are summarized in Table 3 in the hard copy.

See the attached excel file (TableS8)

Table S9. Signed p-values of computed Pearson’s correlation coefficients for all enzyme-metabolite pairs, where the metabolite is a direct substrate or product of the enzyme. The values depicted represent the signed p-values, i.e. probabilities of negative correlations are given as negative p-values, whereas the probabilities of positive correlations are depicted as positive (without sign) p-values.. Significant and positive correlations with $p < 0.05$ are marked yellow whereas $p < 0.01$ labeled red.

name	sucrose	glucose	fructose	glucose 6-P	fructose 6-P	malate	succinate	a-kt-glutarate	aconitate	citrate	isocitrate	glutamate	aspartate	alanine	shikimate
Invertase	- 0.47	- 0.93	- 0.85	0.48	0.62	- 0.17	- 0.40	- 0.17	- 0.50	- 0.24	- 0.32	- 0.97	- 0.90	0.36	0.30
FruK	0.51	0.38	0.65	0.34	0.19	0.80	0.10	0.12	0.13	0.20	0.36	0.15	0.61	0.67	0.34
SPS	0.37	0.52	0.69	0.41	0.37	0.19	0.34	0.02	0.13	0.11	0.15	0.72	0.47	0.98	0.15
PGM	0.08	0.64	0.45	0.47	0.30	0.88	0.05	0.13	0.14	0.05	0.02	0.02	0.23	0.26	0.72
PGI	0.51	0.44	0.32	0.97	0.87	0.61	0.51	0.57	0.66	0.47	0.22	0.48	0.99	0.98	0.73
SuSy	0.46	0.52	0.84	0.44	0.29	0.75	0.11	0.08	0.15	0.18	0.24	0.24	0.50	0.86	0.35
G6PDH	0.56	0.78	0.60	0.90	0.79	1.00	0.54	0.28	0.49	0.29	0.14	0.57	0.81	0.84	0.93
PPi-PFK	0.16	0.84	0.81	0.11	0.05	0.85	0.03	0.08	0.06	0.07	0.08	0.02	0.79	0.16	0.43
ATP-PFK	0.21	0.47	0.42	0.22	0.21	0.00	0.26	0.00	0.01	0.01	0.07	0.93	0.61	0.65	0.00
Aconitase	0.85	0.69	0.31	0.96	0.86	0.04	0.46	0.42	0.60	0.65	0.82	0.05	0.45	0.20	0.18
NADP-IcDH	0.60	0.60	0.52	0.65	0.50	0.28	0.39	0.74	0.79	0.84	0.87	0.05	0.22	0.11	0.43
SCS	0.17	0.61	0.53	0.58	0.61	0.18	0.31	0.24	0.41	0.59	0.45	0.80	0.99	0.61	0.13
NAD-MDH	0.52	0.30	0.46	0.11	0.08	0.46	0.26	0.07	0.07	0.14	0.23	0.39	0.49	0.66	0.19
NAD-GIDH	0.82	0.26	0.18	0.59	0.62	0.90	0.83	0.52	0.79	0.42	0.26	0.74	0.66	0.21	0.97
AlaAT	0.15	0.76	0.77	0.15	0.13	0.02	0.29	0.00	0.01	0.00	0.00	0.51	0.64	0.79	0.05
AspAT	0.17	0.69	0.41	0.46	0.36	0.40	0.07	0.03	0.08	0.02	0.01	0.26	0.92	0.95	0.29
ShkDH	0.88	0.93	0.96	0.93	0.94	0.24	0.97	0.17	0.40	0.20	0.18	0.58	0.56	0.24	0.39

