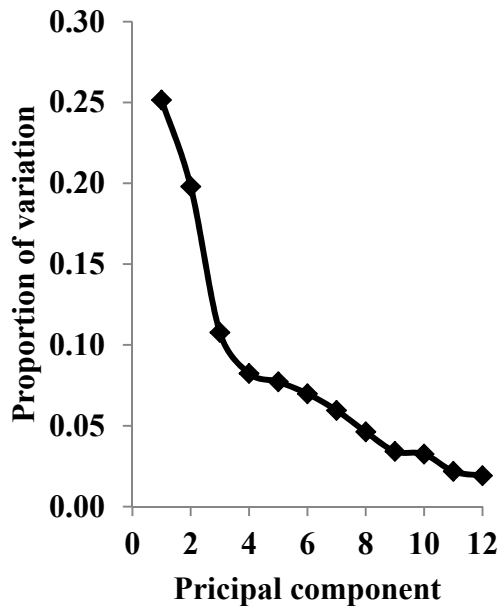


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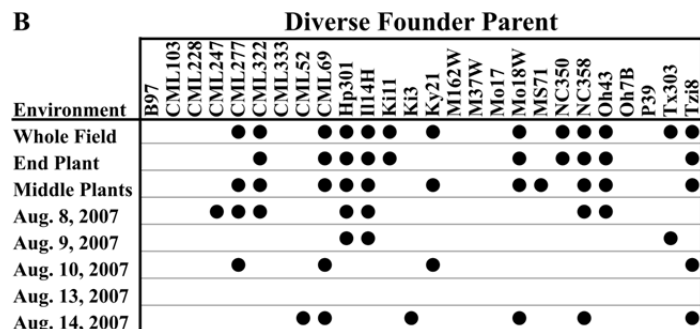
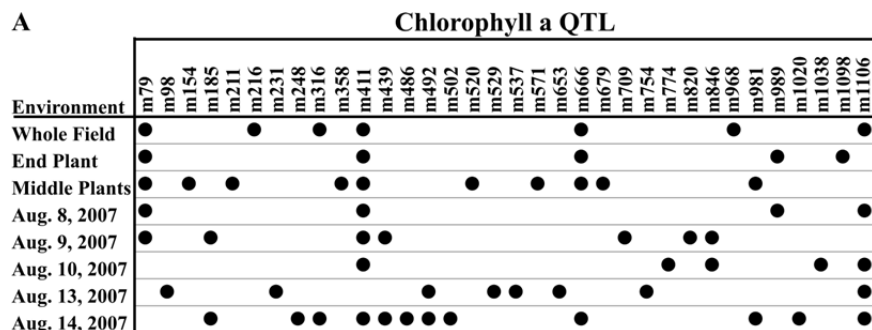
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15 B
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Metabolite	Prin1	Prin2
Chla	0.39	-0.17
Chlb	0.19	0.04
Mala	0.39	0.12
Fuma	0.33	0.25
Glut	0.47	0.15
AA	0.26	-0.06
Prot	0.24	-0.13
Nitr	0.37	-0.05
Star	-0.05	0.49
Sucr	0.18	0.36
Gluc	-0.17	0.48
Fruc	-0.07	0.50
% of variation	25	20

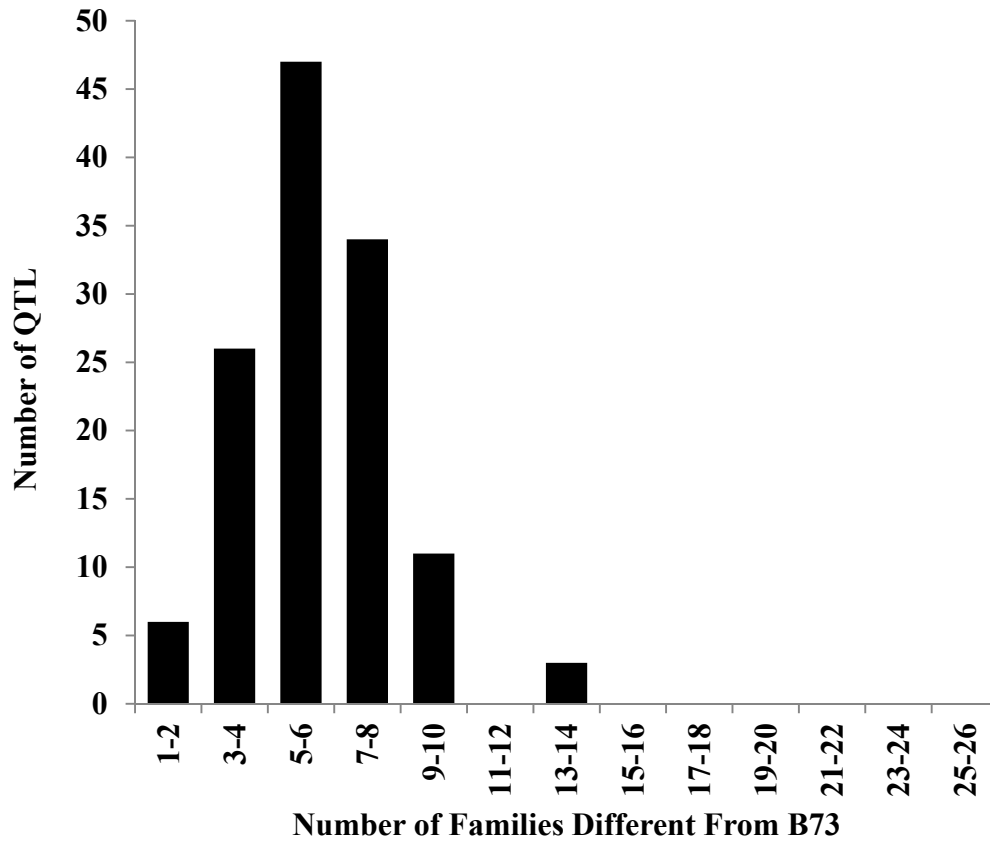
18 **Figure S1.** Principal component analysis of the 12 metabolites. **(A)** Proportion of variation
19 explained by each individual principal component. **(B)** Contribution of different metabolites to
20 the first two principal components. Prin1, first principal component; Prin2, second principal
21 component; Mala, malate; Fuma, fumarate; Glut, glutamate; Chla, chlorophyll a; Chlb,
22 chlorophyll b; Nitr, nitrate; Sucr, sucrose; Gluc, glucose; Fruc, fructose; Star, starch; AA, total
23 amino acids; Prot, protein.

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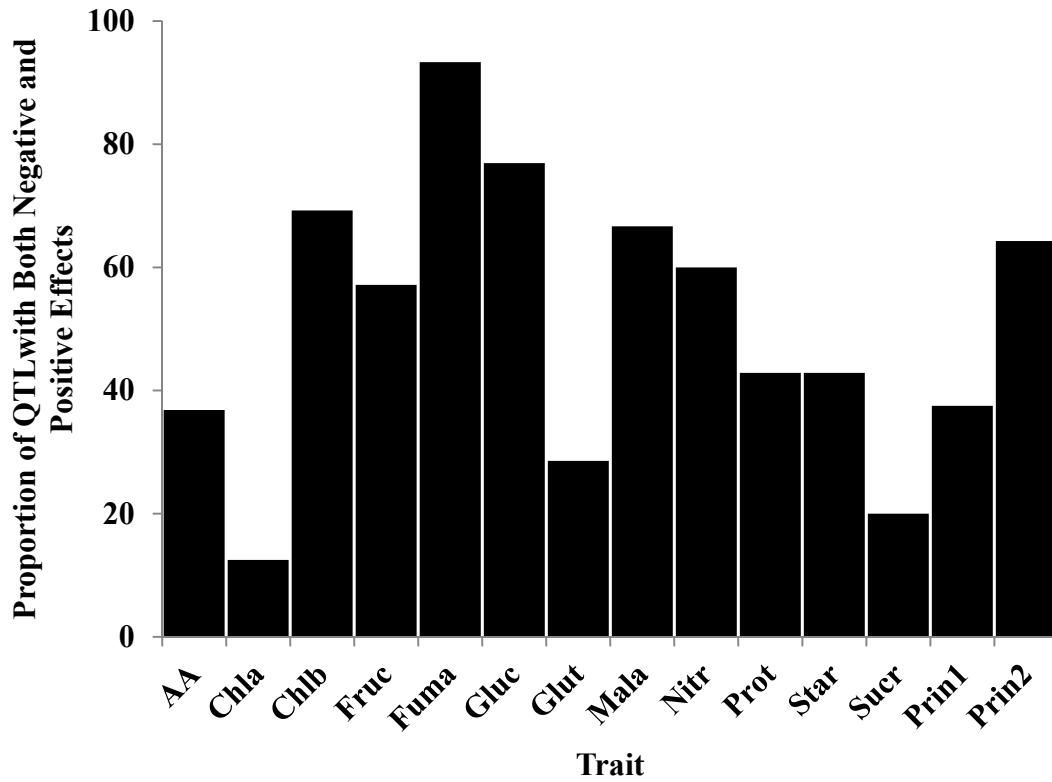
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Figure S2. Sampling effects of subenvironments on QTL discovery. To show how the robustness of QTL discovery, QTL analysis was performed for Chla based on data derived from the whole field or from subenvironments based on the end plant in a row, the middle plants in a row, or the different days of sampling. (A) QTL distribution of Chla derived from different subenvironments. (B) QTL m411 distribution in different NAM families in different subenvironments.



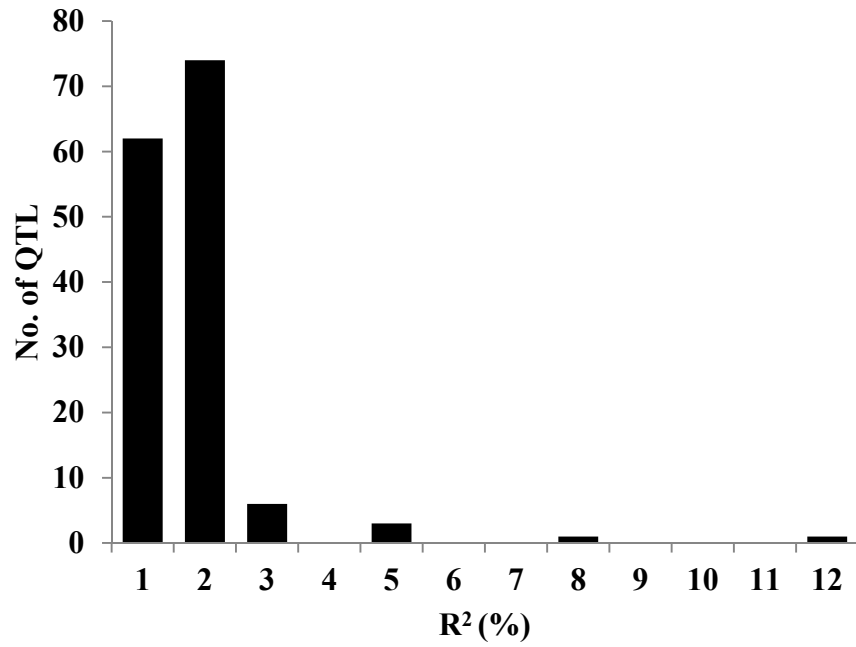
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Figure S3. Distribution of metabolic QTL shared by different families.



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Figure S4. Proportion of metabolite and principal component QTL with both negative and positive effects. Mala, malate; Fuma, fumarate; Glut, glutamate; Chla, chlorophyll a; Chlb, chlorophyll b; Nitr, nitrate; Sucr, sucrose; Gluc, glucose; Fruc, fructose; Star, starch; AA, total amino acids; Prot, protein; Prin1, first principal component; Prin2, second principal component

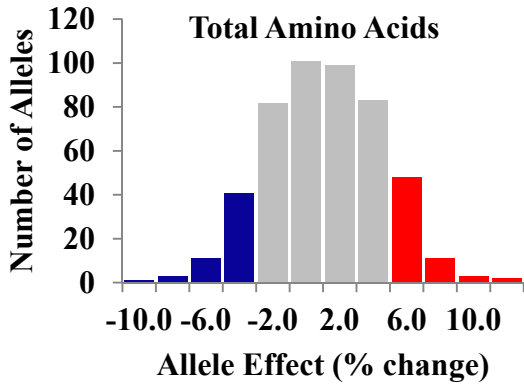


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Figure S5. Histogram of QTL effects for all the 12 metabolites and the first two principal components.

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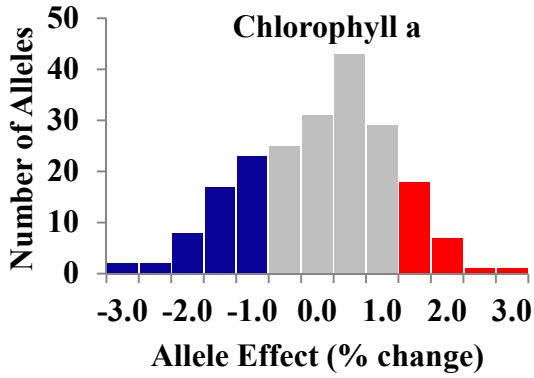
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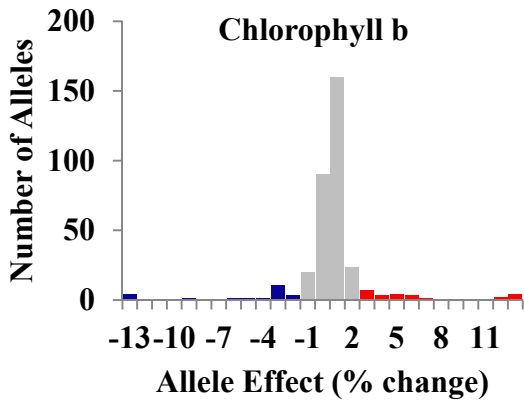
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66 C



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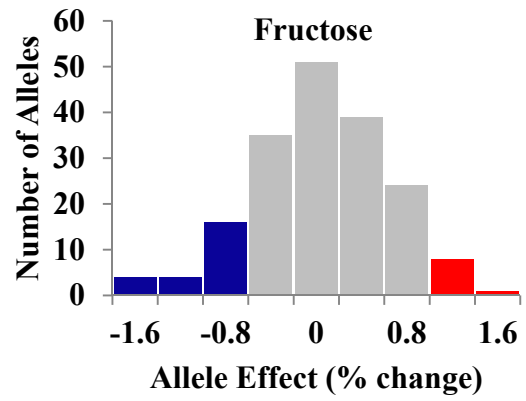
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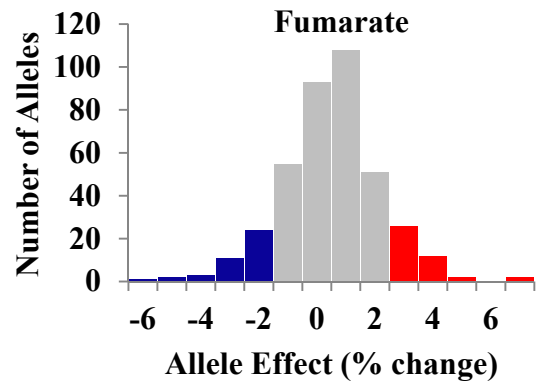
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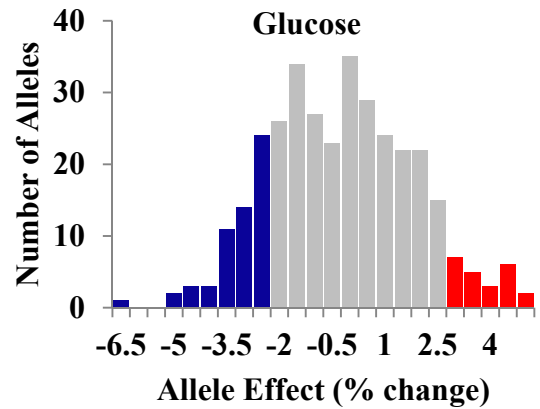
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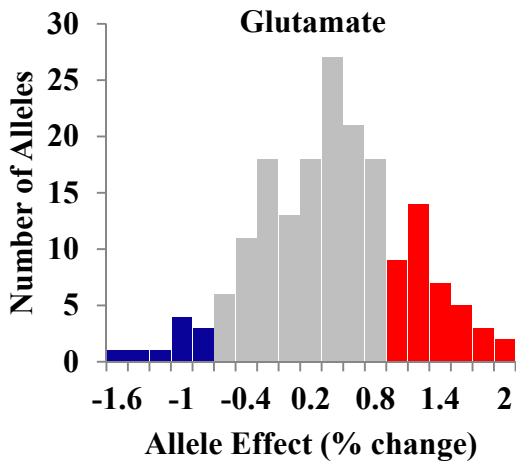
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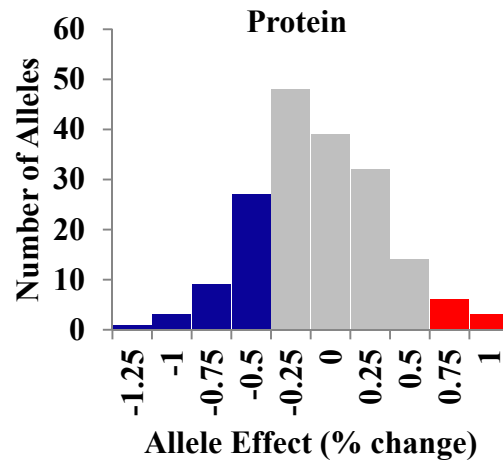


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81 G



89 J



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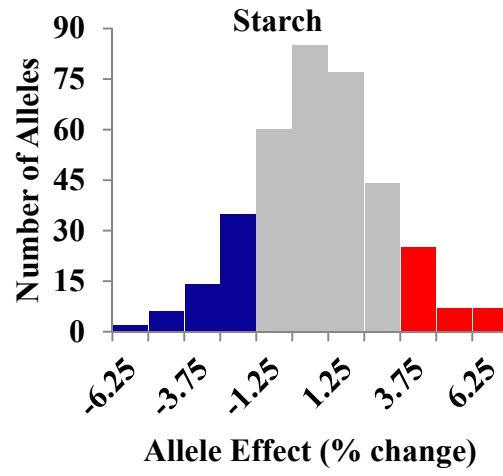
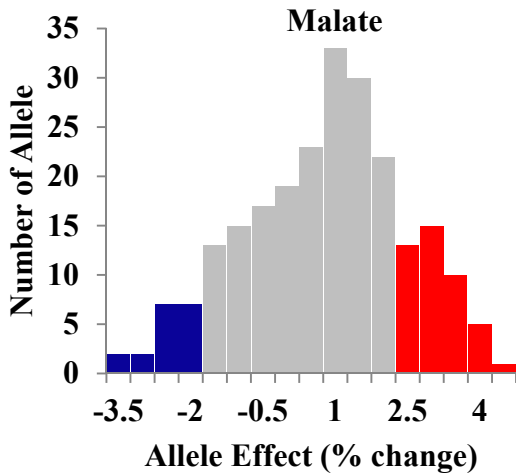
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84 H

92 K



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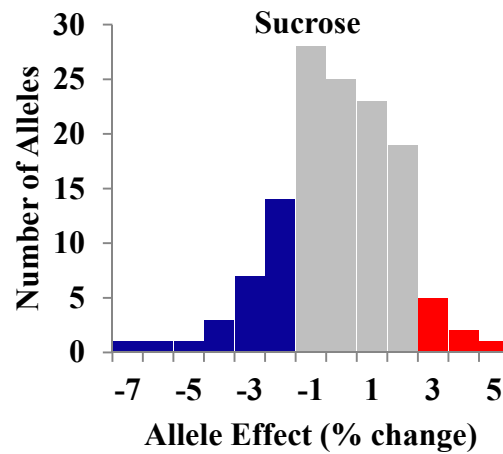
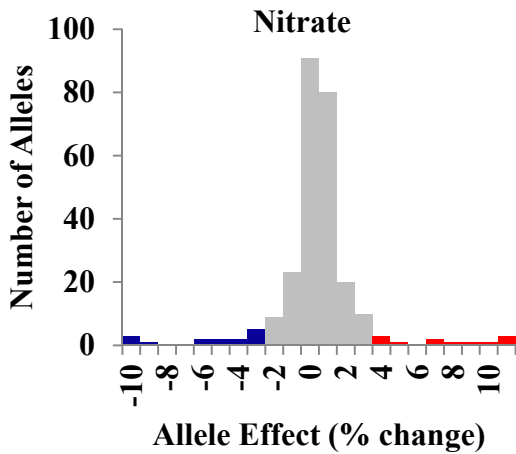
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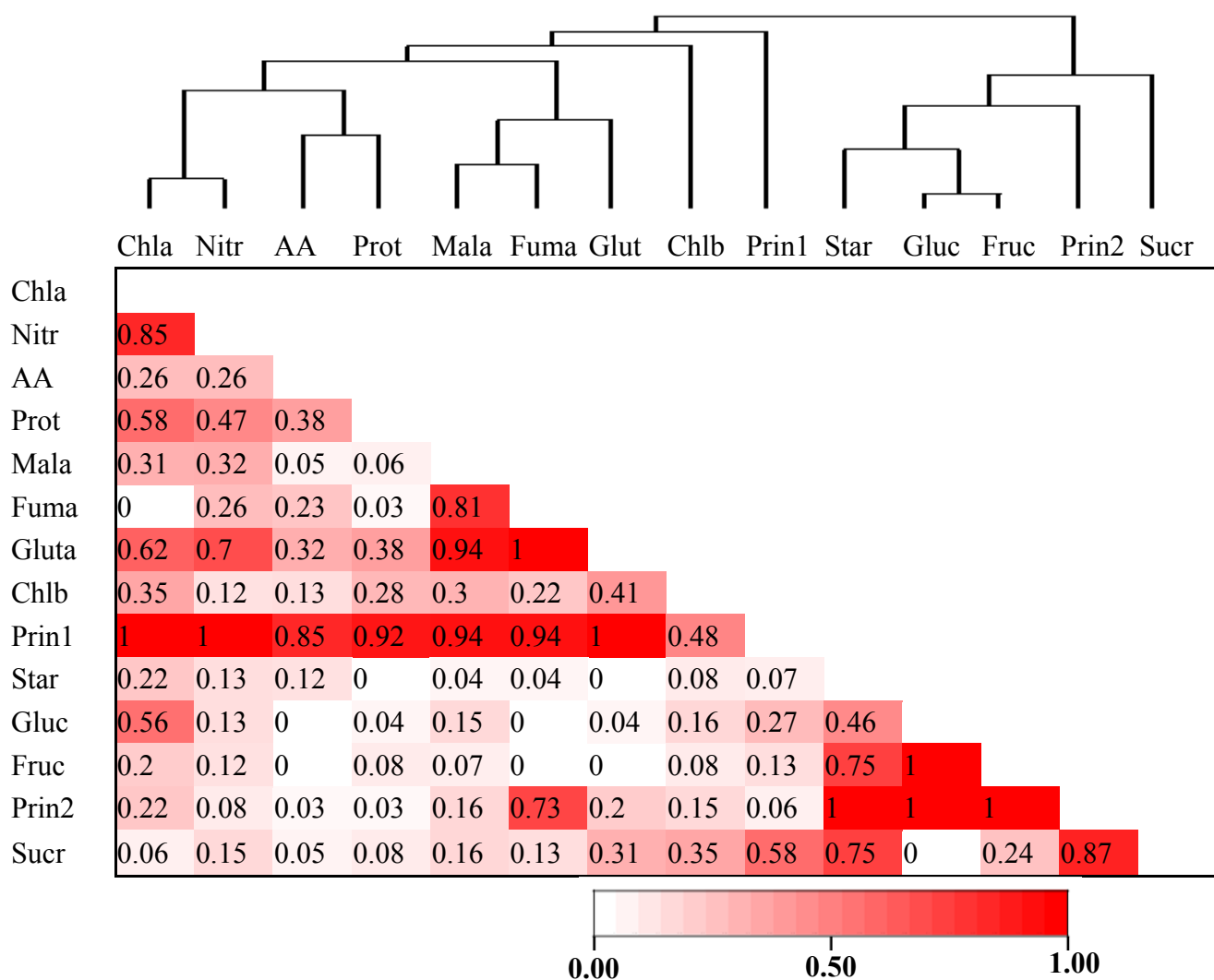
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97 **Figure S6.** Distribution of QTL allele effects for the 12 metabolites. **(A)** Total amino acids. **(B)**
98 Chlorophyll a. **(C)** Chlorophyll b. **(D)** Fructose. **(E)** Fumarate. **(F)** Glucose. **(G)** Glutamate. **(E)**
99 **(H)** Malate. **(I)** Nitrate. **(J)** Protein. **(K)** Starch. **(L)** Sucrose. Most of the allele effects are
100 estimated to be non-significant (grey). The significant positive and negative allele effects are
101 shown in blue and red.
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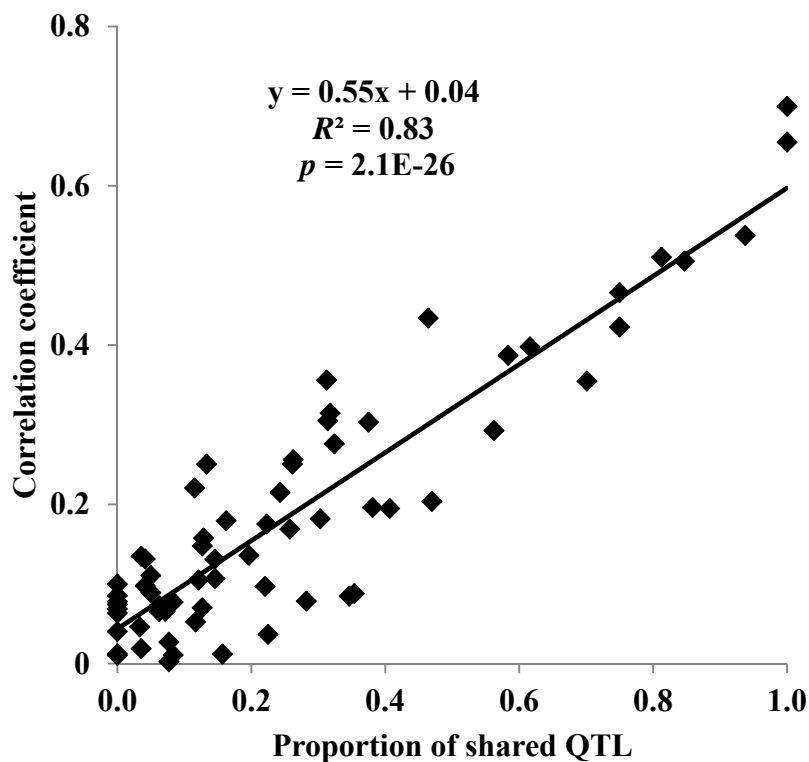
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108 **Figure S7.** Proportion of shared QTL matrix and clusters of the 12 carbon and nitrogen
 109 metabolites and the first two principal components. Mala, malate; Fuma, fumarate; Glut,
 110 glutamate; Chla, chlorophyll a; Chlb, chlorophyll b; Nitr, nitrate; Sucr, sucrose; Gluc, glucose;
 111 Fruc, fructose; Star, starch; AA, total amino acids; Prot, protein; Prin1, first principal component;
 112 Prin2, second principal component

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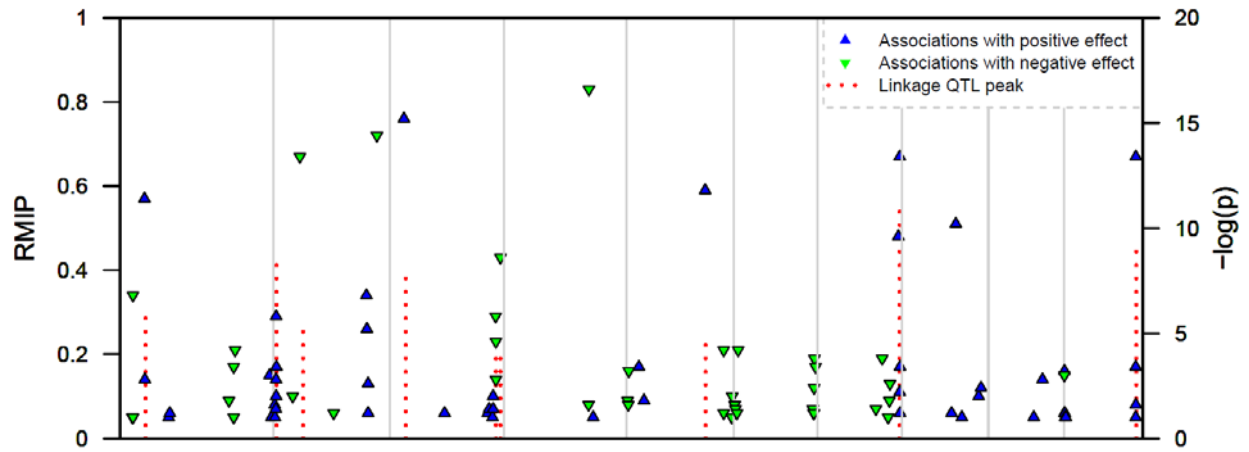
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Figure S8. Relationship between the correlation coefficients of the 12 carbon and nitrogen metabolites and the corresponding proportion of shared QTL. The results demonstrated that metabolites that show correlated changes across the entire NAM population had a significantly higher frequency of shared QTL, which is not the pattern we see in other traits (3, 19).

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A

Malate



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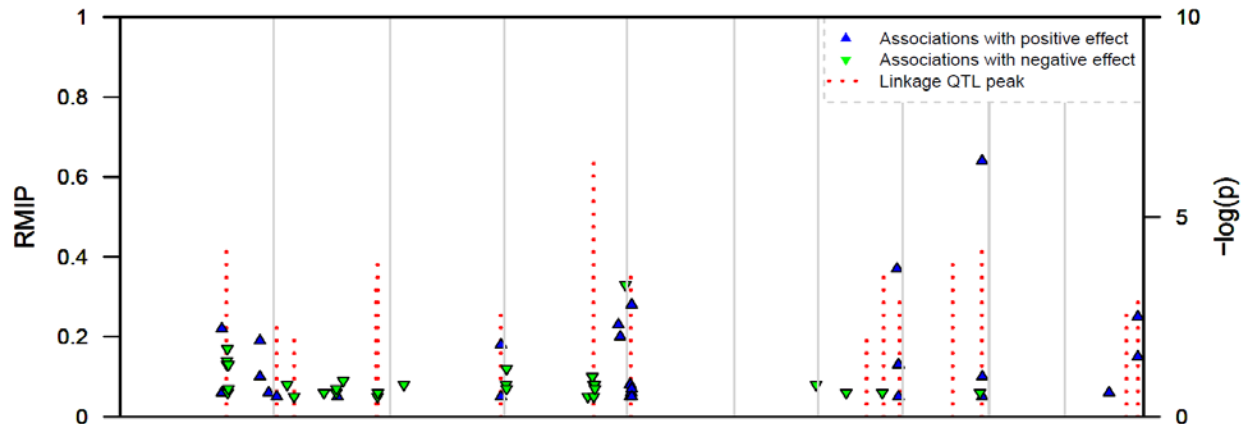
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B

Fumarate



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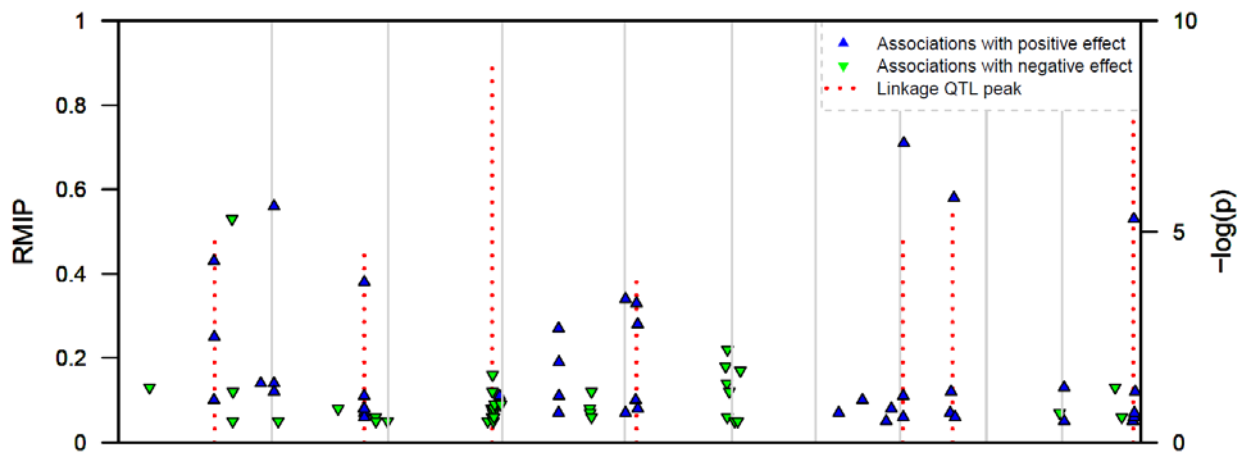
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C

Glutamate



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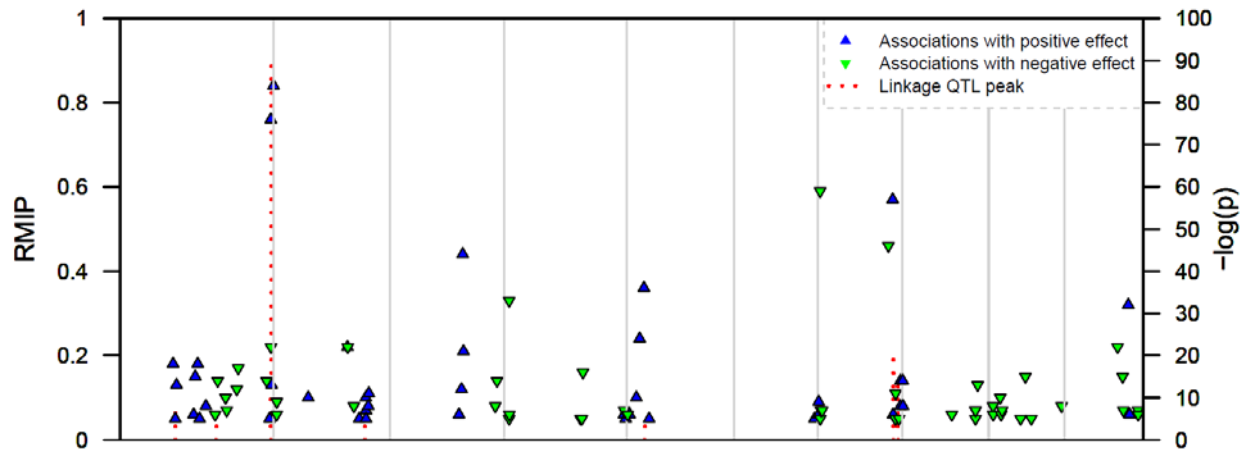
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D

Chlorophyll b



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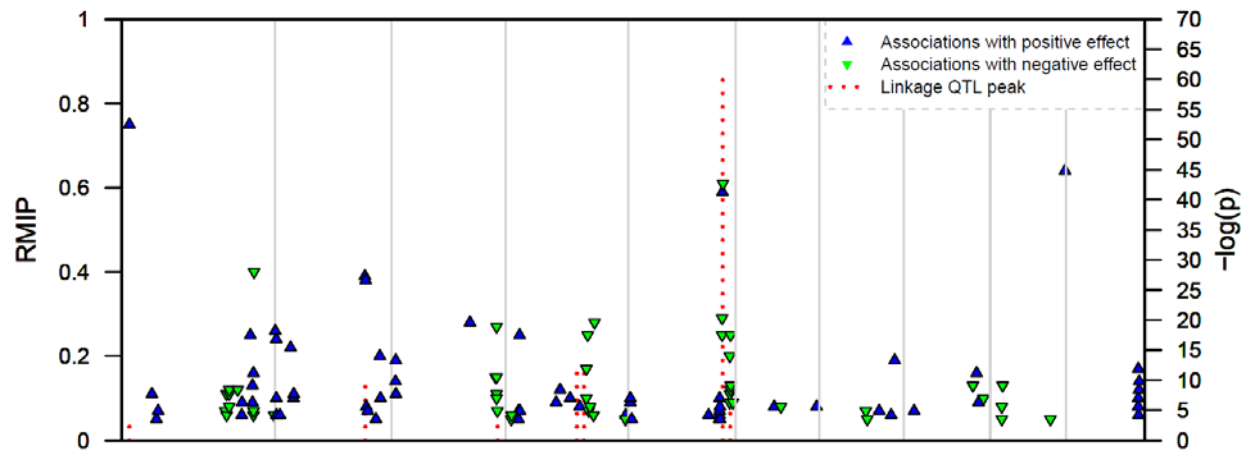
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E

Nitrate



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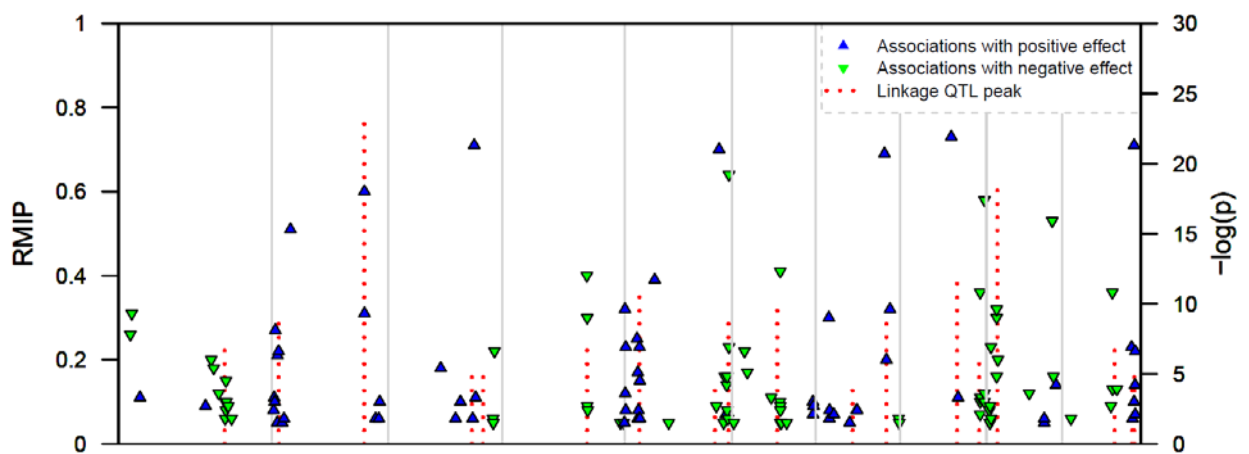
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F

Total Amino Acids



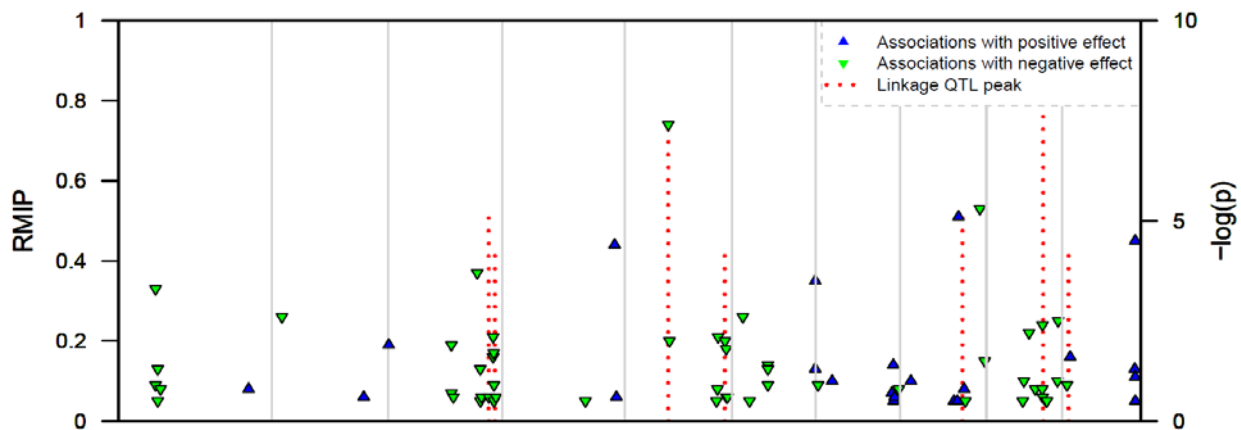
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G

Protein



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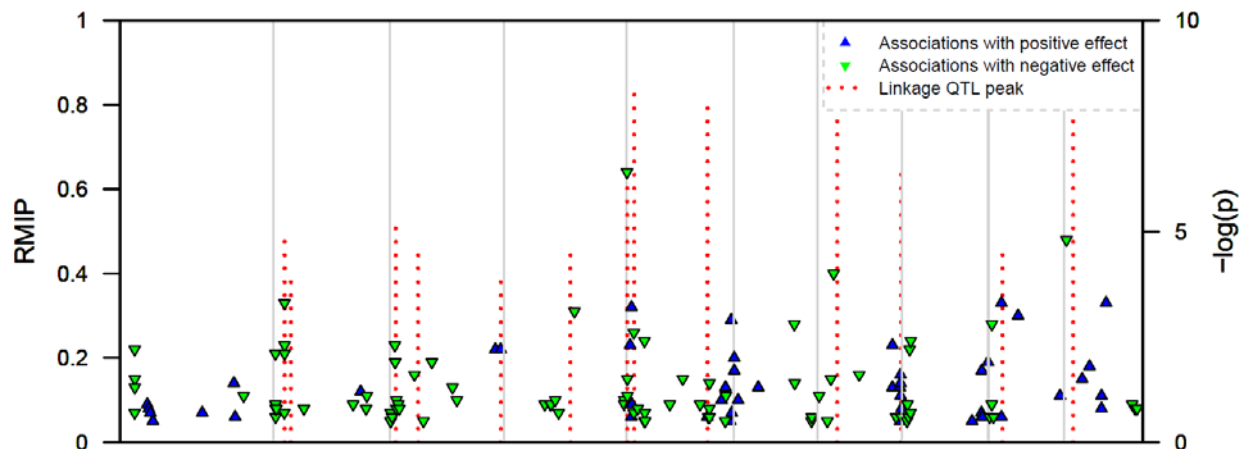
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H

Glucose



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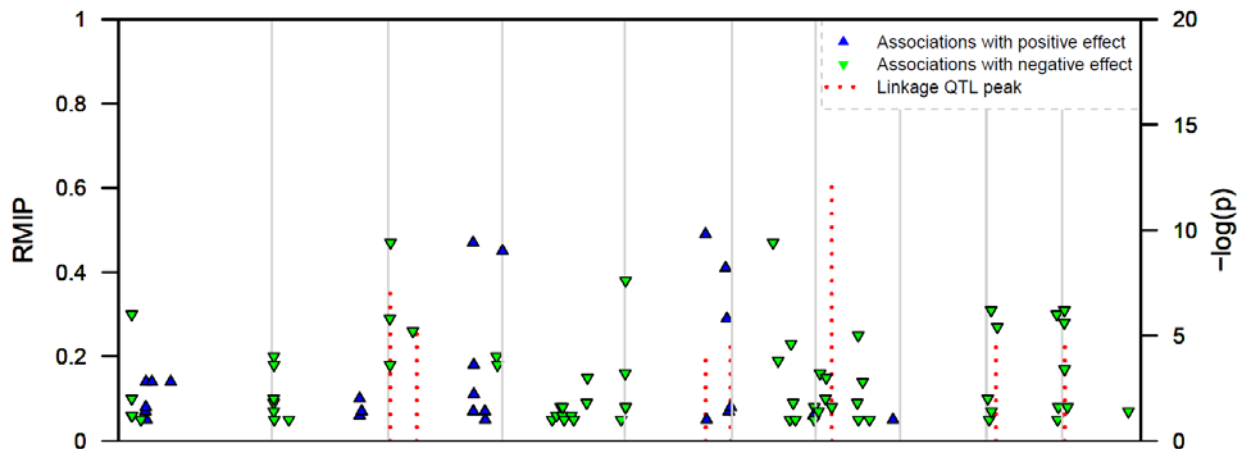
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I

Fructose



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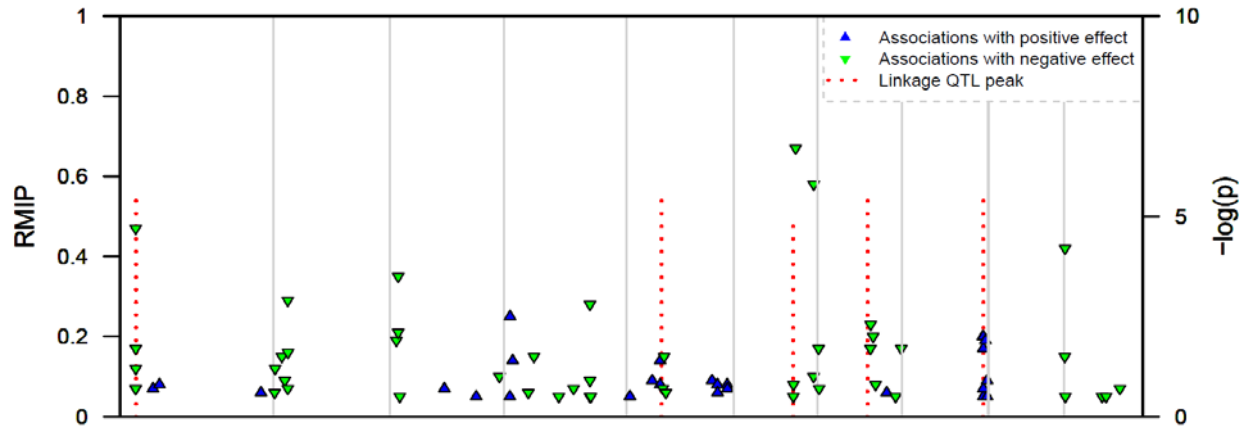
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J

Sucrose



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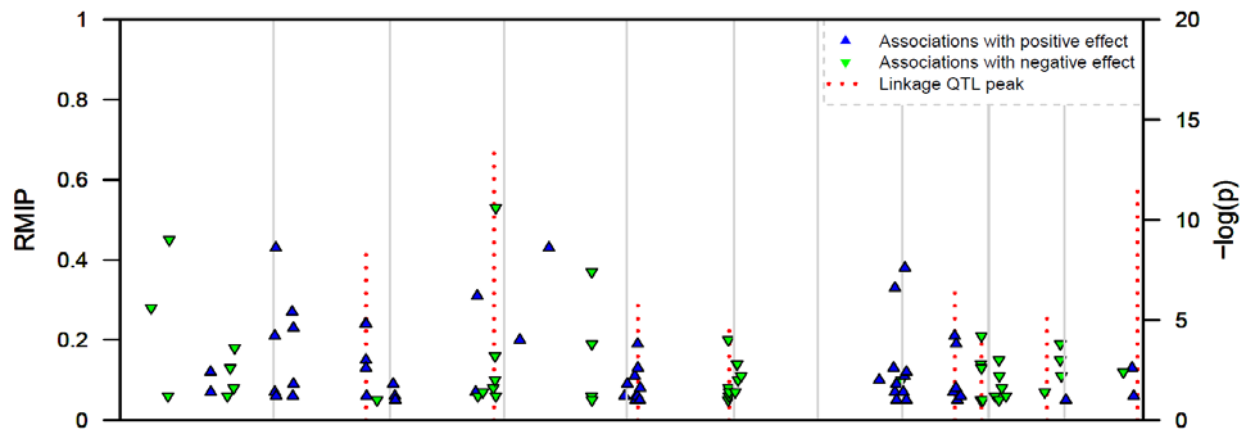
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K

Prin1



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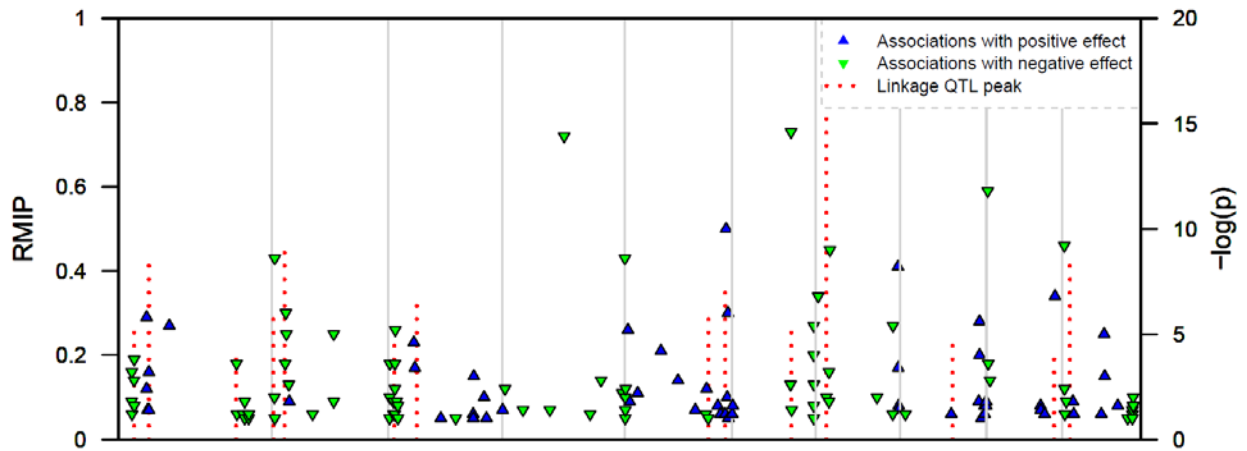
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L

Prin2



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183 **Figure S9.** Overview of GWAS results. All SNPs detected as significant in at least 5 subsamples
184 are triangles (blue with positive effect and green with negative effect) relative to their physical
185 sequence position. Vertical positions of triangles represent resample model inclusion probability
186 (RMIP) of the SNP. QTL, red lines whose vertical positions represent their *F*-test $\log(1/P)$ in the
187 final joint linkage QTL model. **(A)** Malate. **(B)** Fumarate. **(C)** Glutamate. **(D)** Chlorophyll b. **(E)**
188 Nitrate. **(F)** Total amino acids. **(G)** Protein. **(H)** glucose. **(I)** Fructose. **(J)** Sucrose **(K)** First
189 principal component (Prin1). **(L)** Second Principal component (Prin2).

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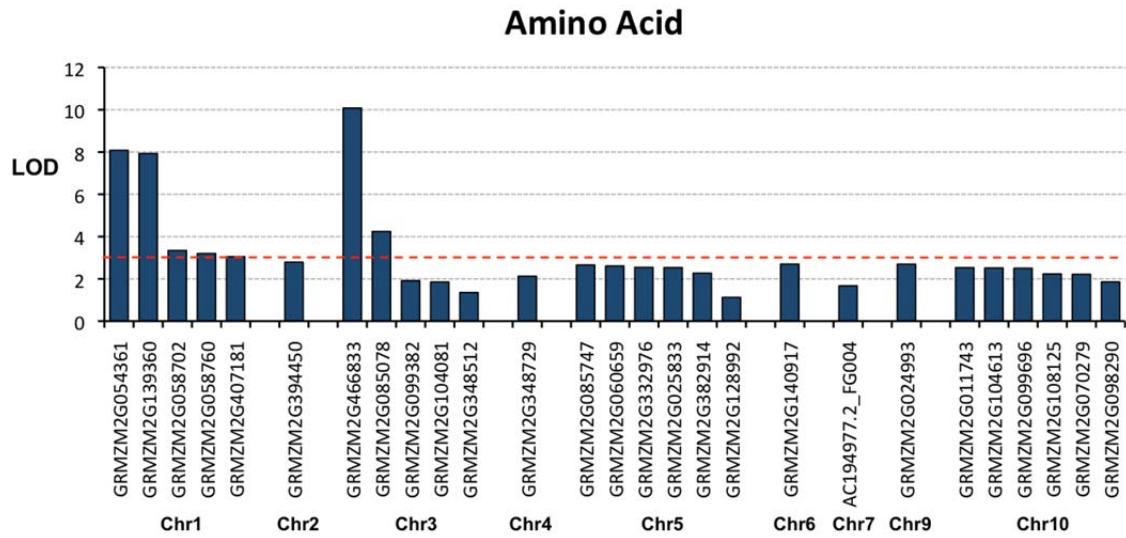
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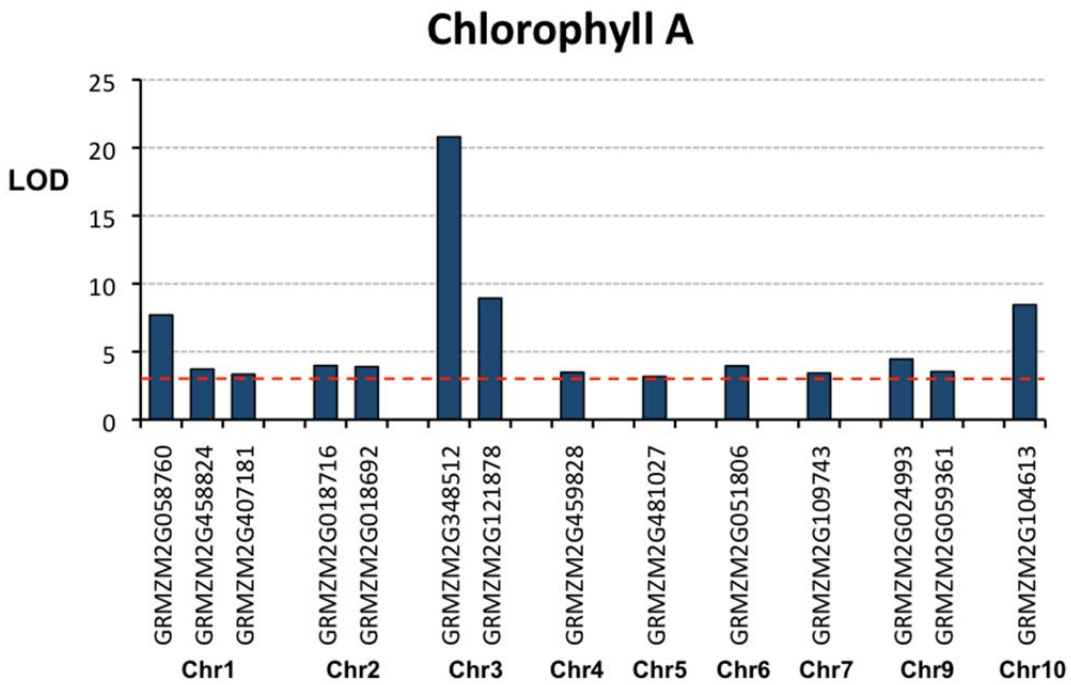
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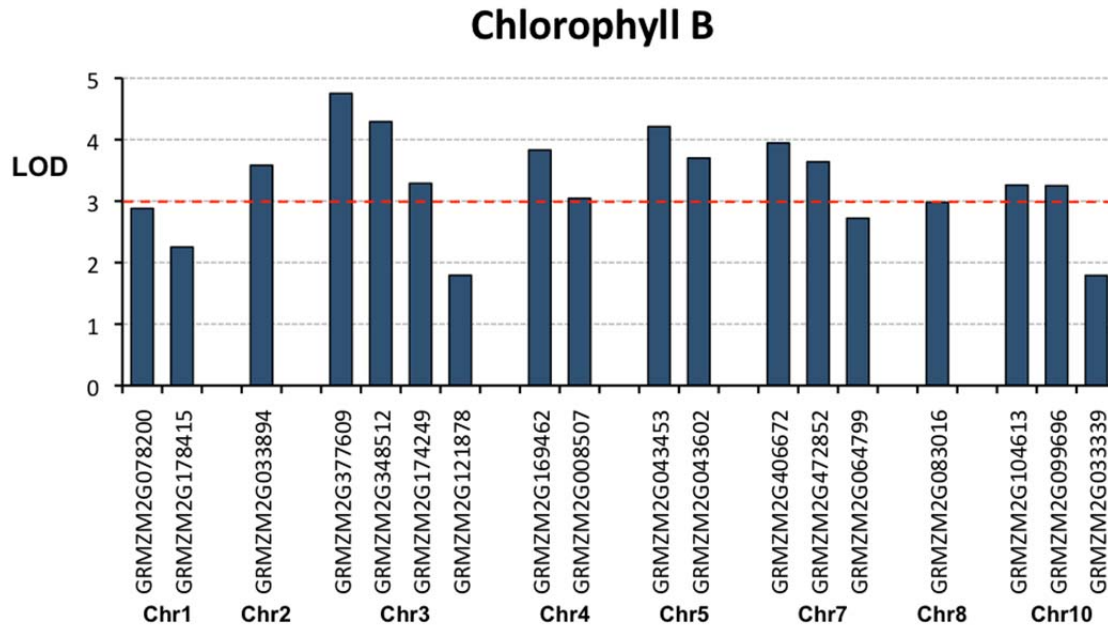


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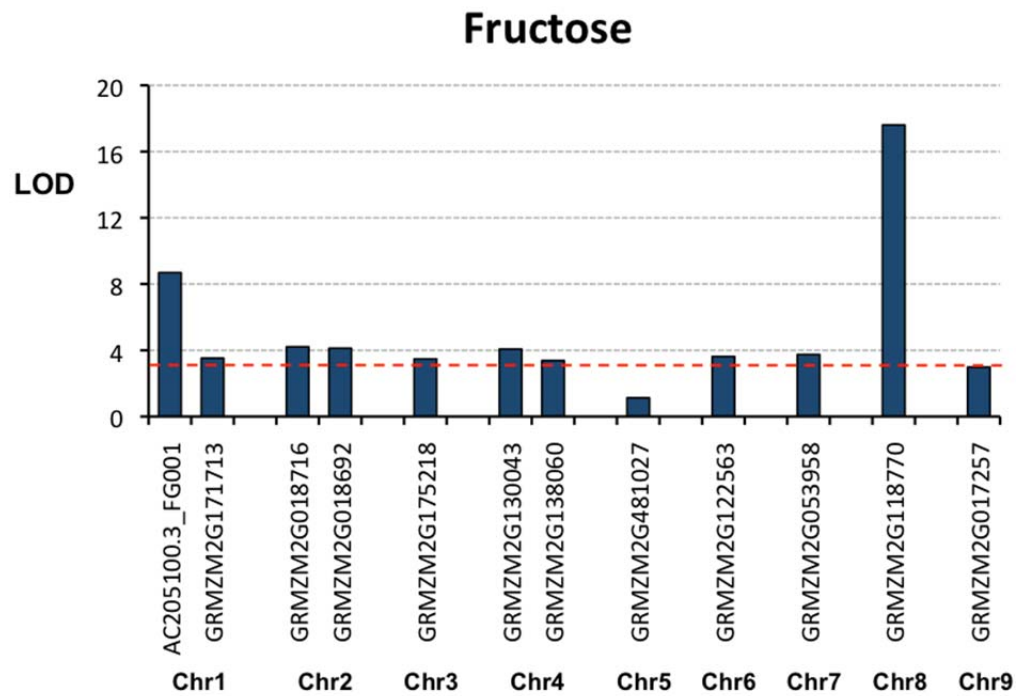


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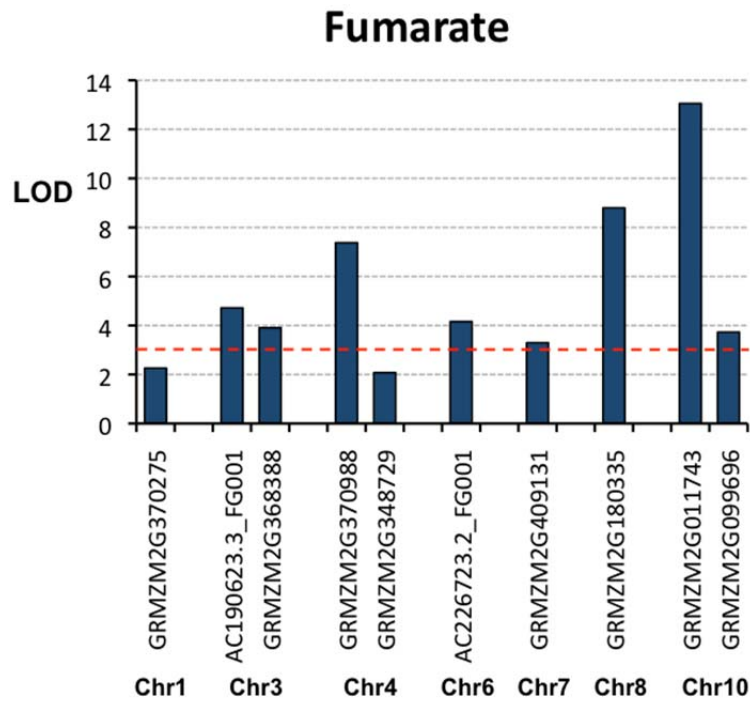


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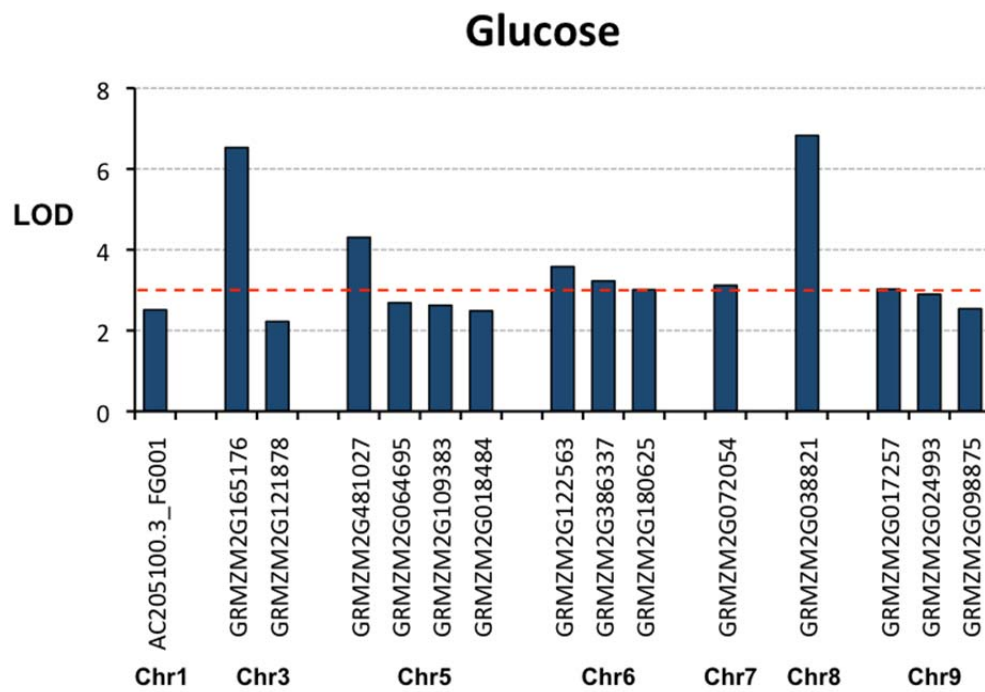


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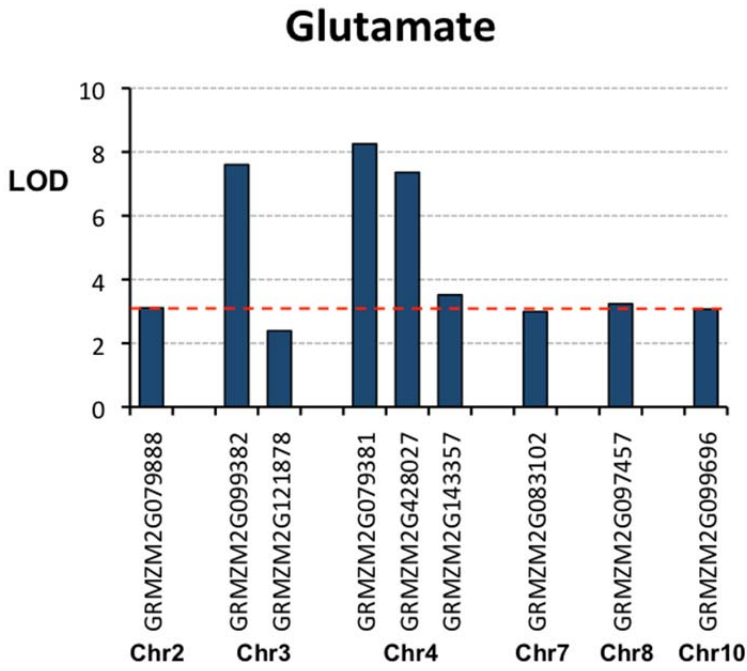


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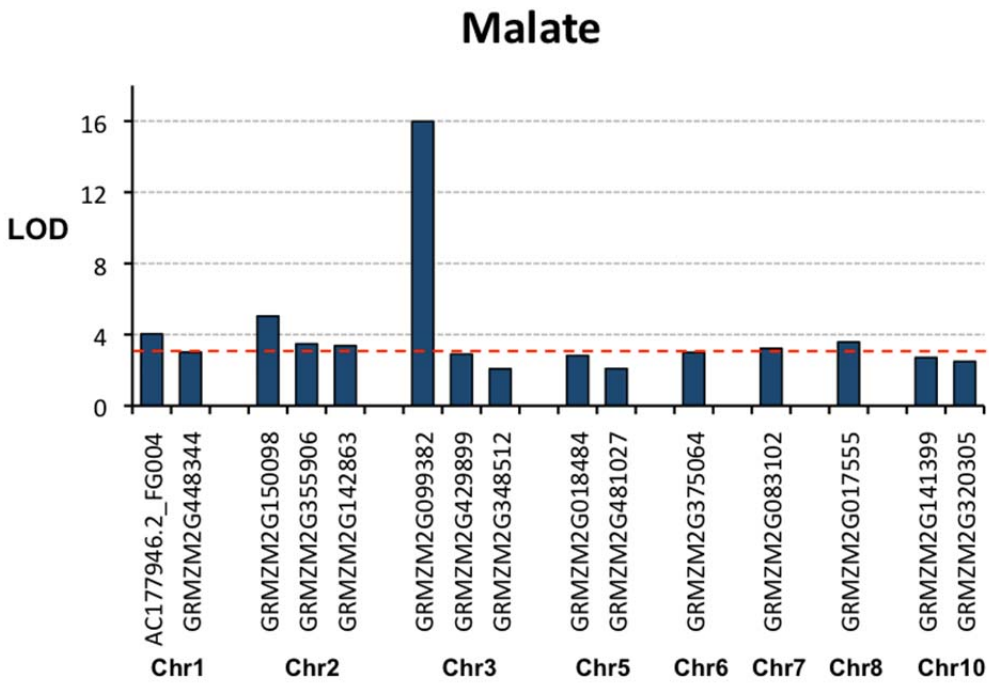


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228 G
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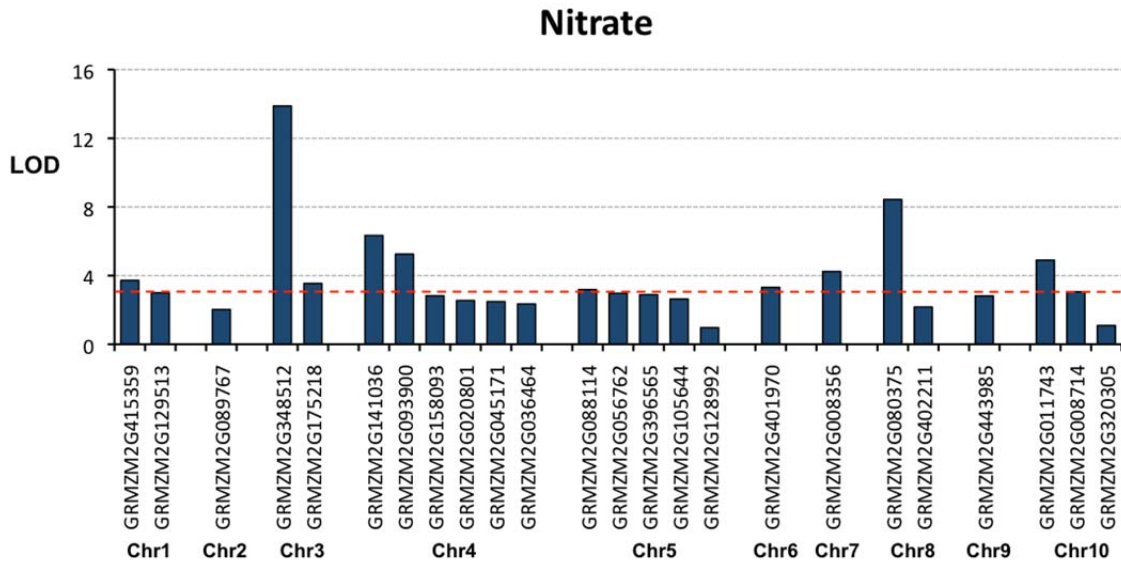


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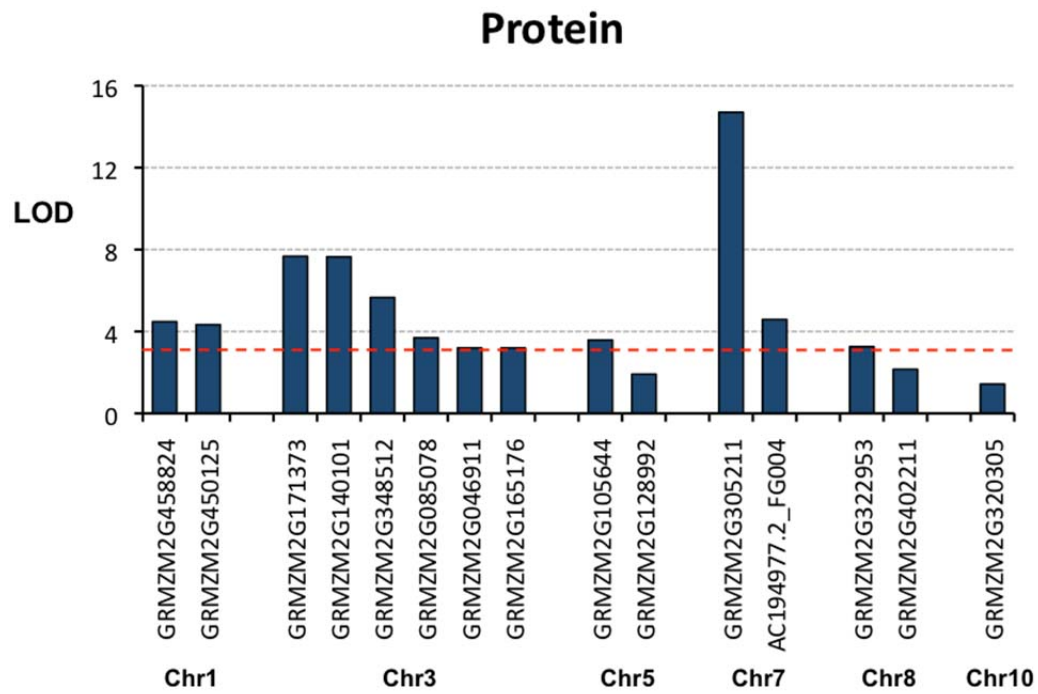


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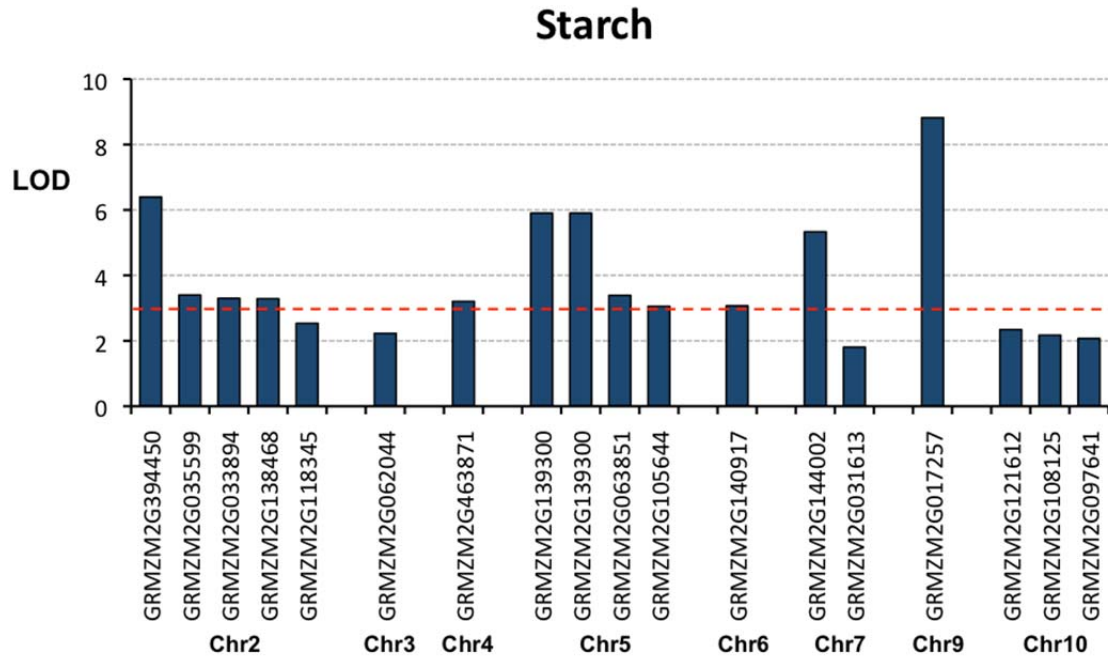


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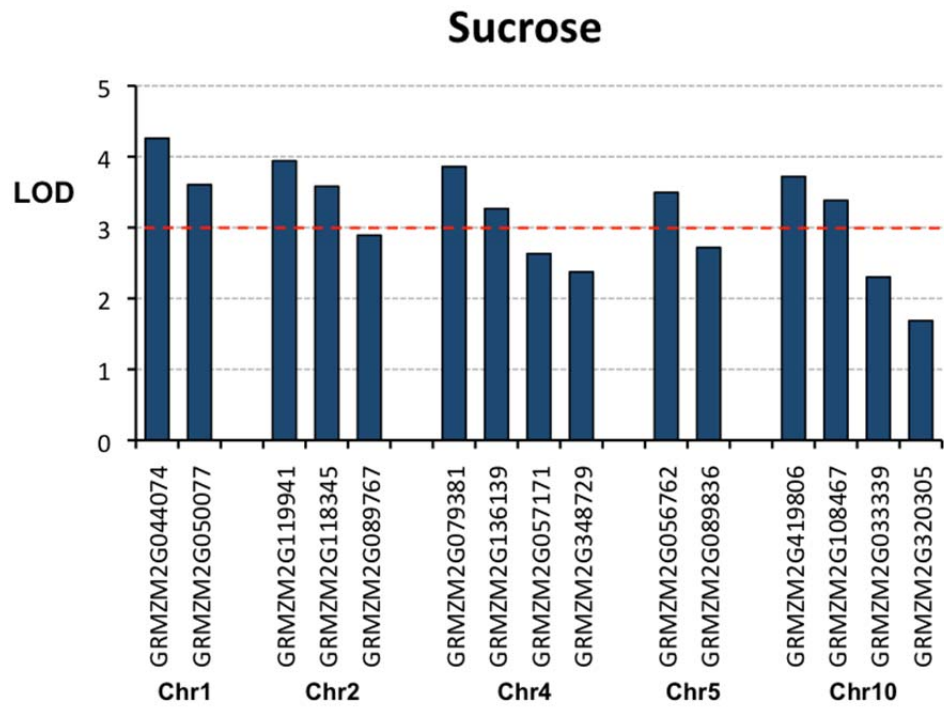


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254 L
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258 **Figure S10.** The overrepresentation of metabolite GWAS in maize priori candidates. The
259 annotations of maize priori candidates shown in this figure can be found in Table S6 and the red
260 dashed line indicates the significance level of LOD = 3. **(A)** Total amino acids. **(B)** Chlorophyll
261 A. **(C)** Chlorophyll B. **(D)** Fructose. **(E)** Fumarate. **(F)** Glucose. **(G)** Glutamate. **(H)** Malate. **(I)**
262 Nitrate. **(J)** Protein. **(K)** Starch. **(L)** Sucrose.

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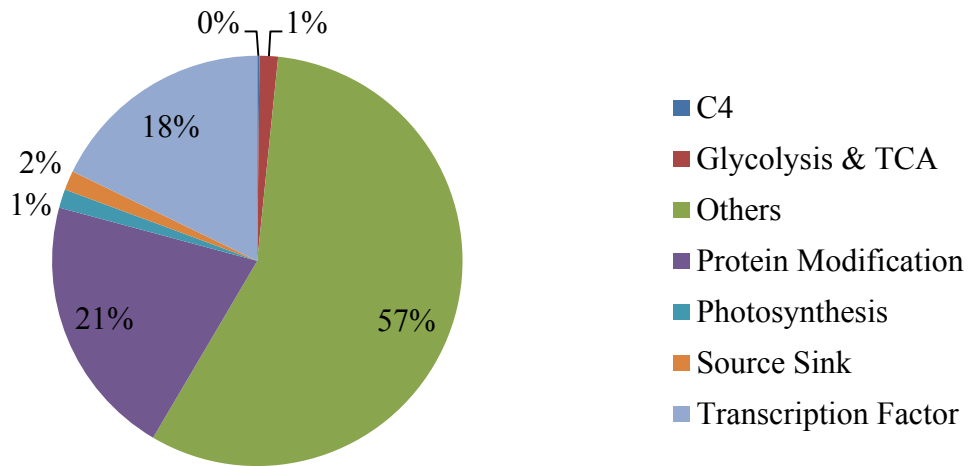
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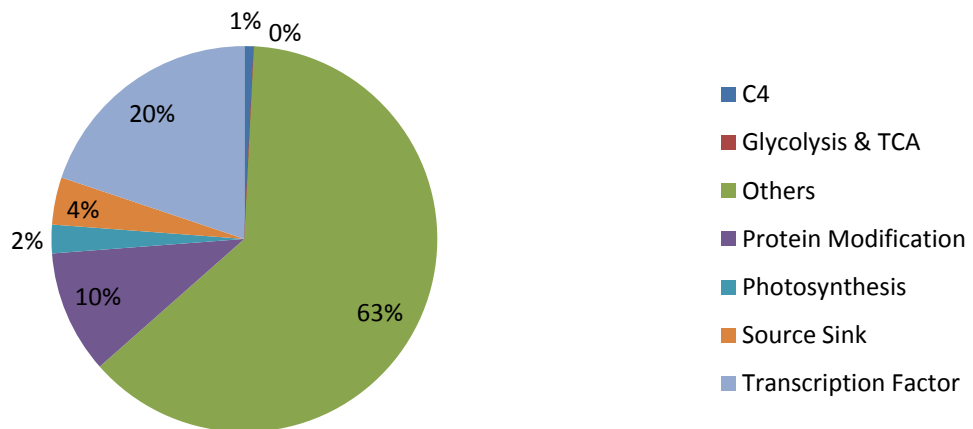
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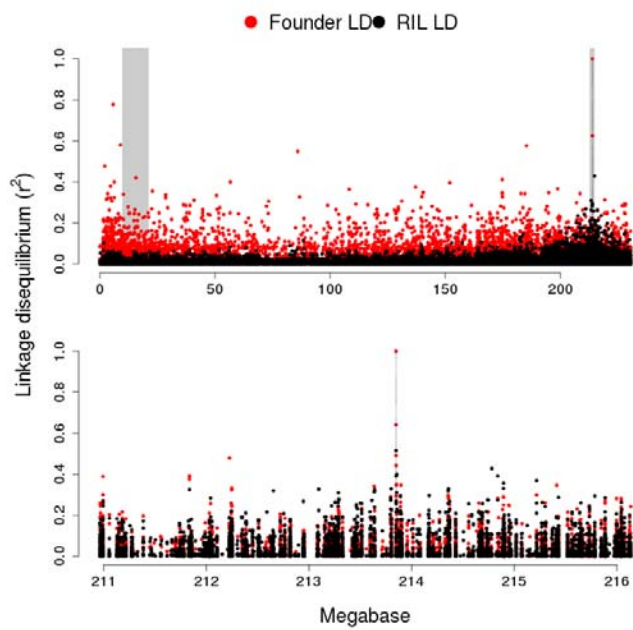


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Figure S11. The distribution of different classes of genes. **(A)** Whole maize genome. **(B)** Genes which the 126 most significantly associated SNPs (RMIP \geq 0.50) sit in or are adjacent to.

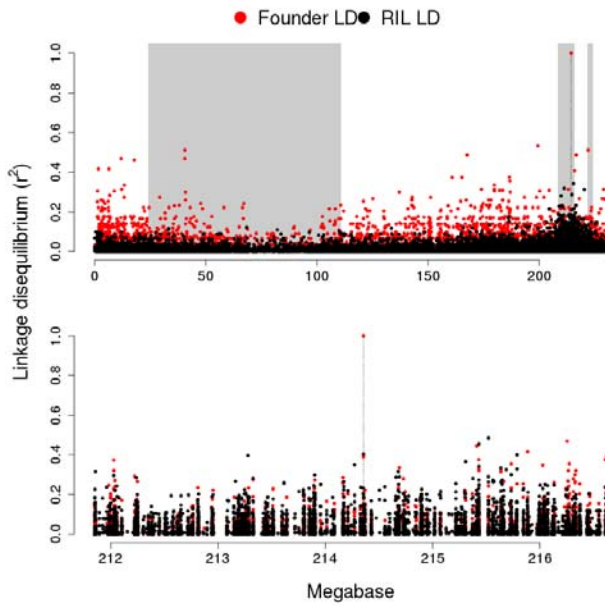
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A. Chrolophyll a, Chromosome 3, 213.8 Mb



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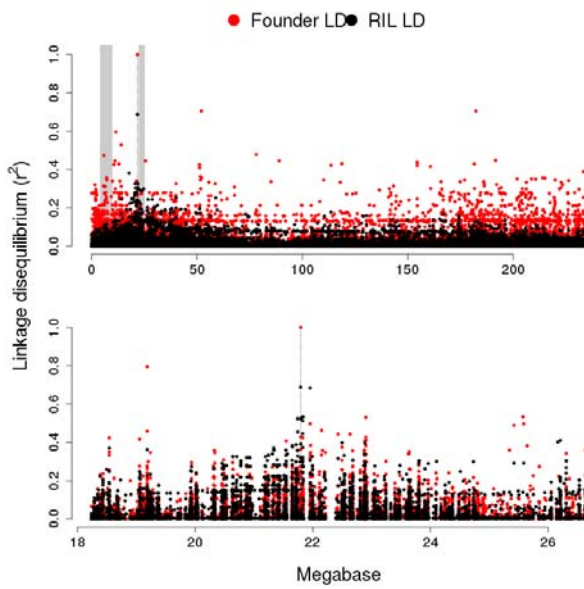
B. Malate, Chromosome 3, 214.3 Mb



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311 **C. Starch, Chromosome 2, 22 Mb**

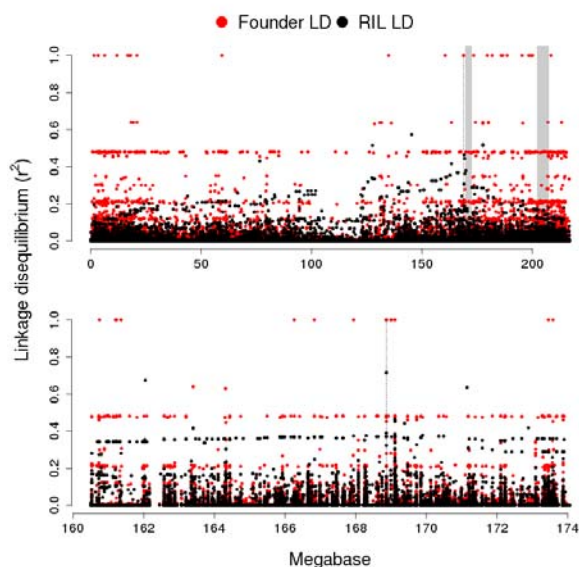
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319 **D. Starch, Chromosome 5, 168.8 Mb**

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323 **Figure S12.** Linkage disequilibrium (r^2) between a portion of SNPs identified as significantly
324 associated with some metabolites (these are SNPs discussed in the text) and all other SNPs on
325 the same chromosome. The position of the significant SNP to which all others are compared is
326 indicated with a dashed vertical line (this SNP has an r^2 of 1.0 with itself). Red circles indicate
327 observed r^2 values in the 25 NAM founders. Black circles indicate expected r^2 values in the
328 NAM RILs. Shaded vertical bars represent all QTL support intervals on a chromosome. Top
329 panels display LD across an entire chromosome. Bottom panels focus on LD across an entire
330 QTL support interval (if the significant SNP is inside of a QTL support interval) or a length of
331 chromosome representing 5 cM of genetic distance to either side of the significant SNP (if the
332 SNP is outside of a QTL support interval).

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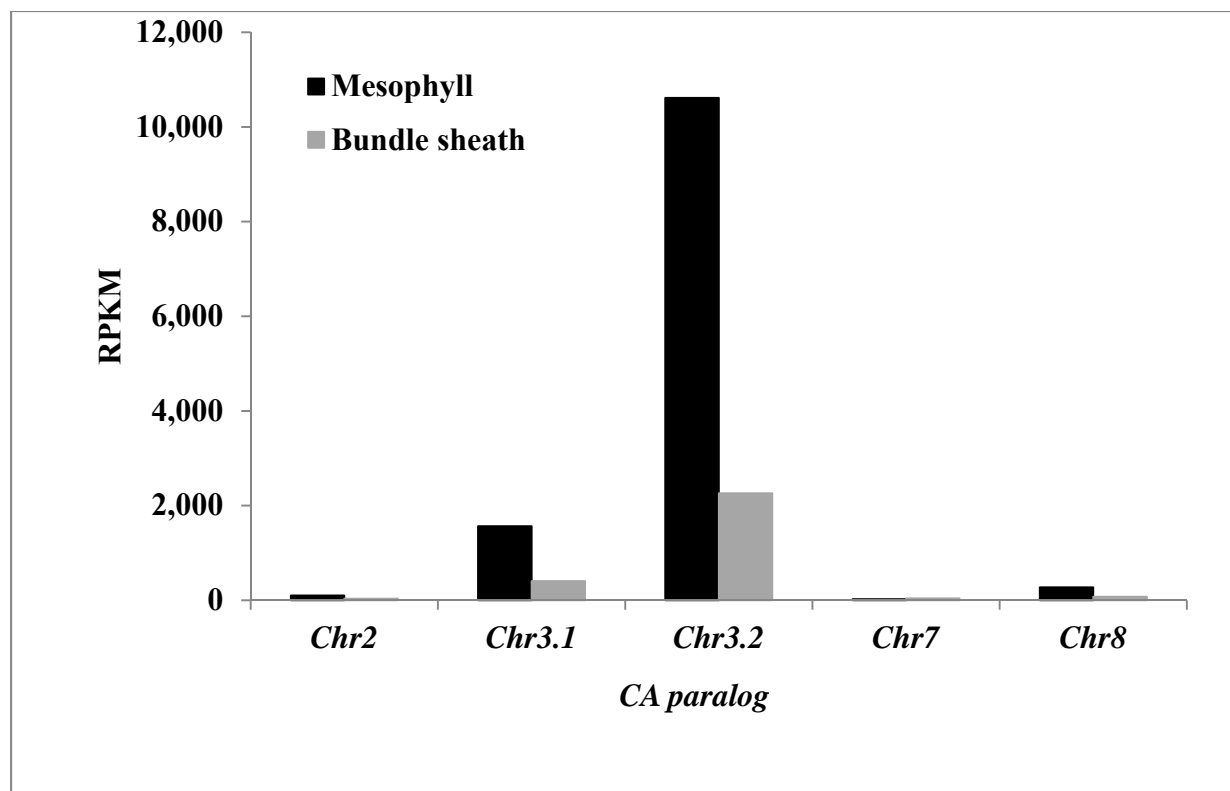
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Figure S13. *carbonic anhydrase (CA)* expression in B73 at 3-leaf stage (35). RPKM: Reads per kilobase per million mapped reads (21).

Table S1. Whole field repeatability, NAM founder line broad-sense heritability, and number of QTL identified by joint linkage mapping.

Trait	Whole-field repeatability	NAM founder line broad-sense heritability	Number of QTL	R ² for model
Sucrose	0.49	0.42	5	0.54
Glucose	0.45	0.28	13	0.41
Fructose	0.44	0.32	7	0.37
Starch	0.53	0.40	14	0.55
Glutamate	0.58	0.45	7	0.33
Protein	0.34	0.26	7	0.34
Chla	0.51	0.49	7	0.41
Chlb	0.50	0.14	9	0.38
Fumarate	0.71	0.36	15	0.32
Malate	0.58	0.68	9	0.44
AA	0.55	0.35	18	0.44
Nitrate	0.15	0.22	7	0.48
Prin1	-	-	8	0.24
Prin2	-	-	14	0.47

Chla, chlorophyll a; Chlb, chlorophyll b; AA, total amino acids; Prin1: first principal component; Prin2: second principal component 2.

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Table S8. Pearson correlations between flowering time and metabolites

Trait	Correlation Coefficient	<i>p</i>-value
Sucr	-0.56	0
Chla	-0.43	5 x 10 ⁻²⁰⁹
Nitr	-0.43	1 x 10 ⁻²⁰¹
Mala	-0.37	1 x 10 ⁻¹⁵⁰
Star	-0.37	2 x 10 ⁻¹⁴⁹
Glut	-0.36	7 x 10 ⁻¹⁴⁰
Fuma	-0.36	3 x 10 ⁻¹³⁵
Fruc	-0.34	1 x 10 ⁻¹²⁴
Prot	-0.21	5 x 10 ⁻⁴⁷
AA	0.11	1 x 10 ⁻¹³
Chlb	-0.07	9 x 10 ⁻⁰⁷
Gluc	-0.06	1 x 10 ⁻⁰⁴

Sucr, sucrose; Chla, chlorophyll a; Nitr, nitrate; Mala, malate; Star, starch; Glut, glutamate; Fuma, fumarate; Fruc, fructose; Prot, protein; AA, total amino acids; Chlb, chlorophyll b; Gluc, glucose

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Parsed Citations

Arnon DI (1949) Copper Enzymes in Isolated Chloroplasts - Polyphenoloxidase in Beta-Vulgaris. Plant Physiol 24: 1-15

Pubmed: [Author and Title](#)

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Bantan-Polak T, Kassai M, Grant KB (2001) A comparison of fluorescamine and naphthalene-2,3-dicarboxaldehyde fluorogenic reagents for microplate-based detection of amino acids. Anal Biochem 297: 128-136

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Bradford MM (1976) Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing Principle of Protein-Dye Binding. Anal Biochem 72: 248-254

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Brautigam A, Weber APM (2011) Do Metabolite Transport Processes Limit Photosynthesis? Plant Physiol 155: 43-48

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Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz JC, et al. (2009) The Genetic Architecture of Maize Flowering Time. Science (80-) 325: 714-718

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