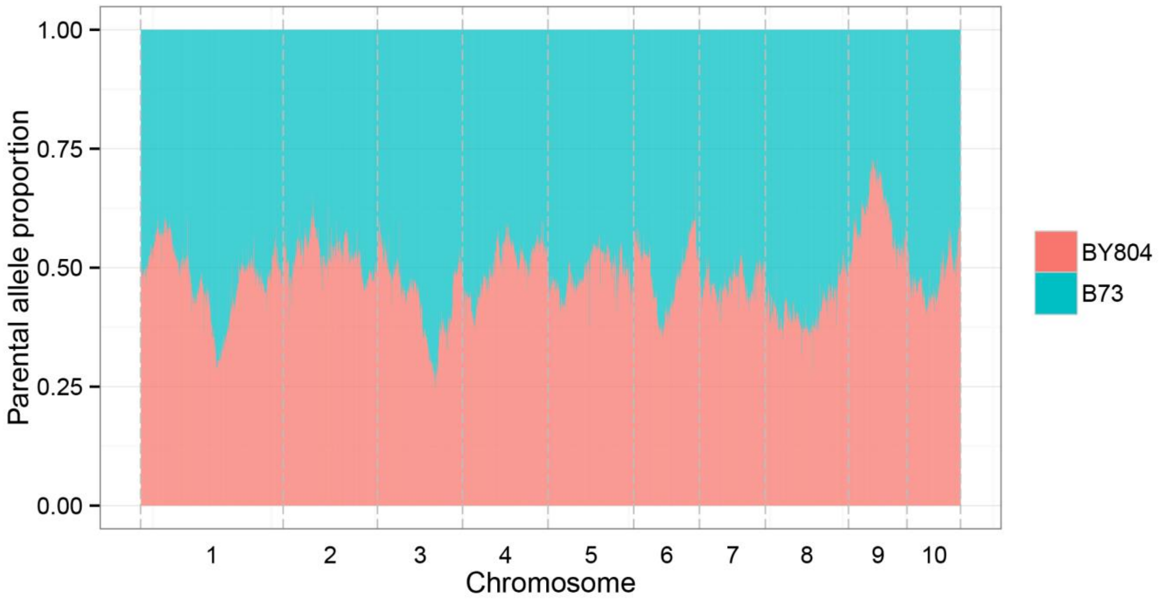
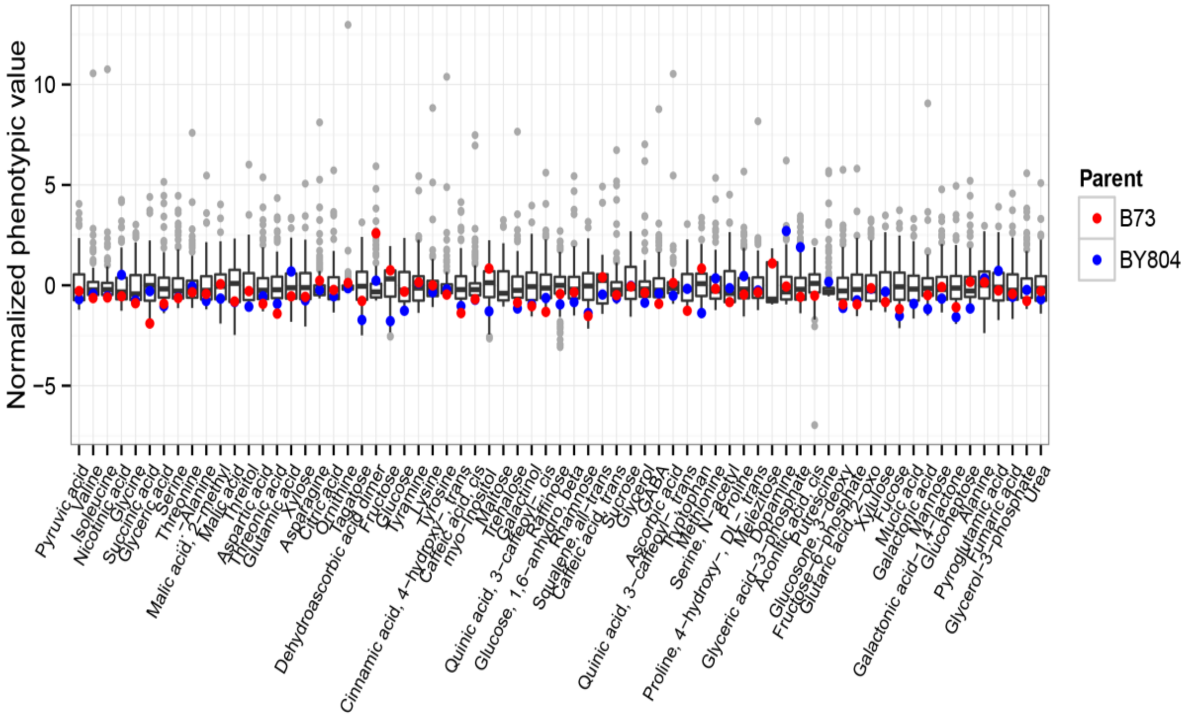
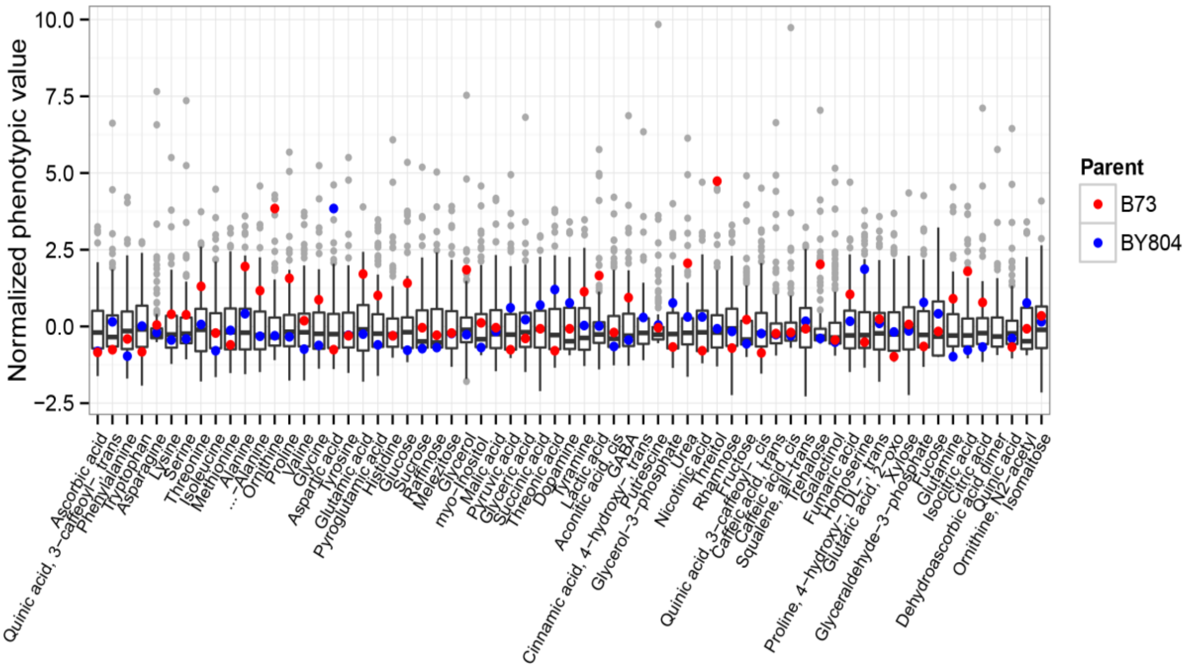


Supplemental Figure 1: Allele distribution within the B73/By804 RIL population (Allele type of each bin for each line within the B73/By804 RIL population). Blue indicates the proportion of lines with a B73 allele and the red indicate the proportion of lines with a By804 allele.

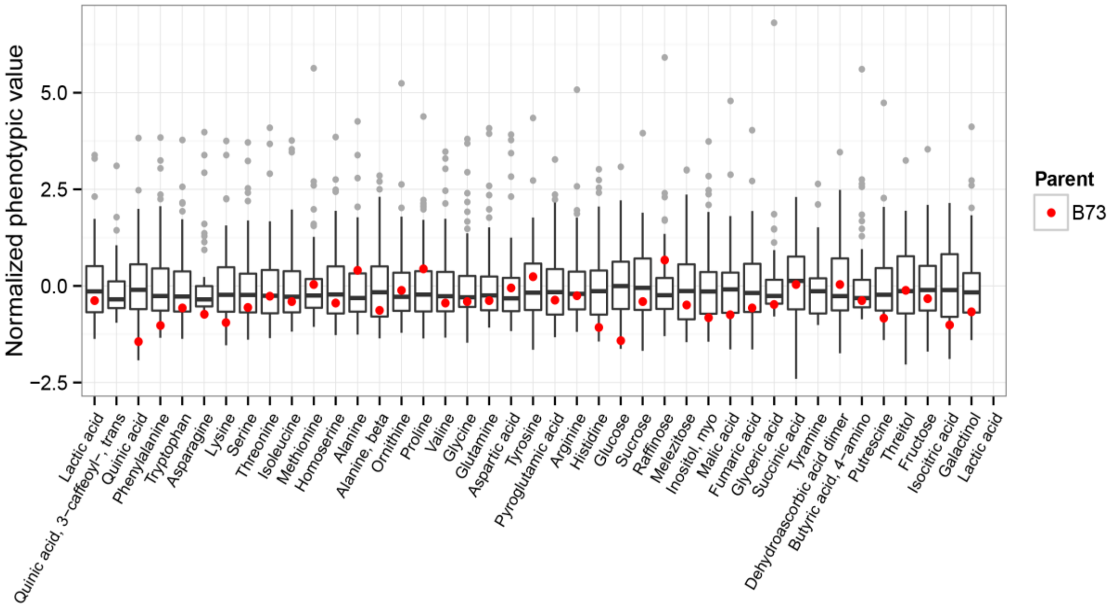
A.


Normalized phenotypic value
10
5
0
-5
Parent
B73
BY804
Pyruvic acid
Valine
Isoleucine
Nicotinic acid
Glycine
Succinic acid
Glyceric acid
Serine
Threonine
...−Alanine
Malic acid, 2−methyl
Malic acid
Threitol
Aspartic acid
Threonic acid
Glutamic acid
Xylose
Asparagine
Citric acid
Ornithine
Tagatose
Dehydroascorbic acid dimer
Fructose
Glucose
Tyramine
Lysine
Tyrosine
Cinnamic acid, 4−hydroxy−, trans
Caffeic acid, cis
myo−Inositol
Maltose
Trehalose
Galactinol
Quinic acid, 3−caffeoyl−, cis
Raffinose
Glucose, 1,6−anhydro, beta
Rhamnose
Squalene, all−trans
Caffeic acid, trans
Sucrose
Glycerol
GABA
Ascorbic acid
Quinic acid, 3−caffeoyl−, trans
Tryptophan
Methionine
Serine, N−acetyl
Proline
Proline, 4−hydroxy−, DL−, trans
Melezitose
Dopamine
Glyceric acid−3−phosphate
Aconitic acid, cis
Putrescine
Glucosone, 3−deoxy
Fructose−6−phosphate
Glutaric acid, 2−oxo
Xylulose
Fucose
Mucic acid
Galactonic acid
Mannose
Galactonic acid−1,4−lactone
Glucoheptose
Alanine
Pyroglutamic acid
Fumaric acid
Glycerol−3−phosphate
Urea

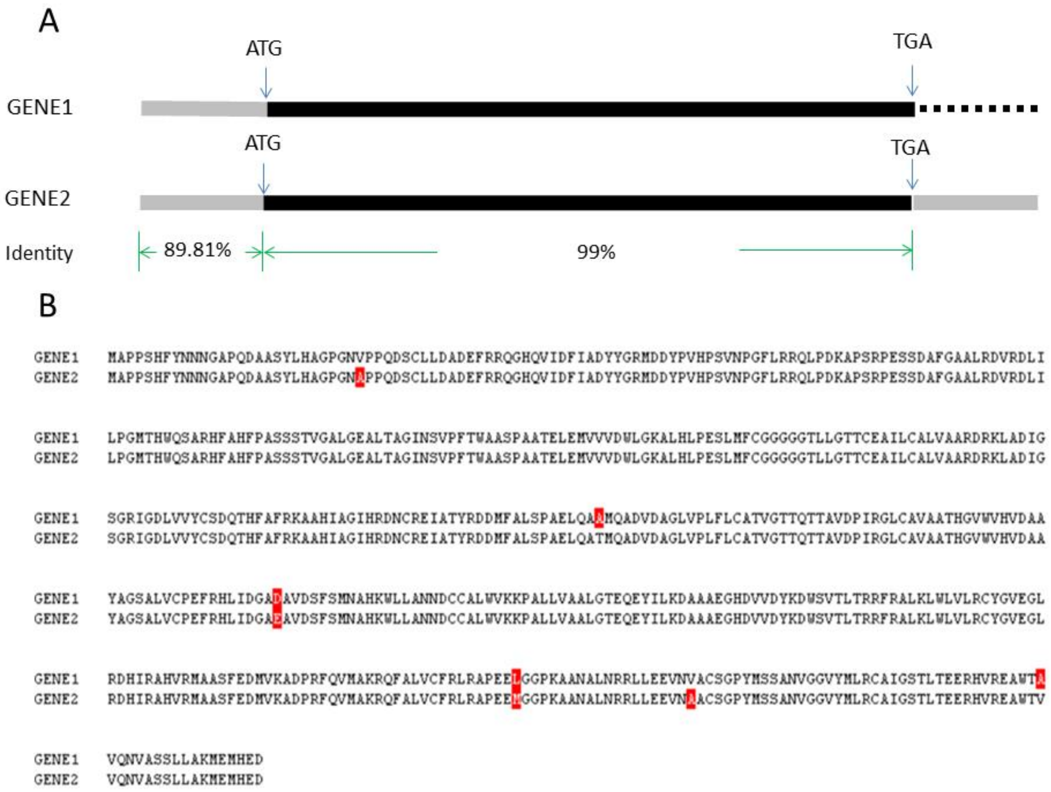
B.


Normalized phenotypic value
10.0
7.5
5.0
2.5
0.0
-2.5
Parent
B73
BY804
Ascorbic acid
Quinic acid, 3−caffeoyl−, trans
Phenylalanine
Tryptophan
Asparagine
Lysine
Serine
Threonine
Isoleucine
Methionine
Alanine
...−Alanine
Ornithine
Proline
Valine
Glycine
Aspartic acid
Tyrosine
Glutamic acid
Pyroglutamic acid
Histidine
Glucose
Sucrose
Raffinose
Melezitose
Glycerol
myo−Inositol
Malic acid
Pyruvic acid
Glyceric acid
Succinic acid
Threonic acid
Dopamine
Tyramine
Lactic acid
Aconitic acid, cis
GABA
Cinnamic acid, 4−hydroxy−, trans
Putrescine
Glycerol−3−phosphate
Urea
Nicotinic acid
Threitol
Rhamnose
Fructose
Quinic acid, 3−caffeoyl−, cis
Caffeic acid, trans
Caffeic acid, cis
Squalene, all−trans
Trehalose
Galactinol
Fumaric acid
Homoserine
Proline, 4−hydroxy−, DL−, trans
Glutaric acid, 2−oxo
Xylose
Glyceraldehyde−3−phosphate
Fucose
Glutamine
Isocitric acid
Citric acid
Dehydroascorbic acid dimer
Quinic acid
Ornithine, N2−acetyl
Isomaltose

C.

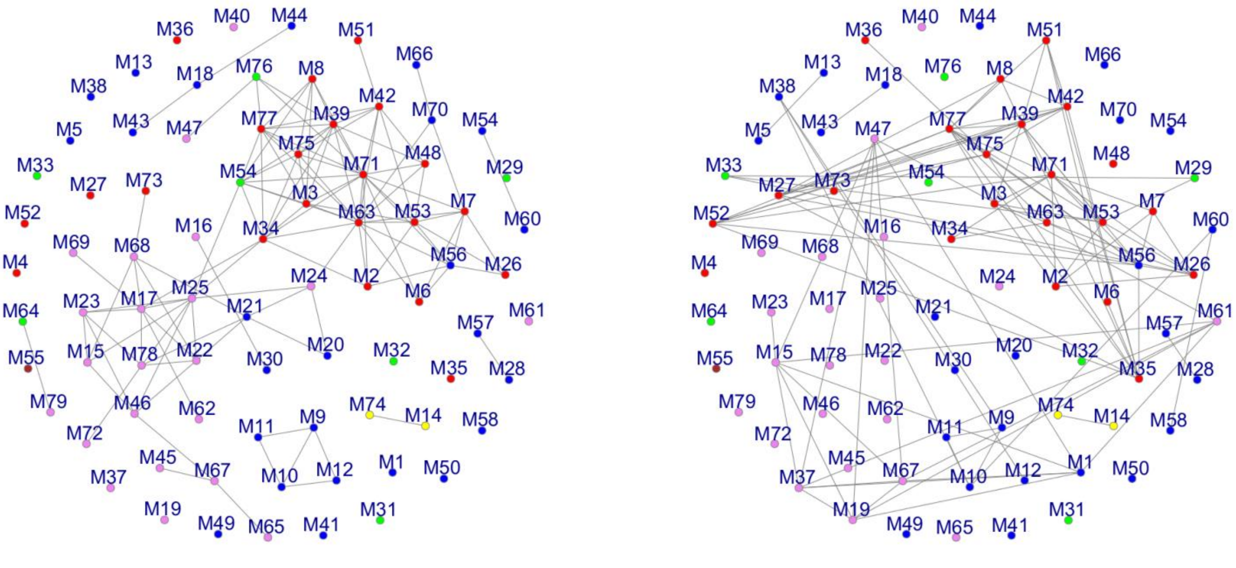


Supplemental Figure 2: Distribution of metabolite level in the B73/By804 recombinant line population. **(A)** Metabolites detected in the seedling leaf of each line. **(B)** Metabolites detected in the leaf at reproductive stage of each line. **(C)** Metabolites detected in the kernel at 15 days after pollination of each line.



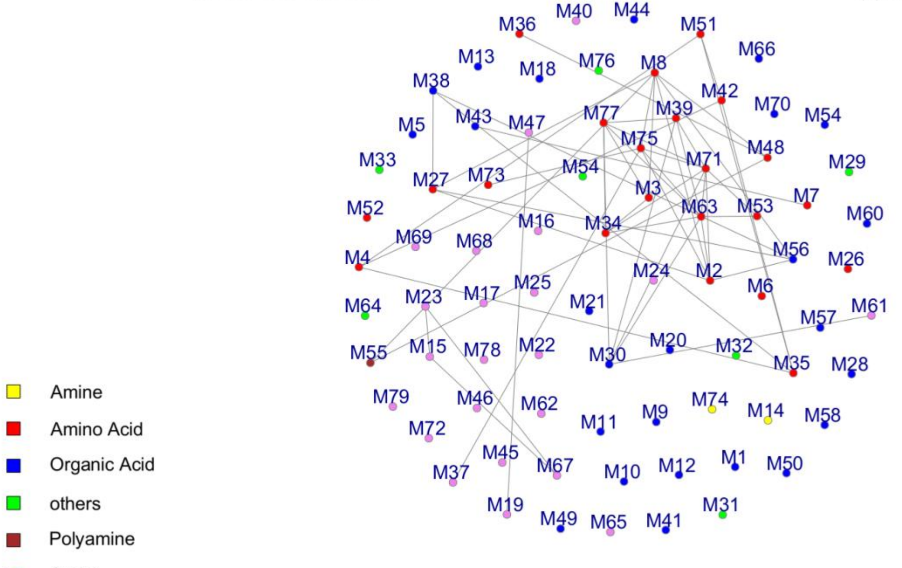
```
GENE1   MAPPSHFYNNNGAPQDAASYLHAGPGNVPPQDSCLLDADEFRRQGHQVIDFIADYYGRMDDYPVHPSVNPGFLRRQLPDKAPSRPESSDAFGAALRDVRDLI
GENE2   MAPPSHFYNNNGAPQDAASYLHAGPGNAPPQDSCLLDADEFRRQGHQVIDFIADYYGRMDDYPVHPSVNPGFLRRQLPDKAPSRPESSDAFGAALRDVRDLI

GENE1   LPGMTHWQSARHFAHFPASSSTVGALGEALTAGINSVPFTWAASPAATELEMVVVDWLGKALHLPESLMFCGGGGGTLLGTTCEAILCALVAARDRKLADIG
GENE2   LPGMTHWQSARHFAHFPASSSTVGALGEALTAGINSVPFTWAASPAATELEMVVVDWLGKALHLPESLMFCGGGGGTLLGTTCEAILCALVAARDRKLADIG

GENE1   SGRIGDLVVYCSDQTHFAFRKAAHIAGIHRDNCREIATYRDDMFALSPAELQAAMQADVDAGLVPLFLCATVGTTQTTAVDPIRGLCAVAATHGVWVHVDAA
GENE2   SGRIGDLVVYCSDQTHFAFRKAAHIAGIHRDNCREIATYRDDMFALSPAELQATMQADVDAGLVPLFLCATVGTTQTTAVDPIRGLCAVAATHGVWVHVDAA

GENE1   YAGSALVCPEFRHLIDGADAVDSFSMNAHKWLLANNDCCALWVKKPALLVAALGTEQEYILKDAAAEGHDVVDYKDWSVTLTRRFRALKLWLVLRCYGVEGL
GENE2   YAGSALVCPEFRHLIDGAEAVDSFSMNAHKWLLANNDCCALWVKKPALLVAALGTEQEYILKDAAAEGHDVVDYKDWSVTLTRRFRALKLWLVLRCYGVEGL

GENE1   RDHIRAHVRMAASFEDMVKADPRFQVMAKRQFALVCFRLRAPEELGGPKAANALNRRLLEEVNVACSGPYMSSANVGGVYMLRCAIGSTLTEERHVREAWTA
GENE2   RDHIRAHVRMAASFEDMVKADPRFQVMAKRQFALVCFRLRAPEEMGGPKAANALNRRLLEEVNAACSGPYMSSANVGGVYMLRCAIGSTLTEERHVREAWTV

GENE1   VQNVASSLLAKMEMHED
GENE2   VQNVASSLLAKMEMHED
```

Supplemental Figure 3: Sequence differences between two TDC genes in B73. **(A)** Schematic of the transcriptional sequence of the two genes. Dashed line represents deletion; the segmental nucleotide sequence identity between the two genes is indicated. **(B)** Amino acid sequence of the two genes. Amino acids that are different between the two genes are marked in red. GENE1 (*TDC1*): GRMZM2G056469; GENE2 (*TDC2*): GRMZM2G093125.



(a) Leaf-seedling stage

(b) Leaf-reproductive stage



(c) Kernel-15 DAP

Supplemental Figure 4: Metabolite relevance networks. Metabolite relevance networks were inferred for **(a)** leaf at seedling stage, **(b)** leaf at reproductive stage, and **(c)** kernel. The red and blue edges imply positive and negative correlation coefficients, respectively. The color of the nodes represents the metabolite classes. M1: M1: cis –Aconitic acid; M2: Alanine; M3: beta-Alanine..; M4: Arginine; M5: Ascorbic acid; M6: Asparagine; M7: Aspartic acid; M8: GABA; M9: Caffeic acid, cis; M10: Caffeic acid, trans; M11: Cinnamic acid-4-hydroxy, trans; M12: Citric acid; M13: Dehydroascorbic acid; M14: Dopamine; M15: Fructose; M16: Fructose-6-phosphate; M17: Fucose; M18: Fumaric acid; M19: Galactinol; M20: Galactonic acid; M21: Galactonic acid-1,4-lactone; M22: Glucoheptose; M23: Glucose; M24:Glucose-1,6-anhydro-beta; M25: Glucosone-3-deoxy; M26: Glutamic acid; M27: Glutamine; M28: 2-oxo-Glutaric acid; M29: Glyceraldehyde-3-phosphate; M30: Glyceric acid; M31: Glyceric acid-3-phosphate; M32: Glycerol; M33: Glycerol-3-phosphate; M34: Glycine; M35: Histidine; M36: Homoserine; M37: Inositol-myo; M38: Isocitric acid; M39: Isoleucine; M40: Isomaltose; M41: Lactic acid; M42: Lysine; M43: Malic.acid; M44: Malic acid-2-methyl; M45: Maltose; M46: Mannose; M47: Melezitose; M48: Methionine; M49: Mucic.acid; M50: Nicotinic.acid; M51: Ornithine; M52: Phenylalanine; M53: Proline; M54: Proline-4-hydroxy-DL, trans; M55: Putrescine; M56: Pyroglutamic acid; M57: Pyruvic acid; M58: Quinic acid; M59: Quinic acid-3-caffeoyl, cis; M60: Quinic acid-3-caffeoyl, trans; M61: Raffinose; M62: Rhamnose; M63: Serine; M64: Serine-N-acetyl; M65: Squalene-all-trans; M66: Succinic acid; M67: Sucrose; M68: Tagatose; M69: Threitol; M70: Threonic acid; M71: Threonine; M72: Trehalose; M73: Tryptophan; M74: Tyramine; M75: Tyrosine; M76: Urea; M77: Valine; M78: Xylose; M79:Xylulose.

Supplemental Table 1. Epistatic interactions between QTLs.

Tissue[a]	Metabolite	QTL_ID[b]	QTL_ID	P value	R^2(%)[c]
leaf1	Threitol	1	2	0.044763	2.51
leaf1	Valine	2	4	0.005097	
leaf1	Valine	3	4	0.043317	3.19
leaf1	Fructose	3	4	0.010592	2.74
leaf1	Inositol, myo	1	2	0.042376	2.57
leaf1	Rhamnose	1	2	0.00796	5.87
leaf1	Nicotinic acid	1	2	0.008464	
leaf1	Nicotinic acid	2	3	0.000454	15.25
leaf1	Ascorbic acid	1	3	0.047734	2.62
leaf1	Serine, N-acetyl	1	2	0.007076	4.48
leaf1	Putrescine	1	2	0.019926	4.24
leaf1	Glutaric acid, 2-oxo	1	2	0.002605	4.97
leaf1	Glucoheptose	2	4	0.025112	2.57
leaf2	Malic acid	2	4	0.008263	
leaf2	Malic acid	3	5	0.048498	
leaf2	Malic acid	5	6	0.004012	5.93
leaf2	Fructose	1	2	0.006004	5.77
leaf2	Caffeic acid, trans	1	2	0.015032	3.85
leaf2	Trehalose	1	2	0.049081	
leaf2	Trehalose	1	3	0.035524	
leaf2	Trehalose	1	4	0.011117	
leaf2	Trehalose	4	6	0.044393	
leaf2	Trehalose	5	6	0.034599	8.95
leaf2	Asparagine	1	2	0.007786	5.21
leaf2	Isocitric acid	2	3	0.008135	6.66
leaf2	Citric acid	1	2	0.044902	1.73
leaf2	Dehydroascorbic acid	1	2	0.030411	4.87
kernel	Homoserine	1	3	0.025924	4.77
kernel	Ornithine	2	5	0.014801	9.54
kernel	Proline	1	3	0.01152	11.97
kernel	Aspartic acid	1	2	0.001607	
kernel	Aspartic acid	2	3	0.022122	
kernel	Aspartic acid	2	4	0.049254	11.71
kernel	Arginine	1	2	0.025086	10.67
kernel	Isocitric acid	1	2	0.0043	12.44
kernel	Galactinol	1	2	0.006988	
kernel	Galactinol	1	3	0.005174	16.64

a) leaf1: leaf at seedling stage; leaf2: leaf at reproductive stage.

b) QTL_ID corresponds to the order of QTL listed in Supplemental Dataset 3.

c) the subtotal of variance explained by epistatic interactions of each metabolic trait PVE: percentage that explains the phenotypic variation.

Supplemental Table 2. Primers used for re-sequencing *AGT* in B73 and By804 and PCR-based InDel detection.

Primers	Forward	Reverse
P1	TGCTGCACATCCCTTGGATAG	GAGTGGAGAGTCGTTTCGTGG
P2	ACGTGCATTTTTCTGGACGC	AGGGTATACCGGCTTCTGGT
P3	CGATGCCCGGTGGAGATG	ACTGCAGCAAACATGTGACT
P4	GTCTGTCTGGGTGCAGTCAT	AATTCCCTGAAACCAGGGGG
P5	ACTGGCAGTCATTACTGGGG	ACACCACCAACATACCACCC
P6	ATTGCGCGCACAAACTAACAA	GGTAGAGGGAACAGGGCATC
Primers for PCR-based InDel detection	CACTCCCACCAGATAGCCAC	GGACAACCAGGAGCGGAATA

Supplemental Table 3. Sequence polymorphisms between B73 and By804 in the candidate gene *AGT* identified by re-sequencing.

Site at *AGT*[a]	Physical Position (bp)[b]	B73/By804[c]
-264	15352982	A/C
-8	15353238	A/C
171	15353417	-/43bp-Insertion
275	15353521	C/T*
331	15353577	C/T*
401	15353647	T/C*
444	15353690	T/C*
458	15353704	A/G*
661	15353907	T/G*
694	15353940	G/A*
721	15353967	T/A*
760	15354006	G/T
764	15354010	A/G
772	15354018	G/A
2077	15355323	-/T-Insertion
2424	15355670	C/T
2539	15355785	A/G*
2814	15356060	-/A-Insertion
3046	15356292	TGG-Insertion/-
3130	15356376	G/A*
3131	15356377	T/C*
3165	15356411	C/T*
3173	15356419	G/A
3201	15356447	G/T*
3202	15356448	T/C*
3218	15356464	C/G*
3232	15356478	A/C
3250	15356496	A/C
3256	15356502	-/GGA-Insertion
3295	15356541	-/GTG-Insertion
3296	15356542	-/GT-Insertion
3314	15356560	A/G
3329	15356575	-/TG-Insertion
3331	15356577	T/C
3337	15356583	-/CC-Insertion
3340	15356586	A/C
3343	15356589	C/T
3358	15356604	T/C
3392	15356638	A/T

a) The location of each polymorphic site in *AGT*

b) The physical position of each polymorphic site in the maize genome

c) Alleles from B73 and By804 at each polymorphic site

“*” represents genomic variants that were also identified by RNA-sequencing

Supplemental Table 4. Sequence polymorphisms between B73 and By804 in the candidate gene *TDC1* identified by re-sequencing.

Site at *TDC1*[a]	Physical Position (bp)[b]	B73/By804[c]	Amino Acid replacement
-475	17831437	G-	
-385	17831347	A/G	
-315	17831277	G/T	
-270	17831232	G/A	
-260	17831222	T/C	
-217	17831179	CTTC+	
-213	17831175	ATGCATG+	
-151	17831113	T/C	
-140	17831102	C/G	
-131	17831093	C/T	
-13	17830975	C/A	
-4	17830966	TCTC-	
34	17830930	G/A	
82	17830882	A/T	
191	17830773	T/C	Val/Ala
243	17830721	C/T	
339	17830625	T/C	
344	17830620	A/C	Asp/Ala
348	17830616	G/T	Lys/Asp
729	17830235	G/A	
966	17829998	G/T	
1029	17829935	C/T	
1066	17829898	T/C	
1146	17829818	T/G	
1368	17829596	G/T	
1483	17829481	G/A	Ala/Thr
1523	17829441	T/C	Val/Ala
1536	17829428	G/A	
1617	17829347	T/C	
1623	17829341	T/C	
1629	17829335	A/T	
1637	17829327	C/T	Ala/Val
1660	17829304	C/A	Leu/Ile

a) The location of each polymorphic site in *TDC1*

b) The physical position of each polymorphic site in the maize genome

c) Alleles from B73 and By804 at each polymorphic site

Supplemental Table 5. Sequence polymorphisms between B73 and By804 in the candidate gene *TDC2* identified by re-sequencing.

Site at *TDC2*[a]	Physical Position (bp)[b]	B73/By804[c]	Amino Acid replacement
-489	17855175	T/G	
-478	17855186	C/83bp-Insertion	
-472	17855192	G/A	
-427	17855237	-/CATG-Insertion	
-384	17855280	-/A-Insertion	
-294	17855370	TAA/-	
-263	17855401	A/T	
-252	17855412	A/C	
-196	17855468	G/C	
-145	17855519	CAC/-	
-135	17855529	T/A	
25	17855689	C/T	
62	17855726	G/TCTCT	
65	17855729	AA/CGC	
72	17855736	G/C	
76	17855740	G/T	
80	17855744	CG/-	
91	17855755	AA/-	
103	17855767	AAAT/-	
113	17855777	-/C-Insertion	
115	17855779	G/A	
123	17855787	TTCACC/-	
138	17855802	A/T	
161	17855825	C/A	
320	17855984	C/T	
398	17856062	C/T	
421	17856085	A/C	Asp/Ala
425	17856089	G/T	Lys/Asn
806	17856470	G/A	
957	17856621	A/G	Thr/Ala
1022	17856686	T/C	
1069	17856733	C/G	Ala/Gly
1094	17856758	G/T	
1106	17856770	C/T	
1160	17856824	A/C	Glu/Asp
1223	17856887	T/G	
1445	17857109	G/T	
1448	17857112	A/G	
1542	17857206	A/T	Met/Leu

1575	17857239	C/A
1586	17857250	A/T
1637	17857301	T/C
1749	17857413	ATGGAGATG/-
1764	17857428	GATTAT/-
1891	17857555	A/G

a) The location of each polymorphic site in *TDC2*

b) The physical position of each polymorphic site in the maize genome

c) Alleles from B73 and By804 at each polymorphic site

Supplemental Table 6. Primers used for re-sequencing *TDC1* and *TDC2* in B73 and By804 and PCR-based InDel detection.

Gene	Primers	Forward	Reverse
TDC1	P1	AACCATCTTTCACTCAGAAGGA	TTCCGACCAGACGCACTTT
	P2	CGGAACAGTAGACGACGAGG	CGAGGCGGCTCGTATTTTTC
	P3	GAGCCGCCTCGTAGCTATTT	TAGAAAACGCGGCAGCCTAA
	P4	GATTAGCCGCACCCCTTCTAC	GCTCCTTTAGCCACATCGTGA
	P5	ATGACATACGCAGTGGCGAA	TTCTGGTGTGCTCTCTACGC
	P6	CCAGTGTCAACCCTGGCTTC	GGAACTCTGGGCAGACGAG
TDC2	P1	GTGTAGTACGTGTACCCCTCG	CGTAGTAGTCGGCGATGAAGT
	P2	GGTACCATGGCCCCGCCATCACACTT	TCTAGATCAATAATCTTCATGCATCTCCATT
	P3	TCCAAGTCATGGCAAAGAGGC	TCTTGAGTGTAGCTTGGGATGC
	Primers for PCR-based InDel Detection	TTGGAAAAGCAGGCAGCCTAA	ATCCTCGCCCTGTCAAGTTT

Supplemental Table 7. Summary of QTL intervals for seven agronomic traits.

Trait	Chromosome	Length of Confidence Interval (cM)	Physical Interval (bp)	LOD	R^2 (%)
Plant height	8	3.6	0-1454311	3.311315	6.8285
Biomass	2	13.5	229414096-234266187	3.615849	8.9016
Biomass	7	10.9	157504609-162259657	4.372863	11.0697
Biomass	10	6.8	138086034-141377442	5.5566	14.0128
Ear height	4	10.1	3140718-4686236	4.067802	8.5249
Ear height	5	18.8	27394919-31860788	3.34793	6.8949
Length of ear leaf	1	4.4	243021141-246472571	4.487848	8.5478
Length of ear leaf	3	18.4	19966911-123658210	4.400415	8.056
Length of ear leaf	6	6.4	0-3988353	5.037573	9.2922
Width of ear leaf	1	9.2	43765452-56573123	3.490645	6.5027
Width of ear leaf	2	17.2	28119790-64390401	5.852753	11.1137
Width of ear leaf	8	14.9	73852614-119721769	3.398459	6.3953
Tassel length	1	6.3	7456817-9058572	8.137053	15.194
Tassel length	3	13.5	9316642-18008789	4.529325	7.6388
Tassel length	3	11.8	204291844-211764342	3.364062	5.708
Tassel length	3	8.3	211950617-215929305	3.379695	5.734
Tassel length	6	16.8	14580546-88907571	5.909388	10.4445
Tassel branch number	1	10.7	199631639-212579074	4.790648	7.7919
Tassel branch number	2	6.5	36891244-44472541	7.728419	13.004
Tassel branch number	4	9.1	188487198-200540299	4.995559	8.2013
Tassel branch number	4	3.5	202075058-218385416	3.948648	6.5641
Tassel branch number	4	4.4	218385416-228662377	3.651538	6.0937
Tassel branch number	8	7	5319559-7624018	4.928994	7.9562