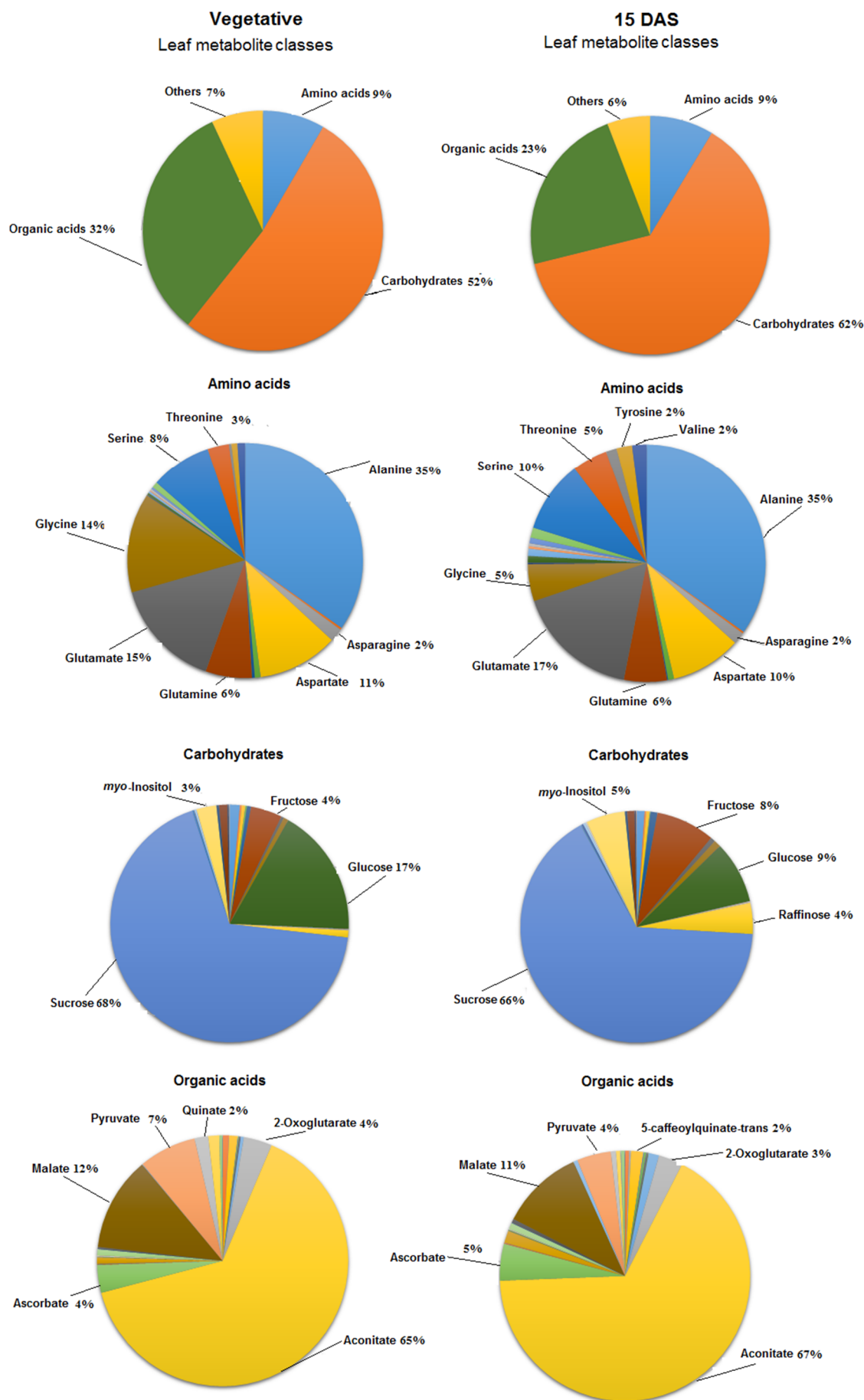


**Supplemental Figure 1.** Genetic relatedness of the 19 maize lines within the origin of the five groups of lines.

(Supports Figures 2, 3, 4.)

The tropical, European Flint, Northern Flint, Corn Belt Dent and Stiff Stalk lines were originally defined by Camus-Kulandaivelu et al. (2006) and classified within the S1P9 genetic diversity panel on the basis of their genetic relatedness using SSR markers (see Supplemental Table 1 for details).



**Supplemental Figure 2A.** Average proportions of different metabolite classes in the 19 maize lines representative of American and European plant diversity.

(Supports Figure 1.)

Plants were grown in the field under non-limiting N feeding conditions. Metabolite content was determined by GC-MS analysis at the vegetative stage (V) on a fully developed young leaf and at the grain filling stage (15 DAS) on the leaf below the ear.

**Amino acids**

■ Alanine	■ β-Alanine	■ Glycine	■ Lysine	■ Proline	■ Tyrosine
■ Arginine	■ GABA	■ Histidine	■ Methionine	■ Serine	■ Valine
■ Asparagine	■ Glutamine	■ Isoleucine	■ O-Acetylserine	■ Threonine	
■ Aspartate	■ Glutamate	■ Leucine	■ Phenylalanine	■ Tryptophan	

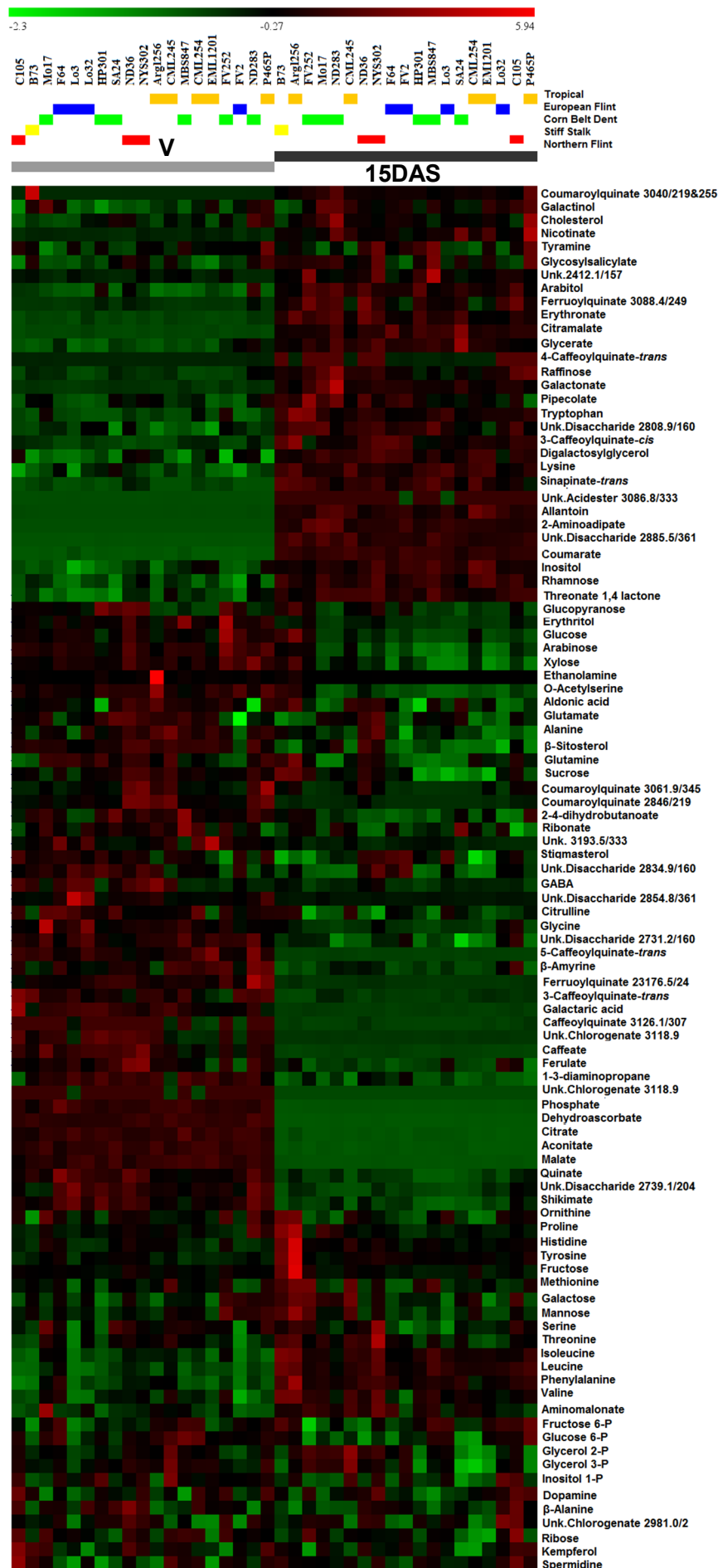
**Organic acids**

■ 3-caffeoylquininate- <i>cis</i>	■ Galactonate	■ Aconitate
■ 4-caffeoylquininate- <i>trans</i>	■ Glycolate	■ Ascorbate
■ U2846/345 (Coumaroylquininate)	■ Itaconate	■ Caffeate
■ U2976.4/345 (3-Coumaroylquininate)	■ Maleate	■ Citrate
■ U3061.9/345 (Coumaroylquininate)	■ Pipecolate	■ Dehydroascorbate
■ U3126.1/307(Cafeoylquininate)	■ Quinate	■ Ferulate - <i>trans</i>
■ U3176.5/249(Ferruloylquininate-2)	■ 3-caffeoylquininate- <i>trans</i>	■ Galactaric acid
■ 2-oxoglutarate	■ 5-caffeoylquininate- <i>trans</i>	■ Glycerate
■ Aminomalonate	■ U2968.9/249 (Ferruloylquininate)	■ Glycosylsalicylate
■ β-hydroxybutyrate	■ U3040/255 (Coumaroylquininate)	■ Malate
■ Citramalate	■ U3088.4/249(Ferruloylquininate)	■ Nicotinate
■ Coumarate	■ U3151/249&255(Ferruloylquininate)	■ Pyruvate
■ Erythronate	■ 2-4-dihydroxybutanoate	■ Shikimate
■ Fumarate		

**Carbohydrates**

■ β-Sitosterol	■ Arabitol	■ Stigmasterol	■ Glucose
■ Phytol-2	■ Glycerol	■ Arabinose	■ Maltose
■ U1824.8/123	■ Mannitol	■ Dihydroxyacetone-P	■ Raffinose
■ Digalactosylglycerol	■ Threitol	■ Glucose-6-P	■ Trehalose
■ Galactinol	■ Fructose-6-P	■ Glycerol-2-P	■ Xylulose
■ Galactosylglycerol	■ Glycerate-3-P	■ <i>myo</i> -Inositol-1-P	■ Kempferol
■ Kestose-1	■ Glycerol-3P	■ Trehalose-6-P	■ Erythriol
■ Mannose	■ Rivose-5-P	■ U532.8/307	■ Inositol-scylo
■ Sucrose	■ U3025.8/204(Galactinol)	■ U1864.3/307(Ketohexose)	■ <i>myo</i> -Inositol
■ Xylose	■ U1860.5/217	■ Fructose	
■ U2412.1/157	■ Campesterol	■ Galactose	

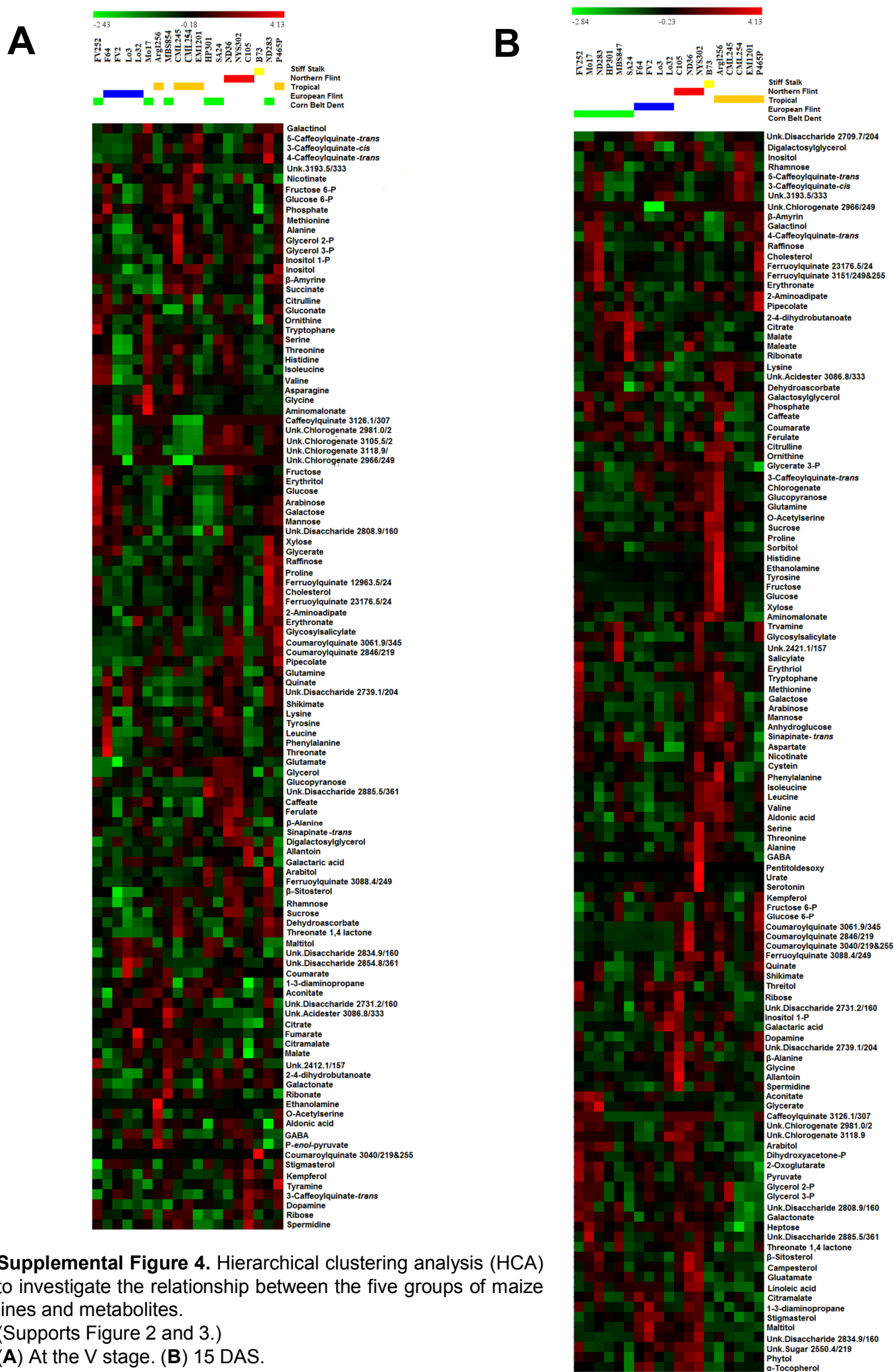
**Supplemental Figure 2B.** List of metabolites and color codes for Supplemental Figure 2A.  
(Supports Figure 1.)

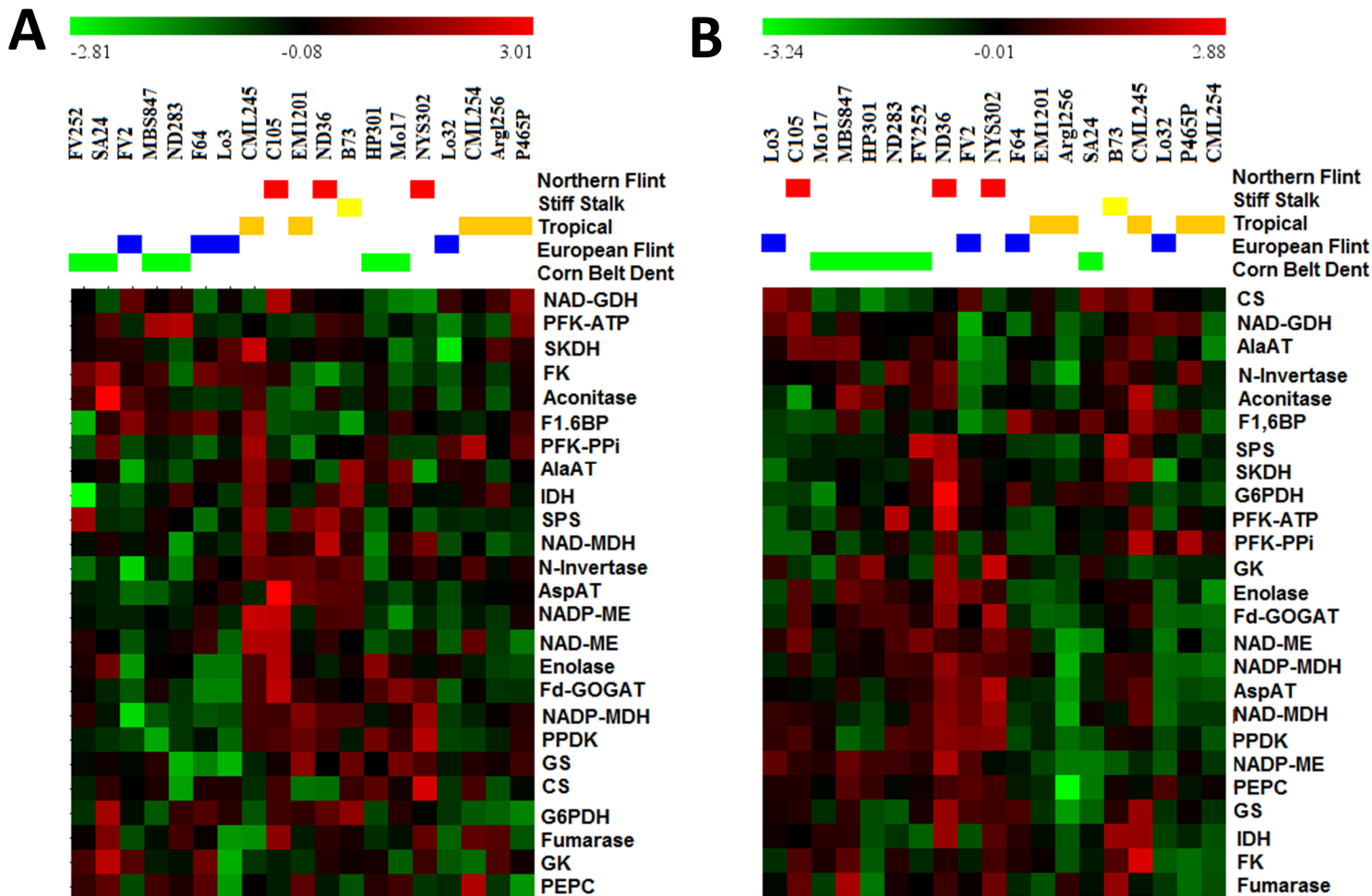


**Supplemental Figure 3.** Hierarchical clustering analysis (HCA) of metabolites and at the V stage and 15 DAS in the 19 maize lines.

(Supports Figure 2 and 3.)

The intensity of the green and red colors correspond to a lower and higher amount of metabolite (scale at the top of the figure).





**Supplemental Figure 5.** Hierarchical clustering analysis (HCA) used to investigate the relationship between the five groups of maize lines of the maize lines and enzyme activities.

(Supports Figure 4.)

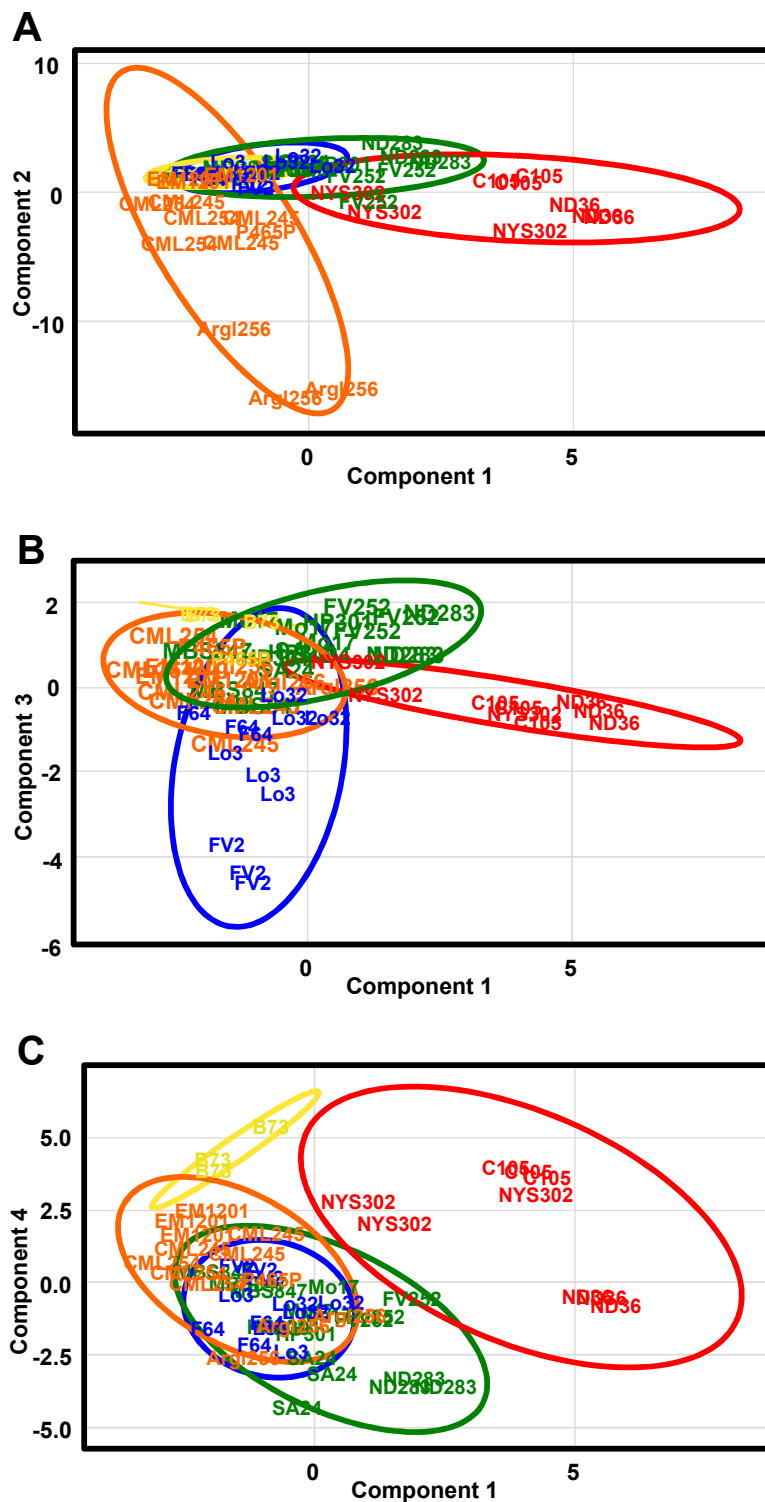
The intensity of the green and red colors corresponds to lower and higher enzyme activities (scale at the top of the figure). **(A)** At the V stage. **(B)** At 15 DAS.

	B73	FV2	Lo3	Lo32	F64	EM1201	CML254	CML245	Argl256	P465P	ND283	FV252	SA24	Mo17	MBS847	HP301	C105	ND36	NYS302	
B73		0.9006	0.9725	0.9721	0.9725	0.9709	0.9721	0.9725	0.9108	0.9831	0.9108	0.9709	0.9725	0.9697	0.9198	0.9941	0.9831	0.9100	0.9013	
FV2			0.9006	0.9006	0.8994	0.8998	0.9006	0.8994	0.9104	0.9006	0.9143	0.9013	0.9006	0.8994	0.9013	0.9006	0.9006	0.9175	0.9281	
Lo3				0.9823	0.9733	0.9717	0.9733	0.9831	0.9104	0.9721	0.9096	0.9713	0.9957	0.9693	0.9194	0.9725	0.9721	0.9100	0.9013	
Lo32					0.9725	0.9717	0.9733	0.9733	0.9100	0.9729	0.9068	0.9697	0.9823	0.9709	0.9194	0.9729	0.9721	0.9100	0.9013	
F64						0.9721	0.9733	0.9725	0.9100	0.9725	0.9084	0.9713	0.9725	0.9709	0.9198	0.9725	0.9725	0.9088	0.9013	
EM1201							0.9717	0.9717	0.9084	0.9721	0.9088	0.9697	0.9717	0.9709	0.9206	0.9721	0.9721	0.9096	0.9009	
CML254								0.9725	0.9104	0.9721	0.9061	0.9705	0.9733	0.9705	0.9194	0.9721	0.9721	0.9072	0.9013	
CML245									0.9104	0.9729	0.9072	0.9701	0.9831	0.9693	0.9194	0.9733	0.9725	0.9100	0.9013	
Argl256										0.9100	0.9289	0.9104	0.9104	0.9068	0.9108	0.9100	0.9100	0.9261	0.9123	
P465P											0.9088	0.9697	0.9721	0.9796	0.9296	0.9733	0.9839	0.9100	0.9013	
ND283												0.9198	0.9068	0.9072	0.9100	0.9092	0.9096	0.9316	0.9159	
FV252													0.9701	0.9689	0.9178	0.9697	0.9697	0.9100	0.9013	
SA24														0.9705	0.9194	0.9725	0.9729	0.9076	0.9013	
Mo17															0.9269	0.9709	0.9697	0.9084	0.9006	
MBS847																				
7																				
HP301																0.9198	0.9198	0.9119	0.9017	
C105																	0.9823	0.9096	0.9006	
ND36																		0.9096	0.9006	
NYS302																			0.9190	

**Supplemental Figure 6.** Metabolism similarity matrix indicating the ratio of active reactions with shared flux ranges between the 19 maize lines in a pairwise manner.

(Supports Figure 5.)

Regulation was imposed on the genome-scale leaf model based on enzyme activities. The flux range of each reaction was determined for each maize line at the time of maximum biomass production. The flux ranges were then compared between two maize lines to determine the number of overlapping flux ranges compared to the total number of reactions active in either maize line. The background color within the figure represents the similarity of each pair of maize lines compared to all other pairs with green indicating very similar, yellow moderately similar, and pale orange dissimilar. The vertical and horizontal green, blue, orange, red and yellow boxes represent the five groups of maize lines in different countries of Europe and America (Stiff Stalk: yellow; European Flint: Blue; Tropical: Orange; Corn Belt Dent: green and Northern Flint: red).

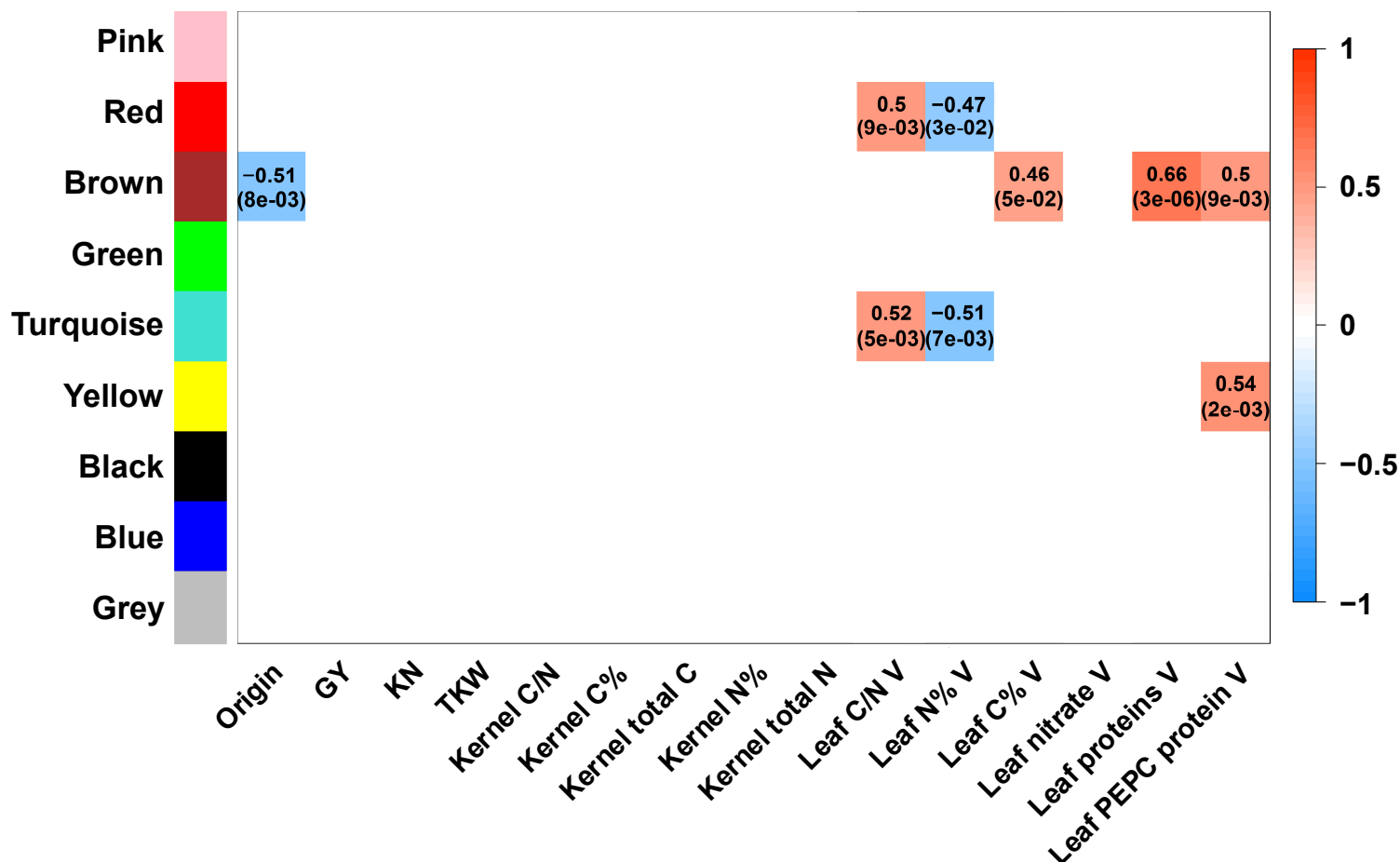


**Supplemental Figure 7.** The five groups of maize lines identified by means of specific leaf metabolic signature during the grain filling period.

(Supports Figure 3.)

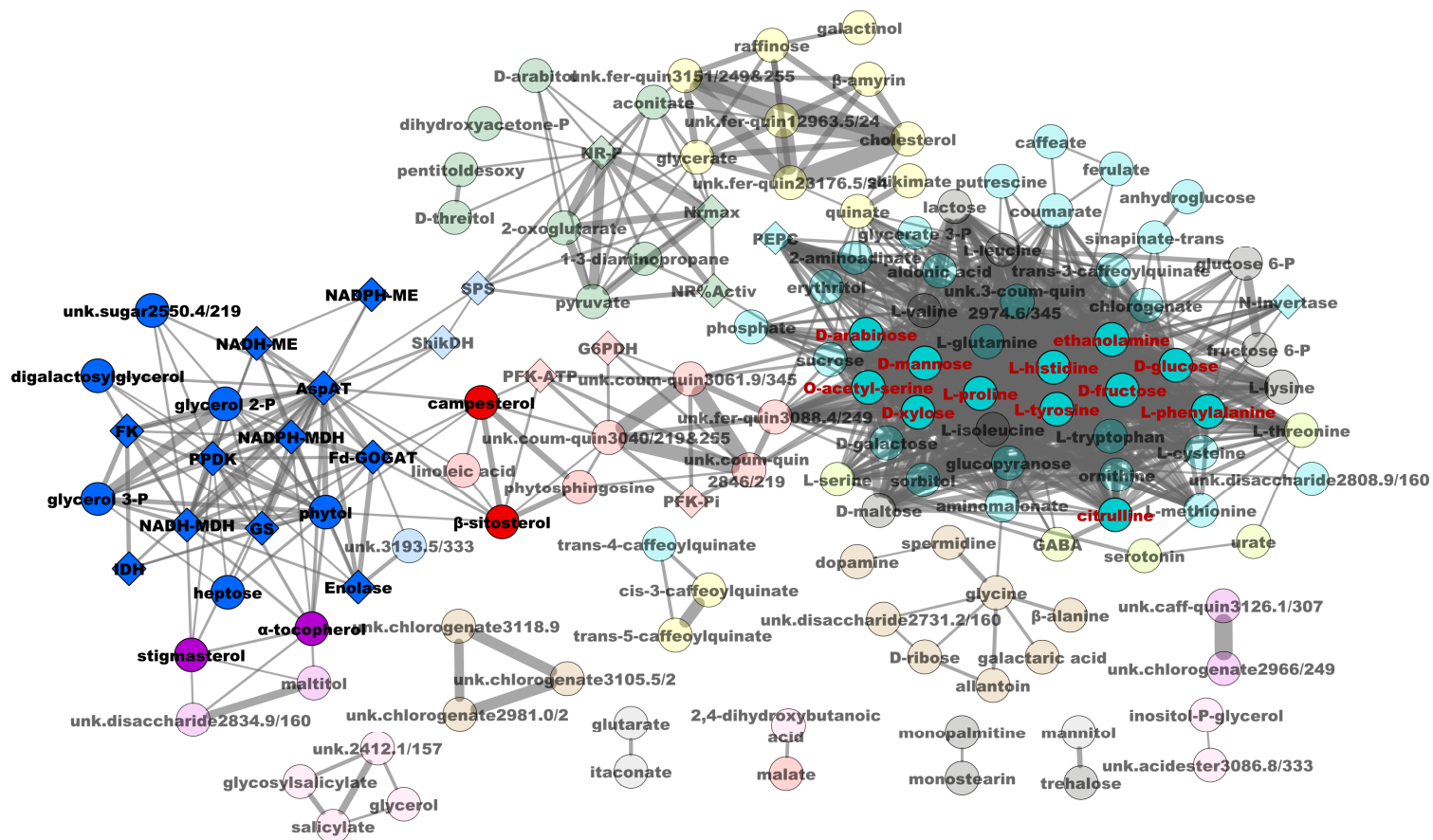
Example of representation in sPLS-DA of metabolites 15 DAS. **(A)** using component 1 and component 2. **(B)** using component 1 and component 3. **(C)** using component 1 and component 4. The green, blue, orange, red and yellow colors represent the five groups of maize lines in different countries of Europe and America (Tropical: Orange; European Flint: Blue; Northern Flint: red; Corn Belt Dent: green and Stiff Stalk: yellow).





**Supplemental Figure 8.** Heatmap and Pearson correlations with the modules and agronomic traits (GY, KN and TKW) and physiological parameters representative of the kernel and leaf physiological status at the V stage. (Supports Figure 7.)

Correlations with the modules containing the generic physiological traits at the V stage. The color names correspond to the 9 modules that were obtained from the V stage metabolite and enzyme activity data set network analyses (Supplemental Data Set 10). Heatmap and Pearson correlations between modules and kernel yield traits (GY, KN and TKW) and physiological parameters representative of the kernel and leaf physiological status (C = carbon, N = nitrogen, C/N ratio, leaf soluble protein, PEPC protein and nitrate contents). Correlations were considered significant with Bonferroni Adjusted-*P*-values <0.05. Adjusted-*P*-values are shown between parentheses. Only significant negative and positive correlation coefficient values are indicated in each colored box. For clarity, only those with a correlation higher than 0.4 have been considered. The box of the heat map using the scale on the right side of the panel. e = exponent base 10. The same analysis was performed at the 15 DAS stage (Figure 7).



**Supplemental Figure 9.** Network diagram of relationships between leaf metabolites and enzyme activities 15 DAS.

(Supports Figure 8.)

Diamond shapes represent enzymes and circles represent metabolites. Colors of the circles and diamonds correspond to the different components within a module. The names of metabolites and enzymes positively or negatively correlated with TKW are highlighted in bold black and in red characters respectively. Only the network connections for which a topological overlap above the threshold of 0.1 are shown. Lines represent a significant correlation between two traits. Thicker lines represent the highest positive or negative correlations. The same analysis was performed at the 15 DAS stage for correlations with GY (Figure 8).

**Supplemental Table 1.** Classification of the 19 maize lines originating from Europe and America on the basis of their genetic relatedness.

(Supports Figures 2, 3, 4.)

Each line belongs to five main structural groups identified on the complete S1P9 diversity panel of 375 lines using 55 (SSR) microsatellites (Camus-Kulandaivelu et al., 2006) and further refined by Bouchet et al. (2013) by admixture based on Panzea Illumina array (SNP) on 347 lines from the S1P9 diversity panel. A value closest to 1 means that the line belongs to one of the 5 defined structural groups. T = Tropical, CBD = Corn Belt Dent, SS = Stiff Stalk, NF = Northern Flint, EF = European Flint. The colors correspond to those used in the figures.

Name	Pedigree	Country	Geographic Origin	Breeder Groups	Kernel Type	SSR Camus-Kulandaivelu et al., 2006						SNP (Bouchet et al., 2013)					
						T	CBD	SS	NF	EF	Groups	T	CBD	SS	NF	EF	Groups
CML254	Pop 21, Tuxpeno Sequia	Mexico	Central America	Tropical	Semi Dent	98%	1%	0%	0%	1%	T	98%	2%	1%	0%	0%	T
CML245	Pop 86, CO	Mexico	Central America	Tropical	Semi Flint	80%	1%	12%	5%	2%	T	Not analyzed due to bad genotyping					
EM1201	Gran Amarillo Andaluz	Spain	Southern Europe	Tropical	Semi Flint	85%	4%	2%	1%	9%	T	73%	0%	0%	0%	27%	Ad T - EF
ARGL256	Double Hybrid Flint	Argentina	Southern America	Argentina Flint	Flint	92%	4%	1%	1%	2%	T	52%	3%	0%	17%	27%	Ad T - EF - NF
P465P	Orange Flint	Argentina	Southern America	Argentina Flint	Flint	41%	38%	0%	5%	17%	Ad T - CBD	33%	49%	4%	6%	8%	Ad T - CBD
FV252	F186 x Co125	France	Western Europe	Early Dent	Dent	1%	97%	1%	1%	1%	CBD	0%	100%	0%	0%	0%	CBD
MBS847	Pioneer 3901	USA	Northern America	Iodent Dent	Dent	1%	95%	1%	2%	1%	CBD	0%	97%	3%	0%	0%	CBD
Mo17	CI187-2 x C103	USA	Northern America	Lancaster Dent	Dent	1%	92%	2%	1%	3%	CBD	0%	99%	0%	1%	0%	CBD
ND283	Haney's Minesota 13	USA	Northern America	Min13 Dent	Dent	3%	94%	2%	1%	1%	CBD	16%	59%	7%	15%	3%	Ad CBD - T/NF
HP301	Supergold	USA	Northern America	Pop-corn	Pop	3%	84%	1%	7%	5%	CBD	21%	39%	4%	35%	0%	Ad NF - CBD - T
SA24	South American	USA	Northern America	Pop-corn	Pop	3%	62%	0%	8%	27%	Ad CBD - EF	27%	33%	3%	37%	0%	Ad NF - CBD - T
B73	BSSC5	USA	Northern America	StiffStalk Dent	Dent	1%	1%	98%	0%	1%	SS	0%	14%	86%	0%	0%	SS
C105	Purple Flour x Early_Yellow_Dent	USA	Northern America	Dent	Semi Dent	8%	42%	0%	49%	1%	Ad NF-CBD	12%	25%	5%	55%	3%	Ad NF - CBD
NYS302	Black Mexican	USA	Northern America	Northern Flint	Sweet	0%	1%	0%	98%	1%	NF	0%	0%	0%	100%	0%	NF
ND36	Manitoba Yellow Flint	USA	Northern America	Northern Flint	Flint	1%	1%	0%	97%	1%	NF	Not analyzed due to bad genotyping					
FV2	Lacaune	France	Western Europe	European Flint	Flint	1%	0%	1%	1%	97%	EF	0%	0%	0%	8%	92%	EF
Lo3	Nostrano dell Isola	Italy	Southern Europe	European Flint	Flint	3%	1%	2%	3%	91%	EF	36%	0%	0%	0%	64%	Ad EF - T
Lo32	Isola Basso	Italy	Southern Europe	European Flint	Flint	17%	2%	1%	2%	79%	EF	38%	0%	0%	0%	62%	Ad EF - T
F64	PI-186223 Argentina	Argentina	Southern America	Argentina Flint	Flint	15%	2%	1%	1%	81%	EF	55%	2%	1%	9%	33%	Ad T-EF

The links providing information on the lines are presented below.

Line	USDA GenBank	CIMMYT GenBank	Maize GDB
CML254	<a href="https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=PI+595542">https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=PI+595542</a>	<a href="http://mgb.cimmyt.org/gringlobal/accessiondetail.aspx?id=17586">http://mgb.cimmyt.org/gringlobal/accessiondetail.aspx?id=17586</a>	<a href="http://www.maizegdb.org/data_center/stock?id=105108">http://www.maizegdb.org/data_center/stock?id=105108</a>
CML245	No	<a href="http://mgb.cimmyt.org/gringlobal/AccessionObservation.aspx?id=20048">http://mgb.cimmyt.org/gringlobal/AccessionObservation.aspx?id=20048</a>	<a href="http://www.maizegdb.org/data_center/stock?id=105098">http://www.maizegdb.org/data_center/stock?id=105098</a>
EM1201	No	No	No
ARGL256	No	No	No
P465P	No	No	No
FV252	No	No	No
MBS847	No	No	No
Mo17	<a href="https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1103586">https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1103586</a>	No	<a href="http://www.maizegdb.org/data_center/stock?id=68498">http://www.maizegdb.org/data_center/stock?id=68498</a>
ND283	<a href="https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=PI+558532">https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=PI+558532</a>	No	<a href="http://www.maizegdb.org/data_center/stock?id=47846">http://www.maizegdb.org/data_center/stock?id=47846</a>
HP301	<a href="https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=PI+587131">https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=PI+587131</a>	No	<a href="http://www.maizegdb.org/data_center/stock?id=47773">http://www.maizegdb.org/data_center/stock?id=47773</a>
SA24	<a href="https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=Ames+27188">https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=Ames+27188</a>	No	<a href="http://www.maizegdb.org/data_center/stock?id=47916">http://www.maizegdb.org/data_center/stock?id=47916</a>
B73	<a href="https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1445409">https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1445409</a>	No	<a href="http://www.maizegdb.org/data_center/stock?id=47638">http://www.maizegdb.org/data_center/stock?id=47638</a>
C105	<a href="https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=NSL+32721">https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?accid=NSL+32721</a>	No	<a href="http://www.maizegdb.org/data_center/stock?id=68505">http://www.maizegdb.org/data_center/stock?id=68505</a>
NYS302	No	No	<a href="http://www.maizegdb.org/data_center/stock?id=70301">http://www.maizegdb.org/data_center/stock?id=70301</a>
ND36	No	No	<a href="http://www.maizegdb.org/data_center/stock?id=68568">http://www.maizegdb.org/data_center/stock?id=68568</a>
FV2	No	No	No
Lo3	No	No	No
Lo32	No	No	No
F64	No	No	No

**Supplemental Table 2.** The enzymes involved in central C and N metabolism used for studying genotypic variation in their activity and for determining the metabolic changes through modelling.

(Supports Figures 4 and 8.)

Their activity was measured in 19 maize lines representative of American and European plant genetic diversity using a robot-based platform according to the protocol described by Gibon et al. (2004).

Enzymes (abbreviation)	Full name	Metabolic pathway	Substrate	Product
PEPC	Phosphoenolpyruvate carboxylase	C4 metabolism	Phosphoenolpyruvate	Oxaloacetate + Pi
PEPCK	Phosphoenolpyruvate carboxykinase	C4 metabolism	Oxaloacetate	Phosphoenolpyruvate, carbon dioxide.
PPDK	Pyruvate, phosphate dikinase	C4 metabolism	ATP, pyruvate, phosphate	ADP, phosphoenolpyruvate, pyrophosphate
NADP-MDH	NADP - Malate dehydrogenase	C4 metabolism	Malate, NADP <sup>+</sup>	Oxaloacetate + NADPH + H <sup>+</sup>
NAD-ME	NAD Malic enzyme	C4 metabolism	Malate, NAD <sup>+</sup>	Pyruvate + CO <sub>2</sub> + H <sup>+</sup>
NADP-ME	NADP Malic enzyme	C4 metabolism	Malate, NADP <sup>+</sup>	Pyruvate + CO <sub>2</sub> + H <sup>+</sup>
N-Invertase	Neutral Invertase	Carbohydrate metabolism	Sucrose	Fructose, Glucose
GK	Glucokinase	Carbohydrate metabolism	Glucose, ATP	Glucose 6-phosphate, AMP
SPS	Sucrose phosphate synthase	Carbohydrate metabolism	UDP-Glucose, fructose 6-phosphate, P <sub>i</sub>	UDP, sucrose 6-phosphate
FK	Fructokinase	Carbohydrate metabolism	Fructose, ATP	Fructose 6-phosphate, AMP
F1,6BP	Fructose 1,6 Bisphosphatase	Glycolysis	Fructose-1,6-bisphosphate	Fructose-6-phosphate, phosphate
PFK-PPi (PFP)	Phosphofructokinase-PPi	Glycolysis	Fructose-6-phosphate, diphosphate	Phosphate, fructose-1,6-bisphosphate
PFK-ATP	ATP-phosphofructokinase	Glycolysis	Diphosphate, fructose-6-phosphate	Phosphate, fructose-1,6-bisphosphate, ATP
Enolase	Enolase	Glycolysis	2-phosphoglycerate	Phosphoenolpyruvate
Fd-GOGAT	Glutamine 2-oxoglutarate amidotransferase	Nitrogen assimilation	Glutamine, 2-oxoglutarate	Glutamate
AlaAT	Alanine Aminotransferase	Nitrogen assimilation	Glutamate, pyruvate	2-oxoglutarate, alanine
AspAT	Aspartate Aminotransferase	Nitrogen assimilation	Aspartate, 2-oxoglutarate	Oxaloacetate, glutamate
GS	Glutamine synthetase	Nitrogen assimilation	Glutamate, ATP, NH <sub>3</sub>	Glutamine, phosphate
NAD-GDH	NAD-Glutamate dehydrogenase	Nitrogen metabolism	Glutamate, NAD	2-oxoglutarate + NH <sub>3</sub> + NADH + H <sup>+</sup>
NRmax	Nitrate Reductase (maximal activity)	Nitrogen assimilation	Nitrate, NADH, H <sup>+</sup>	Nitrite, NAD
NR-P	Nitrate Reductase (inactive NR)	Nitrogen assimilation	Nitrate, NADH, H <sup>+</sup>	Nitrite, NAD
NR%	Nitrate Reductase (% active NR)	Nitrogen assimilation	Nitrate, NADH, H <sup>+</sup>	Nitrite, NAD
G6PDH	Glucose -6 -Phosphate Dehydrogenase	Pentose phosphate pathway	Glucose 6-phosphate, NADP <sup>+</sup>	6-phosphogluconate, NADPH
ShikDH	Shikimate Dehydrogenase	Shikimate pathway	3-dehydroshikimate, NADPH	Shikimate, NADP <sup>+</sup>
Aconitase	Aconitase	Tricarboxylic acid cycle	Citrate, cis-Aconitate	Aconitate, isocitrate
CS	Citrate synthase	Tricarboxylic acid cycle	Acetyl-CoA and oxaloacetate	Citrate
IDH	Isocitrate dehydrogenase	Tricarboxylic acid cycle	Isocitrate, NAD <sup>+</sup>	2-oxoglutarate, NADH
NAD-MDH	NAD-Malate dehydrogenase	Tricarboxylic acid cycle	Malate, NAD <sup>+</sup>	Oxaloacetate + NADH + H <sup>+</sup>
Fumarase	Fumarate hydratase	Tricarboxylic acid cycle	Fumarate	Malate