Evidence of a plant genetic basis for maize roots impacting soil organic matter mineralization

Article

Supplemental Material

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Table S1. Clustering of maize genotypes based on the measured phenotypes. Values are cluster means. Values in parentheses show range of variation within the cluster. Values in Bold indicates the best mean performance of lines among the clusters for specific trait.

Cluster	# of genotypes	SOMC (µg C g^{-1} soil)	CRM (µg C g^{-1} soil)	SBM (g)	RBM (g)	RL (m)	ARD (mm)	SRL (m g^{-1} root biomass)	BM-RS
I	42	17.97 (17.68-18.72)	19.06 (14.84-21.96)	0.40 (0.34-0.51)	0.20 (0.17-0.26)	15.53 (13.39-18.03)	0.51 (0.48-0.52)	80.07 (71.91-86.93)	0.51 (0.48-0.54)
II	24	18.08 (17.15-19.00)	21.34 (16.61-31.77)	0.42 (0.34-0.51)	0.23 (0.17-0.26)	15.80 (13.39-18.03)	0.51 (0.48-0.52)	73.14 (71.91-86.93)	**0.54** **(0.48-0.54)**
III	2	18.28 (18.16-18.40)	12.76 (12.19-13.32)	0.21 (0.19-0.22)	0.10 (0.09-0.11)	9.27 (8.68-9.86)	0.49 (0.48-0.5)	**116.04** **(111.04-121.04)**	0.46 (0.45-0.47)
IV	11	**18.60** **(17.71-19.14)**	17.13 (15.62-19.55)	0.33 (0.29-0.38)	0.18 (0.15-0.21)	13.54 (12.09-15.47)	0.52 (0.51-0.53)	79.18 (68.54-84.95)	0.53 (0.48-0.58)
V	16	18.90 (17.58-19.43)	16.11 (13.06-19.12)	0.30 (0.23-0.34)	0.15 (0.12-0.17)	13.05 (11.5-14.43)	0.49 (0.48-0.51)	87.07 (77.06-96.06)	0.51 (0.48-0.53)
VI	10	18.07 (17.48-18.79)	**22.18** **(18.49-27.34)**	**0.54** **(0.47-0.66)**	**0.29** **(0.27-0.34)**	**16.10** **(13.88-17.41)**	**0.53** **(0.51-0.55)**	66.37 (61.03-71.81)	0.53 (0.5-0.56)

CRM, cumulative root-derived C mineralized; SOMC, cumulative soil organic matter C mineralized; SBM, shoot biomass; RBM, root biomass; RL, root length; ARD, average root diameter; SRL, specific root length; BM-RS, root-to-shoot biomass ratio.

Table S2. Stepwise multiple linear regression to explain soil organic matter C mineralization under combined maize genotypes (lines and hybrids) and maize lines alone. Only the variables in the model that explain a significant ($P < 0.05$) amount of variance or additional variance in soil organic matter C mineralization are presented. No variable could significantly explain the variance in soil organic matter C mineralization in the hybrids alone.

Variables	Model	R^2	*P*-value	RMSE
Lines and hybrids				
RL	SOMC = 25.65 - 0.49xRL	0.161	<0.0001	4.770
ARD	SOMC = -0.88 + 38.35xARD	0.127	<0.0001	4.865
CRM	SOMC = 16.20 + 0.12xCRM	0.058	0.0003	5.055
RL, ARD	SOMC = 8.71 - 0.42xRL + 31.76xARD	0.245	<0.0001	4.534
RL, CRM	SOMC = 25.24 + 0.27xCRM - 0.82xRL	0.406	<0.0001	4.024
ARD, CRM	SOMC = -1.55 + 0.10xCRM + 35.90xARD	0.168	<0.0001	4.761
RL, ARD, CRM	SOMC = 14.35 + 0.25xCRM - 0.75xRL + 20.47xARD	0.439	<0.0001	3.918
Lines				
RL	SOMC = 25.74 - 0.50xRL	0.172	<0.0001	4.844
ARD	SOMC = -5.66 + 47.96xARD	0.154	<0.0001	4.898
CRM	SOMC = 16.09 + 0.12xCRM	0.055	0.0008	5.175
RL, ARD	SOMC = 5.25 - 0.42xRL + 38.51xARD	0.266	<0.0001	4.572
RL, CRM	SOMC = 25.30 + 0.28xCRM - 0.83xRL	0.416	<0.0001	4.078
ARD, CRM	SOMC = -7.13 + 0.11xCRM + 46.82xARD	0.201	<0.0001	4.770
RL, ARD, CRM	SOMC = 10.17 + 0.26xCRM - 0.74xRL + 28.50xARD	0.466	<0.0001	3.909

RL, root length (m); ARD, average root diameter (mm); CRM, cumulative root-derived C mineralized (µg C g^{-1} soil); SOMC, cumulative soil organic matter C mineralized (µg C g^{-1} soil), RMSE, root mean squared error

Table S3. Mean, variance components and least significant difference (LSD) at 5% for nine traits for maize inbred lines in the DTMA association panel.

Traits	**Mean**	$\boldsymbol{\sigma^2_G}$	$\boldsymbol{\sigma^2_e}$	$\boldsymbol{H^2}$	$\mathbf{LSD_{0.05}}$	**CV (%)**
SOM-C	18.09	1.28*	12.22	0.18	2.89	8.06
CRM	19.14	25.46**	89.52	0.36	11.22	49.44
SBM	0.39	0.01*	0.01	0.64	0.16	26.49
RBM	0.20	0.003*	0.003	0.64	0.09	27.59
RL	14.94	5.063**	9.74	0.51	4.40	20.88
ARD	0.51	0.0001*	0.001	0.41	0.04	6.19
SRL	78.08	161.02**	367.41	0.47	25.91	24.31
BM_RS	0.52	0.002*	0.01	0.35	0.15	15.20
Stover	0.60	0.02**	0.02	0.65	0.25	26.02

$\boldsymbol{\sigma^2_G}$, Genotypic variance; $\boldsymbol{\sigma^2_e}$, residual error variance; H^2, broad sense heritability; CRM, cumulative root-derived C mineralized; SOMC, cumulative soil organic matter C mineralized; SBM, shoot biomass; RBM, root biomass; Stover, total plant biomass (root and shoot biomass); RL, root length; ARD, average root diameter; SRL, specific root length; BM_RS, root-to-shoot biomass ratio. * and ** indicate significance at $P<0.05$ and $P<0.01$, respectively.

Table S4. Genome-wide association mapping results for nine root traits in the DTMA panel of maize inbred lines. A positive or negative value indicates that the minor, or major allele, respectively, was favorable for enhanced trait performance. Bold words indicate the two candidate genes we identify with the regulation and active release of root exudates into the rhizosphere.

Trait	SNP[a]	MLM P_value	MAF	Allele effect	Candidate gene	Predicted function
SOMC	S4_181781176	3.23E-05	0.12	-1.27	GRMZM2G060495	Protein coding
	S5_205925931	**2.87E-05**	**0.15**	**-1.13**	**GRMZM2G114362**	**proteolyses, peptidase activity**
	S6_148591382	2.75E-05	0.13	-1.35	GRMZM2G111984	hypothetical protein
	S8_111195562	4.78E-05	0.08	1.43	GRMZM2G308999	Protein coding
	S8_161480811	**2.30E-05**	**0.13**	**-1.19**	**GRMZM2G170962**	**proton-transporting ATP synthase complex, ATP synthesis**
	S8_161480826	2.30E-05	0.13	1.19	GRMZM2G170962	proton-transporting ATP synthase complex, ATP synthesis
CRM	S1_985248	3.12E-05	0.06	4.97	GRMZM2G172177	unknown
	S2_9549764	2.79E-05	0.06	-5.26	GRMZM2G094255	imidazoleglycerol-phosphate dehydratase activity, histidine biosynthetic process
	S2_37569109	2.54E-05	0.05	-5.22	GRMZM2G323309	unknown
	S2_210039092	5.42E-06	0.19	-4.38	GRMZM2G156257	peroxidase activity, heme binding
	S2_210059740	1.21E-05	0.22	-4.08	GRMZM2G102903	unknown
	S3_19942217	2.35E-05	0.06	-5.75	GRMZM2G112756	Protein coding
	S3_19942491	1.57E-05	0.08	-5.13	GRMZM2G112756	Protein coding
	S3_217294167	3.86E-05	0.28	-3.05	GRMZM2G074097	Thiamin biosynthetic process, oxidoreductase
	S3_218189653	3.99E-05	0.25	-3.48	GRMZM2G424380	Histone decetylation
	S3_225898259	2.59E-05	0.15	-4.99	GRMZM2G523266	unknown
	S4_162291835	2.05E-05	0.08	-4.56	GRMZM2G126865	Integral to membrane
	S4_200032025	3.61E-05	0.11	-3.97	AC190609.3_FG001	DNA binding, transcription regulation, TF activity
	S5_2128484	3.04E-05	0.12	-4.18	GRMZM2G554621	Protein coding
	S5_210020164	3.92E-05	0.1	-3.99	GRMZM2G142136	unknown
	S6_96126721	4.03E-05	0.12	-8	GRMZM2G489954	unknown
	S6_151453142	4.84E-05	0.25	-2.7	GRMZM2G093250	unknown
	S7_140817634	1.21E-05	0.16	-5.77	GRMZM2G102243	Zinc ion binding, protein binding
	S8_5330169	3.47E-05	0.12	-4.2	GRMZM2G178635	unknown
	S4_200032049	3.61E-05	0.11	-3.97	AC190609.3_FG001	DNA binding, transcription regulation, TF activity
	S4_200032051	3.61E-05	0.11	-3.97	AC190609.3_FG001	DNA binding, transcription regulation, TF activity
	S9_114066095	6.36E-06	0.17	-3.48	GRMZM2G393330	unknown
	S9_114066168	2.43E-05	0.15	-3.39	GRMZM2G393330	unknown
	S9_114075231	1.15E-05	0.18	-3.45	GRMZM2G393330	unknown
	S9_136558897	2.86E-05	0.2	-4.56	GRMZM2G307906	Zinc ion binding,G-protein coupled receptor protein signaling pathway

	S10_5588101	1.12E-05	0.25	-3.12	AC207755.3_FG006	DNA binding, transcription regulation
SBM	S5_108626300	1.25E-05	0.17	0.12	GRMZM2G494874	Protein coding
	S4_184857626	1.61E-05	0.3	-0.05	GRMZM2G588688	Protein coding
	S2_51286162	3.09E-05	0.29	0.07	GRMZM2G078650	Phosphoric diester hydrolase activity
RBM	S1_167896676	4.60E-05	0.17	0.03	AC202879.3_FG001	Zinc ion binding
	S2_51286162	2.96E-05	0.29	0.04	GRMZM2G078650	Phosphoric diester hydrolase activity
	S4_120643548	1.25E-05	0.28	-0.03	GRMZM2G024038	unknown
	S5_106827724	5.04E-05	0.18	-0.05	AC199575.4_FG001	unknown
	S5_108626300	5.42E-06	0.17	0.07	GRMZM2G494874	Protein coding
	S6_162676771	4.37E-05	0.21	0.03	GRMZM2G517650	Protein coding
	S10_5721642	4.47E-05	0.26	-0.04	AC207755.3_FG003	unknown
RL	S1_163955113	1.98E-05	0.25	1.65	GRMZM2G443525	ATP binding, nucleoside-triphosphatase activity, apoptosis
	S2_1455653	2.44E-05	0.1	2.5	GRMZM2G084938	enterobactin biosynthetic process, 2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase activity
	S2_42003323	3.59E-05	0.2	2.64	GRMZM2G114619	Actin binding
	S2_185112021	2.00E-05	0.07	3	GRMZM2G508000	Protein coding
	S5_14609315	3.71E-05	0.19	2.63	GRMZM2G078436	unknown
	S5_106827724	2.74E-05	0.18	-2.91	AC199575.4_FG001	unknown
	S5_108454858	3.45E-05	0.16	-3.42	GRMZM2G523758	Protein coding
	S5_108626300	1.64E-06	0.17	3.7	GRMZM2G494874	Protein coding
	S5_184053803	2.50E-05	0.18	3.28	GRMZM2G446162	unknown
	S5_197709795	2.63E-05	0.08	2.85	GRMZM2G181273	proteolyses, hydrolase activity, metallo peptidase activity
	S5_205386496	2.05E-05	0.08	-2.52	GRMZM2G076822	unknown
	S5_208076145	1.03E-05	0.09	2.63	AC220927.3_FG001	ORF
	S5_212653116	1.01E-06	0.08	2.87	GRMZM2G152842	unknown
	S5_212748815	9.65E-07	0.06	3.75	GRMZM2G178933	Proteolyses, serine-type carboxypeptidase activity
	S6_149679764	1.38E-05	0.21	2.29	GRMZM2G000842	DNA binding, transcription regulation, TF activity
	S7_167505164	5.01E-05	0.09	2.57	GRMZM2G381404	Zinc ion binding
	S7_168745476	5.49E-07	0.07	-3.29	GRMZM2G113036	Ice binding, homoiothermy, response to freezing, hsp binding
	S10_13471625	1.50E-05	0.08	2.56	GRMZM2G459636	unknown
	S10_33350094	3.84E-05	0.07	2.85	AC213605.3_FG006	ORF
	S10_33350095	3.84E-05	0.07	2.85	AC213605.3_FG006	ORF
	S10_33350099	3.84E-05	0.07	2.85	AC213605.3_FG006	ORF
	S10_33350101	3.84E-05	0.07	2.85	AC213605.3_FG006	ORF
	S10_33350102	3.84E-05	0.07	2.85	AC213605.3_FG006	ORF
	S10_33350093	3.84E-05	0.07	2.85	AC213605.3_FG006	ORF
ARD	S1_54277289	4.19E-05	0.06	0.02	GRMZM2G484075	hypothetical protein

	S1_170476442	7.13E-06	0.3	0.01	GRMZM2G069410	inorganic diphosphatase activity, H translocating phosphatase activity
	S2_194490634	2.96E-05	0.39	0.01	GRMZM2G114650	Ice binding, homoiothermy, response to freezing
	S2_233674088	8.04E-06	0.29	0.01	GRMZM2G104032	galactosidase 2-alpha-L-fucosyltransferase activity, cell wall biogenesis
	S7_25507724	1.45E-05	0.5	-0.01	GRMZM2G343504	unknown
	S3_178410942	4.17E-05	0.07	0.02	AC209855.3_FG002	unknown
	S6_150376054	2.50E-05	0.19	0.01	GRMZM2G037855	hypothetical protein
	S7_25505913	1.74E-06	0.46	0.01	GRMZM2G343504	unknown
	S7_139692066	2.84E-05	0.14	0.02	GRMZM2G114818	Proteolysis, cysteine-type peptidase activity
	S2_194490745	4.08E-05	0.48	-0.01	GRMZM2G114650	Ice binding, homoiothermy, response to freezing
	S7_173750491	4.53E-05	0.11	-0.02	GRMZM2G481983	unknown
	S10_148120002	6.41E-06	0.46	-0.01	GRMZM2G074773	DNA binding, intracellular
	S10_149557722	1.75E-05	0.13	-0.03	GRMZM2G178025	hydrolase activity, hydrolyzing O-glycosyl compounds
SRL	S1_17681223	1.34E-05	0.09	-7.27	GRMZM2G109431	transferase activity, transferring glycosyl groups
	S2_185112021	5.04E-06	0.07	-8.33	GRMZM2G508000	Protein coding
	S2_193815827	2.90E-06	0.13	-6.22	AC197242.3_FG001	ORF
	S3_173022359	1.08E-06	0.11	6.92	GRMZM2G305746	DNA binding
	S3_228448530	1.82E-05	0.17	-4.69	GRMZM2G006130	alpha-N-arabinofuranosidase activity
	S5_34966369	4.70E-10	0.11	-31	GRMZM2G539921	unknown
	S5_214281612	2.90E-05	0.29	3.74	GRMZM2G141456	hydrolase activity, chitinase activity, carbohydrate metabolic process
	S6_36089154	1.95E-08	0.23	-6.13	GRMZM2G124509	nucleic acid binding, ribonuclease H activity
BM_RS	S4_2488289	4.78E-05	0.41	-0.03	GRMZM2G441115	TF, Transcription regulation
	S4_2488290	9.16E-06	0.36	0.04	GRMZM2G441115	TF, Transcription regulation
	S9_124758697	2.34E-05	0.3	-0.03	GRMZM2G450685	unknown
Stover	S2_51286162	2.00E-05	0.29	0.1	GRMZM2G078650	Phosphoric diester hydrolase activity
	S2_185112021	4.58E-05	0.07	0.15	GRMZM2G508000	Protein coding
	S4_120643548	2.56E-05	0.28	-0.09	GRMZM2G024038	unknown
	S4_184857626	2.86E-05	0.3	-0.08	GRMZM2G588688	Protein coding
	S5_106827724	3.42E-05	0.18	-0.15	AC199575.4_FG001	unknown
	S5_108626300	5.73E-06	0.17	0.18	GRMZM2G494874	Protein coding
	S7_168745476	5.00E-05	0.07	-0.14	GRMZM2G113036	Ice binding, homoiothermy, response to freezing, hsp binding

MAF - Minor allele frequency; MLM- Mixed linear model; [a] The exact physical position of the SNP can be inferred from marker's name, for example, S1_82702920: chromosome 1; 82,702,920 bp; CRM, cumulative root-derived C mineralized; SOMC, cumulative soil organic matter C mineralized; SBM, shoot biomass; RBM, root biomass; Stover, total plant biomass (root and shoot biomass); RL, root length; ARD, average root diameter; SRL, specific root length; BM-RS, root-to-shoot biomass ratio.

Table S5. Name of the genotypes with different number of replications in the experiment

No. of replications			
1	2	3	4
DT137	DT137	DT19	SC513
30G19	30G19	DT134	PAN53
DT1	DT1	DT24	
DT10	DT10	DT44	
DT101	DT101	DT8	
DT109	DT109	DT29	
DT11	DT11	SC513	
DT115	DT115	DT14	
DT118	DT118	PAN53	
DT12	DT12	DT136	
DT13	DT13	Pristine601	
DT134	DT134	DT85	
DT135	DT135		
DT136	DT136		
DT14	DT14		
DT143	DT143		
DT148	DT148		
DT160	DT160		
DT163	DT163		
DT166	DT166		
DT17	DT17		
DT175	DT175		
DT178	DT178		
DT180	DT180		
DT181	DT181		
DT183	DT183		
DT19	DT19		

DT190	DT190
DT191	DT191
DT193	DT193
DT194	DT194
DT195	DT195
DT197	DT197
DT199	DT199
DT2	DT2
DT20	DT20
DT200	DT200
DT201	DT201
DT208	DT208
DT209	DT209
DT21	DT21
DT211	DT211
DT214	DT214
DT215	DT215
DT217	DT217
DT22	DT22
DT231	DT231
DT232	DT232
DT238	DT238
DT239	DT239
DT24	DT24
DT25	DT25
DT253	DT253
DT269	DT269
DT28	DT28
DT283	DT283
DT284	DT284
DT29	DT29
DT292	DT292

DT299	DT299
DT3	DT3
DT300	DT300
DT31	DT31
DT33	DT33
DT34	DT34
DT36	DT36
DT37	DT37
DT38	DT38
DT40	DT40
DT41	DT41
DT42	DT42
DT44	DT44
DT45	DT45
DT48	DT48
DT49	DT49
DT52	DT52
DT57	DT57
DT6	DT6
DT60	DT60
DT62	DT62
DT65	DT65
DT66	DT66
DT67	DT67
DT68	DT68
DT69	DT69
DT7	DT7
DT72	DT72
DT73	DT73
DT8	DT8
DT80	DT80
DT82	DT82

DT83	DT83
DT85	DT85
DT88	DT88
DT89	DT89
DT9	DT9
DT218	DT218
DT245	DT245
PAN53	PAN53
PGS61	PGS61
Pristine601	Pristine601
SC513	SC513
SC633	SC633
ZAP55	ZAP55
ZAP61	ZAP61

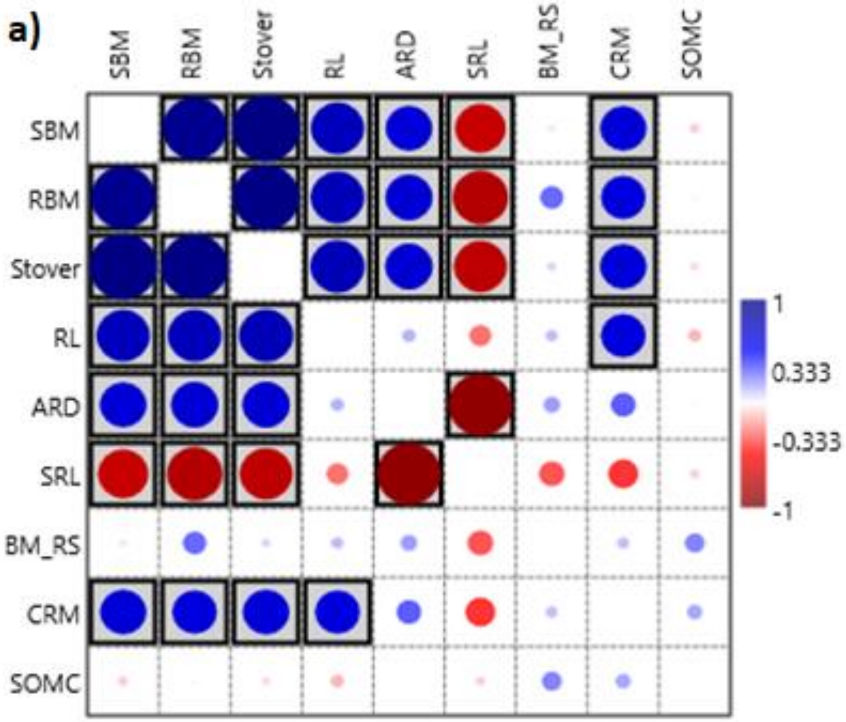

Figure S1a. Pearson correlation analysis of C mineralization in soil and plant traits for maize hybrids (a). SBM, shoot biomass; RBM, root biomass; Stover, total plant biomass (root and shoot biomass); RL, root length; ARD, average root diameter; SRL, specific root length; BM_RS, root-to-shoot biomass ratio; CRM, cumulative root-derived C mineralized; SOMC, cumulative soil organic matter C mineralized. Boxed squares show significant (*P* < 0.05) correlations.

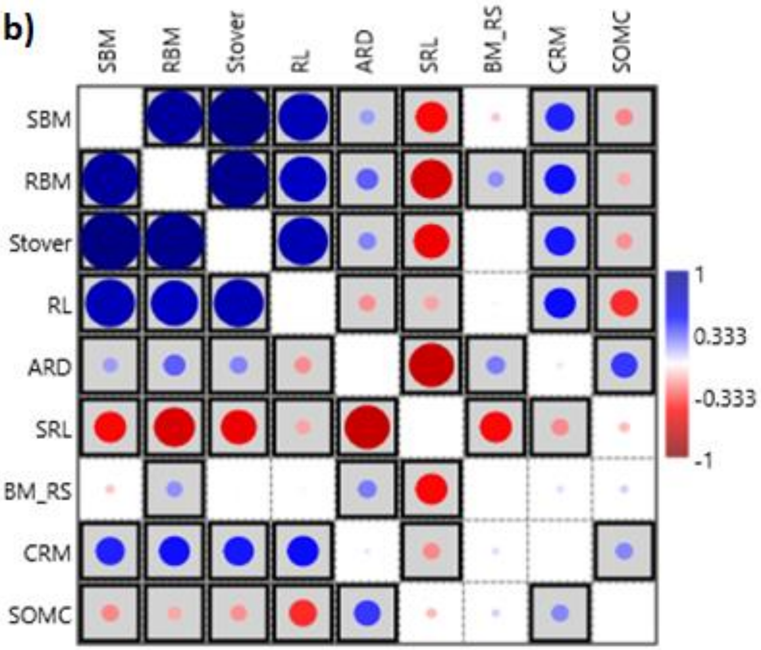

Figure S1b. Pearson correlation analysis of C mineralization in soil and plant traits for maize lines (b). SBM, shoot biomass; RBM, root biomass; Stover, total plant biomass (root and shoot biomass); RL, root length; ARD, average root diameter; SRL, specific root length; BM_RS, root-to-shoot biomass ratio; CRM, cumulative root-derived C mineralized; SOMC, cumulative soil organic matter C mineralized. Boxed squares show significant (*P* < 0.05) correlations.

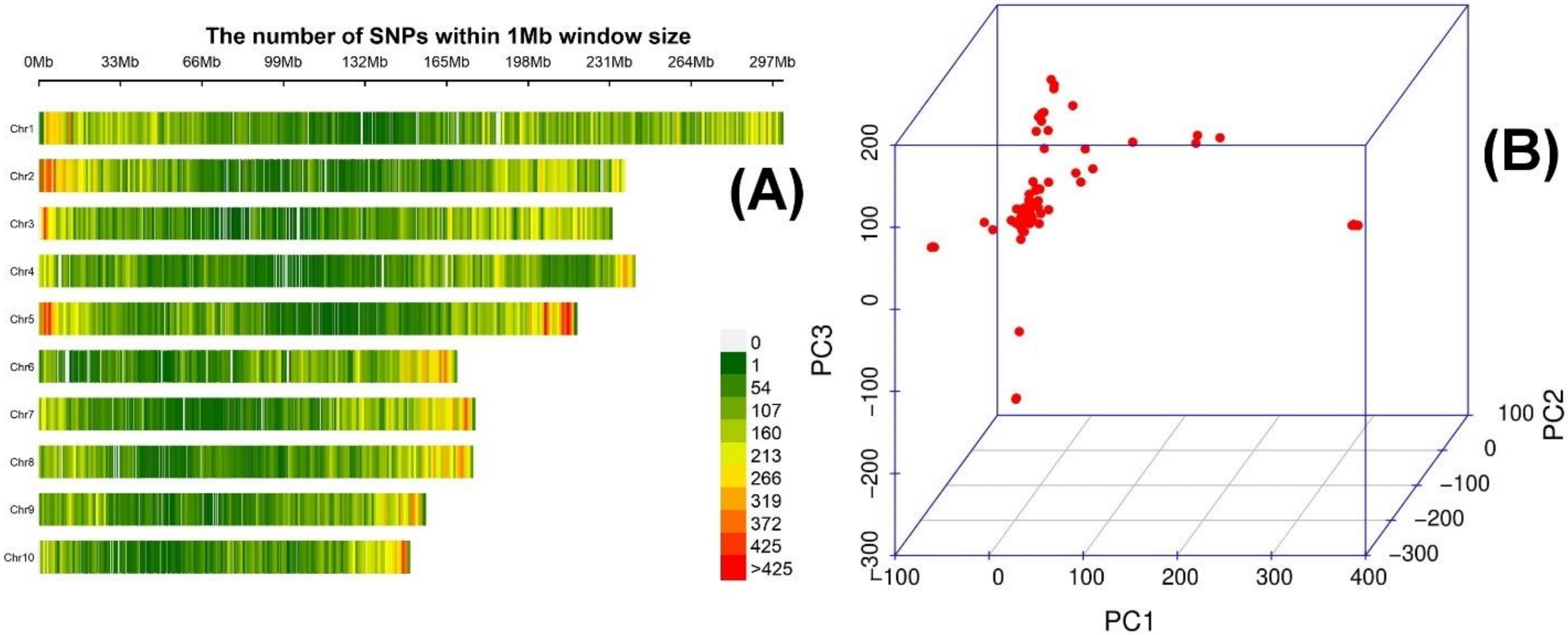

Figure S2. Distribution of 199,755 genotyping-by-sequencing markers in the maize genome (A), the colour key with marker densities indicates the number of markers within a window size of 1 Mb. Principal component analysis of the 97 genotypes (B). Principal components were calculated with TASSEL.

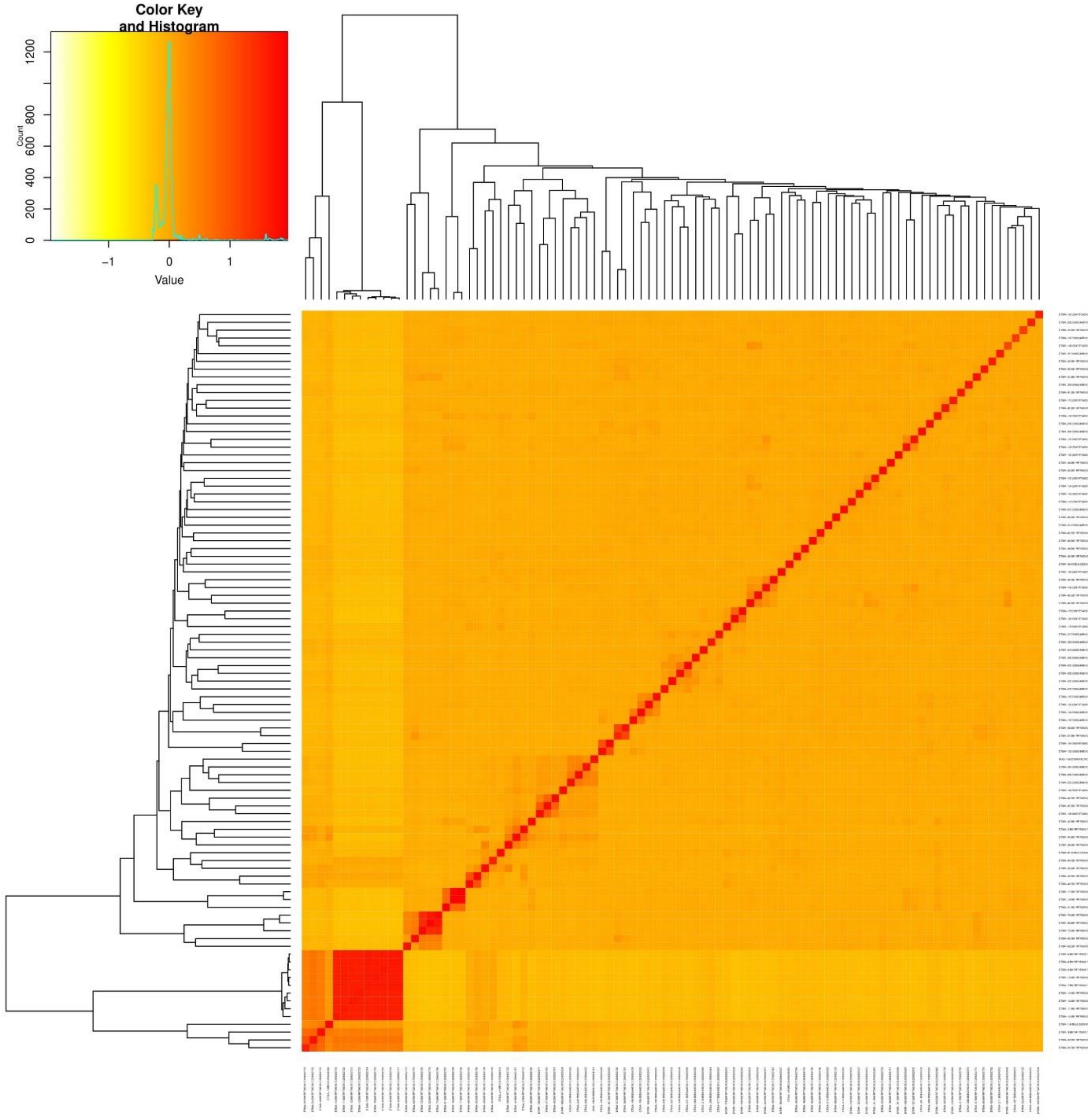

Figure S3. A heatmap of the kinship matrix of the 97 DTMA maize association panel accessions calculated from 199,755 SNPs used in the GWAS suggests moderate levels of relatedness among the 97 lines.