

**Article title:** Shared genetic control of root system architecture between *Zea mays* and *Sorghum bicolor*

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The following Supporting Information is available for this article:

**Supplemental Text S1: CREAMD COFE protocols**

- Step 1: plant labeling and cutting
  - Label plants to be collected with tags, spray paint the north side of stalk. Record if the collected plants are competitive or lodged. Cut the stems about 50-70cm above the soil to make it easy to carry the excavated root balls.
- Step 2: system setup
  - Use a spade to excavate the core root system with a diameter of 30 cm and a depth of 30 cm. This process can be facilitated by first etching a 30-cm circle encompassing the base of the stalk using the AirSpade® Pneumatic Soil Excavation tool (GuardAir Corporation, Chicopee, MA, USA). A towable air compressor (Atlas Copco, Nacka, Sweden. Built: 2011, 85.7 kW, output of 10600 L/min at 6089 kPa) is capable of supporting the simultaneous use of two AirSpade® tools attached by 32.3 m of 5.08 cm diameter hoses (Atlas Copco, Nacka, Sweden).
- Step 3: root cleaning
  - Place excavated root balls on a tarp for cleaning. The power of the air compressor should be adjusted to the minimum air flow necessary to clean the roots. Rotate the root ball during cleaning until the soil has been removed. Place the cleaned roots upside down in an empty bucket to avoid compression of the core root system while transporting to the phenotyping station.

**2. COFE Protocol**

- COFE has several advantages for extracting RSA traits relative to other image analysis pipelines. In contrast to GiA Roots (Galkovskyi et al., 2012), which was developed for trait extraction from seedling root systems grown in a laboratory setting, COFE is capable of phenotyping core root systems of mature, field-grown plants. In contrast to DIRT (Das et al., 2015), COFE uses a blue background, which increases the accuracy of image segmentation relative to the black background recommended by DIRT. In addition, COFE uses automated digital trimming of outlier roots to improve the estimation of root angles, as compared to the RANSAC (random sample consensus) approach employed by DIRT to exclude outliers.

- Step 1: Preprocessing of Images

- The user should identify the lowest node visible above the soil. Then the software automatically crops out distinct components from the image: the marker, the tag and the root *per se*. The cropping is performed on predefined coordinate values because the size and location of the marker and tag are consistent for all images. Each image is then converted from native RGB to hue-saturation-value format to efficiently segment the foreground (the root) from the blue background. Most of the background is removed by excluding pixels that have blue hue values within a predefined range. Once segmentation has been completed, the connected components method (Robert and Linda, 1992) is used to remove spurious outliers and noise (e.g., some parts of the background may have appeared white in the image due to variability in the lighting and thus contains other hues along with blue). The software then enables the user to adjust image segmentation parameters (blue hue range) if predefined settings fail. Finally, the preprocessed segmented binary image of the root and the marker are saved in MATLAB native file format.

- Step 2: Trimming and Trait Extraction

- Once images have been pre-processed, they are trimmed, and traits are extracted in a batch and parallel process from the saved MATLAB files. For trimming, segmented binary images are blurred using a 2D Gaussian filter with a standard

deviation 100 and then thresholded with a cut-off value of 0.25; the thresholded image is used as a mask on the original binary image. This process removes aberrantly sticking out roots and results in trimmed core root systems.

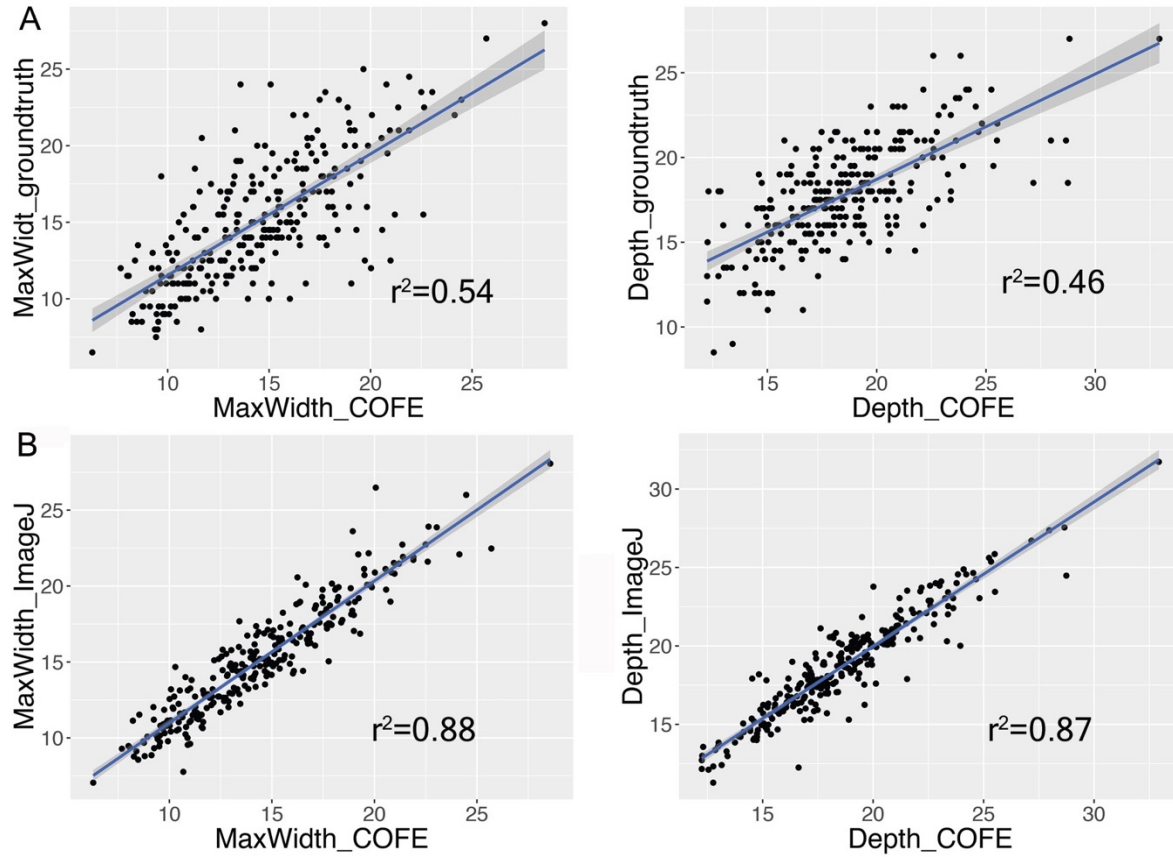
Next the software evaluates global traits including root depth, root area, convex hull, width-profile angle, solidity, root density, maximum width, a modified density based on maximum width, and modified root depth. Because we captured both north (front view) and west (side view) images, and the software registered the smaller depth between the depths evaluated on the two images as the root depth and crops out the top part of the taller image.

#### **Supplemental Text S2: Definition of Width-Profile Angle (WPA)**

To capture the effect of the base width and root width profile together, we defined a trait designated Width-Profile Angle (WPA). WPA is measured from the root-width profile as a function of depth (y-direction, i.e., the direction of gravity), where the root-width is the distance between the left-most and the right-most non-zero pixels at every y-position.

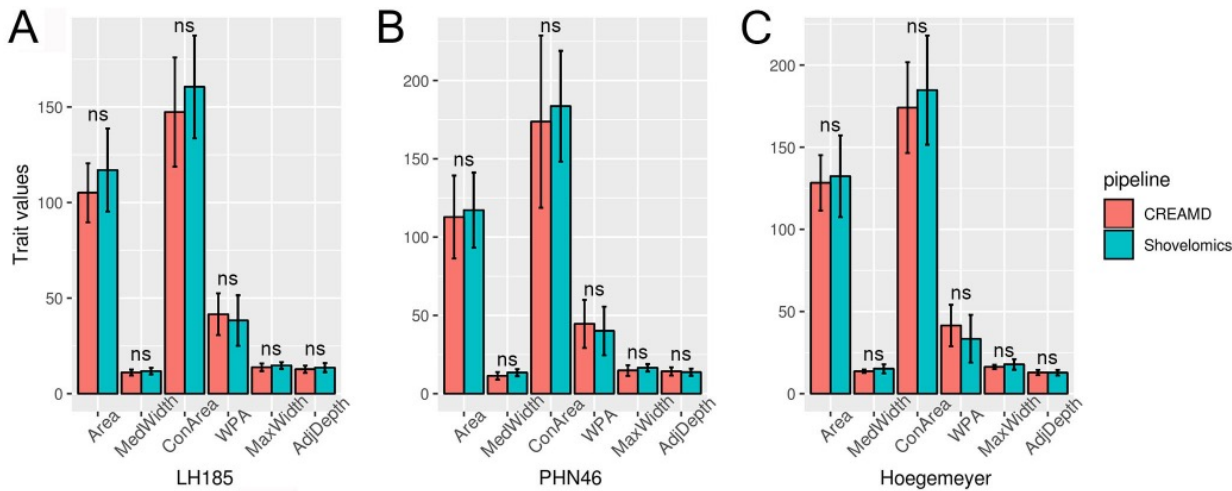
First, the average root width is calculated for every 100 pixels in the y-direction and the slope of the best fitted straight line passing through the origin and the averaged root width value at y=100 pixels is evaluated. Then slopes were reevaluated by successively including averaged root width values at 200, 300, ... 1000 pixels. Finally, the WPA was calculated from the mean value of the evaluated slopes (see Fig. S6).

Supplemental Figure S1:



Supplemental Figure S1: Ground truth validation for trait values extracted from COFE. 298 images from 149 maize plants were analyzed. A) Correlations between ground truth (manual measurements) and trait values extracted from images using COFE; B) Correlations between ImageJ and trait values extracted from images using COFE.  $P < 0.01$  for all correlations.

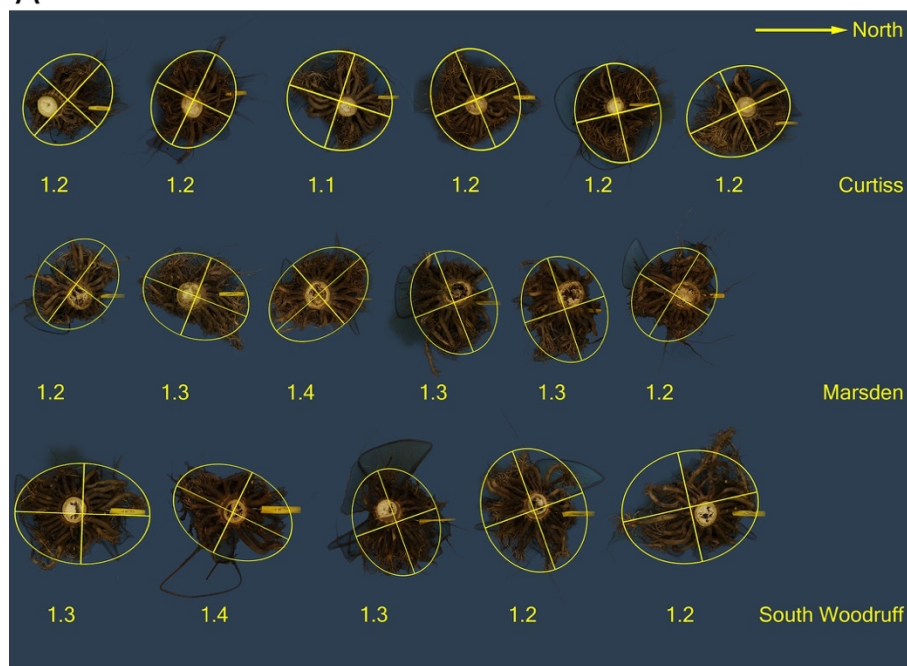
Supplemental Figure S2:



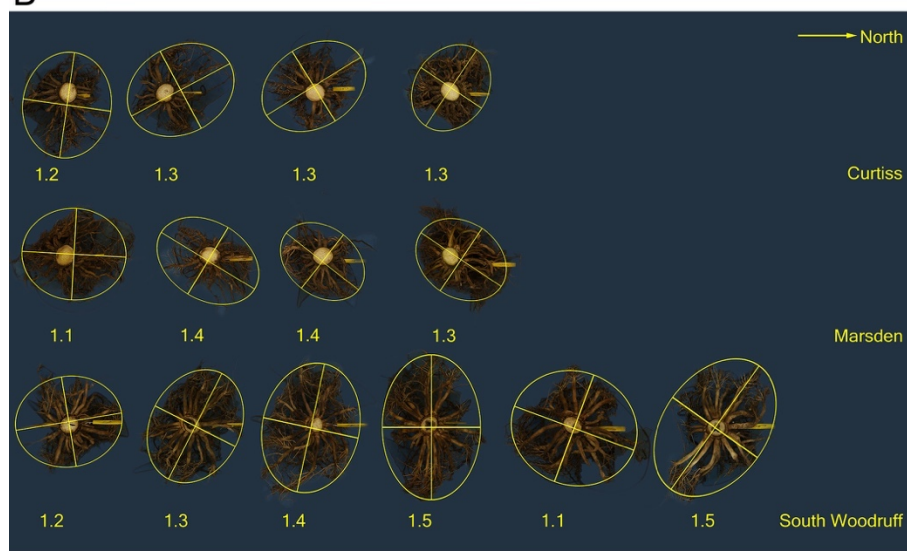
Supplemental Figure S2: Comparisons of trait values extracted using COFE from roots of three genotypes. A) LH185 B) PHN46, and C) Hoegemeyer 7089) collected via CREAMD vs. water-based root cleaning. Bars indicate mean  $\pm$  SD; ns indicates p-value > 0.05 in Student's t-test; n=15.

Supplemental Figure S3:

A

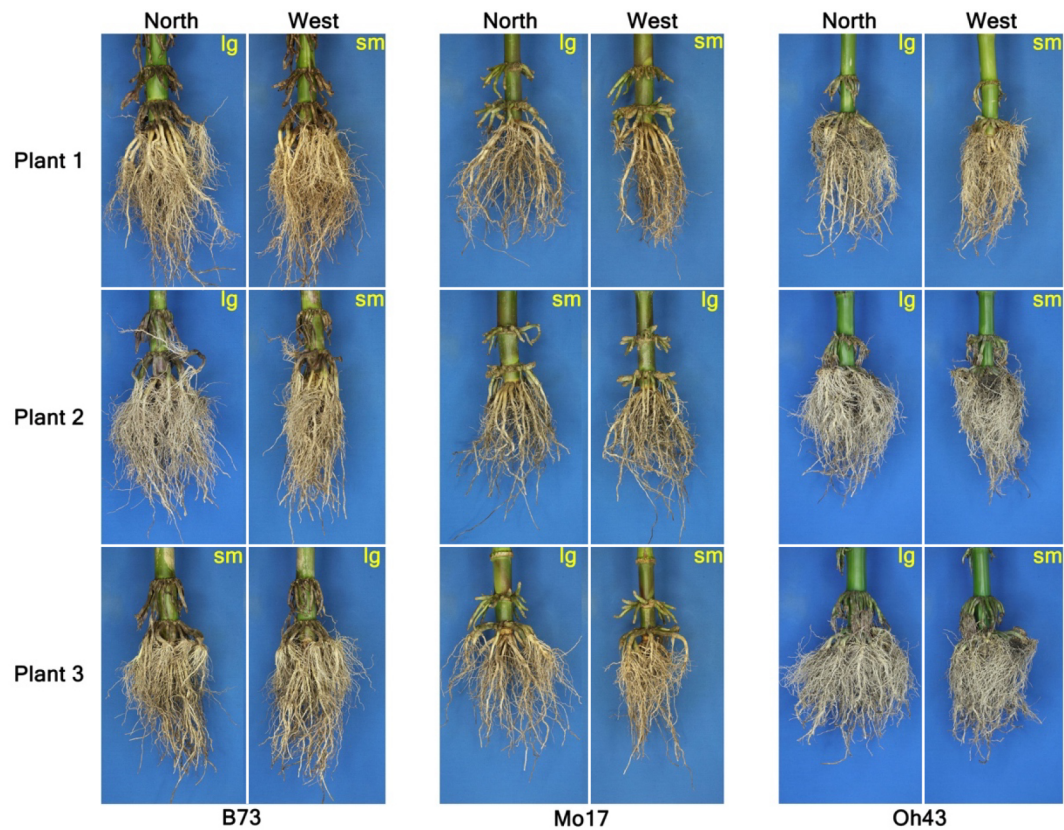


B



Supplemental Figure S3: Maize core root systems grown in three environments (Curtiss, Marsden, and South Woodruff farms) exhibit a lack of radial symmetry. A) The inbred line B73; B) The inbred line Mo17. Roots were imaged from the top and are arranged in the images as they were in the field with the right side of the images being north. Ratios of the length of the long axis and short axis of each ellipse are shown below each core root system.

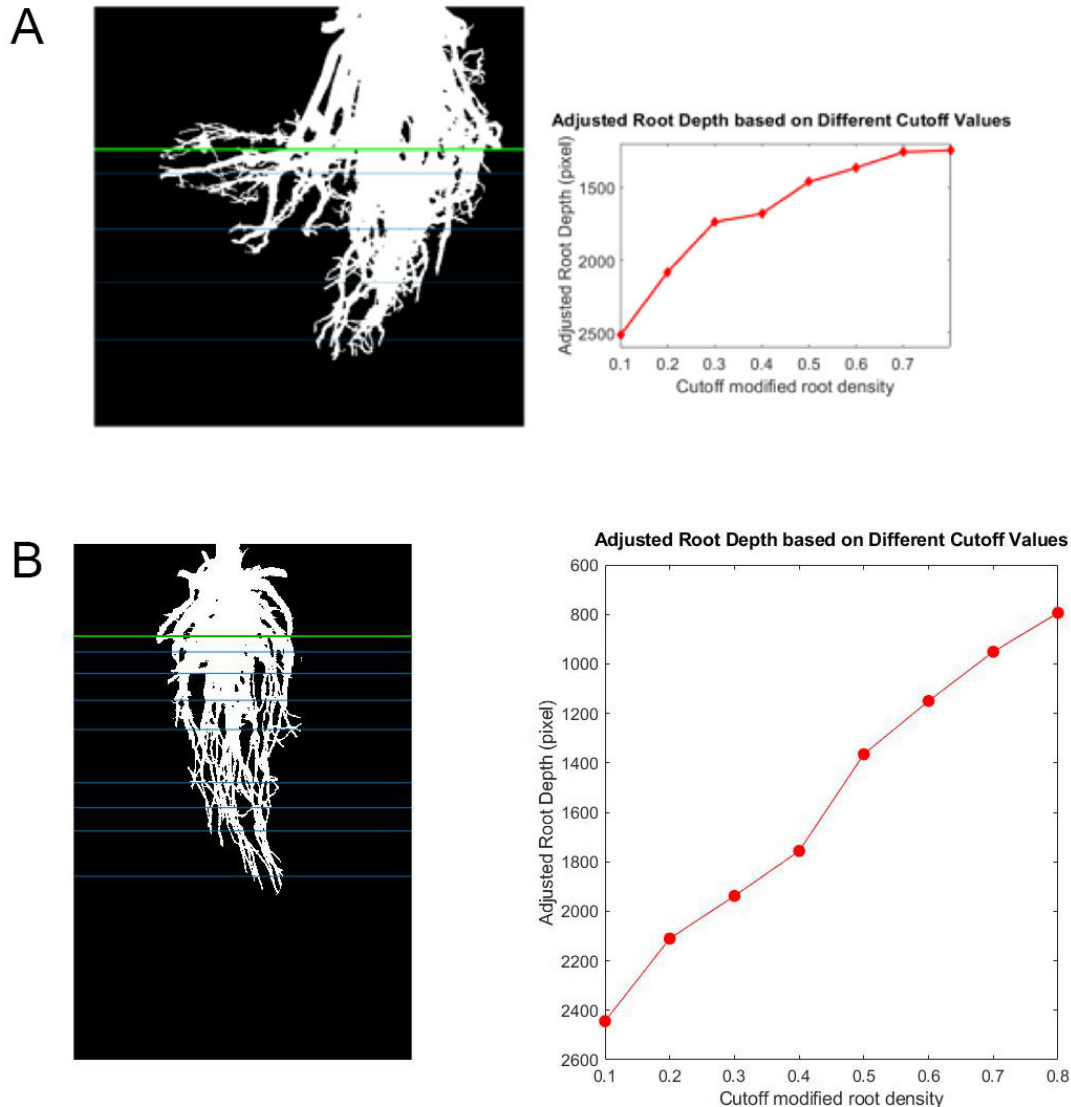
Supplemental Figure S4:



Supplemental Figure S4: Classification of images taken from two angles (North and West) into larger and smaller views on a *per trait* basis. To illustrate that angles do not exhibit a one-to-one correspondence to views, the relationships of angles and views of three plants are shown for three genotypes (left: B73, middle: Mo17, right: Oh43) using root area (Area) as an example. The trait value for each image is shown in Supplemental Table S3.



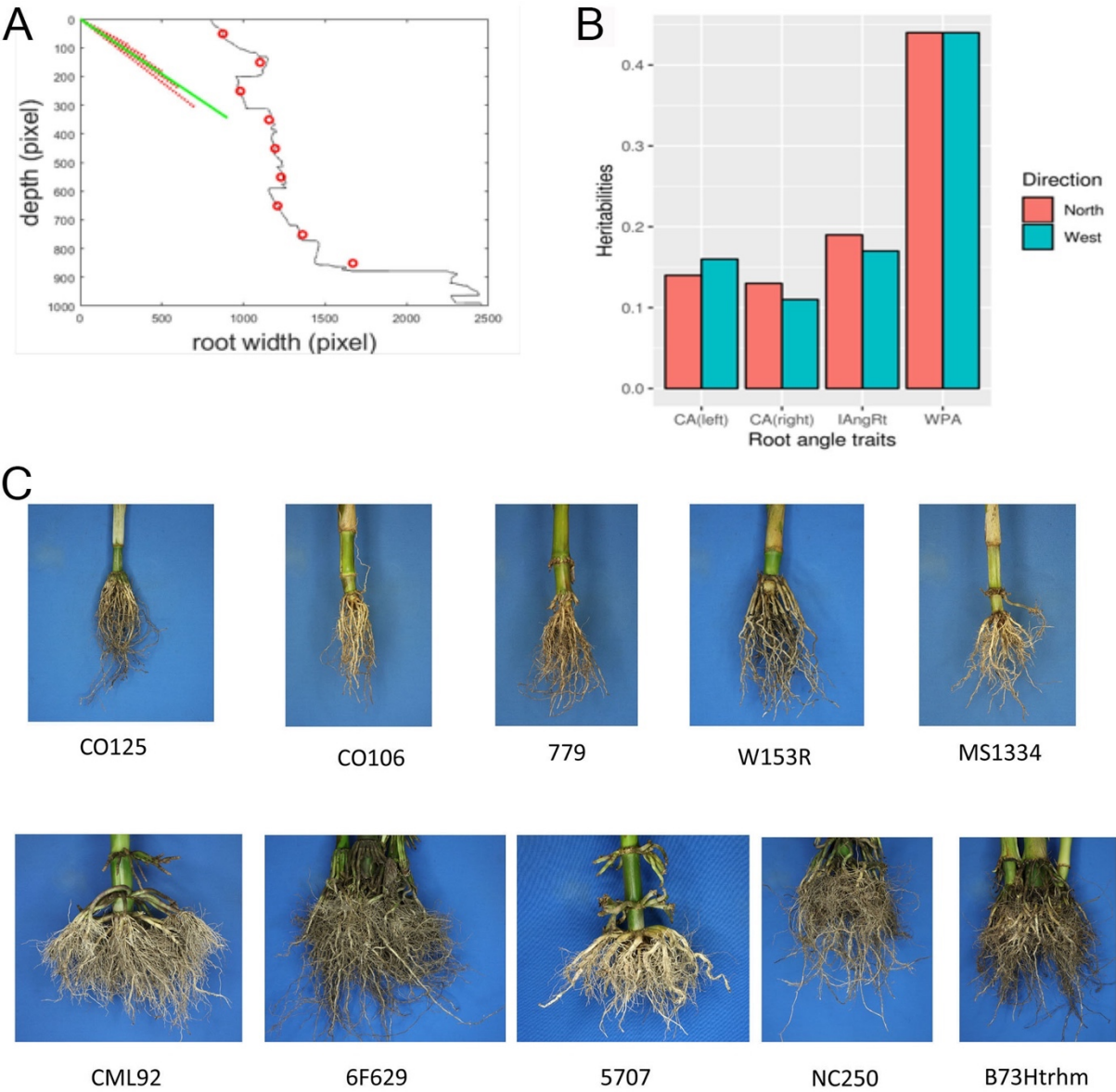
Supplemental Figure S5:



Supplemental Figure S5: Illustration of algorithm for determining root depth (*AdjDepth*) trait values: The ratios of the number of white and black pixels at any depth (y value) at or below the maximum width were determined. The Y value at which this ratio drops below a defined cutoff is defined as the adjusted depth, i.e., *AdjDepth*. In the left panel, the green line indicates the location of the maximum width and blue lines indicate the locations of *AdjDepth* based on the modified root density cut-offs of 0.6, 0.4, 0.2 and 0.1. Right panel shows the *AdjDepths* in pixels at increasing cutoff values. Because root depths at the density cutoffs of 0.5 for maize (A) and 0.3 for sorghum (B) resulted in the highest heritabilities they were selected for further analyses.

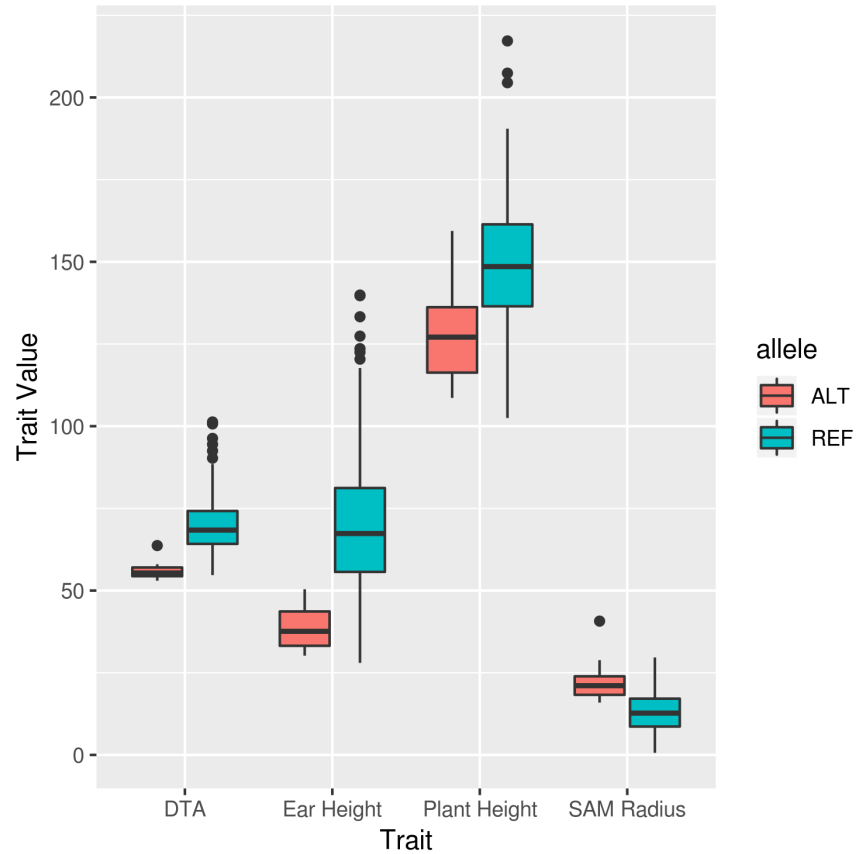


Supplemental Figure S6:



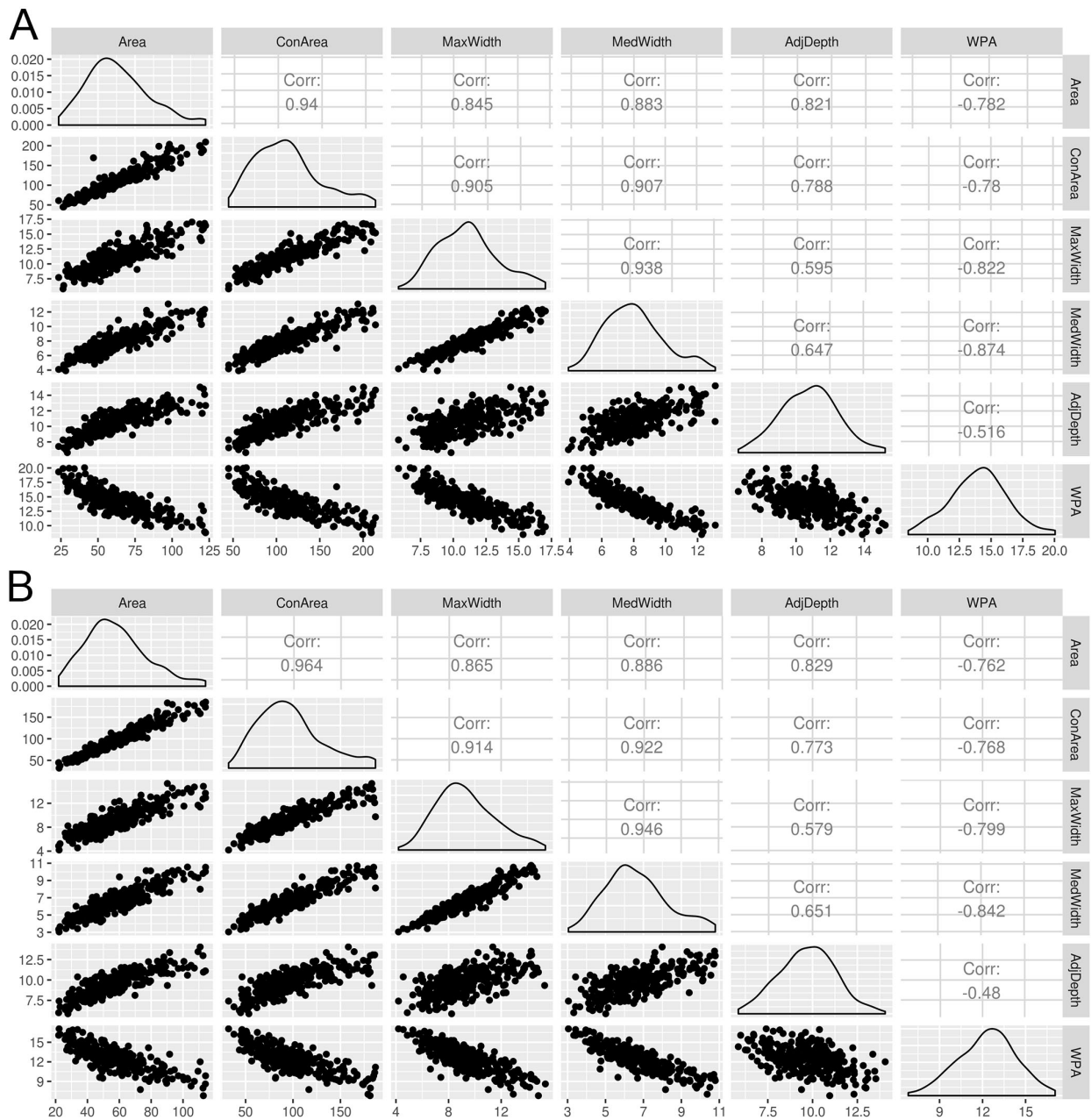
Supplemental Figure S6: Width-Profile Angle (*WPA*) was used to measure root angle. *WPA* is defined in supplemental Text 2. A) Illustration of calculation of *WPA* (see supplemental Text 2; B) Comparison of heritabilities of *WPA* and other published root angle traits (see main text); C) Illustration of root with large (upper panels) and small values (lower panels) of *WPA*.

**Supplemental Figure S7:**



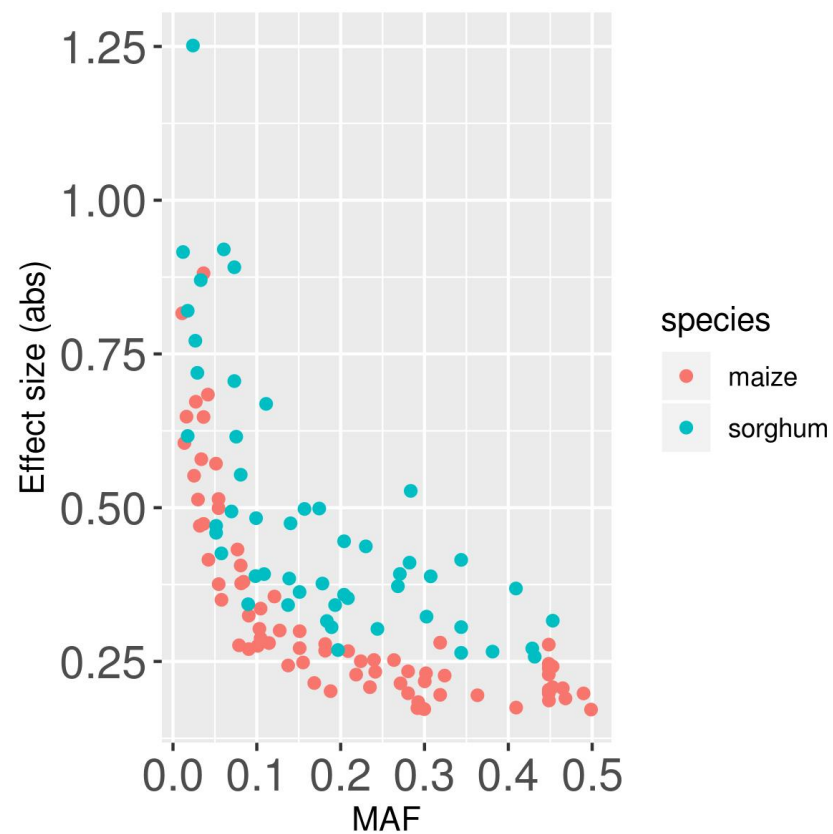
Supplemental Figure S7: Above-ground trait values of inbred lines homozygous for the ALT and REF alleles of *bige1*. Boxplots show days to anthesis (DTA), ear height (cm), plant height (cm), shoot apical meristem (SAM) radius ( $\mu\text{m}$ ),  $n=293$ .

Supplemental Figure S8:



Supplemental Figure S8: Correlations among RSA traits for 294 sorghum inbred lines. A) Correlations among larger views of RSA traits; B) Correlations among smaller views of RSA traits. Each dot represents the average trait value of one inbred line.  $P < 0.01$  for all pairwise correlations.

Supplemental Figure S9:



Supplemental Figure S9: Minor allele frequency (MAF) and the absolute value of effect sizes of maize and sorghum TAS. Red and teal dots indicate maize and sorghum, respectively.

**Supplemental Table S1:** Time required to process 60 core root systems via CREAMD and water-based root cleaning.

Step	Water-based root cleaning	CREAMD
Excavation (incl. tagging)	1 h	1 h
System setup	45 min	10 min
Cleaning	2h 25 min	22 min

**Supplemental Table S2:** RSA traits do not exhibit statistically different values between two orthogonal views (North and West) of the maize SAM panel. ns indicates  $p>0.05$  in Student's t-test.

Trait	P-value for t-test
<i>Area</i>	0.8429, ns
<i>ConArea</i>	0.9719, ns
<i>MaxWidth</i>	0.9986, ns
<i>MedWidth</i>	0.8629, ns
<i>WPA</i>	0.0511, ns
<i>adjDepth</i>	0.1906, ns

**Supplemental Table S3:** Classification of trait values of root area (Area) from two angles (North and West) in to larger and smaller view on a *per trait* basis. The trait values of Area for B73, Mo17 and Oh43 were extracted using COFE software from the photos shown in Supplemental Figure S4.

genotype	Plant ID	<i>lgArea</i>	<i>smArea</i>
B73	plant 1	27.32	26.36
	plant 2	28.76	25.15
	plant 3	28.77	28.76
Mo17	plant 1	24.17	22.64
	plant 2	25.65	25.44
	plant 3	26.25	26.09
Oh43	plant 1	22.89	22.15
	plant 2	21.83	21.33
	plant 3	23.07	22.55



**Supplemental Table S4:** Correlation coefficients between larger and smaller views of RSA traits in the maize SAM and sorghum (SAP-RSA) panels.  $P < 0.01$  for all correlations.

Trait	Maize	Sorghum
Area	0.98	0.99
ConArea	0.97	0.99
MedWidth	0.94	0.96
MaxWidth	0.92	0.96
WPA	0.95	0.96
AdjDepth	0.95	0.97

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346 **Supplemental Table S5:** Correlations among RSA traits and above-ground traits in maize.

347 See file: RSA\_above\_ground\_corr.csv

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349 **Supplemental Table S6:** Maize TAS and SNP-genes at FDR < 0.05

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351 See file: maize\_TAS\_fdr005.csv

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**Supplemental Table S7: Arabidopsis homologs with known root-related functions of maize SNP-genes**

SNP	p-value	trait	gene	Arabidopsis homolog
8_169254446	1.69E-11	<i>lgArea</i>	GRMZM2G143756	ABC transporter G family (Landgraf et al., 2014)
4_190440313	1.56E-09	<i>smMaxWidth</i>	GRMZM2G013128	<i>SMXL3</i> (Wallner et al., 2017)
5_179006605	2.54E-08	<i>lgMaxWidth</i>	GRMZM2G013324	<i>SHV3</i> (Hayashi et al., 2008)
3_3448813	8.62E-10	<i>smMedWidth</i>	GRMZM2G400907	<i>GTE4</i> (Airoidi et al., 2010)
1_19791388	1.16E-08	<i>lgMaxWidth</i>	GRMZM2G447551	<i>CAMTA2</i> (Tokizawa et al., 2015)
6_84383118	1.48E-10	<i>smMaxWidth</i>	GRMZM2G173928	CDPK-related kinase (Lu et al., 2016)
9_145513150	1.58E-07	<i>smAdjDepth</i>	GRMZM2G392737	<i>BUDI</i> (Dai et al., 2006)
5_207485174	1.26E-07	<i>lgMaxWidth</i>	GRMZM2G036092	<i>KDR</i> (Zheng et al., 2017)
9_139385600	4.25E-08	<i>lgWPA</i>	GRMZM2G166767	<i>RHMI</i> (Diet et al., 2006)
9_142752809	9.66E-08	<i>lgArea</i>	GRMZM2G150594	<i>LBD18</i> (Berckmans et al., 2011)

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362 **Supplemental Table S8:** List of eRD-genes

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364 See file: eRD\_genes.csv

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**Supplemental Table S9:** Arabidopsis and Medicago homologs with known root-related functions of maize eRD-genes. Model frequency for each gene is provided

eRD-gene	Model Frequency												Homolog
	lgArea	smArea	lgConArea	smConArea	lgMaxWidth	smMaxWidth	lgMedWidth	smMedWidth	lgWPA	smWPA	lgAdjDepth	smAdjDepth	
GRMZM2G086766	ns	ns	0.068	ns	0.175	0.500	0.156	0.121	0.207	0.177	ns	ns	<i>AtTET6</i> (Wang et al., 2012)
GRMZM2G470740	ns	ns	ns	ns	0.058	ns	0.075	ns	0.061	0.154	ns	ns	<i>AtELP1</i> (Zhong et al., 2002)
GRMZM2G309970	0.079	0.118	ns	0.100	ns	0.120	ns	0.087	ns	ns	ns	ns	<i>AtWPP2</i> (Patel et al., 2004)
GRMZM2G356579	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.063	0.068	<i>AtCAMTA1</i> (Pandey et al., 2013)
GRMZM2G057243	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.100	<i>MtCCD1</i> (Floss et al., 2008)
GRMZM2G086934	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.075	0.053	<i>AtRPA70D</i> (Ishibashi et al., 2005)
GRMZM2G105019	ns	0.055	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<i>AtSGT1B</i> (Gray et al., 2003)
GRMZM2G012814	ns	0.087	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	<i>AtSCN1</i> (Carol et al., 2005)
AC217264.3_FG005	ns	ns	ns	ns	ns	ns	0.067	ns	ns	ns	ns	ns	<i>AtMYB101</i> (Xue et al., 2017)
GRMZM2G395749	ns	0.100	ns	0.075	ns	ns	ns	ns	ns	ns	ns	ns	<i>AtALY3</i> (Pfaff et al., 2018)
GRMZM5G853392	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.052	<i>AtGRF1</i> (Omidbakhshfard et al., 2015)
GRMZM5G888263	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.054	ns	ns	<i>AtMARI</i> (Stanga et al., 2009)

**Supplemental Table S10:** List of cis- and trans- eQTL

See File: eQTL\_summary.csv

**Supplemental Table S11:** Percentage of cis- and trans-eQTL for qualified maize RSA-associated genes using different window sizes.

window size	cis (%)	trans (%)
50kb	7.2	92.8
200kb	8.0	92.0
1Mb	11.5	88.5
2Mb	12.1	87.9



**Supplemental Table S12:** List of inbred lines in used in GWAS for maize (SAM diversity panel) and sorghum (SAP-RSA)

See File: SAM\_panel\_and\_SAP\_RSA\_list.csv

**Supplemental Table S13:** Sorghum TAS at FDR < 0.05

See file: sorghum\_TAS\_fdr005.csv

**Supplemental Table S14:** Syntenic maize-sorghum gene pairs detected via comparative GWAS. For windows containing more than one gene, only the gene nearest to the TAS is included in the table

maize TAS	maize trait	maize p-value	maize gene	sorghum gene	sorghum TAS	Sorghum gene-TAS distance (bp)	sorghum trait	sorghum p-value
9_139385600 <sup>a</sup>	lgWPA	4.25E-08	GRMZM2G166767	Sb01g039220	1_62664732	5,151	smConArea	1.55E-10
1_19791388	lgMaxWidth	1.16E-08	GRMZM2G447551	Sb01g044480	1_67446233	130,638	lgMedWidth	2.31E-10
1_10544732	smArea	5.50E-13	GRMZM2G028521	Sb01g047080	1_70277456	110,663	smArea	9.32E-08
2_212645701 <sup>b</sup>	lgMaxWidth	6.08E-10	GRMZM2G064640	Sb02g040080	2_74214205	57,403	lgConArea	2.13E-06
8_157314586	lgMedWidth	1.96E-08	GRMZM2G118644	Sb03g046520	3_73657961	38,159	lgMedWidth	3.06E-06
8_73900637 <sup>c</sup>	lgWPA	2.26E-08	GRMZM2G097768	Sb09g029520	9_58128064	44,180	smArea	1.21E-08
6_94761469	smMedWidth	6.28E-08	GRMZM5G897944	Sb10g026290	10_55601636	66,188	lgMedWidth	6.01E-08

Notes:

a. The other gene in the syntenic region is GRMZM2G166780; the corresponding syntenic gene detected in sorghum GWAS is Sb01g039330.

b. The other genes in the syntenic region are GRMZM2G064630 and GRMZM2G064938; the corresponding syntenic gene detected in sorghum GWAS are Sb02g040050 and Sb02g040140.

c. The other gene in the syntenic region is GRMZM2G097768; the corresponding syntenic gene detected in sorghum GWAS is Sb09g029520.

**Supplemental Table S15:** List of Yan panel and SAM panel TAS for four traits (PH, PEH, DTA, EL)

See file: SAM\_Yan\_TAS.csv

**Supplemental Table S16:** List of TAS for four traits (PH, PEH, DTA, EL) identified via GWAS conducted on the maize273 and SAM273 panels.

See file: maize273\_SAM273\_TAS.csv

**Supplemental Table S17:** RSA trait values (BLUP) of maize SAM panel.

See file: maize\_RSA\_traits\_BLUP.csv

**Supplemental Table S18:** RSA trait values of sorghum SAP-RSA panel.

See file: sorghum\_RSA\_traits.csv

**Supplemental Table S19:** List of syntenic genes. Genes from the Maize1 and Maize2 subgenomes are based on the maize v2 reference genome; sorghum genes are based on the sorghum v1.4 reference genome.

See file: sorghum1maize2\_intelligent.csv

**Supplemental Table S20:** Ear length trait values (BLUP) of of maize SAM panel.

See file: SAM\_panel\_ear\_length.csv

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