RESEARCH

Phenotypic Diversity of Root Anatomical and Architectural Traits in *Zea* Species

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ABSTRACT

We characterized phenotypic variation for root traits in 256 *Zea* spp. accessions, including maize landraces and *Z. mays* L. subsp. *huehuetenangensis* (H. H. Iltis & Doebley) Doebley, subsp. *mexicana* (Schrad.) H. H. Iltis, and subsp. *parviglumis* H. H. Iltis & Doebley, *Z. nicaraguensis* H. H. Iltis & B. F. Benz, *Z. perennis* (Hitchc.) Reeves & Mangelsd., and *Z. luxurians* (Durieu & Asch.) R. M. Bird. Anatomical traits included areas of the cross-section, stele, cortex, aerenchyma, and xylem and number of cortical cells and cell files. Architectural traits included diameters of the nodal root system, individual crown roots, and the stem; numbers of seminal and nodal roots; biomass; and nodal root length and branching. Ranges for anatomical traits were similar for teosintes and landraces, except for aerenchyma and xylem areas, and number of cortical cells. Landraces had greater variation for architectural traits except for nodal root number and branching, and had larger mean stele and xylem areas, longer nodal roots, wider nodal systems, and more seminal roots than teosintes. In contrast, teosintes were smaller but had more nodal roots with greater branching. At a common plant size, teosintes would have lower mean values for all anatomical traits, except for number of cortical cells and cell files. Teosintes had greater scaled values for all architectural traits except average root diameter. Cluster analysis divided accessions into eight root phenotypes. Phenotypic diversity for root traits in the genus *Zea* could be a valuable resource for improving stress tolerance in maize.

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Abbreviations: α, scaling exponent; Forks, number of lateral branch points; PCA, principal component analysis; Tips, number of root tips.

Root architectural and anatomical traits are potential selection criteria for plant breeding because they play a central role in plant growth, resource allocation, and acquisition of soil resources (Lynch and Brown, 2006, 2012). Generally, traditional breeding has focused on shoot traits with only indirect selection of belowground traits. However, root traits are receiving increasing attention as a means of improving tolerance to abiotic stresses such as drought, waterlogging, salinity, and suboptimal nutrient availability (Zhu et al., 2005ab; Araus et al., 2008; Mano et al., 2008; Tuberosa and Silvio, 2009; Bayuelo-Jiménez et al., 2011). Improvements in plant stability and root mechanical strength could enhance lodging resistance and are influenced by root traits such as length and branching, cell arrangements, and cell wall composition (Sanguineti et al., 1998; Striker et al., 2007). Resistance to biotic stress can be improved by increased lignin in root tissues and by more highly branched architectures that permit continued root function after herbivory (Orians et al., 2002; Chen et al., 2005; Lynch, 2005; Johnson et al., 2010). A primary limitation to the use of root traits in breeding programs is the identification and characterization of root phenotypes that influence whole-plant physiological processes and yield.

Within the genus *Zea*, teosintes and landraces are potential sources of traits for improving plant performance and stress tolerance

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in maize (Holland, 2009). *Zea mays* subsp. *mays*, or maize, is the cultivated subspecies of this genus. All other species in the genus are considered wild relatives of maize and are referred to as teosinte. Landraces are cultivated varieties of crop plants that have developed adaptations to specific soil, climate, and biotic stress factors without the influence of formal plant breeding (Newton et al., 2010). Landraces represent important sources of stress tolerance traits because they are generally grown in relatively low-input systems (Ceccarelli, 1996; Hartings et al., 2008; Holland, 2009). Geographic differences in soil P bioavailability have been related to P efficiency among landraces of common bean (*Phaseolus vulgaris* L.) (Beebe et al., 1997) and maize (Bayuelo-Jiménez et al., 2011). In landraces of wheat (*Triticum aestivum* L.), improved performance under drought has been associated with investment of root biomass in deeper soil horizons, with concomitant improvement in water uptake (Reynolds et al., 2007). Similarly, wild relatives of cultivated plants are useful in the identification of novel stress tolerance alleles, due to greater genetic diversity compared to their cultivated relatives (Lynch et al., 1992; Bayuelo-Jiménez et al., 2002; Wright et al., 2005; Hochholdinger, 2009; Tuberosa and Silvio, 2009). The availability of diverse germplasm is important for plant breeding since domestication results in loss of genetic diversity (Wright et al., 2005). Surveys of diversity in cultivated maize indicate that preferred use of hybrid varieties over landraces has decreased allelic diversity in this species (Reif et al., 2005; Warburton et al., 2008). Since root traits have not been well represented in crop science or plant breeding research, the extent to which phenotypic variation in maize root traits has changed over time is unknown.

Characterizing the functional diversity of root traits is an important step in harnessing associated variation for plant breeding. Previous investigations of phenotypic diversity in the genus *Zea* have typically focused on shoot characteristics (Lafitte et al., 1997; Brandolini and Brandolini, 2001; Pressoir and Berthaud, 2004). In the present study, a diverse collection of maize landraces and teosintes was phenotyped for architectural and anatomical root traits. Selection of accessions was based on maximizing genetic diversity while emphasizing accessions from stressful soil environments. Traits are highlighted with potential value as selection criteria for enhanced soil resource acquisition.

MATERIALS AND METHODS

Plant Materials

A diversity panel emphasizing accessions from stressful soil environments was assembled by Mark Millard at the North Central Regional Plant Introduction Station of the United States Department of Agriculture, Ames, IA (USDA-ARS Genetic Resources Information Network). Accessions were selected from diverse soil environments emphasizing regions with dry, acidic, or saline soils. The collection included members of the genus *Zea*, including 195 landraces and 61 teosintes originating primarily from North, Central, and South America (Supplemental Tables S1 and S2, and Supplemental Figures S1 and S2). The genus *Zea* is composed of five species, divided into two sections (Doebley and Iltis, 1980; Iltis and Benz, 2000). In the *Zea* section are *Z. mays* L. subsp. *mays*, *Z. mays* subsp. *huehuetenangensis*, *Z. mays* subsp. *mexicana*, and *Z. mays* subsp. *parviglumis*. In the *Luxuriantes* section are *Z. luxurians*, *Z. diploperennis* H. H. Iltis et al., *Z. nicaraguensis*, and *Z. perennis*. All listed taxa are annuals, except for *Z. perennis*. Landraces used in this study were from *Z. mays* subsp. *mays* while the teosinte group represented one *Zea* hybrid and six of the other seven taxa listed above. *Zea diploperennis* was not included in this collection.

Growth Conditions

Plants were grown in a completely randomized design in a greenhouse located on the campus of The Pennsylvania State University in University Park, PA (40°48′ N, 77°51′ W), from May through July 2009. Three biological replications were grown per accession, and replications were planted 7 d apart in the same greenhouse. Before planting, landrace seeds were soaked for 1 h in a mixture of benomyl (methyl [1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamate) (Benlate fungicide, E.I. DuPont and Company, Wilmington, DE) and 1.3 M metalaxyl (2-[(2,6-dimethylphenyl)-(2-methoxy-1-oxoethyl) amino]propanoic acid methyl ester) (Allegiance fungicide, Bayer CropScience, Monheim am Rhein, Germany). Following the fungicide treatment, five seeds per accession were germinated for 48 h in darkness at 28°C in rolled germination paper (Anchor Paper Company, St. Paul, MN) moistened with 0.5 mM $CaSO_4$, 8 mM benomyl, and 1.3 M metalaxyl. Teosinte seed was scarified with sandpaper and soaked in 0.5 mM $CaSO_4$ for 48 h before germination. For each accession, one seedling with a 6 to 8 cm primary root was selected for each replication. The seed of each seedling was planted at a 4 cm depth although mesocotyl length varied among accessions and individuals. Plants were grown in 10.5 L pots (21 by 40.6 cm, top diameter × height, Nursery Supplies Inc., Chambersburg, PA). The growth medium was composed of 45% peat, 45% vermiculite, and 10% silica sand, limed to pH 6.0. The nutrient solution consisted of the following: 2211 μM NO_3, 777 μM NH_4, 398 μM CH_4N_2O, 411 μM P), 1858 μM K, 1455 μM Ca, 960 μM Mg, 16 μM B, 0.33 μM Cu, 7 μM Zn, 8 μM Mn, 0.85 μM Mo, and 16 μM Fe ethylenediaminetetraacetic acid. Following seedling establishment, 2 to 3 L of nutrient solution were applied as needed to each pot three to four times per week via drip irrigation using a DI-16 Dosatron fertilizer injector (Dosatron International Inc, Dallas, TX). Irrigation volume and frequency increased as plant development proceeded. Sulfuric acid was injected into the water supply to acidify the irrigation water to a pH of 6.0. Environmental data were collected hourly in the greenhouse using a HOBO U10-003 datalogger (Onset Corporation, Pocasset, MA). Mean ambient temperature was 26.5°C ± 5.9 (day) and 21.3°C ± 2.4 (night), and mean relative humidity level was 57% ± 12.2. Maximum photosynthetic flux density was 1200 μmol photons m^{-2} s^{-1}.

Sample Analysis

Plants were harvested 28 d after planting (V6–V7 stage). Stem diameter was measured at the most basal whorl of brace roots using a caliper. The central stem was measured on plants with tillers. Shoots were destructively sampled for dry weight measurement.

Root systems were washed with water, preserved in 75% ethanol, and stored at 4°C until the time of processing and analysis. The following data were collected from the intact root system: crown root system diameter, numbers of seminal and crown roots, root system dry weight, and length of the longest crown root. Crown root system diameter was measured 20 mm below the most basal whorl of brace roots. For root systems with asymmetrical crown root architecture, the widest diameter was measured.

An 8-cm root segment was collected 20 to 28 cm from the base of a representative second whorl crown root on each plant and used to assess lateral branching and average root diameter. These segments were scanned using a flatbed scanner at a resolution of 400 dots per inch (Epson Expression 1680, Seiko Epson Corporation, Suwa, Japan) and analyzed by the root image analysis software WinRhizo (Regent Instruments, 2008). Using the morphological analysis package, average diameter of the crown root segment was measured, and the number of lateral root tips and forks were counted in each segment. The number of root tips (Tips) measurement is a count of the terminal portion of lateral and axial roots while the number of lateral branch points (Forks) measurement indicates the degree of lateral root branching.

A 4-cm tissue segment was collected 5 to 9 cm from the base of a second whorl crown root for hand sectioning. Segments were stored in 75% ethanol at 4°C until they were sectioned. Preserved, unembedded tissue was sectioned using Teflon-coated double-edged stainless steel blades (Electron Microscopy Sciences, Hatfield, PA) and wet mount slides were immediately prepared. Section thickness was between 30 and 50 μm. Sections were examined on a Diaphot inverted light microscope (Nikon, Chiyoda-ku, Japan) at 4x magnification with an additional 0.7x adaptor for a combined magnification of 2.8x. This allowed larger sections to be viewed in their entirety. Three sections were selected for each root segment as subsamples for image capture. Selection of particular cross-sections was based on overall quality of the section, tissue integrity, and the relative perpendicularity of sectioning (uniform thickness across the section). The microscope was fitted with a black and white XC-77 CCD Video Camera Module (Hamamatsu, Iwata-City, Japan). ImageMaster 5.0 software (Photon Technology, 2004) was used to capture and save images.

Images were analyzed in MatLab 7.6 2008a (MathWorks, 2008) using the program RootScan, which was created for this purpose (Burton et al., 2012). The following measurements were made via pixel counting: areas of the total cross-section, aerenchyma lacunae, total stele, and xylem vessels. Some of these primary measurements were used to calculate secondary measurements in MatLab: area of the cortex (cross-section area − stele area), cortical cell area (cortical area − aerenchyma area), percent cortical cell area (cortical cell area/cross-sectional area), percent aerenchyma (total aerenchyma area/cortex area), and area ratios of stele to cross-section and stele to cortex. Count data included number of cortical cells and cortical cell files. Area measurements were in square millimeters and were calibrated using an image of a 1-mm micrometer taken at the same magnification as the analyzed images (1 linear mm equals 204 pixels).

Statistical Analysis

Statistical analyses were performed using the R program, version 2.9.2 (R Development Core Team, 2010). One- and two-way ANOVA was used to evaluate phenotypic differences within the landraces and teosintes and between these two groups for all traits. A Pearson correlation analysis was performed on corresponding traits between and within the landrace group and the teosinte group using raw data. A principal component analysis (PCA) using a varimax rotation was performed within each group and for pooled data for the two groups. The first two components were characterized based on variable eigenvalues and on vector clustering within plots of components 1 and 2. Based on Kaiser's Criterion and Cattell's method of component retention, only components with eigenvalues greater than 3.0 were retained (Kaiser, 1960; Cattell, 1966). A hierarchical cluster analysis was performed using Ward's method, in which cluster creation is based on minimizing the squared Euclidean distance between points (Ward, 1963). Characteristics defining each cluster were evaluated based on the summary statistics of mean, minimum, and maximum values.

In addition to analysis of raw data, allometric analysis was performed to compare trait values between the teosintes and landraces in the absence of the effect of size, using the method of Lleonart et al. (2000). Seminal root number was excluded from this analysis, because many teosinte accessions had a value of zero for this trait. The allometric analysis included two parts. First, regression analyses were performed to obtain the allometric coefficients [R^2 and α (the scaling exponent)] for each trait in each group. Second, using the allometric coefficients, raw data were scaled based on plant dry weight to create a new data set. Within the landrace and teosinte groups, a reduced major axis regression was performed of the natural logarithm of each trait against the natural logarithm of total plant dry weight. Log transformations are used in allometric analysis to reveal the underlying exponential relationship between size traits and biomass. From these regression analyses, the coefficient of determination (R^2) and the slope of the regression line (α) were recorded (Niklas, 1994). Using the original data sets and the y-intercepts from the log-log regressions, original trait values were scaled based on the mean plant dry weight across the two groups. Significance of slopes for traits in each group was evaluated before scaling of data. The following equation was used to create the scaled data set:

$$Y \times I = Yi\ (Xo/Xi)^b$$

in which Yi is the ith value of trait in question, Xo is the mean plant dry weight across all accessions, Xi is the ith value for plant dry weight, and b is the intercept from log-log regressions in each group.

Finally, ANOVA was performed for each trait to compare the scaled data of the landraces and teosintes.

RESULTS

Significant phenotypic variation was observed for anatomical and architectural traits within the landraces and teosintes and between these two groups (Table 1). The two groups were significantly different for five of the 12 anatomical traits. Between the two groups, there were significant differences for all of the architectural traits, except root to shoot ratio. Within each group, significant variation was observed for all anatomical traits, with the exception of aerenchyma area among the landraces (p = 0.12). For architectural traits, significant variation was

Table 1. Summary of ANOVA between and within the maize landrace (*Zea mays* subsp. *mays*) and teosinte (*Zea* spp.) groups showing mean square (MS), *F* values, and associated significance for all traits based on main effect within groups (Landraces and Teosinte) and between these two groups.

		Landraces			Teosinte			Between groups		
Abbreviation	Description	MS	*F*	*p*-value	MS	*F*	*p*-value	MS	*F*	*p*-value
				Anatomical traits						
RXSA	Root cross-section area, mm^2	0.03	1.52	<0.001	0.10	7.07	<0.001	0.01	0.51	NS[†]
TCA	Total cortical area, mm^2	0.02	1.49	<0.01	0.06	6.90	<0.001	0.02	3.32	NS
TSA	Total stele area, mm^2	0.00	1.52	<0.001	0.01	6.84	<0.001	0.05	35.77	<0.001
TSA:RXSA	Total stele area:root cross-section area	0.00	1.43	<0.01	0.00	6.03	<0.001	0.15	179.92	<0.001
TSA:TCA	Total stele area:total cortical area	0.01	1.44	<0.01	0.01	5.93	<0.001	0.52	176.16	<0.001
AA	Aerenchyma area, mm^2	0.00	1.17	NS	0.00	4.39	<0.001	0.00	2.54	NS
%A	Percent of cortex as aerenchyma	61.00	1.25	<0.05	167.00	4.20	<0.001	12.00	0.30	NS
CCA	Cortical cell area, mm^2	0.01	1.47	<0.05	0.04	6.75	<0.001	0.01	1.32	NS
%CCA	Percent of cortex as cells	0.01	1.28	<0.05	0.01	4.98	<0.001	0.15	57.17	<0.001
XVA	Xylem vessel area, mm^2	0.00	1.96	<0.001	0.00	3.96	<0.001	0.02	411.75	<0.001
#CC	Number of cortical cells	43,001.00	1.29	<0.05	105,684.00	6.15	<0.001	15,995.00	0.63	NS
#CF	Number of cortical cell files	2.64	1.41	<0.01	5.78	5.17	<0.001	2.83	1.88	NS
				Architectural traits						
RDW	Root system dry weight, g	15.45	2.17	<0.001	3.89	1.49	NS	285.60	34.47	<0.001
SDW	Shoot dry weight, g	146.60	2.03	<0.001	41.71	3.68	<0.01	15,843.00	184.25	<0.001
RDW:SDW	Root system dry weight:shoot dry weight	0.20	0.41	NS	1.00	111.40	<0.001	0.30	1.02	NS
StemDia	Stem diameter, mm	28.40	1.70	<0.001	35.30	3.80	<0.01	4,422.30	200.00	<0.001
Sem#	Number of seminal roots	3.30	1.40	<0.05	0.40	1.10	NS	1,268.70	570.60	<0.001
Nod#	Number of nodal roots	39.80	1.60	<0.01	80.30	1.70	NS	1,691.70	41.50	<0.001
NodLen	Longest nodal root length, cm	366.00	1.40	<0.05	346.90	3.70	<0.01	14,033.00	48.30	<0.001
SysDia	Crown root system diameter, mm	463.00	1.70	<0.01	453.50	2.40	<0.05	27,976.00	76.60	<0.001
AvgDia	Average crown root diameter, mm	0.02	1.50	<0.01	0.04	1.42	NS	0.13	5.68	<0.05
Tips	Number of root tips	8,725.00	1.50	<0.01	15,389.00	1.90	<0.05	289,888.00	30.90	<0.001
Forks	Number of lateral branch points	115,457.00	1.70	<0.001	231,389.00	2.60	<0.05	6,097,340.00	46.30	<0.001

[†]NS, not significant.

observed for all traits within the landrace group except root to shoot ratio while the teosinte group showed significant phenotypic variation for seven of the 11 architectural traits. No strong correlations (Pearson $R > 0.60$) were observed between the teosinte and landrace groups for corresponding traits. Phenotypic variation in the landraces and teosintes is shown for selected traits in Fig. 1.

In the landrace group, both anatomical and architectural traits showed substantial variation (Table 2). Among anatomical traits, traits with a 10-fold or greater range of variation included areas of the stele, aerenchyma, and xylem and the number of cortical cells. Among architectural traits, landraces were highly variable in the number of nodal and seminal roots. One of the more broadly variable architectural traits was root system diameter, for which the maximum value was 18.5 times greater than the minimum value. Considerable phenotypic variation was also observed for the two branching traits in the landraces (Tips and Forks).

As with the landraces, anatomical and architectural traits displayed considerable variation in the teosinte group (Table 2). Stele, xylem, and aerenchyma area varied 10-fold among accessions while most other anatomical traits had less variation. Among architectural traits, low variation for seminal root number was noteworthy. For all teosintes sampled, 62% of the plants did not have seminal roots. A lack of seminal roots did not appear to influence the magnitude of variation in other traits. For instance, among teosintes lacking seminal roots, the number of crown roots varied from 5 to 45. Root system diameter was highly variable, with the maximum value 22 times greater than the minimum. The most variable architectural traits in the teosinte group were those for branching. The maximum number of branching forks was 126 times greater than the minimum phenotypic value for that trait.

Mean values and ranges for anatomical and architectural traits highlight differences between the two groups (Table 2). Landraces had greater xylem area and greater values for the ratios of stele to cross-section area and stele to cortical area. For anatomical traits, both groups had similar ranges for all variables, with the exception of xylem vessel area, percent aerenchyma, and cortical cell number. For these traits, the landrace group showed a greater magnitude of phenotypic variation than the teosintes. For aerenchyma area, the mean value in both groups was similar, but the magnitude of variation for the landraces was almost half that of the teosinte group. Still, the frequency distribution of values was similar (Fig. 1). Teosinte plants were smaller,

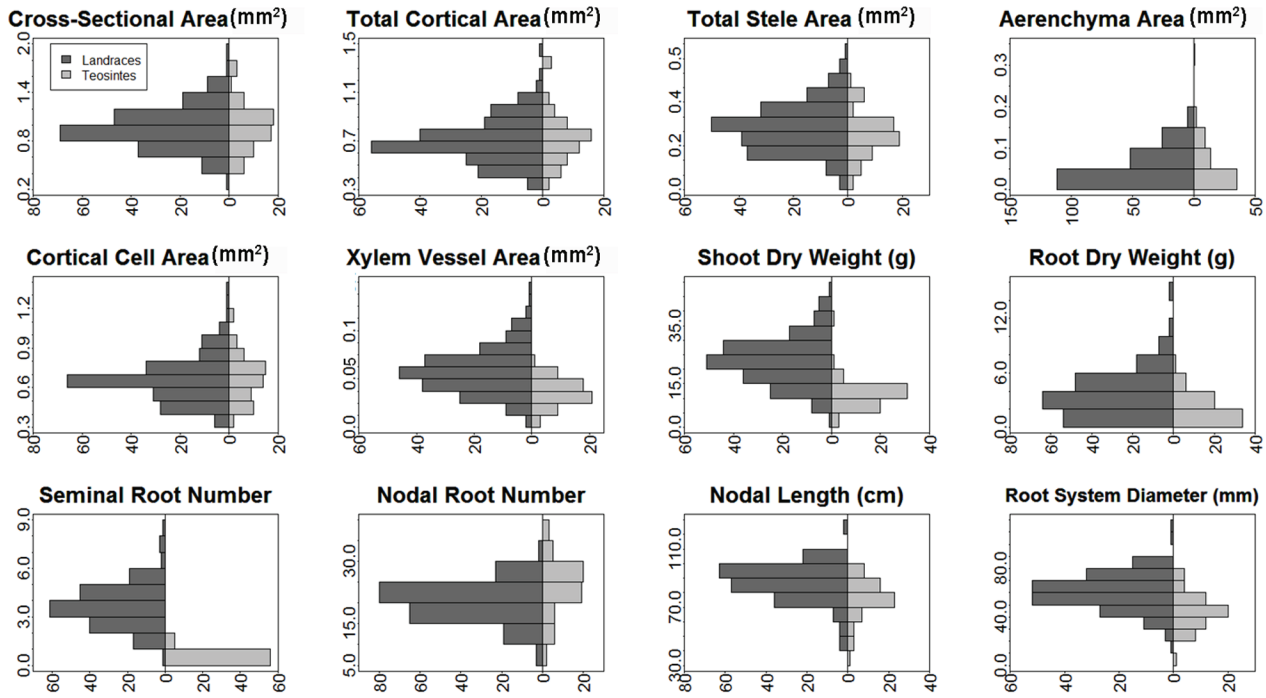

Figure 1. Histogram panel showing phenotypic distribution of selected architectural and anatomical traits for the landrace (*Zea mays* subsp. *mays*) and teosinte (*Zea* spp.) groups.

Table 2. Summary of descriptive statistics for anatomical and architectural traits measured in the maize landrace (*Zea mays* subsp. *mays*) and teosinte (*Zea* spp.) groups.

		Landraces					Teosinte				
Abbreviation	Description	Mean	Median	SD	Min.	Max.	Mean	Median	SD	Min.	Max.
					Anatomical traits						
RXSA	Cross-section area, mm^2	0.966	0.940	0.258	0.298	2.338	0.966	0.949	0.280	0.353	2.451
TCA	Total cortical area, mm^2	0.708	0.682	0.187	0.217	1.693	0.727	0.710	0.211	0.293	1.899
TSA	Total stele area, mm^2	0.258	0.252	0.079	0.069	0.765	0.239	0.220	0.075	0.060	0.606
TSA:RXSA	Total stele area:root cross-section area	0.263	0.271	0.030	0.111	0.368	0.245	0.246	0.027	0.151	0.374
TSA:TCA	Total stele area:total cortical area	0.361	0.373	0.055	0.124	0.582	0.327	0.327	0.048	0.178	0.597
AA	Aerenchyma area, mm^2	0.051	0.023	0.047	0	0.298	0.057	0.021	0.058	0	0.564
%A	Percent of cortex as aerenchyma	6.49	3.31	5.25	0	37.80	6.57	3.20	5.34	0	30.19
CCA	Cortical cell area, mm^2	0.658	0.630	0.167	0.216	1.693	0.671	0.621	0.177	0.293	1.553
%CCA	Percent of cortex as cells	0.686	0.693	0.051	0.428	0.824	0.701	0.710	0.05	0.507	0.846
XVA	Xylem vessel area, mm^2	0.053	0.055	0.019	0.009	0.143	0.039	0.039	0.010	0.008	0.080
#CC	Number of cortical cells	596.0	581.0	141.0	134.3	1524.0	595.7	580.3	134.5	279.0	1192.0
#CF	Number of cortical cell files	9.96	10.00	1.09	6.00	15.67	10.04	10.00	0.98	7.33	13.67
					Architectural traits						
RDW	Root system dry weight, g	3.77	3.39	2.50	0.29	29.57	2.32	1.83	1.84	0.20	13.02
SDW	Shoot dry weight, g	24.01	23.34	9.90	2.61	57.94	12.57	10.77	5.17	5.08	36.96
RDW:SDW	Root system dry weight:shoot dry weight	0.16	0.15	0.07	0.11	0.51	0.18	0.17	0.07	0.04	0.35
StemDia	Stem diameter, mm	22.8	23.1	4.5	10.0	37.0	17.1	17.0	4.5	10.0	30.0
Sem#	Number of seminal roots	3.9	4.0	1.3	1.0	11.0	0.5	0	0.5	0	3.0
Nod#	Number of nodal roots	20.6	20.0	4.2	6.0	45.0	24.0	25.0	6.9	5.0	51.0
NodLen	Longest nodal root length, cm	87.2	89.5	12.8	25.0	175.0	75.5	76.8	13.3	24.0	116.5
SysDia	Crown root system diameter, mm	60.9	61.0	14.5	7.0	130.0	45.1	46.5	13.9	5.0	110.0
AvgDia	Average crown root diameter, mm	0.50	0.47	0.09	0.29	1.25	0.52	0.480	0.14	0.34	1.110
Tips	Number of root tips	181.2	164.0	60.5	53.0	510.0	240.3	195.5	90.7	20.0	763.0
Forks	Number of lateral branch points	475.5	406.0	221.4	79.0	2210.0	742.1	564.5	357.1	22.0	2778.0

based on mean shoot and root dry weight and stem diameter. Landraces had 61% greater root dry weight and 52% greater shoot dry weight than teosintes. Landraces had a greater mean number of seminal roots, but teosintes had a greater mean number of nodal roots. Landraces had longer nodal roots and wider nodal root system diameter. Teosintes had

Table 3. Summary of allometric analyses of root architectural and anatomical traits in landraces (*Zea mays* subsp. *mays*), teosintes (*Zea* spp.), and data pooled from these two groups. Table lists coefficient of determination (R^2) and slope (α) from regression of the natural logarithm of each trait and the natural logarithm of total plant dry weight. All slopes were significant at $p < 0.05$ except those that are not significant (NS).

		Landraces		Teosinte		Together	
Trait	Description	R^2	α	R^2	α	R^2	α
RXSA†	Root cross-section area, mm^2	0.089	0.196	0.087	0.070 NS	0.045	0.128
TCA†	Total cortical area, mm^2	0.080	0.180	0.005	0.050 NS	0.032	0.105
TSA†	Total stele area, mm^2	0.098	0.245	0.027	0.139	0.077	0.196
TSA:RXSA‡	Total stele area:root cross-section area	0.072	0.069	0.235	0.126	0.197	0.100
TSA:TCA‡	Total stele area:total cortical area	0.068	0.091	0.230	0.165	0.192	0.134
AA†	Aerenchyma area, mm^2	0.096	0.932	0.167	1.288	0.065	0.634
%A‡	Percent of cortex as aerenchyma	0.077	0.750	0.191	1.165	0.064	0.557
CCA†	Cortical cell area, mm^2	0.067	0.162	0.000	0.0123 NS	0.024	0.087
%CCA‡	Percent of cortex as cells	0.047	−0.038	0.107	−0.056	0.108	−0.048
XVA†	Xylem vessel area, mm^2	0.081	0.236	0.102	0.236	0.152	0.293
#CC‡	Number of cortical cells	0.069	0.153	0.019	0.073 NS	0.032	0.084
#CF‡	Number of cortical cell files	0.061	0.067	0.019	0.032 NS	0.021	0.031
StemDia‡	Stem diameter, mm	0.427	0.261	0.455	0.518	0.568	0.401
Sem#‡	Number of seminal roots	0.002	0.045	–	–	–	–
Nod#‡	Number of nodal roots	0.476	0.369	0.452	0.582	0.189	0.232
NodLen‡	Longest nodal root length, cm	0.390	0.242	0.460	0.342	0.475	0.249
SysDia‡	Crown root system diameter, mm	0.351	0.361	0.402	0.586	0.472	0.442
AvgDia‡	Average crown root diameter, mm	0.031	−0.074	0.002	−0.023 NS	0.046	−0.079
Tips‡	Number of root tips	0.156	0.311	0.159	0.366	0.016	0.089
Forks‡	Number of lateral branch points	0.134	0.395	0.130	0.405	0.002	0.045 NS

†Area traits.

‡Linear traits.

greater nodal root branching, based on the mean number of tips and forks.

Isometric and anisometric relationships were observed for architectural and anatomical traits among and between the landraces and teosintes (Table 3). Isometric relationships are those in which growth of a tissue or organ occurs in proportion to increases in total dry weight. Anisometric relationships are those in which growth is *not* proportional to increases in total dry weight. Based on comparison to biomass, isometric scaling exponents are expected to be 0.33 for linear dimension traits (e.g., counts, percents, ratios, linear measurements) and 0.67 for area traits. Scaling exponents near these values indicate isometric (proportional) growth of the organ or tissue with respect to biomass. Scaling exponents that differ from these values indicate anisometric growth. Area traits were predominantly anisometric among and between landraces and teosintes, with the exception of aerenchyma area for pooled data. Among these area traits, scaling exponents were mostly below the expected isometric scaling exponent (0.67), except for aerenchyma area in both the landraces and teosintes. For linear dimension traits, scaling exponents indicated isometric relationships for nodal number, system diameter, tips, and forks in the landraces and nodal length and tips in the teosintes. The remaining scaling exponents indicated anisometry in the linear dimension traits, with values that were either higher or lower than the expected isometric scaling exponent (0.33). In the teosintes, scaling exponents for nodal number (0.582) and system diameter (0.586) approached two times the isometric scaling exponent. All traits in the landraces had significant scaling exponents. In the teosintes, scaling exponents were insignificant for five anatomical traits (root cross-section area, total cortical area, cortical cell area, number of cortical cells, and number of cortical cell files) and one architectural trait (average crown root diameter). For pooled data, all scaling exponents were significant except for the Forks trait.

When data were allometrically scaled to normalize for size, mean values in the teosinte group were less than those in the landraces for all anatomical traits, except the number of cortical cells and cell files (Table 4). The teosintes had greater scaled mean values for most architectural traits, except for the average diameter of a crown root, but a smaller range of variation in scaled data for most traits.

Principal component analyses were performed separately on the landraces and teosintes and with data pooled from the two groups. Overall, the trait clustering and component loading in the pooled PCA were not substantially different from those seen in the separate analyses. In the three analyses, architectural and anatomical traits generally clustered together, and the magnitude and direction of loading values was similar. Despite differences between the groups, multivariate analysis revealed a similar trait structure. Due to

Table 4. Summary of mean, minimum. and maximum values for root anatomical and architectural traits in the landraces (*Zea mays* subsp. *mays*) and teosintes (*Zea* spp.) following allometric scaling to remove the effect of plant size.

		Mean		Minimum		Maximum		
Abbreviation	Description	Landraces	Teosintes	Landraces	Teosintes	Landraces	Teosintes	*p*-value
		Anatomical traits						
RXSA	Root cross-section area, mm^2	1.1963	0.5086	0.0840	0.0604	4.3499	1.6959	<0.001
TCA	Total cortical area, mm^2	0.9156	0.3509	0.0432	0.0347	3.9325	1.5167	<0.001
TSA	Total stele area, mm^2	0.5773	0.0812	0.0014	0.0008	5.0725	0.9741	<0.001
TSA:RXSA	Total stele area:root cross-section area	0.3585	0.1132	0.0181	0.0123	1.3647	0.5729	<0.001
TSA:TCA	Total stele area:total cortical area	0.4703	0.1635	0.0356	0.0219	1.5826	0.6396	<0.001
AA	Aerenchyma area, mm^2	0.5639	0.0840	0.0000	0.0000	14.1133	4.3916	<0.05
%A	Percent of cortex as aerenchyma	6.66	5.75	0.00	0.06	26.21	19.21	NS†
CCA	Cortical cell area, mm^2	0.8568	0.3168	0.0394	0.0330	3.8778	1.2919	<0.001
%CCA	Percent of cortex as cells	0.6900	0.6100	0.4400	0.4500	0.8900	0.7600	<0.001
XVA	Xylem vessel area, mm^2	0.3527	0.0209	0.0000	4.4×10^{-6}	8.0865	0.8233	<0.01
#CC	Number of cortical cells	78,261.9	663,839.4	2.5	11.1	13,127,953.9	14,286,723.9	<0.01
#CF	Number of cortical cell files	15.3	64.2	1.4	2.5	344.5	375.2	<0.001
		Architectural traits						
StemDia	Stem diameter, mm	27.1	55.8	4.3	5.4	428.3	203.2	<0.001
Nod#	Number of nodal roots	29.1	139.2	2.8	7.8	752.5	696.1	<0.001
NodLen	Longest nodal root length, cm	364.9	2,224.3	3.3	8.6	28,260.4	21,460.8	<0.001
SysDia	Crown root system diameter, mm	107.7	370.7	6.8	15.7	4,410.7	3,120.5	<0.001
AvgDia	Average crown root diameter, mm	0.5	0.4	0.2	0.2	1.0	0.9	<0.001
Tips	Number of root tips	4,003.1	38,155.5	2.0	19.6	568,519.4	470,405.3	<0.001
Forks	Number of lateral branch points	50,051.4	620,218.3	1.8	29.7	8,323,951.1	13,844,316.1	<0.001

†NS, not significant.

this, we present only the separate analyses. For the landraces, the first two components accounted for 51.5% of the total variation in the data (Table 5). For the teosintes, the first two components explained 57.2% of the total variation in the data (Table 6). Both groups showed high loading scores on the first and second components for similar sets of variables. Based on the variable loadings, the first component can be interpreted as "tissue-level carbon investment" in both the landrace and teosinte groups. Variables most strongly associated with this component included root cross-sectional area, total stele area, total cortical area, and cortical cell area. The second component can be interpreted as "whole-plant carbon allocation" in both groups. Variables most strongly associated with this component included shoot and root dry weight, nodal number, crown root system diameter, stem diameter, and nodal root length. Biplots of the first and second components showed similar trends in trait structure in both groups (Fig. 2). In the landrace group, vectors for the two trait categories grouped closely, with the exception of the percent of cortex as cells (Fig. 2A). In the teosinte group, a similar clustering of anatomical and architectural traits was observed (Fig. 2B). However, vectors for some traits were isolated from their respective clusters, including xylem vessel area, percent aerenchyma, and percent cortical cell area. When data were pooled for the two groups, trait structure, loading values, and biplots were similar to those of the isolated groups (data not shown).

In the hierarchical cluster analysis, accessions were divided into eight clusters (Table 7). Six of the clusters contained both teosintes and landraces while the remaining two clusters each contained members of a single group. Cluster membership was not influenced by seed provenance since clusters included accessions from a variety of geographic locations. Root architectural and anatomical phenotypes differed among the clusters. Each cluster was typically defined by a few traits, including both anatomical and architectural characteristics. Certain traits appeared to be more influential in determining cluster membership, due to pronounced differences in phenotypic means and ranges across the clusters. Influential anatomical traits included the areas of the cross-section, cortex, stele, aerenchyma, and xylem vessels. Architectural traits determining membership in one or more clusters included root number, crown root length, root system diameter, branching, and biomass (Fig. 3).

DISCUSSION

Since its domestication, maize has been cultivated in many different environments, thereby developing phenotypic and functional diversity. Races of domesticated plants are phenotypically distinct and, in many cases, diverge from one another due to geographic, climatic, and agronomic factors (Iltis and Doebley, 1980; Chacón et al., 2005; Corral et al., 2008; Brown et al., 2011). Maize originated from annual teosinte (*Z. mays* subsp. *parviglumis*) in a single domestication event in Central America followed by intensive diversification as it spread throughout the Americas (Matsuoka et al., 2002). Rapid spread of its cultivation involved adaptation to common edaphic

Table 5. Loading scores and variance for components 1 and 2 in the principal components analysis of anatomical and architectural traits measured in the maize landrace (*Zea mays* subsp. *mays*) group.

Landraces		Component	
Trait		1	2
RXSA	Root cross-section area, mm^2	0.328	−0.204
TCA	Total cortical area, mm^2	0.315	−0.223
%CCA	Percent of cortex as cells	−0.142	−0.203
TSA	Total stele area, mm^2	0.333	−0.144
TSA:RXSA	Total stele area:root cross-section area	0.161	0.105
TSA:TCA	Total stele area:total cortical area	0.156	0.106
AA	Aerenchyma area, mm^2	0.144	0.102
%A	Percent of cortex as aerenchyma	0.083	0.168
CCA	Cortical cell area, mm^2	0.305	−0.270
XVA	Xylem vessel area, mm^2	0.308	−0.116
#CC	Number of cortical cells	0.260	−0.283
#CF	Number of cortical cell files	0.257	−0.294
SDW	Shoot dry weight, g	0.215	0.309
RDW	Root system dry weight, g	0.215	0.278
StemDia	Stem diameter, mm	0.173	0.278
Sem#	Number of seminal roots	0.015	0.009
Nod#	Number of nodal roots	0.134	0.327
NodLen	Longest nodal root length, cm	0.191	0.247
SysDia	Crown root system diameter, mm	0.192	0.275
Tips	Number of root tips	0.148	0.155
Forks	Number of lateral branch points	0.162	0.125
Proportion of variance		0.362	0.152
Cumulative proportion		0.362	0.515

Table 6. Component loading scores and variance for components 1 and 2 in the principal components analysis of anatomical and architectural traits measured in the teosinte (*Zea* spp.) group.

Teosintes		Component	
Trait	Description	1	2
RXSA	Root cross-section area, mm^2	0.306	−0.247
TCA	Total cortical area, mm^2	0.289	−0.270
%CCA	Percent of cortex as cells	−0.278	−0.077
TSA	Total stele area, mm^2	0.326	−0.162
TSA:RXSA	Total stele area:root cross-section area	0.164	0.188
TSA:TCA	Total stele area:total cortical area	0.161	0.188
AA	Aerenchyma area, mm^2	0.228	−0.140
%A	Percent of cortex as aerechyma	0.213	−0.003
CCA	Cortical cell area, mm^2	0.271	−0.276
XVA	Xylem vessel area, mm^2	0.307	0.013
#CC	Number of cortical cells	0.212	−0.246
#CF	Number of cortical cell files	0.216	−0.250
SDW	Shoot dry weight, g	0.163	0.330
RDW	Root system dry weight, g	0.191	0.317
StemDia	Stem diameter, mm	0.172	0.211
Sem#	Number of seminal roots	0.044	0.217
Nod#	Number of nodal roots	0.135	0.265
NodLen	Longest nodal root length, cm	0.218	0.198
SysDia	Crown root system diameter, mm	0.147	0.262
Tips	Number of root tips	0.147	0.195
Forks	Number of lateral branch points	0.162	0.165
Proportion of variance		0.349	0.224
Cumulative proportion		0.349	0.572

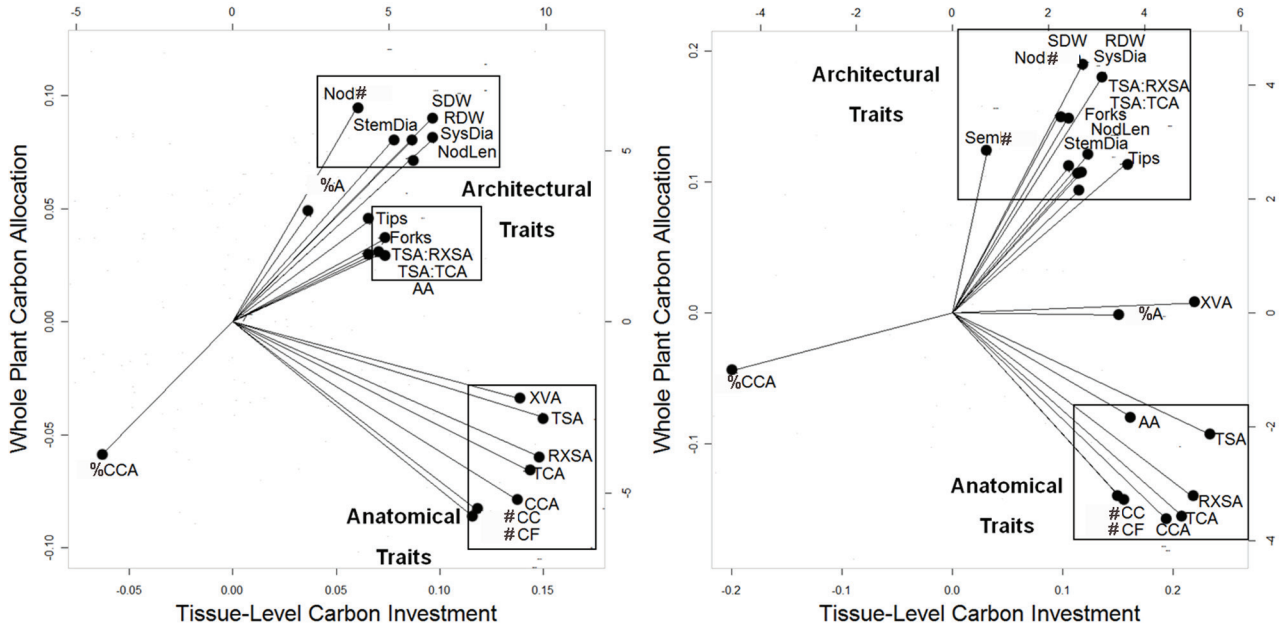

Figure 2. Biplot of principal components analysis for anatomical and architectural traits in a diverse set of accessions of (A) maize landraces (*Zea mays* subsp. *mays*) and (B) teosinte (*Zea* spp.). Based on traits with the highest loading values, component 1 can be characterized as "tissue level carbon investment" and component 2 as "whole plant carbon allocation." Boxes show trait clustering by trait type. %A, percent aerenchyma; AA, aerenchyma area; AvgDia, average crown root diameter; #CC, number of cortical cells; %CCA, percent of cellular area in cortex; CCA, cortical cell area; #CF, number of cortical cell files; Forks, number of lateral branch points; Nod#, number of nodal roots; NodLen, longest nodal root length; RDW, root system dry weight; RXSA, root cross-section area; TCA, total cortical area; Tips, number of root tips; TSA, total stele area; SDW, shoot dry weight; Sem#, number of seminal roots; StemDia, stem diameter; SysDia, diameter of the crown root system; XVA, xylem vessel area.

Table 7. Summary of hierarchical cluster analysis of accessions of *Zea* species, showing species (landrace [*Zea mays* subsp. *mays*] or teosinte [*Zea* spp.]) and number of accessions in each group (in parentheses in Species column). Membership in each cluster was based on anatomical and architectural characteristics, and phenotypic descriptions are based on the mean and range for each trait within a cluster.

Cluster	Landraces	Teosintes	Species	Anatomical summary	Architectural summary
1	3	8	*Z. mays* subsp. *parviglumis* (5), *Z. mays* subsp. *mays* (3), and *Z. perennis* (3)	Lowest values for all anatomical traits among the eight clusters	Smallest biomass and stem diameter, few seminal and nodal roots, and shortest crown roots with small diameter, little branching, and steep angles of growth
2	0	3	*Z. mays* subsp. *mexicana* (1), *Z. mays* subsp. *parviglumis* (1), and *Z. nicaraguensis* (1)	Largest tissue region areas (root cross-section area, Total stele area, and total cortical area) and greatest amount of aerenchyma among the clusters, large values for number of cortical cells and cell files, and moderate xylem vessel area	Small biomass, few seminal roots and moderate number of nodal roots, and large diameter, highly branched crown roots of moderate length growing at shallow angles
3	10	1	*Z. mays* subsp. *mays* (10) and *Z. mays* subsp. *parviglumis* (1)	Moderate values for anatomical traits, with a few exceptions. Stele and xylem vessel areas were large and the amount of aerenchyma was low, and cortical cells and cell files were numerous	Moderate to large biomass, numerous seminal roots, and moderate number of long, highly branched nodal roots of low diameter with shallow growth
4	10	9	*Z. mays* subsp. *mays* (10), *Z. mays* subsp. *parviglumis* (6), and *Z. mays* subsp. *mexicana* (3)	Moderate values for anatomical traits, except for stele and xylem areas, which were relatively large	Moderate values for most architectural traits, except for branching, which was moderate to high
5	61	0	*Z. mays* subsp. *mays* (61)	Low to moderate values on anatomical traits except xylem vessel area, which was moderate to large	Large shoot and root biomass, large stem diameter, numerous seminal roots, and moderate number of long, small diameter, and shallow growing crown roots with moderate branching
6	74	32	*Z. mays* subsp. *mays* (74), *Z. mays* subsp. *parviglumis* (19), *Z. mays* subsp. *mexicana* (9), *Z. perennis* (2), *Z. mays* subsp. *huehuetenangensis* (1), and *Z. luxurians* (1)	Small to moderate values for all anatomical traits	Large values for root and shoot biomass, moderate values for seminal and nodal root numbers, and average crown root diameter, root system diameter, and branching
7	7	3	*Z. mays* subsp. *mays* (7), *Z. mays* subsp. *parviglumis* (2), and *Zea* hybrid (1)	Smaller values for anatomical traits, except for aerenchyma area, which was relatively large	Largest biomass values, moderate to large number of seminal roots, greatest number of nodal roots, shallowest nodal root growth, and long nodals with small diameter that possessed the most branching of all clusters
8	27	5	*Z. mays* subsp. *mays* (27), *Z. mays* subsp. *mexicana* (3), and *Z. mays* subsp. *parviglumis* (2)	Small values for all anatomical traits	Small biomass, numerous seminal roots and moderate number of nodal roots, and nodal roots of a moderate length, small diameter, with steep angles of growth and lower amounts of branching

stresses such as drought, salinity, and nutrient deficiency (Vigouroux et al., 2008). Adaptation to edaphic stress has been suggested as an influential factor in the domestication of maize (Vielle-Calzada et al., 2009). The results of the present study show that considerable variation exists in the genus *Zea* for architectural and anatomical root traits related to acquisition of water and nutrients.

Root architectural traits influence plant fitness by regulating access to soil resources (Lynch and Brown, 2008). Root traits such as branching, length, number, and growth angle determine root distribution among soil horizons (Lynch and Brown, 2006). In maize, nodal roots are important in the scavenging of both mobile and immobile soil resources (McCully, 1999; Lynch, 2011; Postma and Lynch, 2011a, 2011b). Between the landrace and teosinte groups, differences were observed in nodal root system characteristics, with considerable phenotypic variation seen for several architectural traits within each group (Table 2). For unscaled data, landraces had longer nodal roots, a wider nodal root system diameter, and less branching. Longer nodal roots have been shown to assist in the capture of mobile resources in the soil and are considered to be a primary determinant of drought tolerance in maize (Ribaut et al., 2009; Zhu et al., 2010). A wide crown root system indicates a relatively broad region of soil exploration, allowing enhanced access to soil resources. In contrast,

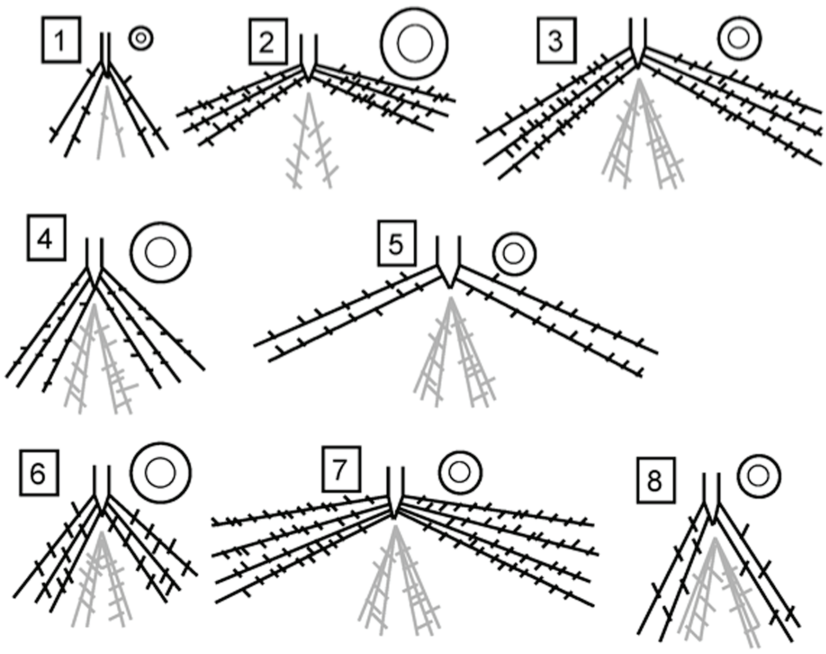

Figure 3. Schematic drawings of eight root architectural types, based on cluster analysis of maize landraces (*Zea mays* subsp. *mays*) and teosintes (*Zea* spp.). Gray lines represent crown roots and red lines represent seminal roots. Primary root is not depicted. Hash marks indicate degree of lateral root branching. The circular drawings are representative of crown root diameter.

teosintes had shallower and more highly branched root systems compared to the landraces in the unscaled data set. This root phenotype is more efficient at P acquisition by favoring root growth in the P-rich epipedon (Lynch and Brown, 2001; Zhu et al., 2005a; Lynch, 2007b). In maize, P acquisition efficiency has been associated with several root characteristics, such as greater nodal root number, branching, length and root hair density (Bayuelo-Jiménez et al., 2011), longer root hairs on the tap root (Zhu et al., 2005b), and increased lateral root branching in the embryonic root system (Zhu and Lynch, 2004). Our results suggest that typical teosinte root systems are better equipped to deal with low soil P while landraces have developed root phenotypes suited to drought tolerance.

In maize, seminal roots play a central role in plant establishment and therefore influence the likelihood of survival to reproduction (Tuberosa and Silvio, 2009). Their relative importance during early vegetative growth is directly related to the number, length, and branching of seminal roots as well as their growth rate (Zhu and Lynch, 2004; Enns et al., 2006). The difference in seminal root number between the two groups may be related to smaller seed size in the teosintes. Larger seed size was selected during the domestication of maize, providing more seed resources for early growth (Pickersgill, 2007). A lack of seminal roots in some teosintes may reflect favored use of limited seed resources for rapid downward growth by the primary root to access water and provide early anchorage. Given the role of the embryonic root system in establishing anchorage, numerous seminal roots might be unnecessary to prevent lodging of the low-stature teosinte.

Anatomical variation has been related to differences in functional efficiency and stress response in several crop species, including maize (Zhu et al., 2010), wheat (Richards and Passioura, 1981), common bean (Peña-Valdivia et al., 2010) and rice (*Oryza sativa* L.) (Uga et al., 2008). Roots of landraces and teosintes displayed variable expression of anatomical traits, with the most prominent differences observed in the vascular cylinder (Table 2; Fig. 4). Greater mean and range for xylem vessel areas in landraces indicate selection during domestication for accessions that were better equipped for rapid or efficient resource transport from the root to support the larger shoots in landraces. Based on the Hagen-Poiseuille equation, the hydraulic conductance of a xylem vessel is proportional to the vessel radius to the fourth power. The availability of extra water with irrigation could have favored selection for larger xylem vessels.

Landraces also had a slightly greater mean stele area and greater ratios of stele area to cross-section and cortex areas. The proportion of stele area in the cross-section is likely to influence root system cost and function. While larger steles in landraces can accommodate larger xylem vessels for increased water transport, the initial C investment for growth of a larger stele is greater on a per root basis. Based on the results of this study, increased C investment in the root system may have been a trade-off for increased resource transport in larger xylem vessels during the domestication of maize.

Allometry is not typically considered in phenotypic analyses, despite the influence of organ scale and shape on biological function. Generally speaking, allometry is the study of the relationship between the growth of individual

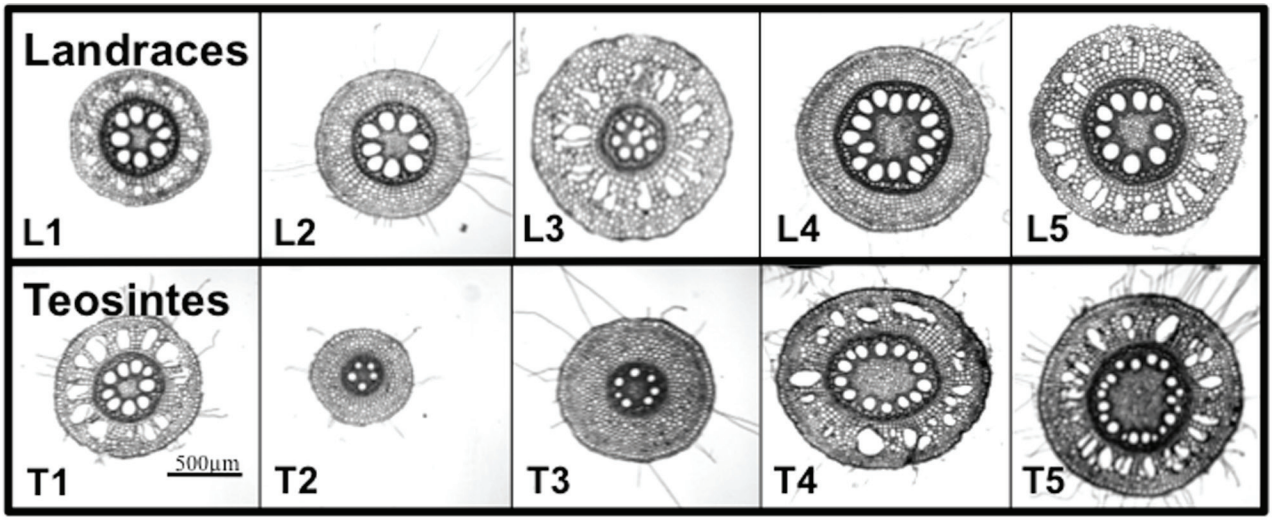

Figure 4. Images showing phenotypic variation in anatomical traits for the landrace (*Zea mays* subsp. *mays*) (upper panel) and teosinte (*Zea* spp.) (lower panel) groups. Scale at lower left applies to all images. Landrace (L) accession and cluster (C) number: L1, PI 583932, in C7; L2, PI 503566, in C4; L3, PI 571465, in C6; L4, PI 474205, in C5; and L5, PI 628480, in C4. Teosinte (T) accession and cluster number: T1, Ames 21798, in C6; T2, Ames 21872, in C1; T3, Ames 21869, in C6; T4, Ames 21793, in C6; and T5, Ames 21789, in C6.

organs or tissues and increases in total biomass of an organism (Niklas, 1994). Within that, one can examine whether the relationship is isometric (tissue growth is in proportion to increases in biomass) or anisometric (tissue growth is *not* in proportion to biomass increases). In the present study, allometry influenced architectural and anatomical traits in each group, including tissue and aerenchyma areas, root length, number, and branching, and root system diameter (Table 3). Slope values (i.e., scaling exponents) from allometric analysis indicate whether growth of a particular organ or tissue is isometric or anisometric with respect to total biomass. Anisometric growth occurs when growth of a tissue or organ results in a change in relative proportions within the organism while proportional relationships are maintained during isometric growth (Yang et al., 2009). For linear traits, isometric growth is expected with slopes of 0.33, and area traits are considered isometric with slopes of 0.67. Slope values below these values predict growth less than expected from isometric growth ("negative anisometry") while greater values predict growth that exceeds expected isometric growth ("positive anisometry").

Differences in scaling exponents suggest that landraces and teosintes exhibit distinct growth patterns (Table 3). Most area traits had low scaling exponents, indicating weak allometric relationships for anatomical area traits in both groups. A notable exception was for aerenchyma area, for which both groups displayed strong positive anisometry. Based on this, as growth proceeds, the proportion of aerenchyma would be greater than that expected with isometric growth in both groups. Teosintes had a greater slope for aerenchyma area, and therefore, this effect would be expected to be greater in that group compared to the landraces. Aerenchyma has been shown to reduce the respiratory cost of maize roots (Fan et al., 2003; Zhu et al., 2010). The progressive loss of cortical cells during aerenchyma formation and cortical senescence may indicate that the cortex plays a diminishing role or represents an increasing burden as development proceeds in cereals. By reducing the metabolic costs of soil exploration, aerenchyma improves drought tolerance in maize (Zhu et al., 2010), and functional–structural modeling suggests that aerenchyma could improve crop growth in soils with suboptimal availability if N, P, and K (Postma and Lynch, 2011a, 2011b). Strategies to optimize the metabolic costs of soil exploration are important aspects of plant adaptation to edaphic stress.

For linear traits such as nodal number and crown root system diameter, scaling exponents for the landraces were isometric while anisometric slopes for the teosintes indicated that development of these traits would greatly exceed concomitant biomass increases (Table 3). As landrace root systems grow, they would be expected to initiate nodal roots and expand root system diameters proportionally to their biomass while teosintes would be expected to do so at almost twice the isometric rate. Within the teosintes, this would result in greater root investment in shallow soil than if growth for those traits were isometric. Despite these differences in nodal root growth, lateral rooting traits in both groups were close to isometric as was nodal root length in the teosintes. For teosintes, proportional (isometric) growth in nodal root length could represent a tradeoff for an increased investment in the number of nodal roots caused by strong positive anisometry in the latter trait. In the landraces, nodal root length displayed negative anisometry with a scaling exponent of 0.242 (Table 3). Therefore, nodal roots in that group would be shorter than expected for growth proportional to plant size. These differences in underlying patterns of belowground C allocation may reflect indirect selection for root traits during maize domestication. Selection for desirable traits has been

shown to change allometric relationships in oat (*Avena sativa* L.), even in the relatively short timeframe of a breeding program (Semchenko and Zobel, 2005). In teosintes, strong scaling exponents show that this group preferentially allocates biomass to numerous shallow nodal roots during development but that nodal root length increases at a comparatively slower rate. This indicates that wild *Zea* species are well suited to resource scavenging in the epipedon. In contrast, the isometric or negatively anisometric pattern of nodal root growth and expansion in landraces may be a more balanced strategy, allowing such plants to obtain multiple scarce resources in both surface and subsurface soil horizons.

Allometric scaling enhanced the ability to observe differences between landraces and teosintes by correcting for the effect of plant size on trait values (Lleonart et al., 2000). Allometric scaling adjusts trait values and therefore can alter comparisons among groups (Niklas and Marler, 2007). In allometric scaling, a new data set is created based on mean biomass across two groups and differs from evaluation of anisometry vs. isometry in which mean biomass is calculated for each group separately. Allometric scaling is particularly relevant to comparisons between crop species and their wild relatives because changes in tissue and organ size are likely to occur during domestication (Bretting, 1986). In the present study, teosintes had a slightly greater mean number of nodal roots when data were unscaled. However, scaled data showed that at a common plant size, landraces would have considerably fewer nodal roots than teosintes. The nodal root system is established in maize beginning 2 to 3 wk after germination, with the initiation of belowground crown roots followed by aboveground brace roots (Hochholdinger, 2009). Due to greater root length and branching, the nodal root system plays a more prominent role in resource acquisition and plant anchorage over the life of the plant compared to the seminal system (McCully, 1999; Doussan et al., 2003; Hochholdinger, 2009).

Phylogenetic analyses of the *Zea* genus have revealed population structures based on geography or climate (Reif et al., 2004; Fukunaga et al., 2005; Vigouroux et al., 2008). In the present study, cluster membership was not determined by these factors. Our analysis emphasizes the differing objectives of cluster analyses based on genetic and phenotypic data. Clustering of genetic data is more likely to reveal population structure since the data are not influenced by environmental factors. Phenotypic clustering is influenced by environment and therefore can highlight groups based on plant performance. Based on the cluster analysis, root architectural phenotypes may be used to identify functional similarities among maize accessions (Table 7; Fig. 3). Accessions in clusters 3 and 7 had long and highly branched nodal roots, which would aid in resistance to lodging. Phenotypes with steeply growing nodal roots, such as in clusters 1 and 8, would be at an advantage in acquiring mobile resources such as N and water. Many clusters had shallow-rooted phenotypes, which would favor acquisition of nutrients found in the epipedon, such as P and K (e.g., clusters 2, 3, 5, and 7). Greater levels of branching would enhance this advantage by increasing the root surface area, as in clusters 2, 3, and 7.

In summary, we observed substantial phenotypic variation for root architecture and anatomy between and within maize landraces and related wild *Zea* species. This study emphasizes the importance of allometric scaling in phenotypic studies, particularly those that assess differences among species. Root phenotypic variation is likely to have functional importance in adaptation to edaphic stress and soil resource acquisition. In general, landraces offer a better source of genetic variation for root anatomical and architectural traits than teosintes.

Supplemental Information Available

Supplemental material is included with this manuscript.

Supplemental Figure S1. Choropleth map showing seed provenance of 173 of the maize landraces (*Zea mays* subsp. *mays*) used in this study. Color intensity represents the number of different landraces found in each area. Additional landraces of unknown or imprecise origin are not included in this map. Map created by Gregory Luna (Pennsylvania State University).

Supplemental Figure S2. Choropleth map of the states of Mexico showing seed provenance of 46 of the maize landraces (*Zea mays* subsp. *mays*) and 35 of the teosintes (*Zea* spp.) used in this study. Color intensity represents the number of different landraces or teosintes found in each area. Additional teosintes of unknown or imprecise origin are not included in this map. Map created by Gregory Luna (Pennsylvania State University).

Supplemental Table S1. List of landrace (*Zea mays* subsp. *mays*) accessions used in this study showing accession number, geographic origin, species, subspecies, and data for each variable.

Supplemental Table S2. List of teosinte (*Zea* spp.) accessions used in this study showing accession number, geographic origin, species, subspecies, and data for each variable.

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