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ORIGINAL PAPER

QTL mapping and phenotypic variation of root anatomical traits in maize (*Zea mays* L.)

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Abstract
Key message **Root anatomical trait variation is described for three maize RIL populations. Six quantitative trait loci (QTL) are presented for anatomical traits: root cross-sectional area, % living cortical area, aerenchyma area, and stele area.**
Abstract Root anatomy is directly related to plant performance, influencing resource acquisition and transport, the metabolic cost of growth, and the mechanical strength of the root system. Ten root anatomical traits were measured in greenhouse-grown plants from three recombinant inbred populations of maize [intermated B73 × Mo17 (IBM), Oh43 × W64a (OhW), and Ny821 × H99 (NyH)]. Traits included areas of cross section, stele, cortex, aerenchyma, and cortical cells, percentages of the cortex occupied by aerenchyma, and cortical cell file number. Significant phenotypic variation was observed for each of the traits, with maximum values typically seven to ten times greater than minimum values. Means and ranges were similar for the OhW and NyH populations for all traits, while the IBM population had lower mean values for the majority of traits, but a 50 % greater range of variation for aerenchyma area. A principal component analysis showed a similar trait structure for the three families, with clustering of area and count traits. Strong correlations were observed among area traits in the cortex, stele, and cross-section. The aerenchyma and percent living cortical area traits were independent of other traits. Six QTL were identified for four of the traits. The phenotypic variation explained by the QTL ranged from 4.7 % (root cross-sectional area, OhW population) to 12.0 % (percent living cortical area, IBM population). Genetic variation for root anatomical traits can be harnessed to increase abiotic stress tolerance and provide insights into mechanisms controlling phenotypic variation for root anatomy.

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Introduction

Traditionally, plant breeding has focused on aboveground characteristics due to the challenges of in situ examination of roots and the complex influence of the environment on root growth (Tuberosa et al. 2003). However, breeding for superior root traits has the potential to enhance yield and stress tolerance in a variety of crops (Tuberosa et al. 2011; Wasson et al. 2012; York et al. 2013; Jung and McCouch 2013; Lynch 2013). Root anatomical traits influence important plant functions, including acquisition and transport of water and nutrients, anchorage, and tolerance of abiotic and biotic stresses, but have received little attention as selection criteria in crop breeding. To fully explore their potential, a better understanding of the variation and genetic control of such traits is necessary.

A variety of root anatomical traits may be useful as breeding targets for improving plant performance. Traits such as cell size, number, arrangement, and density determine pathways by which water and nutrients enter and travel through the root (Marschner 1995). The number of cortical cells or files of cells can change root diameter and the distance required for resources to travel to vascular tissues (Justin and Armstrong 1987). The area or diameter of

the xylem vessels affects the axial hydraulic conductivity (Tombesi et al. 2010). This trait has been used to improve water use efficiency in wheat and holds potential for similar applications in other crops (Richards and Passioura 1989). Finally, traits such as cell wall thickness and cell density influence the mechanical strength of the root system (Justin and Armstrong 1987; Crook et al. 1994; Striker et al. 2007). Breeding programs targeting traits related to mechanical strength could be useful in improving resistance to lodging or encouraging root growth in compacted soils.

Root traits can be used to improve tolerance to abiotic soil stresses, such as drought and nutrient deficiency, by enhancing the metabolic efficiency of soil exploration (Lynch 2011, 2013). The growth and maintenance of root tissue requires an investment of respiratory carbon by the plant. Strategic investment of carbon resources results in more efficient root system function (Lynch and Ho 2005). This can be accomplished by elimination of unnecessary tissues and their associated maintenance costs, or by preferential investment in tissues that require less carbon for construction (Lynch 2011, 2013). Root systems that are more metabolically efficient have a greater ability to grow into new soil domains to obtain limiting resources and support greater yields (Lynch and Ho 2005; Lynch 2007). Root cortical traits have been shown to reduce the metabolic costs of root growth and maintenance (Fan et al. 2003; Zhu et al. 2010; Jaramillo et al. 2013). Root cortical aerenchyma reduces root respiration, and improves plant performance and yield of drought-stressed maize (Zhu et al. 2010). However, the amount of living tissue in the cortex has been shown to be a better predictor of root respiration, soil exploration, and drought tolerance than aerenchyma or root diameter (Jaramillo et al. 2013). Development of genotypes with such carbon-economizing traits in more expensive root classes could influence the long-term cost of the root system.

In addition to resource acquisition efficiency, anatomical traits may affect the way roots interact with soil biota. The degree of infection or damage by pathogenic organisms has been related to cell arrangement, cell wall structure, and composition (Deacon and Lewis 1982; Thomas et al. 2007; Johnson et al. 2010). Since many fungal endophytes colonize root cortical cells (Sieber and Grunig 2013), cortical traits may also affect these relationships, though this is yet to be investigated.

Marker-assisted selection of genotypes based on quantitative trait loci (QTL) is an approach to improve target traits (Collins et al. 2008). QTL have been identified for only a small number of plant anatomical traits. QTL identified for shoot traits include wheat stem traits related to lodging resistance (Hai et al. 2005), leaf stomatal and epidermal traits believed to vary in response to elevated carbon dioxide in poplar (Ferris et al. 2002), and stem vascular traits in tomato (Coaker et al. 2002) and apple (Lauri et al. 2011). QTL for root anatomical traits have included those for root stele and xylem vessel diameter in rice (Uga et al. 2008, 2010), xylem vessel characteristics in wheat (Sharma et al. 2010), root cortical aerenchyma in *Zea* species (Mano et al. 2005, 2007; Mano and Omori 2008), and various root anatomical characteristics in tomato (Ron et al. 2013). Modern phenotyping techniques, including faster image analysis methods for anatomical traits such as *RootScan* (Burton et al. 2012b) should make these traits more accessible in the future.

Root anatomical traits are strongly related to plant performance and yield in stress and non-stress environments, but have received relatively little attention as target traits in plant breeding. Phenotypic characterization of relevant traits is an important first step, followed by identification of loci controlling these traits. In this study, phenotypic variation is described for ten anatomical root traits in three recombinant inbred line populations of maize. QTL are reported for four of the ten traits.

Materials and methods

Mapping populations

Two hundred recombinant inbred lines (RIL) of *Zea mays* L. ssp. *mays* were randomly selected from each of three recombinant inbred populations: Intermated B73 × Mo17 (IBM), OH43 × W64a (OhW), and NY821 × H99 (NyH). The IBM, NyH, and OhW populations are publicly available from the Maize Genetics Cooperation Stock Center (Urbana, IL, USA). Details regarding the populations and genetic maps are provided in (Burton et al. 2014).

Experimental conditions

Greenhouse

Plants were grown in a greenhouse located in University Park, PA (40°48′N, 77°51′W), from May–August 2008 as previously described (Burton et al. 2014). Briefly, three replications were grown per genotype, planted 7 days apart. Maize plants were grown in 10.5 L pots (21 × 40.6 cm, top diameter × height, Nursery Supplies Inc., Chambersburg, PA, USA). One plant was grown per pot. 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 (in μM): NO_3 (2,211), NH_4 (777), CH_4N_20 (398), P (410), K (1,857), Ca (1454), Mg (960), B (16), Cu (0.33), Zn (7), Mn (7), Mo (0.85), Fe-EDTA (16). Three liters of nutrient solution was applied to each pot, three to four times per week via drip

Table 1 Anatomical root traits, listing their abbreviation and explanation of traits

Abbreviation	Trait explanation
RXSA	Root cross-sectional area = TSA + TCA
TCA	Total cortical area = RXSA−TSA
TSA	Total stele area = RXSA−TCA
AA	Area of aerenchyma lacunae = TCA−LCA
%Aer	Percentage of cortex as aerenchyma = (AA/TCA) × 100
LCA	Living cortical area = TCA−AA
%LCA	Percentage of cross section occupied by cortical cells = (LCA/RXSA) × 100
XVA	Xylem vessel area (total of all metaxylem vessels)
CCC	Cortical cell count = number of cells in the cortex
CCFN	Cortical cell file number counted radially

irrigation. Mean ambient temperature was 26.5 °C ± 5.9 (day)/21.3 °C ± 2.4 (night) and mean relative humidity was 57 % ± 12.2. Plants were harvested 28 days after planting (V6–V7 stage), and root systems were washed with water to remove growth media.

Field

A subset of 31 IBM genotypes was grown in a Murrill silt loam soil (fine-loamy, mixed, semi-active, medic Typic Hapludult) at the Russell Larsen Agricultural Research Station of The Pennsylvania State University (40°44′N, 77°53′W) May–August 2008. These plants were grown for comparison with the 4-week-old plants grown in the greenhouse (described above). Soil pH was 6.8, and soil nutrients were optimized for maize cultivation based on soil tests prior to planting. Irrigation was delivered bi-weekly via water gun (6.3 cm/hectare). Genotypes were randomly assigned to plots in a completely randomized design. Each plot contained ten biological replicates of a single genotype, planted in a 2.1 m row (planting density: 5.74 plants/m^2). Plots were not replicated in this design. Plants were grown to the tasseling stage (8 weeks, V12 stage), at which point three representative plants were chosen for harvest. Root crowns were excavated from the soil using the "shovelomics" method (Trachsel et al. 2011) and washed with soap and water.

Sectioning and image analysis

For greenhouse- and field-grown plants, a 4-cm tissue segment was collected 5 cm from the base of a second whorl crown root for anatomical analysis. Previous research had shown that this segment provided a good representation of the overall anatomical features of the crown root system for maize plants at this stage of development (Burton et al. 2012a). Root segments were preserved in 75 % ethanol. Preserved, unembedded tissue was cross-sectioned using Teflon-coated double-edged stainless steel blades (Electron Microscopy Sciences, Hatfield, PA, USA) 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 4× magnification with an additional 0.7× adapter, for a combined magnification of 2.8×. This allowed larger sections to be viewed in their entirety. The three best sections on a slide were selected as subsamples for image capture. Selection of particular cross sections was based on tissue integrity and the relative perpendicularity of sectioning (i.e., 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 International, Birmingham, NJ, USA) was used to capture and save images.

Analysis of images was performed in MatLab 7.6 2008a (The MathWorks Company, Natick, MA, USA), using the program *RootScan* which was created for this purpose (Burton et al. 2012b). The following measurements were made via pixel-counting: areas of the cross section (RXSA), aerenchyma lacunae (AA), stele (TSA), and xylem vessels (XVA). Some of these primary measurements were used to calculate a set of secondary measurements: total area of the cortex [TCA, (cross-sectional area)-(stele area)], percent aerenchyma [%Aer, (total aerenchyma area)/(cortex area)], living cortical area [LCA, (total cortex area)-(aerenchyma area)], and percent living cortical area [%LCA, (living cortical area)/(total cross-sectional area)]. Count data included number of cortical cells (CCC) and cortical cell files (CCFN). Area measurements were in mm^2 and calibrated from pixels using an image of a 1-mm micrometer taken at the same magnification as the analyzed images (1 linear mm = 204 pixels). Trait abbreviations and explanations are summarized in Table 1.

Statistical analysis

Statistical analysis was performed in the R Program, version 2.9.2 (R Development Core Team 2010). Data points

that were ±3 standard deviations from the mean for a given trait were examined as potential outliers by evaluating related root images and other trait values for a given individual. Data points were removed only when there was clear evidence for error in data entry or image analysis. Correlation and principal component analyses (PCA) were performed separately for each family and for all families together. For the subset of 31 IBM genotypes, least squares regressions were performed to compare data from greenhouse- and field-grown plants for each trait. Allometric analysis was performed by plotting a linear regression of the natural logarithm of each trait against the natural logarithm of total plant dry weight, and recording the coefficient of determination (R^2) and the slope of the regression line ("allometric scaling exponent", α) (Niklas 1994). Prior to the QTL analysis, variation within each trait was assessed using two-way analysis of variance, with repetition and genotype as factors. Square root transformations were performed on the number of cortical cells in OhW and number of cortical cell files in NyH prior to analysis of variance of those traits. For each trait in a given family, repeatability estimates were calculated as $\sigma^2_G/(\sigma^2_G + (\sigma^2_E/n))$ where σ^2_G = genotype variance, σ^2_E = error variance, and n = number of repetitions. Variance components were obtained using linear mixed model analyses in which genotype and repetition were treated as random effects.

QTL analysis

Quality analysis on the marker information and map construction was performed in R and revealed no apparent problems regarding similarity of individuals, marker redundancy, marker order, and segregation distortion (e.g., plots of pairwise recombination fraction, genotype frequency, number of matching genotypes) (Burton et al. 2014). The QTL analysis was performed separately for each family. Identification of quantitative trait loci (QTL) was carried out using composite interval mapping (Zeng 1994; Wang et al. 2006) with five marker covariates, a window size of 10 cM in R/qtl (Broman et al. 2003). The LOD threshold was established separately for each trait using 1,000 permutations at a significance threshold of 0.05. The position and effect of significant QTL were refined using the Haley–Knott regression method and then assessed for additive effects and percent variation explained by fitting a model containing all QTL identified for a given trait in R/qtl. The physical position of markers underlying a QTL was used in conjunction with the known chromosome lengths of maize to create a single map of QTL identified using the plot package in R (Fig. 1). Marker number, map length, and average and maximum spacing of the genetic maps of the three populations can be found in the supplemental materials of our previous paper describing these maps (Burton et al. 2014).

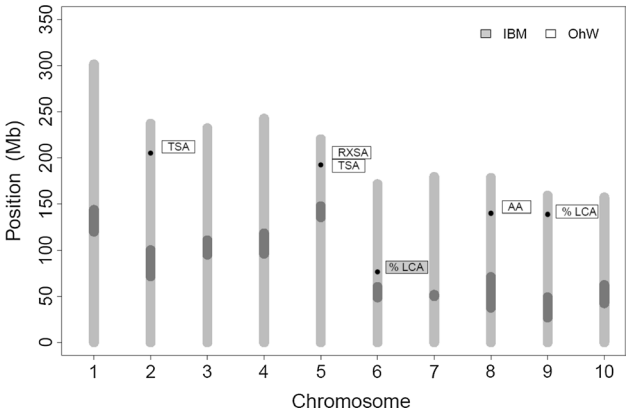

Fig. 1 Chromosome map, showing significant QTL for two inbred populations of maize. The trait in the *gray box* refers to QTL for Intermated B73 × Mo17 (IBM), and traits in the *white boxes* are for OH43 × W64a (OhW). Trait abbreviations can be found in Table 1

Results

Phenotypic variation

Anatomical traits in the three populations showed significant phenotypic variation (Table 2). Mean values were similar across all three populations for area of the cross section, area of the cortex, and area of the stele. The IBM population had a slightly narrower range of phenotypic variation for cross section and cortical areas compared to the other two populations. For the aerenchyma traits (AA, %Aer), the IBM population had lower means but greater ranges of phenotypic variation than the other two populations. Mean values and ranges for xylem vessel area were similar for all three populations. Plants in the IBM population had a lower mean number of cortical cells and cell files, while NyH had the greatest mean for both traits. Repeatability values exceeded 50 % for all traits in the three families, except for number of cell files in IBM (Table 2). Repeatabilities were generally lower for the count variables (CCFN, CCC). Repeatabilities exceeded 70 % in the OhW population for a majority of the traits.

The pattern of phenotypic variation differed by trait and by family (Figs. 2, 3, 4). For most traits in the three families, the distribution of phenotypes was close to normal, or slightly skewed to smaller trait values. The distributions of values for the aerenchyma traits were skewed to smaller values in all three populations, but this pattern was most pronounced in the IBM population. For living cortical area, about 65 % of the genotypes in the NyH population had values in the interval of 0.3–0.5 mm^2, whereas values in the other two families were more dispersed across the total range. Distributions for number of cortical cells and cell files were close to normal in all three populations, though

Table 2 Phenotypic and repeatability values for IBM, OhW, and NyH recombinant inbred populations of *Zea mays*

	RXSA	TCA	TSA	AA	%Aer	LCA	%LCA	XVA	CCC	CCFN
B73	1.16	0.85	0.31	0.01	0.83	0.84	72.4	0.04	688.3	10.0
Mo17	1.44	1.10	0.34	0.08	7.78	1.02	70.8	0.03	671.7	10.3
Population mean	1.32	0.97	0.35	0.03	3.61	0.94	71.2	0.04	591.2	9.8
Min value	0.42	0.32	0.07	0.00	0.00	0.27	61.8	0.01	105.0	4.0
Max value	3.40	2.45	0.99	0.62	39.51	2.35	80.7	0.10	1,699.0	17.0
Repeatability (%)	69.2	68.2	70.6	60.4	59.3	68.2	63.9	64.2	53.9	47.3
OH43	0.59	0.44	0.15	0.03	7.65	0.41	68.0	0.02	312.0	7.0
W64a	1.27	0.87	0.40	0.00	0.51	0.87	68.0	0.03	681.7	10.0
Population mean	1.41	1.01	0.41	0.07	7.23	0.93	66.0	0.04	760.0	10.6
Min value	0.42	0.30	0.07	0.00	0.00	0.27	45.0	0.01	158.0	5.0
Max value	3.84	2.77	1.17	0.43	37.40	2.75	86.0	0.09	1,704.0	16.0
Repeatability (%)	76.8	78.1	75.0	73.8	75.4	77.3	78.0	66.8	63.2	62.3
NY821	1.35	0.92	0.42	0.00	0.00	0.92	68.0	0.05	791.5	12.0
H99	1.56	1.03	0.52	0.01	0.65	1.03	66.0	0.06	838.7	11.7
Population mean	1.37	1.02	0.35	0.06	6.30	0.96	70.0	0.04	793.7	11.7
Min value	0.42	0.34	0.08	0.00	0.00	0.24	46.0	0.01	239.0	6.0
Max value	3.82	2.90	0.95	0.43	32.80	2.84	83.0	0.10	2,510.0	22.0
Repeatability (%)	60.9	63.5	55.8	64.4	63.4	62.5	69.3	50.8	51.1	52.8

Phenotypic values for parent lines represent mean values across biological replicates. Population mean, minimum, and maximum values were based on raw data from all RILs and both parents for a given family following removal of outliers. With the exception of percentages and counts, all measurements are in mm^2 . Explanations of abbreviations are listed in Table 1. All traits varied significantly within each population at $p < 0.05$ except CCC in OhW, where $p < 0.10$

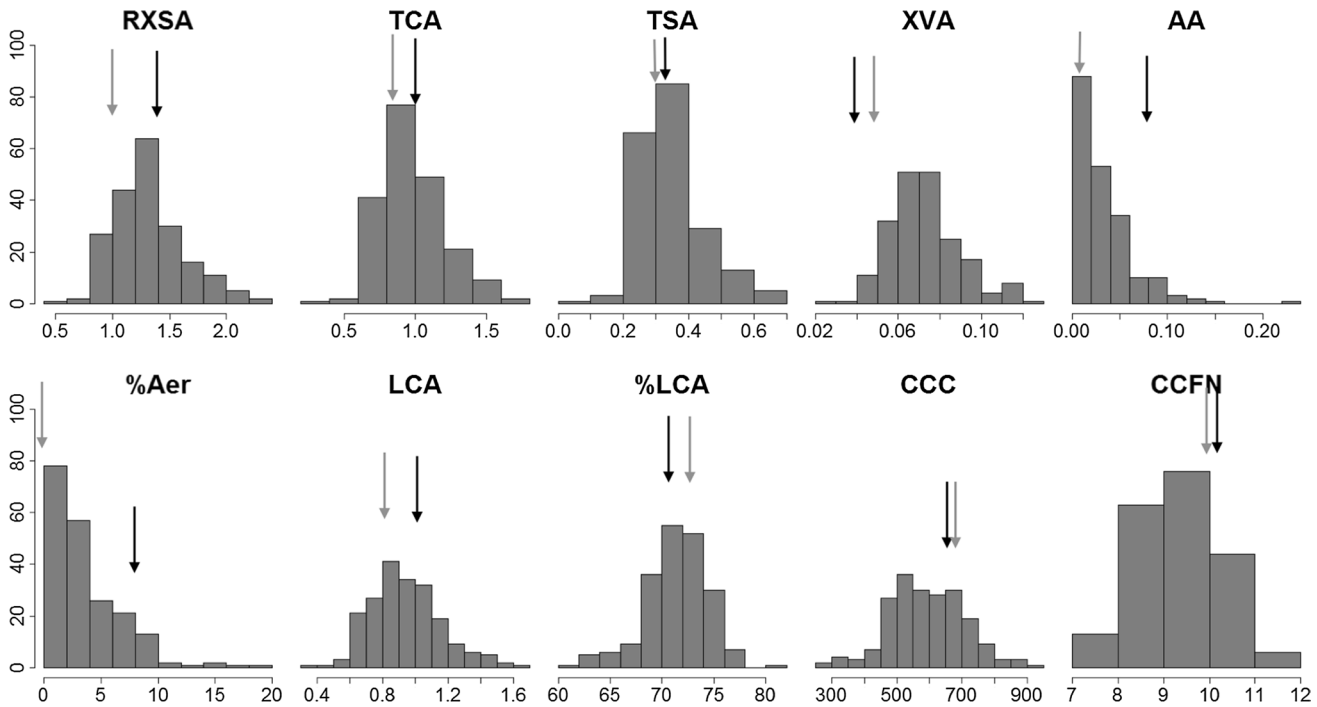

Fig. 2 Phenotypic variation in ten anatomical root traits in the recombinant inbred population of *Zea mays*, Intermated B73 × Mo17. All measurements are in mm^2, except for count and percent variables. *Arrows* indicate phenotypic values of the parent lines. *Gray arrows* are for B73 and *black arrows* are for Mo17

values in the NyH population were more strongly skewed to the left than those in the other two populations.

Within the three families, similar patterns of trait correlations were observed (Table 3). Strong and significant correlations ($r > 0.95$) were observed among tissue area traits in the cortex, stele, and cross-section, and between each of these traits and living cortical area. Also, strong correlations were observed in each of the three families between the number of cortical cells and the number of cortical cell files. This general pattern was observed in all three families,

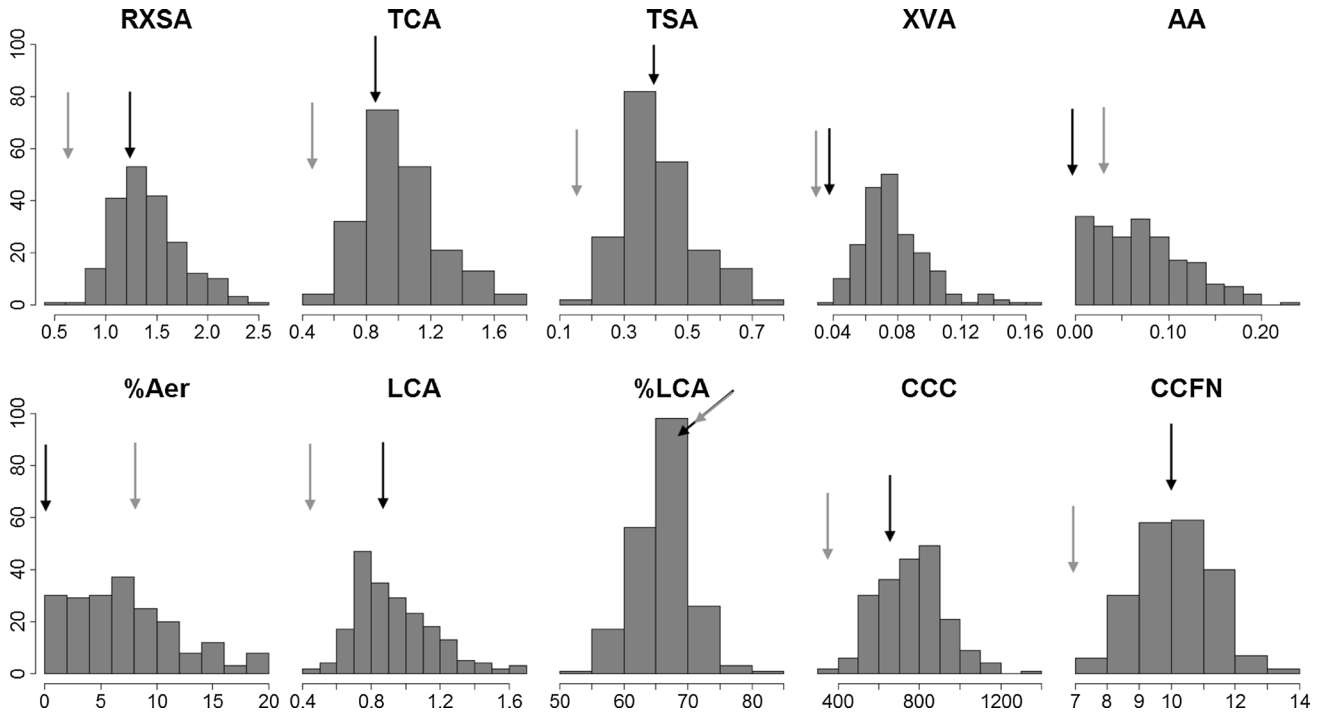

Fig. 3 Phenotypic variation in ten anatomical root traits in the recombinant inbred population of *Zea mays*, OH43 × W64a (OhW). All measurements are in mm^2, except for count and percent variables. *Arrows* indicate phenotypic values of the parent lines. *Gray arrows* are for OH43 and *black arrows* are for W64a

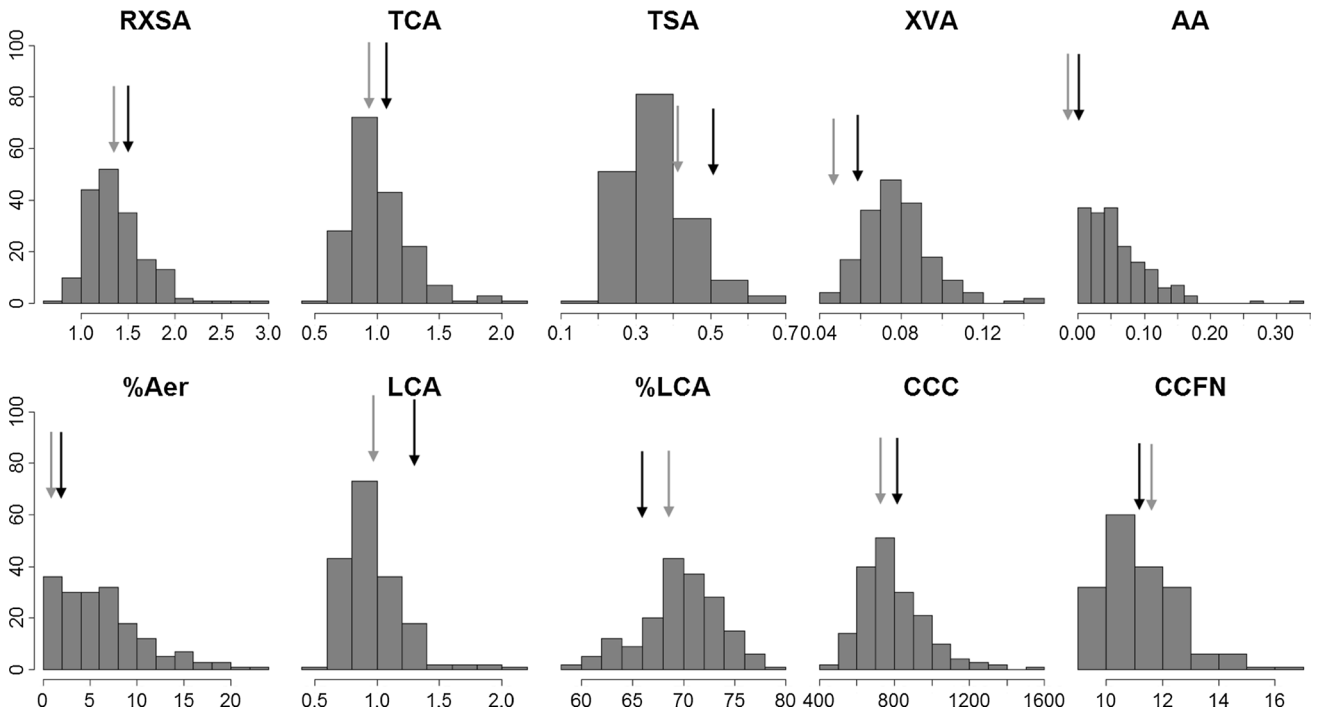

Fig. 4 Phenotypic variation in ten anatomical root traits in the recombinant inbred population of *Zea mays*, NY821 × H99 (NyH). All measurements are in mm^2, except for count and percent variables. *Arrows* indicate phenotypic values of the parent lines. *Gray arrows* are for NY821 and *black arrows* are for H99

Table 3 Pearson correlation coefficients among root anatomical traits in three recombinant inbred populations of *Zea mays*

IBM									
TCA	*0.991**								
TSA	*0.957**	*0.91**							
AA	0.069	0.093	0.009						
%Aer	−0.149*	−0.134	−0.171*	*0.92**					
LCA	*0.985**	*0.99**	*0.912**	−0.046	−0.263*				
%LCA	−0.174*	−0.102	−0.323*	−0.657*	−0.698*	−0.011			
XVA	0.801*	0.763*	0.833*	0.114	−0.083	0.75*	−0.338*		
CCC	0.848*	0.847*	0.796*	−0.118	−0.315*	0.866*	0.026	0.691*	
CCFN	0.767*	0.798*	0.65*	−0.084	−0.287*	0.813*	0.173*	0.573*	0.89*
	RXSA	TCA	TSA	AA	%Aer	LCA	%LCA	XVA	CCC
OHW									
TCA	*0.975**								
TSA	0.871*	0.74*							
AA	0.211*	0.252*	0.081						
%Aer	−0.128	−0.096	−0.175*	*0.91**					
LCA	*0.962**	*0.979**	0.747*	0.051	−0.289*				
%LCA	0.081	0.214*	−0.228*	−0.59*	−0.692*	0.344*			
XVA	0.421*	0.321*	0.567*	0.136	0.012	0.303*	−0.316*		
CCC	0.767*	0.728*	0.714*	−0.183*	−0.45*	0.789*	0.285*	0.389*	
CCFN	0.762*	0.778*	0.588*	−0.097	−0.395*	0.823*	0.419*	0.299*	*0.923**
	RXSA	TCA	TSA	AA	%Aer	CCA	%CCA	XVA	CCC
NYH									
TCA	*0.99**								
TSA	*0.915**	0.851*							
AA	0.157*	0.188*	0.05						
%Aer	−0.095	−0.07	−0.16	*0.92**					
LCA	*0.977**	*0.981**	0.856*	−0.008	−0.254*				
%LCA	0.137	0.196*	−0.048	−0.706*	−0.775*	0.34*			
XVA	0.761*	0.708*	0.829*	0.148*	−0.058	0.691*	−0.119		
CCC	0.864*	0.862*	0.773*	−0.093	−0.315*	0.896*	0.354*	0.611*	
CCFN	0.857*	0.877*	0.7*	−0.064	−0.293*	*0.906**	0.44*	0.57*	*0.954**
	RXSA	TCA	TSA	AA	%Aer	CCA	%CCA	XVA	CCC

Correlation coefficients greater than 0.90 are highlighted in italics, and statistically significant relationships are indicated by an asterisk ($p < 0.05$). Explanations of abbreviations are listed in Table 1

although some r values in the OhW and NyH populations were slightly lower than those in IBM. In general, strong correlations ($r > 0.70$) were observed between tissue area traits (RXSA, TSA, TCA, LCA) and xylem vessel area, cortical cell count, and number of cortical files. Notable exceptions to this included low correlations between xylem vessel area and most other traits in the OhW population. In the IBM and NyH populations, moderate correlations ($0.57 < r < 0.70$) were observed between xylem vessel area and the numbers of cortical cells and cell files. The traits of %LCA, AA, and %Aer did not correlate highly with any other traits. Among families, correlation coefficients for corresponding traits were low (ranging from −0.192 to 0.180) (data not shown).

Based on a subset of genotypes, weak but significant linear relationships were observed between greenhouse and field data for six of the ten traits (Fig. 5). Field versus greenhouse comparisons were not significant for percent living cortical area and the numbers of cortical cells, and were significant only at $p < 0.07$ for xylem vessel area and cortical cell file number. Among these regressions, the greatest R^2 value observed was for percent aerenchyma (0.224).

Based on low scaling exponents (α) compared to isometric values, most trait values in IBM and OhW were less than would be expected if trait values were proportionate to biomass (Table 4). Notable exceptions to this were observed for the aerenchyma traits (%Aer, AA), especially

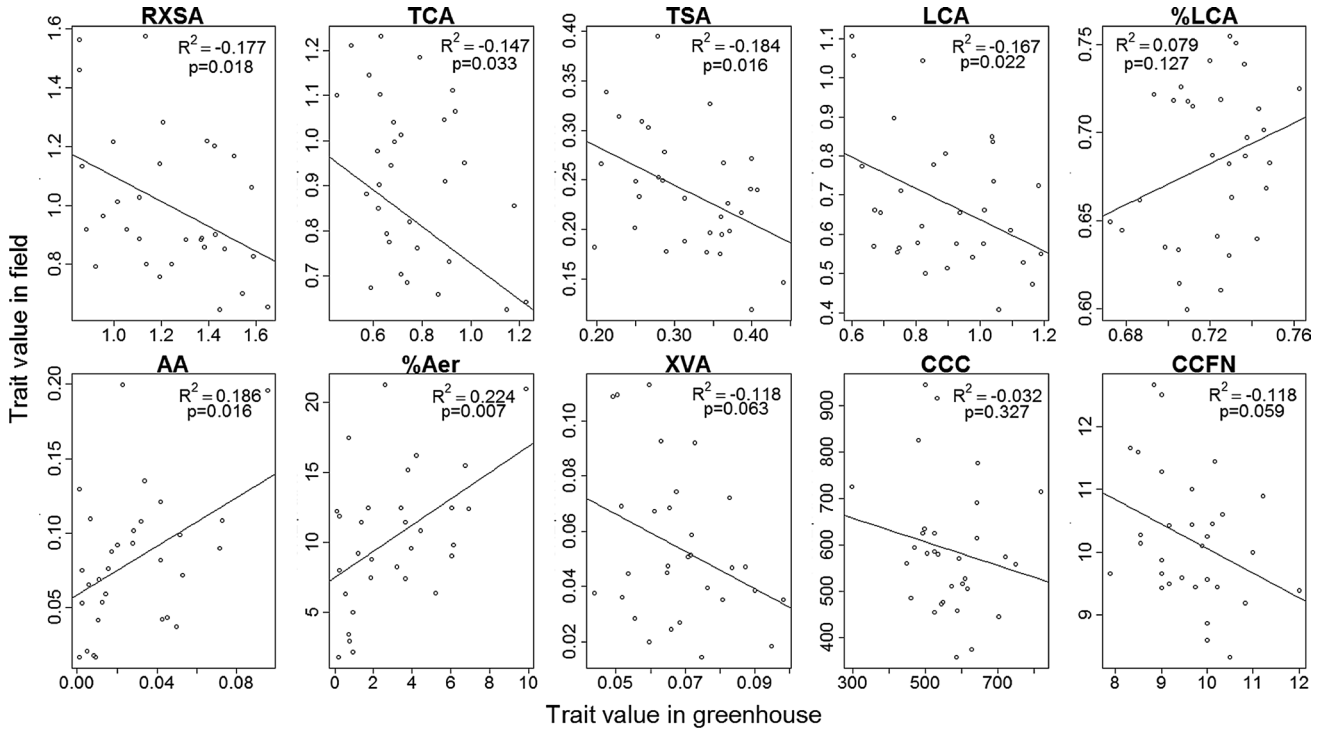

Fig. 5 Scatter plots showing relationship between measurements of ten anatomical root traits in greenhouse-grown (4 WAP, V6–7) vs. field-grown (8 WAP, V12) maize plants. Coefficients of determination (R^2) and p values are based on least squares regression of data from a subset of recombinant inbred lines in the Inbred B73 × Mo17 (IBM) population

Table 4 Summary of allometric analysis of root anatomical traits for individual populations of recombinant inbred lines of maize (IBM, OhW, NyH), showing R^2 value and slope of the regression line ("allometric scaling exponent", α) for regression of the natural logarithm of each trait (y-axis) against the natural logarithm of total plant dry weight

Trait	IBM		OhW		NyH		Isometric value of α
	R^2	α	R^2	α	R^2	α	
RXSA	0.132	0.251	0.003 ^{NS}	0.042	0.072	0.145	0.67
TCA	0.133	0.240	0.001^{NS}	−0.025	0.073	0.149	0.67
TSA	0.115	0.280	0.071	0.227	0.053	0.133	0.67
AA	0.034	0.552	0.011^{NS}	0.347	0.169	0.938	0.67
%Aer[a]	0.013^{NS}	0.342	0.015^{NS}	0.400	0.122	0.773	0.33
LCA	0.115	0.230	0.002	−0.038	0.044	0.120	0.67
%LCA[a]	0.032	−0.021	0.151	−0.080	0.033	−0.025	0.33
XVA	0.109	0.225	0.120	0.286	0.045	0.108	0.67
CCC[a]	0.125	0.212	0.035	0.135	0.054	0.117	0.33
CCFN[a]	0.055	0.069	0.006^{NS}	0.030	0.072	0.075	0.33

Explanations of abbreviations are listed in Table 1

[a] These traits are linear; other traits are area measurements

in the NyH population. With these traits, scaling exponents for NyH far exceeded the linear isometric values, i.e., in NyH roots, the amount of aerenchyma was much greater than that expected based on plant biomass. In OhW and IBM, the values were approximately isometric, i.e., as expected in relation to plant biomass. NyH had higher than isometric values for most anatomic variables, with the exception of %LCA and CCFN.

Principal component analysis showed a comparable trait structure within each of the three families, despite

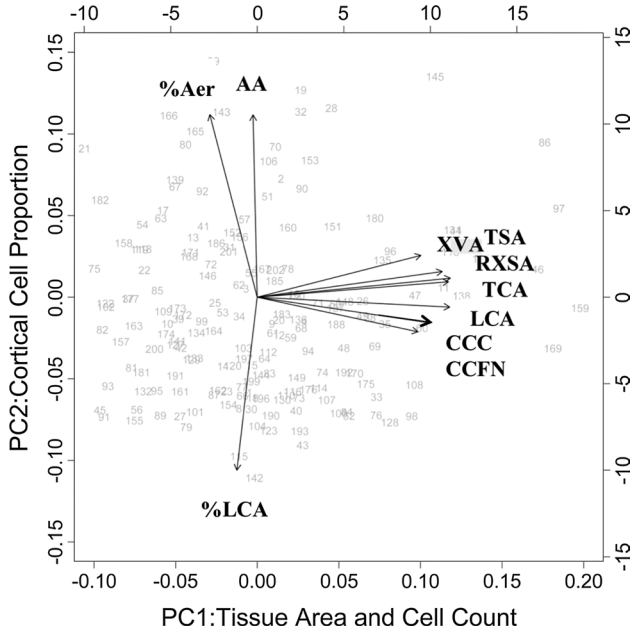

Fig. 6 Principal component analysis biplot of ten anatomical root traits in the recombinant inbred population of *Zea mays*, Intermated B73 × Mo17. The *x* and *y* axes are components 1 and 2, respectively. Explanations of abbreviations are listed in Table 1

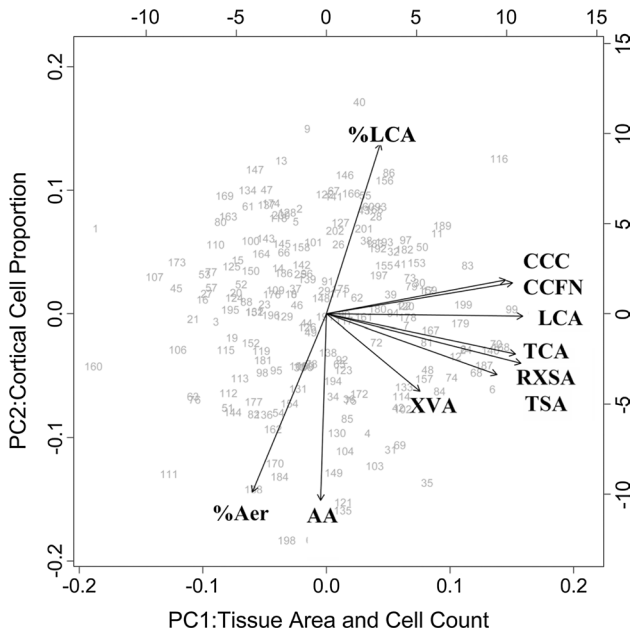

Fig. 7 Principal component analysis biplot of ten anatomical root traits in the recombinant inbred population of *Zea mays*, OH43 × W64a (OhW). The *x* and *y* axes are components 1 and 2, respectively. Explanations of abbreviations are listed in Table 1

phenotypic differences and distinct genetic backgrounds (Figs. 6, 7, 8). When PCA was performed on pooled data for all three families, the same general trait structure was

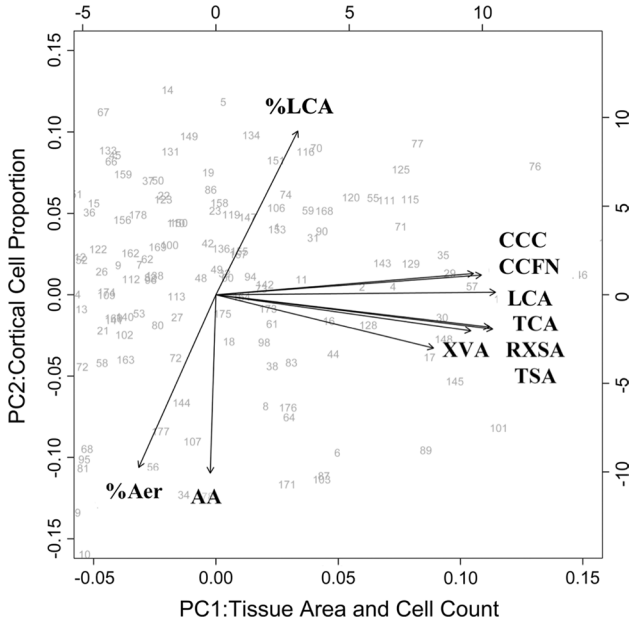

Fig. 8 Principal component analysis biplot of ten anatomical root traits in the recombinant inbred population of *Zea mays*, NY821 × H99 (NyH). The *x* and *y* axes are components 1 and 2, respectively. Explanations of abbreviations are listed in Table 1

observed (data not shown). The clustering of trait vectors indicates correlation in PCA biplots, while the length and orientation of the vectors show an association with a particular component. Since most area and count variables fell on the PC1 axis, we named this axis "tissue area and cell count", and PC2 variables were called "cortical cell proportion" because variation there was mostly restricted to alternate fates of the cortical cells, aerenchyma formation, or living cortical area. The aerenchyma traits and percent living cortical area had strong loading values on PC2, but these eigenvalues had opposite signs (±0.50–0.60), reflecting the fact that aerenchyma and living cortical area are mutually exclusive fates. Vectors for the aerenchyma traits (%Aer and AA) and percent living cortical area were separated from other traits in the PCA plot. The remaining traits clustered together and were related to the relative size or number of tissue features. These traits had similar loading values for the first component, most of which were 0.38–0.41 within and across the three families.

Detection of QTL

Quantitative trait loci were detected for four of the ten traits: %LCA, AA, RXSA, and TSA (Table 5). QTL were identified on chromosomes 2, 5, 6, 8, and 9 explaining between 4.74 and 12.07 percent of the phenotypic variation (Fig. 1; Table 5). Among the QTL, one was identified in the IBM population and five in OhW. The majority of identified

Table 5 Summary of QTL for root anatomical traits in the inbred maize populations Intermated B73 × Mo17 (IBM) and OH43 × W64a (OhW), listing the population, trait abbreviation, significant marker, chromosome, position of the QTL (cM and bp), markers, positions, and width of the 1.5 LOD confidence interval, logarithm of the odds value (LOD), additive contribution to the phenotype, and the % variation explained by the QTL

Population	Trait	Significant Marker	Chr	position (Mb)	1.5 LOD Interval				LOD	*p* value	Estimate (a)	% Variation explained
					Marker	Position (Mb)	Marker	Position (Mb)	Width (Mb)				
IBM	%LCA	bin_5182	6	76.8	bin_5166	59.8	bin_5192	80.4	20.6	6.024	0.07	1.1	12.07
OhW	%LCA	bin_5139	9	138.8	bin_5130	137.4	bin_5147	139.6	2.2	7.559	0.002	1.4	9.91
OhW	AA	bin_4646	8	140	bin_4636	137.9	bin_4661	144.1	6.25	5.338	0.088	0.02	9.76
OhW	RXSA	bin_3184	5	192.6	bin_3178	189.5	bin_3187	194.1	4.55	5.435	0.058	0.07	4.74
OhW	TSA	bin_1382	2	205.1	bin_1374	203.8	bin_1391	206.8	3.05	5.845	0.039	0.04	10.81
OhW	TSA	bin_3184	5	192.6	bin_3178	189.5	bin_3187	194.1	4.55	5.138	0.093	0.02	5.06

Explanations of abbreviations are listed in Table 1. Markers refer to maps presented in (Burton et al. 2014)

QTL had r^2 values below 0.10, though two QTL of greater effect were identified (Table 5). The QTL with the largest effect was for percent living cortical area ($r^2 = 0.12$) and was identified on chromosome 6 in the IBM population. The QTL for RXSA and TSA in the OhW population are co-localized on chromosome 5.

Discussion

Root anatomical traits have not been widely used by plant breeders, in spite of their potential for improving stress tolerance in crop plants. An important first step in utilizing such traits is to evaluate their phenotypic variation and identify related QTL. We have previously reported phenotypic variation of anatomical traits in maize landraces and teosintes (Burton et al. 2013). In this study, significant phenotypic variation was observed for ten anatomical traits in each of three unrelated recombinant inbred populations of maize (Table 2). QTL are reported for four of the ten traits, including living cortical area, aerenchyma area, root cross-sectional area, and stele area.

In grasses such as maize, cortical traits are of interest because the cortex typically occupies a large proportion of the root volume and is subject to either aerenchyma development (e.g., in maize and rice) or cortical senescence (e.g., in barley and wheat) at later stages of growth (Wenzel and McCully 1991; Mano et al. 2006). The size of the root cortex, in particular the cortical cells remaining after aerenchyma formation (LCA), is directly related to respiratory cost and performance under drought stress (Jaramillo et al. 2013). Root systems that minimize the amount of metabolically active tissue are more efficient at soil exploration, since they use more available resources for new growth rather than maintenance of existing tissue (Lynch and Ho 2005). We identified two QTL for %LCA in two different families (Table 5), the first published loci for this trait. This trait had similarly broad phenotypic variation within each of the three populations, with maximum values approximately ten times greater than minimum values. Since LCA has strong phenotypic variation, good repeatability, and good evidence for functional significance, this trait will be a useful breeding target.

One QTL for AA was identified on chromosome 8 in the OhW population. QTL have been previously identified for aerenchyma in F_2 populations of maize × *Zea* spp. grown under flooded and non-flooded conditions (Mano et al. 2007, 2008; Mano and Omori 2008). The QTL for AA reported here did not co-localize with these previously identified loci, which could be due to alleles from the wild species *Zea nicaraguensis* and *Zea luxurians* present in those studies that may be absent in *Zea mays*. Although aerenchyma formation is often associated with flooding,

it is known to form in response to other edaphic stresses including deficiency of nitrogen (Konings and Verschuren 1980), sulfur (Bouranis et al. 2003), phosphorus (Fan et al. 2003), and water (Zhu et al. 2010). High aerenchyma is associated with lower root respiration per unit length, greater root depth, better water capture, and higher yield in maize under drought (Chimungu et al. 2014b; Zhu et al. 2010). Similarly, high aerenchyma is associated with reduced respiration, reduced root N content, greater rooting depth, greater leaf N content, and higher yield under low N field conditions in maize (Saengwilai et al. 2014). %Aer and %LCA are usually negatively correlated (Table 3), but are separately regulated, as indicated by the distinct QTL (Table 5) and very low correlations between AA and LCA (i.e., when expressed as area rather than percent, Table 3).

Aerenchyma traits had the highest allometric scaling exponents of any of the anatomical traits, indicating a stronger relationship with overall plant size (Table 4). These results are consistent with our previous allometric analyses of anatomical traits in maize landraces and teosinte (Burton et al. 2013). Of the three populations, NyH had the highest allometric scaling exponents for aerenchyma traits. In the previous study, both teosintes and landraces had higher allometric scaling exponents than what we report here for recombinant inbred populations, and those for teosintes were higher than for landraces (Burton et al. 2013). These results indicate that the genetic factors underlying allometric relationships vary among populations.

Within each family, low correlations were observed between %LCA, AA, and %Aer and all other traits, meaning that the expression of these traits is independent of the other anatomical traits. A similar pattern of trait correlations was observed in each of the three populations, with strong correlations among areas of the cortex, stele, cross section, and living cortical area, and between the number of cortical cells and cell files (Table 3). These relationships were also reflected in the clustering of vectors in the PCA plots (Figs. 6, 7, 8). To a certain degree, strong correlations would be expected among these traits since they are either mathematically or spatially related. For instance, a larger cortex is likely to have a greater number of cortical cells and cell files.

Some strong correlations were observed between traits that are not mathematically related. For example, areas of the stele and xylem vessels are not mathematically related, but are functionally and spatially related. These two traits were strongly correlated in the IBM and NyH populations, but less so in the OhW population (Table 3). In a previous study of anatomical traits in maize landraces and teosinte, the PCA plots showed that xylem vessel area was independent of other traits in the teosinte group, but not in the landrace group (Burton et al. 2013). In a diverse set of rice accessions, there was a high correlation between metaxylem vessel area and total stele area (Uga et al. 2009), probably related to differences in root and stele diameters between *Indica* and *Japonica* varietal groups (Kondo et al. 2000). The relationships among traits differ among and within populations, providing opportunities to disconnect trait associations in breeding programs.

Cortical cells and cortical cell files showed wide distribution ranges in all three populations. Value ranges for IBM and OhW were similar to the ranges previously found for maize landraces and teosintes (Burton et al. 2013), while ranges for NyH were larger. These traits had low allometric scaling exponents, probably because the cortical cell organization is determined in the meristem. Since we always measured anatomical traits in the second whorl crown roots, our measurements did not reflect the increased cortical cell numbers that would be found in the larger-diameter roots of succeeding crown root whorls. CCFN has been recently shown to affect the metabolic cost of roots, and maize genotypes with lower CCFN had reduced specific root respiration, greater root depth, and better yield under water stress than genotypes with higher CCFN (Chimungu et al. 2014a).

Two QTL for TSA were identified, both in OhW and one at the same position as a QTL for RXSA on chromosome 5 (Table 5). These traits were highly correlated in all three populations (Table 3). Two QTL for TSA (called STA for stele transversal area) have been identified in rice (Uga et al. 2008); one of these has been fine-mapped (Uga et al. 2010). The region containing our coinciding QTL for TSA and RXSA on chromosome 5 in OhW is syntenic to the QTL for stele area and root thickness (RTH), qSTA-2 and qRTH-2, on chromosome 2 in rice (Uga et al. 2008). The coincidence of these QTL in two different species suggests that there may be common genetic factors regulating both traits in the grasses.

It was interesting that five of the six QTL we discovered were found in OhW. Except for TSA, where OhW had about 17-fold variation compared to 14-fold for IBM and 12-fold for Nyh, OhW had ranges of trait variation similar to the other two populations, but repeatability was somewhat higher (Table 2). We previously found that OhW also had high variation for root architectural traits (Burton et al. 2014). In field experiments, OhW lines had steeper brace and crown roots and more brace roots than lines from IBM and NyH (Trachsel et al. 2011). Both parents of OhW are noted for root traits, Oh43 for resistance to lodging and drought, and W64a for good root strength (Stringfield 1959; Troyer 2004).

In our studies of the value of specific anatomical phenes for maize productivity under abiotic stress conditions, we have typically found a small or no significant impact of the anatomical phene on yield under non-stressed conditions, but significant improvements in growth and yield under stress. For example, low LCA and high AA (called RCA

for root cortical aerenchyma in the cited studies) improved maize growth and yield under drought stress (Zhu et al. 2010; Jaramillo et al. 2013; Chimungu et al. 2014b) and low nitrogen stress (Saengwilai et al. 2014), but usually had no significant effect under non-stressed conditions. Fewer and larger cortical cells improved yield under drought stress, but not under well-watered conditions in the field (Chimungu et al. 2014c). Therefore, we would not necessarily expect to find our QTL for these anatomical traits coinciding with strong QTL for yield under non-stress conditions. In comparing our results with a meta-analysis of QTL for grain yield under stress, we found that the QTL on chromosome 5 for RXSA and TSA coincides with the consensus QTL DCQ19/WCQ19 associated with grain yield under both drought and non-stressed conditions, and our QTL for %LCA on chromosome 9 overlaps with consensus QTL called DCQ36 for grain yield under drought stress (Hao et al. 2009). The same %LCA QTL on chromosome 9 also overlaps with a consensus QTL in another study called mQTL9.5, associated with both grain yield under well-watered conditions and anthesis–silking interval under water stress (Semagn et al. 2013).

Root phenotypes may differ among experimental settings since edaphic factors influence the expression of root traits (Lynch and Brown 2012). In this study, relationships between phenotypes in greenhouse vs. field environments indicated that several of these traits possess considerable plasticity in their expression (Fig. 5). For most of the traits, linear relationships were weak between younger greenhouse- and older field-grown plants, though several were statistically significant. Negative linear relationships indicated a general trend for field-grown plants to have smaller areas of the stele, cortex, cross section, xylem vessels, and fewer numbers of cortical cells and cell files. This pattern may be attributable to physical and chemical differences between field soil and greenhouse media, such as bulk density and moisture holding capacity. In previous greenhouse and field studies, we and others have found that aerenchyma and LCA vary in response to nutrient and water availability (Drew et al. 1979; Konings and Verschuren 1980; Justin and Armstrong 1987; Fan et al. 2003; Zhu et al. 2010; Jaramillo et al. 2013; Saengwilai et al. 2014). Plastic responses of these traits must be taken into account when breeding crops for target stress environments.

Positive slopes in the greenhouse–field correlations for the aerenchyma traits (%Aer, AA) provided further evidence that the amount of aerenchyma increases with plant growth, since AA and %Aer were greater in the older field-grown plants (Fig. 5) in addition to having relatively large allometric scaling exponents (Table 4). The point in development at which plants are harvested influences anatomical phenotypes for traits that change over time, such as aerenchyma. We evaluated the nodal root system rather than embryonic roots, a strategy designed to minimize variation due to ontogeny. Previous work on aerenchyma has shown that the sampling location used in this study (the base of a 2nd whorl crown root) is representative of the mean amount of aerenchyma in the root system in greenhouse-grown plants (Burton et al. 2012a).

Although the use of root anatomical traits has been limited in plant breeding, there are a few notable examples. Breeding for smaller diameter xylem vessels has been employed as a means of improving water use efficiency in wheat (Richards and Passioura 1989). Cultivars with narrow xylem vessels had 3–11 % greater grain yield than controls in dry environments where water conservation was important for grain development. Another anatomical trait, suberin content in the cortical exodermis, has been examined as a potential breeding target for tolerance to *Phytophthora* in red raspberry (Valenzuela-Estrada et al. 2012) and soybean (Ranathunge et al. 2008). These applications demonstrate that root anatomical traits can be utilized to address significant problems in plant breeding. The development of advanced phenotyping platforms, the demonstration of the value of anatomical traits for stress tolerance, and the identification of genetic markers for anatomical traits should make these traits more accessible to breeding programs in the future.

Conclusions

Anatomical traits influence the uptake and transport of water and nutrients, and the mechanical strength and functional efficiency of the root system. The results of this study demonstrate that significant and substantial phenotypic variation exists for a set of root anatomical traits in three recombinant inbred populations of maize. Similar trait relationships were observed among traits in each of the three families. Strong trait correlations were observed among tissue area traits, but the aerenchyma traits and percent living cortical area were found to be independent of the other traits. Quantitative trait loci were identified for four of the ten traits examined. This is the first report of QTL for several novel traits important to agriculture, including total cortical area, living cortical area, and percent living cortical area. Knowledge of phenotypic variation and quantitative trait loci for root anatomical traits could be used in the development of cereal varieties with superior performance under stress.

Author contributions All authors participated in the review and editing of the manuscript. A.B. carried out collection, analysis, and interpretation of data, and drafted the manuscript. J.J participated in construction of the genetic map, and data analysis. J.F. and M.H. participated in data

analysis. S.K. participated in conception of the experiment, resource generation, and data analysis. J.L. and K.B. contributed in conception of the study, experimental design, trait identification, and data interpretation.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The research described in this paper complies with the current laws of the country in which it was performed.

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