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ORIGINAL PAPER

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

Amy L. Burton · James M. Johnson · Jillian M. Foerster · Candice N. Hirsch · C. R. Buell · Meredith T. Hanlon · Shawn M. Kaeppler · Kathleen M. Brown · Jonathan P. Lynch

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Abstract

Key message QTL were identified for root architectural traits in maize.

Abstract Root architectural traits, including the number, length, orientation, and branching of the principal root classes, influence plant function by determining the spatial and temporal domains of soil exploration. To characterize phenotypic patterns and their genetic control, three recombinant inbred populations of maize were grown for 28 days in solid media in a greenhouse and evaluated for 21 root architectural traits, including length, number, diameter, and branching of seminal, primary and nodal roots, dry weight of embryonic and nodal systems, and diameter of the nodal root system. Significant phenotypic variation was observed for all traits. Strong correlations were observed among traits in the same root class, particularly for the length of the main root axis and the length of lateral roots. In a principal component analysis, relationships among traits differed slightly for the three families, though vectors grouped together for traits within a given root class, indicating opportunities for more efficient phenotyping. Allometric analysis showed that trajectories of growth for specific traits differ in the three populations. In total, 15 quantitative trait loci (QTL) were identified. QTL are reported for length in multiple root classes, diameter and number of seminal roots, and dry weight of the embryonic and nodal root systems. Phenotypic variation explained by individual QTL ranged from 0.44 % (number of seminal roots, NyH population) to 13.5 % (shoot dry weight, OhW population). Identification of QTL for root architectural traits may be useful for developing genotypes that are better suited to specific soil environments.

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A. L. Burton · M. T. Hanlon · K. M. Brown · J. P. Lynch (✉)
Department of Plant Science, The Pennsylvania State University, 102 Tyson Building, University Park, PA 16801, USA
e-mail: jpl4@psu.edu

J. M. Johnson · J. M. Foerster · S. M. Kaeppler
Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706, USA

C. N. Hirsch · C. R. Buell
Department of Plant Biology, Michigan State University, 612 Wilson Road, East Lansing, MI 48824, USA

C. N. Hirsch · C. R. Buell
DOE Great Lakes Bioenergy Research Center, Michigan State University, 612 Wilson Road, East Lansing, MI 48824, USA

Introduction

Root architectural traits, including the number, length, orientation, and branching of several root classes, influence the performance and yield of agronomic crops (Jansen et al. 2005; Lynch 2005). Identification of quantitative trait loci (QTL) for root traits can offer new insights into breeding crop varieties that are better suited to tolerating abiotic and biotic stress (de Dorlodot et al. 2007). In breeding programs, QTL are used in marker-assisted selection to identify genotypes with desirable traits (Miklas et al. 2006; Collins et al. 2008). However, traditional breeding has not typically focused on root traits, despite their central role in plant function (Tuberosa et al. 2003). While root traits may be employed in breeding programs using molecular markers, direct phenotypic selection can also be effective

(Gowda et al. 2011). Identification and characterization of physiologically relevant root traits represents a limitation in the development of plant varieties tolerant to a variety of soils (Gregory et al. 2009). Phenotypic screening in breeding programs is used to identify genotypes possessing trait combinations with agronomic value, and to characterize the genetic control of trait expression.

While root architectural traits have potential for breeding improved crop varieties, their use has been limited due to difficulty in directly accessing roots for phenotyping (Zhu et al. 2011). Quantitative trait loci have been identified for root traits in several agronomic species, e.g., QTL controlling root diameter (Clark et al. 2008) length (Kamoshita et al. 2008; Li et al. 2009b; Gamuyao et al. 2012; Uga et al. 2013a) and angle (Uga et al. 2011, 2013b) in rice (*Oryza sativa*), and adventitious root formation (Ochoa et al. 2006), root architecture and morphology (Beebe et al. 2006), and basal root gravitropism under P stress (Liao et al. 2004) in common bean (*Phaseolus vulgaris*). In maize, identification of QTL for root architectural traits has been limited, with most work focused on young plants (V1–V2) (e.g., Tuberosa et al. 2002; Hund et al. 2004; Zhu et al. 2006; Trachsel et al. 2009; Ruta et al. 2010; Cai et al. 2012; Zhu et al. 2005b). Few QTL studies have examined the post-embryonic root system due to the practical aspects of growing and excavating larger plants. While examination of the seedling root system is relatively easy, root traits in young plants may be poor predictors of mature root system architecture or plant performance (Zhu et al. 2011). A few QTL for architectural traits have been identified in mature maize root systems, including those for adventitious root formation (Mano et al. 2005), brace root whorl number (Ku et al. 2012), and several crown root traits including number (Lebreton et al. 1995; Guingo et al. 1998; Liu et al. 2008a; Cai et al. 2012), diameter (Guingo et al. 1998), angle (Guingo et al. 1998), and length (Liu et al. 2008a; Cai et al. 2012). A more detailed analysis of QTL for architectural traits of mature maize plants would enable breeding of crops with improved stress tolerance.

Architectural traits are central to the primary roles of the root system, including anchorage, defense, symbiosis, and resource acquisition (Bailey et al. 2002; Lynch 2005; Zhu et al. 2005a; Mata et al. 2006). Greater root branching can allow for continued root system function following herbivory (Chen et al. 2005). Increased branching and length can provide better plant anchorage, and increase the ability of the plant to resist lodging (Hebert et al. 1996; Mickovski et al. 2007). The formation of symbiotic relationships with mycorrhizal fungi may either alter or be enhanced by root architecture (Liu et al. 2008b; Gutjahr et al. 2009). Finally, the overall shape of the root system determines the volume of soil the plant can exploit for nutrients and water. Deeper roots may assist in the capture of mobile resources such as nitrogen and water, while topsoil foraging can enhance phosphorus acquisition (Lynch and Brown 2012; Lynch 2013).

Root architectural traits merit further attention by plant breeders. In order to make use of root traits for improving crop performance under stress in modern breeding programs, it is essential to understand individual traits and their relationships, and to develop convenient selection tools such as QTL that can be used in marker-assisted selection. In this paper, we employ three recombinant inbred populations of maize to examine relationships among a large collection of root architectural traits and identify QTL for root architectural traits.

Materials and methods

Mapping populations

Two biparental populations were developed by crossing Oh43 × W64a (OhW) and Ny821 × H99 (NyH) with 200 OhW and 176 NyH independently derived $S_{4:6}$ RILs analyzed in this study. The 200 IBM lines were obtained from the Maize Genetics Stock Center and utilized for this study. The parental genotypes (Ny821, H99, Oh43, W64A, Mo17, and B73) represent a sampling of Midwest dent germplasm across heterotic patterns. Populations were chosen for this study based on parental variation for various root traits. The Oh43 and W64a contrast was initially chosen based on response to mycorrhiza under low P and because they differ in root cortical aerenchyma. Ny821 and H99 were chosen because they differ in lateral root branching. Mo17 and B73 were chosen because they differ in root hair length and density and root angle. The populations were subsequently determined to be variable for a large number of traits.

DNA isolation, read mapping, single nucleotide polymorphism detection and bin map construction

For each of the OhW, NyH, and IBM RILs and the respective parents, above ground seedling tissue was harvested from 5–10 plants and pooled. DNA was isolated using a modified CTAB method (Saghai-Maroof et al. 1984), and subsequently barcoded and pooled according to the GBS protocol (Elshire et al. 2011) with an additional size selection for fragments approximately 300 base pairs in length. Parental and RIL DNA samples were pooled with 16–48 barcoded DNA samples per library. Sequencing was done using the Illumina Genome Analyzer II (San Diego, CA) and the Illumina HiSeq 2000 (San Diego, CA) at the University of Wisconsin Biotechnology Center (Madison, WI). Single end reads between 74 and 100 bp were generated. Read quality in the multiplexed libraries was evaluated

based on phred-like quality scores. GBS sequence reads from the RILs and parents are available at the National Center for Biotechnology Information (accession number PRJNA172919).

To produce the genetic map, pooled reads were cleaned using the fastx_clipper program within the FASTX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html). The minimum sequence length was set to 15 bp after clipping using both Illumina single end adapter sequences. Sequence reads were parsed into individual genotype files requiring a perfect match to the barcode and *Ape*KI cut site (GC[A/T]GC) and the barcode sequences were removed. Reads from each genotype were mapped to the maize B73 v2 pseudomolecules (AGPv2, http://ftp.maizesequence.org/; (Schnable et al. 2009) using Bowtie version 0.12.7 (Langmead et al. 2009) requiring a unique alignment and allowing for up to two mismatches. SAMtools version 0.1.7 (Li et al. 2009a) was used to generate unfiltered pileup files. At each locus, at least one of the two parents had to have read coverage, and the reads had to support a single consensus base (greater than 70 % of reads supporting a single base). If there was a single consensus base in both parents the locus needed to be polymorphic between the parents. Within the population, at least five of the RILs were required to have information. Additionally, only two alleles could be present at greater than 10 % frequency across the population and the two alleles were required to be congruent with the parental consensus calls. When information was present in only one of the parents the alternate parent genotype score was inferred from the population. Using this method, 68,246, 40,959 and 19,970 SNP markers were identified for the IBM, OhW and NyH mapping populations, respectively.

The genotypic data were recoded as either parent1 or parent2 and formatted using a custom Perl script for downstream imputation and bin map construction. A sliding-window approach was used to construct genetic maps built from genotyping-by-sequencing (GBS) based single nucleotide polymorphisms (SNPs) to account for sequencing error and missing individual data at a given SNP site (Huang et al. 2009; Zhao et al. 2010). The sliding-window imputation method utilizes the available marker data to determine the origin of each individual's marker genotype relative to its parents, thus resulting in a genetic map with no missing data. Individuals that lacked phenotypic data were not used in the QTL analysis. After imputation and identification of recombination break points, the maps contained 8,224, 5,683 and 5,320 informative bin markers at an average marker density of 0.7, 0.6 and 0.7 cM for the IBM, OhW and NyH populations, respectively (Online resources 1–28). The subsequent maps resulted in 822, 568 and 532 markers per chromosome on average for the IBM, OhW and NyH populations, respectively. To maintain the nomenclature required for R/qtl, the markers were assigned A or B corresponding to parent 1 or parent 2, respectively (Parent 2–IBM: Mo17, NyH: H99, OhW: W64a). Quality analysis on the marker information was performed in R (R Development Core Team, 2013), 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) (Broman et al. 2003).

Among OhW RILs, 47.9 % of all marker genotypes were inherited from Oh43, 48.3 % were inherited from W64a, and 3.8 % were heterozygous. Among the NyH RILs, 44.5 % of all marker genotypes were inherited from H99, 46.9 % were inherited from Ny821, and 8.6 % remained heterozygous. The residual heterozygosity in these populations is slightly higher than expected. Heterozygosity is expected to be <1 % after selfing six generations or more.

Phenotyping conditions

Plants were grown in a greenhouse located on the campus of The Pennsylvania State University in University Park, PA (40°48′N, 77°51′W), from May to August 2008. Three replications were grown per genotype, and replications were planted 7 days apart. Prior to planting, seeds were soaked for 1 h in a mixture of benomyl (Benlate fungicide, E.I. DuPont and Company, Wilmington, DE, USA) and 1.3 M metalaxyl (Allegiance fungicide, Bayer CropScience, Monheim am Rhein, Germany). Following the fungicide treatment, seeds were germinated for 48 h in darkness at 28 °C in rolled germination paper (Anchor Paper Company, St. Paul, MN, USA) moistened with 0.5 mM CaSO4, 8 mM benomyl and 1.3 M metalaxyl. Plants were grown in 10.5 L pots (21 × 40.6 cm, top diameter × height). The growth medium was composed of 45 % peat, 45 % vermiculite, 10 % silica sand, and was 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 (1,454), Mg (960), B (16), Cu (0.33), Zn (7), Mn (7), Mo (0.85), Fe-EDTA (15). Three liters of nutrient solution were applied to each pot, 3–4 times per week via drip irrigation using a DI-16 Dosatron fertilizer injector (Dosatron International Inc, Dallas, TX, USA). Sulfuric acid was injected into the water line at an application rate of 1.44 mM, to maintain a pH of 6.0. Environmental data were collected hourly in the greenhouse using a HOBO U10-003 datalogger (Onset Corporation, Pocasset, MA, USA). Mean ambient temperature was 26.5 ± 5.9 °C (day)/21.3 ± 2.4 °C (night), and mean relative humidity level was 57 ± 12.2 %. Maximum photosynthetic flux density was 1,200 μmol photons m^{-2} s^{-1}, measured at mid-day.

Data collection

Plants were harvested 28 days after planting (V6–V7 stage). 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 root system: root system diameter, numbers of seminal and nodal roots, dry weights of the embryonic and nodal root systems, and the sum of these two root dry weight fractions ("root dry weight"). The embryonic root system included the seminal and primary roots. Root classes considered part of the nodal root system included crown roots (belowground nodal roots) and brace roots (nodal roots initiating aboveground). The count of nodal roots included all crown roots and any brace roots that extended below the surface of the soil. Root system diameter was measured 20 mm below the most basal whorl of brace roots. The roots had not reached the side of the pot at this point. For root systems with asymmetrical root system architecture, the widest diameter was measured. Three roots were removed from each root system for measurement of length and average diameter of individual roots: the primary root, and one representative seminal and second whorl crown root (i.e., second whorl from the bottom). Representative roots were selected as those that typified a given class for a given individual plant, based on visual examination of the length and diameter of all roots within a root class (Online resources). These segments were scanned using a flatbed scanner (Epson Expression 1680, Seiko Epson Corporation, Suwa, Japan), and analyzed by the program WinRhizoPro (Regent Instruments, Quebec, Canada). Using the diameter binning function in WinRhizoPro, total root length in each scan was separated into total lengths of the main axis, first-order and second-order laterals. Trait abbreviations and explanations are summarized in Table 1. Figure 1 illustrates the root classes studied.

Statistical analysis

Statistical analysis was performed in the *R* Program, version 2.9.2 (R Development Core Team 2012). 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. Pearson correlation analysis was performed within families, and between pairs of families for all traits. Principal component analyses (PCA) using varimax rotation were performed separately for all traits within each family, and for all traits on data pooled for the three families. The first two components were characterized based on variable eigenvalues and on vector clustering within plots of components 1

Table 1 Architectural and morphological root traits, listing their abbreviation and explanation of traits

Trait abbreviation	Trait explanation
PriLen	Total length of primary root (main axis+laterals)
PriDia	Average diameter of primary root (main axis+laterals)
MAPriLen	Total length of the main axis of the primary root
FOLPriLen	Total length of first-order laterals, primary root
SOLPriLen	Total length of second-order laterals, primary root
SemLen	Total length of seminal root (main axis+laterals)
SemDia	Average diameter of seminal root (main axis+laterals)
MASemLen	Total length of main axis of seminal root
FOLSemLen	Total length of first-order laterals, seminal root
SOLSemLen	Total length of second-order laterals, seminal root
CrnLen	Total length of crown root (main axis+laterals)
CrnDia	Average diameter of crown root (main axis+laterals)
MACrnLen	Total length of main axis of crown root
FOLCrnLen	Total length of first-order laterals, crown root
SOLCrnLen	Total length of second-order laterals, crown root
EDW	Embryonic root system dry weight
NDW	Nodal root system dry weight
RDW	Total root dry weight (embryonic+nodal)
SemNum	Number of seminal roots
NodNum	Number of nodal roots
SysDia	Diameter of the root system

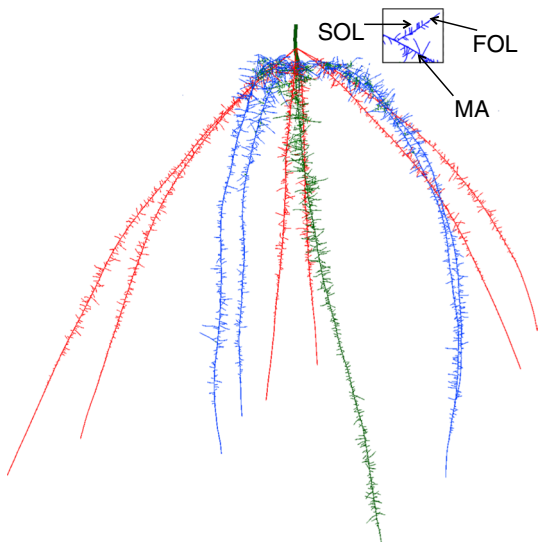

Fig. 1 Illustration of root classes in a typical maize root system 30 days after germination, with axial root classes shown in different colors: the primary root (*green*), seminal roots (*blue*), and crown roots (*red*). *Inset* contains a magnified view of a crown root, showing the main axis (MA) of the root, and the first-order (FO) and second-order (SO) lateral roots (color figure online)

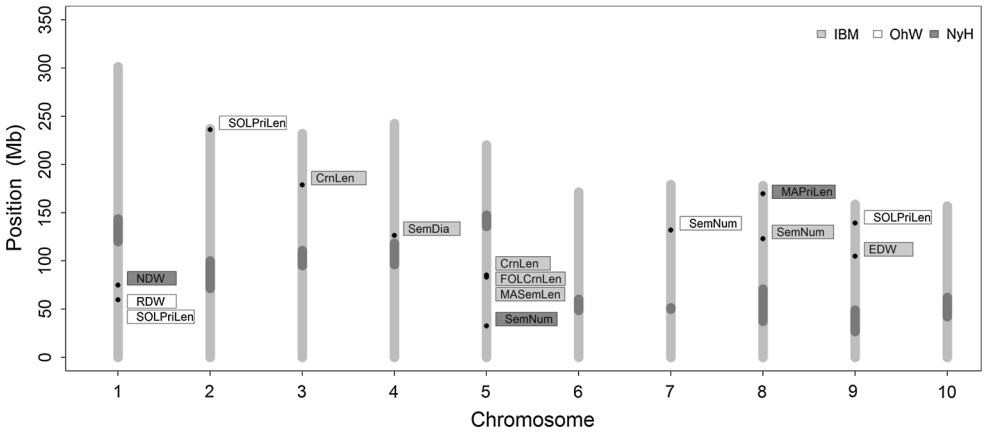

Fig. 2 Chromosome map, showing significant QTL for three inbred populations of maize. Traits in *light grey boxes* refer to QTL for Intermated B73 × Mo17 (IBM), in *white boxes* for OH43 × W64a (OhW), and in *dark grey boxes* for NY821 × H99 (NyH). Trait abbreviations can be found in Table 1

and 2. Based on Kaiser's Criterion and Cattell's method of component retention, only components with eigenvalues greater than 2.5 were retained (Kaiser 1960; Cattell 1966) (Online resources). 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, known as the allometric scaling exponent (α) (Niklas 1994). Prior to the QTL analysis, variation within each trait was assessed separately in each family using a standard two-way analysis of variance, where repetition and genotype were independent variables. For each trait in a given family, repeatability estimates were calculated as $\sigma_G^2/\left(\sigma_G^2 + \left(\sigma_E^2/n\right)\right)$ where σ_G^2 = genotype variance, σ_E^2 = error variance, and n = number of repetitions. Variance components were obtained from random effects model analyses, using the lmer package in *R* in which genotype and repetition were treated as random effects [Trait ~ (1|Genotype) + (1|Repetition)].

QTL analysis

QTL analysis was conducted using composite interval mapping with the qtl package of *R* (Broman et al. 2003) (Online resources 1–28). Identification of quantitative trait loci (QTL) was carried out using composite interval mapping (Zeng 1994) with 5 marker covariates, a window size of 10 in R/qtl (Broman, 2003). A HMM (hidden Markov model) was used to calculate the probabilities of the true underlying genotypes given the observed multipoint marker data with an assumed error probability of 0.0001. Haley–Knott regression as an approximation to the EM (expectation maximization) algorithm was utilized as Haley–Knott offers a great increase in computational speed with similar results when marker data are sufficient. 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. 2).

Results

Phenotypic variation

Continuous phenotypic variation among genotypes was observed at a significance level of $p < 0.10$ for seminal root diameter in IBM, and seminal main axis length and primary root diameter in NyH. All other traits in the three families showed continuous phenotypic variation among genotypes at a significance level of $p < 0.05$ (data not shown). Within all three populations, mean diameter and total length of crown roots were greater than corresponding values in both classes of embryonic roots (Table 2), except for primary root length in OhW. In each of the three populations, repeatability values were mostly in the range of 0.20–0.50 (Table 2). Repeatabilities in the OhW population were greater than those in the other two populations, with the exception of seminal root number and diameter, nodal root number, and root system diameter.

Among the three families, differences were observed in the phenotypic distribution of traits (Figs. 3, 4, 5). Generally, less variation was observed for total root length and diameter traits in the embryonic root classes than for corresponding traits in nodal roots. In the IBM population, primary and seminal length values were more evenly distributed across the phenotypic range than were values for crown root length (Fig. 3). Primary and seminal root diameters had a broader range than crown root diameter in that population. In the OhW population, primary and seminal

Table 2 Phenotypic values for inbred populations of maize: Intermated B73xMo17 (IBM), OH43xW64a (OhW), and NY821xH99 (NyH)

	IBM						OhW					
	B73	Mo17	Population Mean	Min Value	Max Value	Repeatability	OH43	W64a	Population Mean	Min Value	Max Value	Repeatability
PriLen	90.6	630.9	483.9	47.4	1183.5	44.7	626.6	608.4	664.5	52.2	1518.3	47.6
PriDia	0.26	0.42	0.44	0.26	0.83	10.9	0.44	0.41	0.44	0.34	0.53	41.4
MAPriLen	290.3	184.2	287.8	24.8	874.2	12.4	191.7	166.8	170.6	10.7	445.1	48.3
FOLPriLen	72.9	90.8	142.5	9.4	412.1	10.4	359.6	368.5	393.8	20.8	857.1	40.4
SOLPriLen	111.0	62.5	83.7	3.2	251.7	22.3	75.3	73.0	93.2	10.2	219.2	60.2
SemLen	114.1	433.2	303.8	36.3	775.3	36.5	318.3	518.5	440.3	17.5	1148.3	37.7
SemDia	0.28	0.45	0.47	0.27	1.05	18.0	0.44	0.44	0.44	0.29	0.57	46.0
MASemLen	48.16	120.3	66.1	3.1	233.0	18.5	113.7	136.1	123.8	3.3	351.3	38.4
FOLSemLen	59.7	210.1	147.3	11.8	388.9	33.1	131.4	238.9	208.5	0.5	557. 9	37.4
SOLSemLen	6.3	102.8	89.2	5.3	244.7	31.4	73.1	143.4	106.4	13.4	247.1	35.7
CrnLen	157.9	740.1	597.8	115.1	1479.7	40.7	477.3	849.4	625.4	61.3	1429.5	45.5
CrnDia	0.41	0.53	0.57	0.38	0.78	34.6	0.52	0.46	0.51	0.38	0.79	67.4
MACrnLen	102.2	270.3	148.0	7.6	540.0	27.2	149.5	287.4	165.6	6.4	422.7	37.4
FOLCrnLen	41.9	409.6	400.8	27.2	1072.7	40.1	297.4	529.2	410.8	35.5	909.2	48.7
SOLCrnLen	13.9	60.2	53.7	8.3	102.9	30.2	30.4	32.7	44.2	6.6	85.6	45.0
EDW	0.04	0.11	0.05	0.02	0.11	42.8	0.02	0.12	0.08	0.03	0.15	15.1
NDW	0.61	0.47	1.12	0.09	2.40	21.3	0.25	0.57	0.94	0.15	1.92	42.7
RDW	0.65	0.58	1.16	0.05	2.46	18.4	0.27	0.69	0.99	0.22	1.91	37.0
SemNum	2.3	3.7	3.2	0.5	7.5	50.7	4.0	4.0	3.0	1.0	5.7	21.1
NodNum	22.3	14.3	15.8	7.3	24.0	58.3	18.0	21.0	14.1	7.0	20.3	51.3
SysDia	39.0	36.0	34.8	14.0	54.5	43.2	30.0	42.0	27.7	16.0	43.0	42.7

	NyH					
	NY821	H99	Population Mean	Min Value	Max Value	Repeatability
PriLen	244.1	81.9	479.4	25.0	1077.0	29.2
PriDia	0.48	0.82	0.46	0.37	0.59	15.6
MAPriLen	58.7	20.2	184.2	9.7	475.3	29.5
FOLPriLen	146.7	46.7	221.4	9.8	452.5	29.2
SOLPriLen	38.7	15.0	66.7	5.4	132.4	29.3
SemLen	181.8	356.5	285.4	37.9	670.6	15.9
SemDia	0.37	0.55	0.46	0.31	0.66	43.7
MASemLen	74.3	78.0	75.0	10.3	185.9	22.1
FOLSemLen	80.9	170.9	131.0	8.0	313.0	14.9
SOLSemLen	26.6	107.6	79.4	17.4	159.4	17.9

Table 2 continued

	NyH					
	NY821	H99	Population Mean	Min Value	Max Value	Repeatability
CrnLen	1027.8	361.2	574.2	157.6	1154.9	36.5
CrnDia	0.48	0.57	0.56	0.45	0.73	28.6
MACrnLen	301.7	70.5	130.4	14.2	341.6	32.7
FOLCrnLen	688.6	245.3	399.0	113.9	773.4	36.1
SOLCrnLen	37.5	45.4	44.7	17.6	85.3	38.4
EDW	0.05	0.07	0.06	0.02	0.12	47.3
NDW	3.38	1.33	1.79	0.39	3.63	26.1
RDW	3.42	1.40	1.83	0.41	3.73	23.2
SemNum	2.3	2.0	3.2	1.0	6.0	43.6
NodNum	19.0	24.0	18.2	11.7	27.7	40.8
SysDia	38.5	48.0	33.7	18.0	51.5	19.5

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. Units: "PriLen"-"SOLCrnLen" (cm), "EDW"-"RDW" (g), "SemNum"-"NodNum" (count), and "SysDia" (mm)

root diameters, and seminal root length were less variable than length and diameter traits of the crown roots (Fig. 4). In the NyH population, seminal root length was less variable than in the other two root classes (Fig. 5). All three populations had similar proportions between main axis and lateral root lengths in a particular root class (Table 2). In each of the three root classes, the total length of first-order lateral roots exceeded the total length of either the main axis or second-order lateral roots. The only exception was the primary root in the IBM population, in which the greatest length was observed for the main root axis.

The NyH population had the greatest mean nodal and total root dry weights, while the OhW population had the greatest mean and range for embryonic root dry weight (Table 2). The numbers of seminal and nodal roots were highly variable in all three families (Table 2). The NyH population had the greatest mean number of nodal roots, but the fewest seminal roots. The greatest variation in root number was observed in the IBM population. Maximum values for nodal root number in the IBM population were more than three times greater than the minimum, while maximum seminal root number was 15 times greater than the minimum. The mean diameter of the root system (SysDia) was highly variable in all three populations. The mean value for this trait was similar for the IBM and NyH populations, but the magnitude of phenotypic variation in IBM was greater than in NyH.

Moderate-to-strong trait correlations ($r > 0.60$) were observed among traits in each family, and generally were among length traits in a given root class, or between length and dry weight traits (Tables 3, 4, 5). Moderate negative correlations were observed between the diameter and the length of the main axis of the crown root in the IBM and OhW families. In IBM, root length traits in the crown root correlated moderately with the length of the seminal root.

Significant allometric relationships were observed for root length and dry weight traits (Table 6). Allometric scaling exponents (α) indicated that root growth in these populations did not commonly occur in proportion to increases in total plant biomass. This was true of root traits where allometric scaling exponents differed from the isometric value of 0.33; allometric scaling exponents above this threshold indicate growth that exceeds proportional increases in biomass. In the IBM and NyH populations, root length traits in the embryonic and nodal root systems had allometric scaling exponents that exceeded the isometric value, and included main axis and lateral root traits. In the OhW population, allometric scaling exponents exceeded the isometric value for length traits in seminal and crown roots but not in primary roots. In all three populations, allometric scaling exponents were low for root number and system diameter traits, but were greater than 1.00 for nodal and total root dry weights.

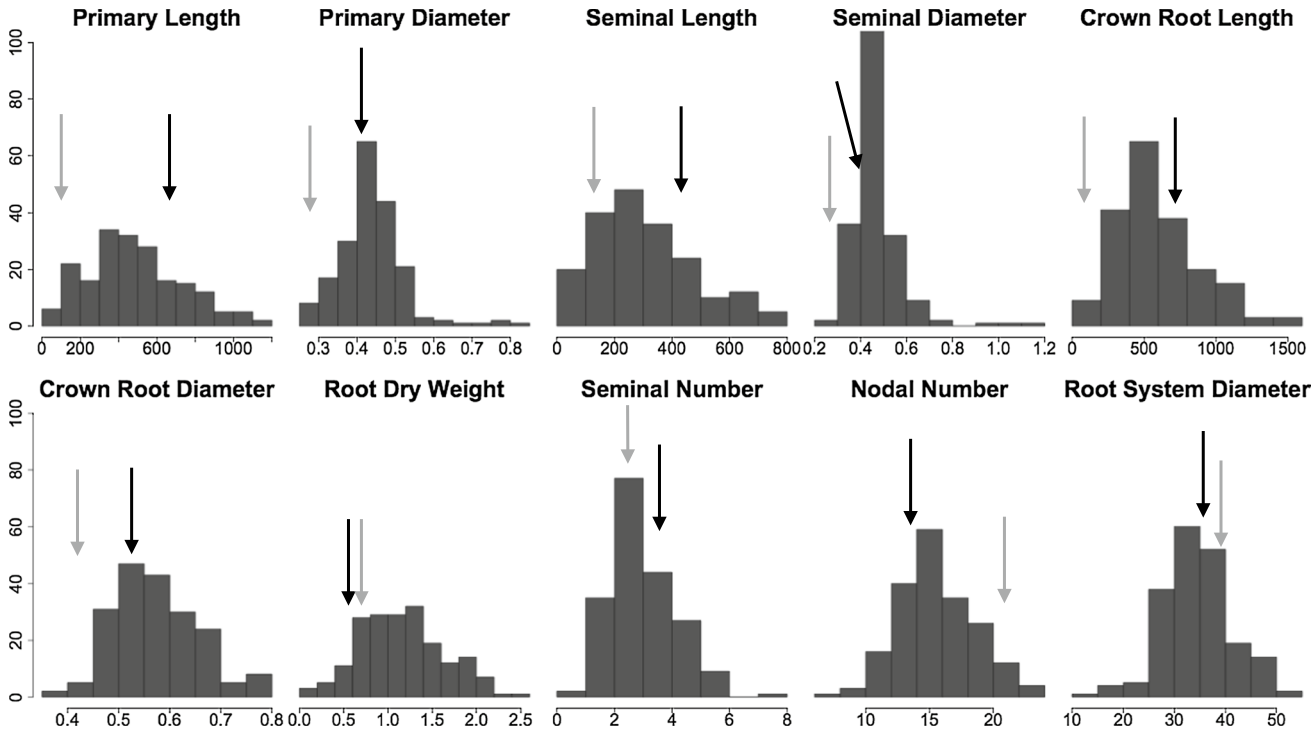

Fig. 3 Phenotypic variation in 10 root architectural traits in the recombinant inbred population of *Zea mays*, Intermated B73 × Mo17 (IBM). *Y axis* shows number of individuals. Units of measurement were as follows: length and diameter of the three root classes (cm), root dry weight (g), seminal and nodal number (count), and root system diameter (mm). *Grey arrows* are for B73 and *black arrows* are for Mo17

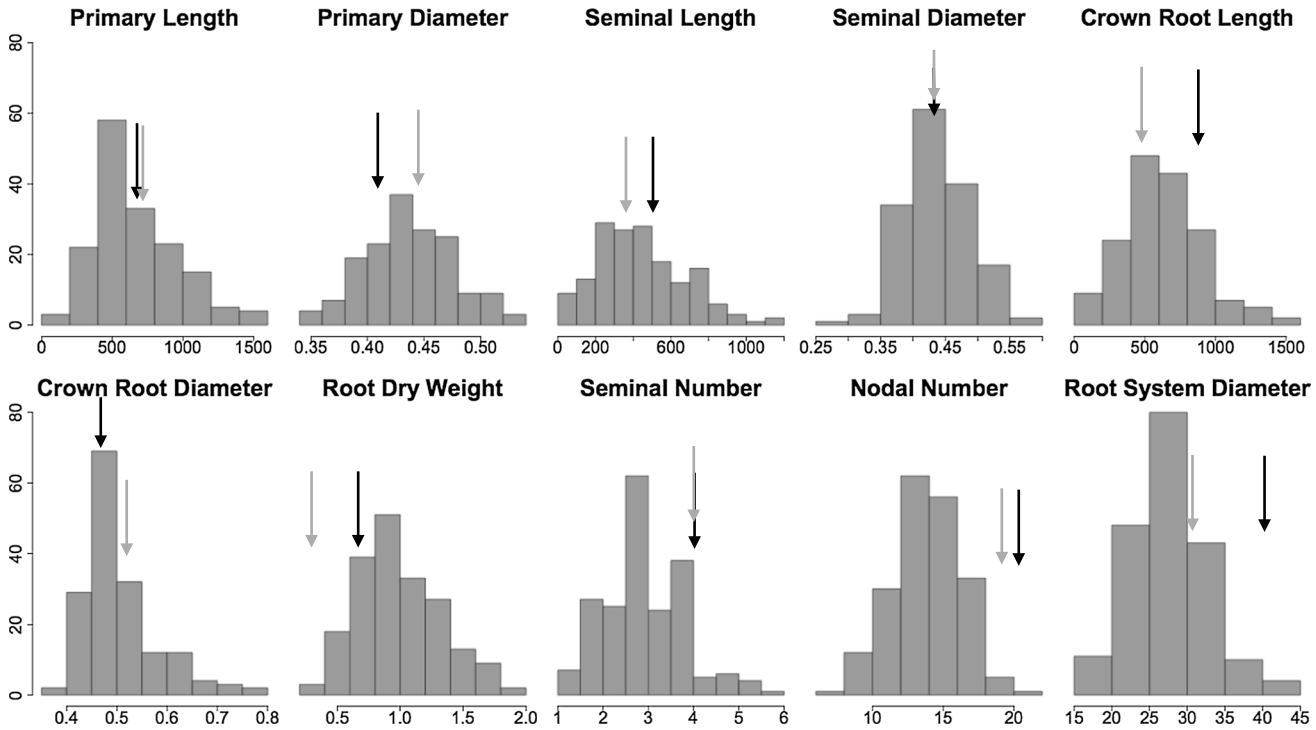

Fig. 4 Phenotypic variation in 10 root architectural traits in the recombinant inbred population of *Zea mays*, OH43 × W64a (OhW). Units of measurement were as follows: length and diameter of the three root classes (cm), root dry weight (g), seminal and nodal number (count), and root system diameter (mm). *Grey arrows* are for OH43 and *black arrows* are for W64a

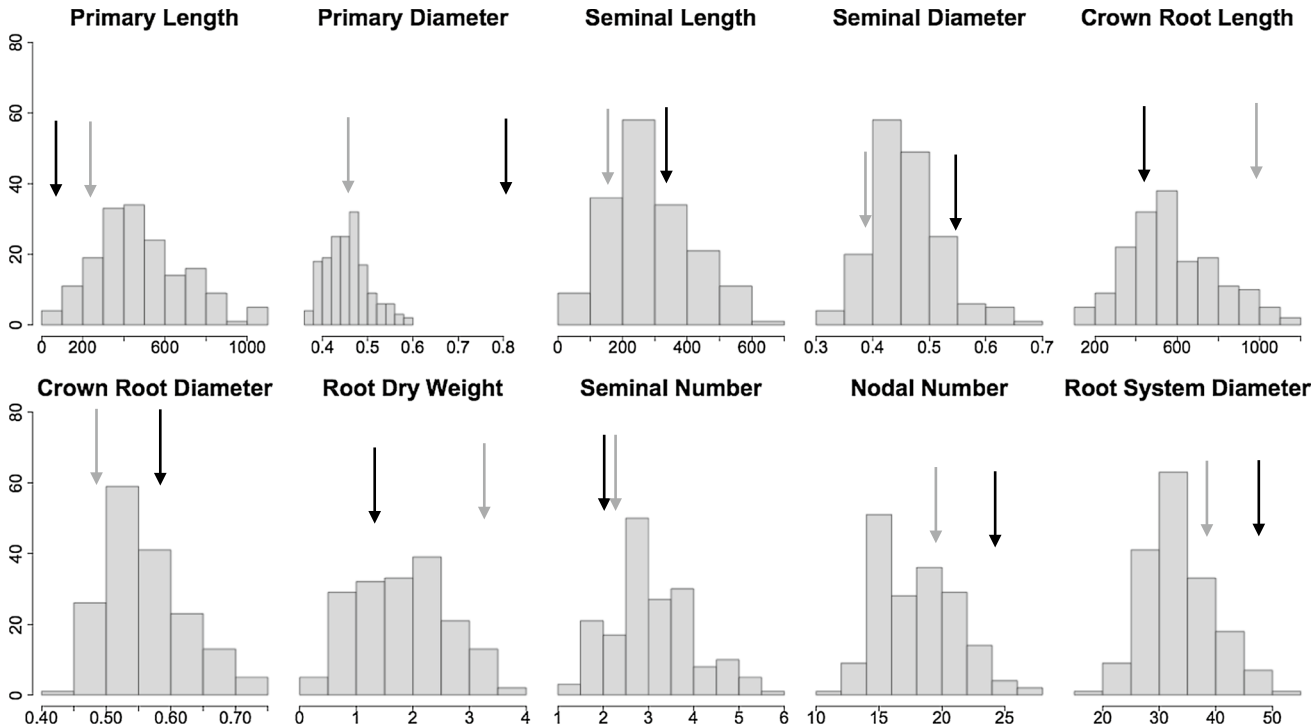

Fig. 5 Phenotypic variation in 10 root architectural traits in the recombinant inbred population of *Zea mays*, NY821 × H99 (NyH). Units of measurement were as follows: length and diameter of the three root classes (cm), root dry weight (g), seminal and nodal number (count), and root system diameter (mm). *Grey arrows* are for NY821 and *black arrows* are for H99

Principal component analysis showed that the overall pattern of trait clustering was similar in the three families (Figs. 6, 7, 8). The first two components in each family explained 45 % (IBM), 47 % (OhW), and 51 % (NyH) of variation in each family's dataset (data not shown). Components 3 and greater each explained less than 10 % of the variation in the datasets of each family. In principal component analysis plots, clustering of vectors is an indication of correlation among traits, while vector length and direction indicate association with a particular component. In each of the three families, dry weight and root count variables generally clustered together in biplots of components 1 and 2, as did root length variables. In the OhW plot, root number, crown root system diameter, and two of the dry weight traits clustered together in a set of vectors separated from the length variables (Fig. 7). In the same plot, length variables clustered together by root class. In the IBM and NyH plots (Figs. 6, 8), vector clusters were observed for length traits by root class, but vectors were less closely grouped together than in the OhW plot. Separation of vector clusters for length traits and dry weight, diameter and number traits was also less pronounced in the NyH and IBM plots, compared to the OhW plot. When data from the three families were grouped for PCA analysis, component loading values were generally low (<0.30), which was reflected in a lack of trait clustering in the plotting of components (data not shown). This was due to a highly variable pooled data set, and indicates the degree of diversity across the three populations. Overall, the PCA of the combined data of all three families showed lower values for component loading and variance explained by each component than individual analyses of the three families.

Detection of QTL

For 10 of the 21 traits measured, a total of 15 significant QTL were identified across the three inbred populations (Table 7). QTL were identified on eight out of ten chromosomes (Fig. 2). Ten of the QTL were for traits in the embryonic root system, including diameter and number of seminal roots, primary root length, length of second-order laterals on the primary root, and embryonic system dry weight. In the IBM population, three traits showed overlapping QTL on chromosome 5 (crown root length, first-order lateral length on the crown root, seminal root length). Two QTL for crown root length in the IBM population were identified on chromosome 5. Together, these two QTL explained 17.1 % of the variation in crown root length in this population. In the OhW population, three QTL for length of second-order laterals on the primary root were identified on chromosomes 1, 2, and 9. These QTL explained 27.9 % of the variation of that trait in the OhW

Table 3 Pearson correlation coefficients between traits in the recombinant inbred population of *Zea mays*, Intermated B73 × Mo17 (IBM)

IBM																			
PriDia	−0.077																		
FOLPriLen	0.041	−0.035																	
SOLPriLen	0.079	−0.064	**0.613***																
SemLen	0.390*	−0.042	0.018	0.087															
SemDia	0.126	0.060	−0.158*	−0.144*	−0.067														
MASemLen	0.410*	−0.041	0.092	0.115	**0.747***	−0.209*													
FOLSemLen	0.339*	−0.037	−0.002	0.063	**0.968***	−0.028	0.574*												
SOLSemLen	0.304*	−0.035	−0.033	0.068	**0.927***	0.045	0.494*	**0.938***											
CrnLen	0.452*	−0.021	−0.067	0.035	0.552*	0.099	0.563*	0.481*	0.449*										
CrnDia	−0.116	0.133	−0.126	−0.136	−0.075	0.193*	−0.312*	−0.007	0.085	−0.428*									
MACrnLen	0.301*	−0.06	−0.007	0.095	0.399*	0.021	0.548*	0.298*	0.266*	**0.880***	−0.566*								
FOLCrnLen	0.482*	−0.001	−0.084	0.005	0.571*	0.120	0.521*	0.522*	0.487*	**0.971***	−0.340*	**0.744***							
SOLCrnLen	0.462*	0.019	−0.162*	−0.045	0.570*	0.232*	0.393*	0.550*	0.569*	**0.759***	0.018	0.504*	**0.792***						
EDW	0.354*	−0.127	0.022	0.051	0.413*	0.015	0.417*	0.342*	0.379*	0.265*	−0.141	0.285*	0.228*	0.217*					
NDW	0.348*	0.028	−0.104	−0.042	0.432*	0.141	0.307*	0.434*	0.386*	**0.644***	−0.153*	0.498*	**0.653***	**0.615***	0.303*				
RDW	0.358*	0.023	−0.099	−0.036	0.442*	0.139	0.319*	0.441*	0.394*	**0.645***	−0.158*	0.503*	**0.652***	**0.614***	0.333*	**0.999***			
SemNum	−0.160*	0.118	0.044	−0.031	0.031	−0.149*	0.088	−0.001	0.018	−0.006	−0.069	0.042	−0.028	−0.047	0.313*	0.022	0.031		
NodNum	0.094	−0.076	0.044	−0.045	0.131	−0.016	0.044	0.149*	0.136	0.136	−0.065	0.100	0.141	0.124	0.166*	0.303*	0.305*	0.142*	
SysDia	−0.015	−0.092	−0.012	−0.053	0.049	−0.031	−0.099	0.104	0.085	0.062	−0.024	0.102	0.034	0.054	0.114	0.141	0.143*	0.060	0.272*
	PriLen	PriDia	FOLPriLen	SOLPriLen	SemLen	SemDia	MASem-Len	FOLSem-Len	SOLSem-Len	CrnLen	CrnDia	MACrn Len	FOLCrn-Len	SOLCrn-Len	EDW	NDW	RDW	SemNum	NodNum

Correlation coefficients greater than 0.60 are highlighted in bold, and statistically significant relationships are indicated by an asterisk ($p < 0.05$)

Table 4 Pearson correlation coefficients between traits in the recombinant inbred population of Zea mays, OH43 × W64a (OhW)

OhW																			
PriDia	−0.311*																		
FOLPriLen	**0.970***	−0.341*																	
SOLPriLen	**0.844***	0.046	**0.766***																
SemLen	0.155*	−0.337*	0.198*	−0.045															
SemDia	0.268*	0.156*	0.221*	0.442*	−0.341*														
MASemLen	0.182*	−0.415*	0.201*	−0.055*	**0.941***	−0.360*													
FOLSemLen	0.137*	−0.325*	0.194*	−0.065*	**0.985***	−0.325*	**0.890***												
SOLSemLen	0.115*	−0.146*	0.155*	0.034	**0.891***	−0.264*	**0.732***	**0.868***											
CrnLen	0.077*	−0.345*	0.079*	−0.084*	0.280*	−0.077*	0.360*	0.282*	0.064*										
CrnDia	0.071*	0.382*	0.033	0.302*	−0.198*	0.129*	−0.278*	−0.207*	0.015	**−0.627***									
MACrnLen	0.060*	−0.343*	0.049	−0.100*	0.304*	−0.102*	0.397*	0.297*	0.084*	**0.962***	−0.586*								
FOLCrnLen	0.094*	−0.339*	0.103*	−0.061*	0.258*	−0.061*	0.328*	0.265*	0.055*	**0.985***	**−0.637***	**0.903***							
SOLCrnLen	−0.085*	−0.111*	−0.079*	−0.166*	0.100*	−0.021	0.148*	0.110*	−0.033	0.558*	−0.237*	0.500*	0.519*						
EDW	0.424*	−0.175*	0.394*	0.395*	0.214*	0.131*	0.225*	0.187*	0.206*	0.105*	−0.028	0.088*	0.125*	−0.108*					
NDW	−0.040	−0.245*	−0.040	−0.177*	0.124*	−0.121*	0.177*	0.143*	−0.043	0.461*	−0.328*	0.419*	0.463*	0.349*	0.006				
RDW	−0.005	−0.249*	−0.014	−0.135*	0.127*	−0.100*	0.180*	0.140*	−0.029	0.470*	−0.308*	0.427*	0.471*	0.361*	0.090*	**0.976***			
SemNum	−0.241*	0.009	−0.236*	−0.218*	0.096*	−0.162*	0.055*	0.090*	0.158*	−0.074*	−0.055*	−0.05	−0.083*	−0.078*	0.091*	−0.001	0.006		
NodNum	−0.088*	−0.005	−0.052	−0.090*	0.075*	−0.122*	0.030	0.107*	0.054*	0.088*	−0.091*	0.078*	0.105*	−0.125*	−0.026	0.397*	0.377*	0.046	
SysDia	−0.088*	0.066*	−0.085*	−0.056*	−0.046	0.003	−0.067*	−0.038	−0.017	−0.027	0.080*	−0.052	−0.023	0.144*	−0.157*	0.298*	0.298*	0.084*	0.355*
	PriLen	PriDia	FOLPriLen	SOLPriLen	SemLen	SemDia	MASem-Len	FOLSem-Len	SOLSem-Len	CrnLen	CrnDia	MACrnLen	FOLCrn-Len	SOLCrn-Len	EDW	NDW	RDW	SemNum	NodNum

Correlation coefficients greater than 0.60 are highlighted in bold, and statistically significant relationships are indicated by an asterisk ($p < 0.05$)

Table 5 Pearson correlation coefficients between traits in the recombinant inbred population of *Zea mays*, NY821 × H99 (NyH)

NyH																				
PriDia	−0.420*																			
FOLPriLen	**0.949***	−0.375*																		
SOLPriLen	**0.809***	−0.096*	**0.804***																	
SemLen	0.416*	−0.306	0.381*	0.196*																
SemDia	0.009	−0.003*	0.061*	0.050	−0.037															
MASemLen	0.452*	−0.396	0.356	0.143	**0.877***	−0.054														
FOLSemLen	0.397*	−0.286*	0.384*	0.192*	**0.983***	−0.042	**0.800***													
SOLSemLen	0.273*	−0.125*	0.280*	0.216*	**0.878***	0.013	**0.602***	**0.866***												
CrnLen	0.478*	−0.328	0.422*	0.231*	0.397*	−0.091	0.476*	0.382*	0.192*											
CrnDia	0.007	0.050*	0.050*	0.090	−0.114	0.007	−0.135	−0.091	−0.102	−0.156*										
MACrnLen	0.454*	−0.334*	0.378	0.186*	0.393*	−0.082	0.498*	0.370*	0.171*	**0.964***	−0.140									
FOLCrnLen	0.472*	−0.317*	0.423*	0.237*	0.386*	−0.102	0.450*	0.376*	0.191*	**0.990***	−0.171*	**0.922***								
SOLCrnLen	0.319*	−0.163*	0.314*	0.240*	0.238*	0.041	0.268*	0.216*	0.174*	0.540*	0.034	0.492*	0.485*							
EDW	**0.603***	−0.233*	0.556*	0.446*	0.542*	0.019	0.519*	0.518*	0.452*	0.490*	−0.068	0.451*	0.486*	0.369*						
NDW	0.408*	−0.263*	0.369*	0.242*	0.341*	−0.049	0.409*	0.306*	0.216*	**0.681***	−0.078	**0.660***	**0.661***	0.493*	0.481*					
RDW	0.392*	−0.259*	0.352*	0.203*	0.345*	−0.134	0.428*	0.308*	0.204*	**0.690***	−0.078	**0.669***	**0.672***	0.469*	0.460*	**0.980***				
SemNum	−0.024	−0.072	−0.032*	−0.097	0.066	−0.045	0.116	0.028	0.067	0.028	−0.100	0.007	0.036	0.040	0.208*	0.148	0.130			
NodNum	0.130	−0.070	0.131	0.094	0.140	−0.058	0.141	0.136	0.101	0.277*	−0.081	0.267*	0.269*	0.209*	0.155*	**0.613***	**0.602***	0.213*		
SysDia	0.115	0.082	0.110	0.132	0.179*	−0.023	0.168*	0.160*	0.185*	0.339*	−0.029	0.325*	0.324*	0.325*	0.184*	0.435*	0.444*	−0.039	0.348*	
	PriLen	PriDia	FOLPriLen	SOLPriLen	SemLen	SemDia	MASemLen	FOLSemLen	SOLSemLen	CrnLen	CrnDia	MACrn-Len	FOLCrn-Len	SOLCrn-Len	EDW	NDW	RDW	SemNum	NodNum	

Correlation coefficients greater than 0.60 are highlighted in bold, and statistically significant relationships are indicated by an asterisk ($p < 0.05$)

Table 6 Summary of allometric analysis for individual recombinant inbred populations 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	
	R^2	α	R^2	α	R^2	α
PriLen	0.125	0.665	0.000^{NS}	−0.008	0.150	0.495
PriDia	0.055	0.126	0.076	−0.081	0.064	−0.068
MAPriLen	0.001^{NS}	−0.063	0.007^{NS}	0.169	0.167	0.656
FOLPriLen	0.005^{NS}	−0.174	0.000^{NS}	0.024	0.140	0.468
SOLPriLen	0.011^{NS}	−0.294	0.049	−0.321	0.059	0.285
SemLen	0.202	0.901	0.024	0.322	0.199	0.597
SemDia	0.023	0.091	0.040	−0.080	0.240	−0.173
MASemLen	0.063	0.659	0.064	0.609	0.275	0.740
FOLSemLen	0.220	1.114	0.025	0.368	0.198	0.772
SOLSemLen	0.187	0.912	0.001^{NS}	0.0591	0.069	0.284
CrnLen	0.419	0.983	0.240	0.842	0.454	0.674
CrnDia	0.001^{NS}	−0.012	0.183	−0.203	0.164	−0.110
MACrnLen	0.224	1.016	0.255	1.224	0.463	0.998
FOLCrnLen	0.374	1.072	0.217	0.772	0.430	0.631
SOLCrnLen	0.376	0.688	0.120	0.503	0.152	0.316
EDW	0.115	0.387	0.015^{NS}	0.127	0.256	0.474
NDW	0.737	1.274	0.708	1.099	0.872	1.222
RDW	0.738	1.216	0.710	1.010	0.854	1.179
SemNum	0.007^{NS}	0.092	0.000^{NS}	−0.011	0.021^{NS}	0.114
NodNum	0.152	0.230	0.000	0.180	0.238	0.213
SysDia	0.130	0.217	0.163	0.269	0.185	0.213

Allometric scaling exponents (α) around 0.33 indicate that growth for linear dimension traits is isometric (i.e., proportional to biomass increases)

NS not significant

population. Overall, the QTL with the greatest effect was for shoot dry weight in the OhW population. This QTL was identified on chromosome 1, with an r^2 value of 0.1348.

Discussion

Among genetically distinct populations, differences in trait expression may represent useful genetic diversity for crop breeding. In contrast, traits that are similarly expressed across genetically distinct lines may be useful for breeding outcomes in which predictable or consistent phenotypes are desirable. Differences were notably present among the three populations for mean length of the primary and seminal roots, and for the lateral roots associated with these root classes (Table 2). The OhW population was characterized by embryonic root systems that possessed relatively long seminal and lateral roots, compared to IBM and NyH. In the IBM population, the main axis of the primary root was longer than in the other two populations. An increased investment in the length of main axis embryonic roots would be beneficial for early establishment of strong plant anchorage and access to deep or highly mobile resources (Lynch 2013). Early investment in embryonic lateral roots

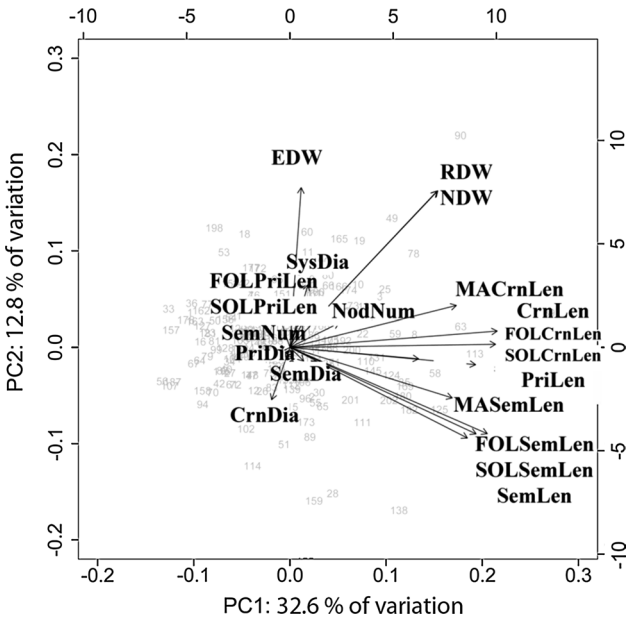

Fig. 6 Principal component analysis of root architectural traits in the recombinant inbred population of *Zea mays*, Intermated B73 × Mo17 (IBM). The x and y axes are components 1 and 2, respectively. Axis labels include the percentage of variation explained by each of these two components

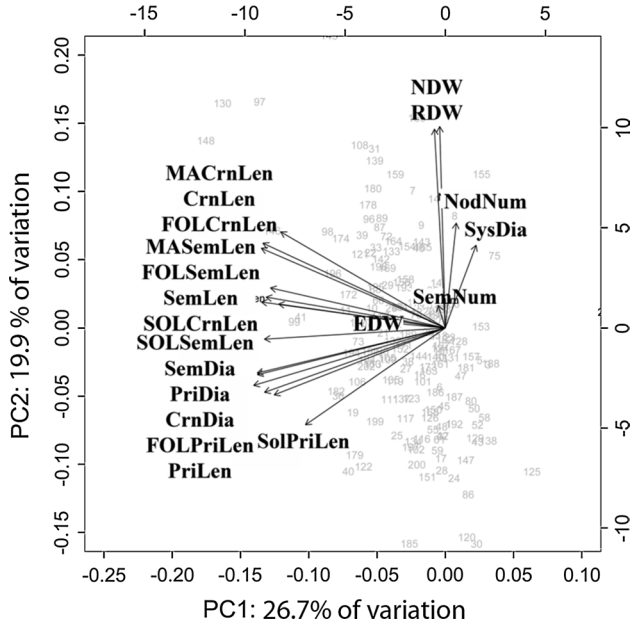

Fig. 7 Principal component analysis of root architectural traits in the recombinant inbred population of *Zea mays*, OH43 × W64a (OhW). The *x* and *y* axes are components 1 and 2, respectively. Axis labels include the percentage of variation explained by each of these two components

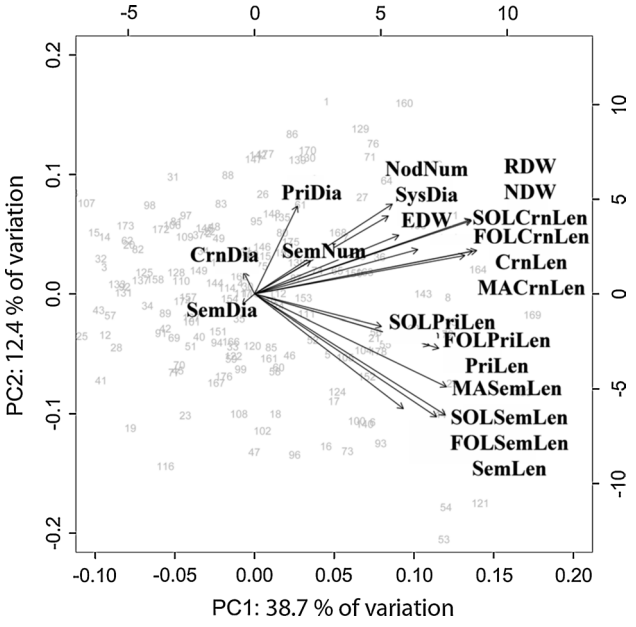

Fig. 8 Principal component analysis of root architectural traits in the recombinant inbred population of *Zea mays*, NY821 × H99 (NyH). The *x* and *y* axes are components 1 and 2, respectively. Axis labels include the percentage of variation explained by each of these two components

would further strengthen plant anchorage, and provide additional root surface area for acquisition of both mobile and immobile resources (Lynch 2013). Plant breeders may also find information on similarities in trait expression to be useful. While seminal root number has been shown to vary widely in teosinte and maize landraces (Burton et al. 2013), mean and range values for this trait were fairly consistent across the three maize populations in the present study. The number of seminal roots represents an early investment by the plant and a drain on the finite carbohydrate reserves of the seed (Lynch 2013). Consistency in seminal number may reflect an optimized state for maize seedling establishment in which seed carbohydrates are preferentially invested in lateral root proliferation or increased primary root length, rather than in growth of additional seminal roots. Whether this investment favors increased root surface area (i.e., lateral root proliferation) or increased embryonic rooting depth represents seedling establishment strategies that would be beneficial in differing environments.

Many of the traits examined were related to root length, specifically of main axes, and first- and second-order lateral roots in three root classes (primary, seminal and crown). Root length affects the ability of the plant to explore the soil for water and nutrients, and influences resistance to lodging (Hebert et al. 1996; Lynch 2005). Mean and range values showed that crown root length traits were similar across the three populations. This was supported by a similar pattern of PCA vector clustering and strong correlations among crown root traits in the three populations. Repeatability values for the crown root length traits were mostly in the 30–40 % range across the three populations (Table 2). The OhW population had considerable variation for total root length in the embryonic root classes (PriLen, SemLen), compared to the other two populations. Total root length included the length of the main axis plus the total length of lateral roots. While main axis length in the seminal and primary roots displayed variation in the OhW population, the large phenotypic range for total root length appears to be primarily due to variation in lateral root length. This was less true for total crown root length, because the length of the main axis occupied a greater proportion of the total root length in that class. Lateral root length, therefore, was an important difference among the three root classes across these populations.

Lateral roots are central to the primary roles of the root system, including nutrient and water acquisition, and anchorage (McCully 1999, Postma et al. 2014). The expression of lateral root traits tends to be highly variable, due to their plasticity in response to abiotic and biotic factors in the soil (Lynch and Brown 2012). For lateral root length traits, repeatability values varied by root class (primary, seminal, crown), and by branching order (first- or second-order laterals) among the three populations (Table 2). Phenotypic variation has been demonstrated for

Table 7 Summary of QTL for root architectural traits in the inbred maize populations Intermated B73 × Mo17 (IBM), OH43 × W64a (OhW) and NY821 × H99 (NyH), listing the population, trait abbreviation, highest significant marker with the corresponding genetic and physical position, 1.5 LOD support interval, width of the support interval, LOD score, additive effect estimate and percent variation explained by the QTL

Population	Trait Abbrev.	Significant marker	Chr	Pos (cM)	Position (bp)	Marker	Position (bp)	Marker	Position (bp)	Width (Mb)	LOD	Estimate (a)	Percent variation
IBM	SemDia	bin_3660	4	268.8	126450000	bin_3658	125500000	bin_3678	132600000	7.1	6.523	0.02714	3.12
IBM	EDW	bin_7405	9	241.1	105050000	bin_7398	101350000	bin_7410	105550000	4.2	6.208	0.005122	6.95
IBM	FOLCrnLen	bin_4477	5	263.1	83450000	bin_4474	83150000	bin_4485	85950000	2.8	7.304	68.16	10.24
IBM	CrnLen	bin_2910	3	430.001	178950000	bin_2900	177750000	bin_2915	179450000	1.7	5.68	84.64	6.93
IBM	CrnLen	bin_4483	5	265.3	85450000	bin_4475	83250000	bin_4485	85950000	2.7	8.105	103.06	10.18
IBM	MASemLen	bin_4475	5	260.9	83250000	bin_4470	81550000	bin_4488	86300000	4.75	8.971	14.167	5.72
IBM	SemNum	bin_6786	8	313.301	123050000	bin_6784	122750000	bin_6787	123150000	0.4	6.113	−0.28157	5.38
NyH	MAPriLen	bin_4510	8	198.1	169700000	bin_4505	168750000	bin_4514	170250000	1.5	5.32	35.9	8.23
NyH	NDW	bin_0265	1	177	74900000	bin_0259	73050000	bin_0276	78650000	5.6	5.49	0.26521	8.04
NyH	SemNum	bin_2836	5	86.7	32750000	bin_2822	26500000	bin_2838	33600000	7.1	7.42	0.0689	0.45
OhW	RDW	bin_0285	1	157.1	59750000	bin_0276	56950000	bin_0289	61100000	4.15	11.68	0.5623	13.48
OhW	SemNum	bin_4097	7	141.5	131900000	bin_4092	130700000	bin_4099	132450000	1.75	6.257	0.17978	4.02
OhW	SOLPriLen	bin_1545	2	347.001	236050000	bin_1542	234950000	bin_1548	236550000	1.6	5.633	−13.735	6.89
OhW	SOLPriLen	bin_5144	9	159.4	139250000	bin_5139	138750000	bin_5146	139450000	0.7	9.079	16.813	9.95
OhW	SOLPriLen	bin_0228	1	130.7	44150000	bin_0218	41500000	bin_0241	46350000	4.85	6.543	−17.568	11.08

Directionality was based on parent2 in each population (IBM: Mo17, NyH: H99, OhW: W64a)

the length of first-order lateral roots on 11-day-old primary roots in a subset of the IBM maize population (Zhu et al. 2005b). The range of phenotypic variation observed in that study was considerably less than that observed for the older IBM plants in the present study (Table 2). In the study with younger plants, QTL were identified on chromosomes 2, 3, 4 and 8. In the present study, QTL for lateral root length were found in two of the three populations (IBM and OhW) on chromosomes 1, 2, 5, and 9. QTL for the length of main axis and lateral roots co-localized on chromosome 5 in the IBM population (Fig. 2). These traits were also highly correlated, indicating a possible common genetic regulation (Table 3). This region on chromosome 5 overlapped with a previously identified QTL for maximal axial root length under low nitrogen conditions (Liu et al. 2008a). Previous reports have shown that QTL for lateral and axial root traits in a given root class can co-localize (Liu et al. 2008a; Ruta et al. 2010).

The number of roots in a particular root class directly affects the soil scavenging ability of the plant (Lynch 2011, Saengwilai et al. 2014). Mean seminal root number was similar in all three families, but the greatest range for this trait was observed in the IBM population. Repeatability values for seminal root number were moderate in NyH (43.6 %) and IBM (50.7 %), but lower in OhW (21.1 %). Along with embryonic root length, the number of seminal roots is likely to influence early plant establishment (Lynch 2013). QTL were identified for seminal root number in all three families, though loci were on different chromosomes (Chr 5, NyH; Chr 7, OhW; Chr 9, IBM). Previously, QTL for seminal number in maize seedlings have been identified on chromosomes 1, 2, and 3 under high phosphorus, and on chromosomes 1, 2, and 6 under low phosphorus (Zhu et al. 2006). Mean number of nodal roots was greatest in the NyH population, and the magnitude of phenotypic variation was also greater compared to the other two populations.

Allometry is not typically considered in phenotyping studies, despite the fundamental importance of scale and proportion in understanding trait expression. The term allometry describes changes in the size of an organ as it relates to the size of the whole organism (Niklas 1994). Allometric relationships influence carbon allocation within the plant over time, and therefore have implications for plant performance. Over the course of a long-term breeding program, direct selection for certain plant characteristics has been shown to change allometric relationships in oat (*Avena sativa*) (Semchenko and Zobel 2005). Allometric analysis allows for a better understanding of comparison among genotypes, by considering the relationship between the magnitude of a phenotypic trait and the size of the plant.

Allometric scaling exponents (α) around 0.33 indicate that growth for linear dimension traits is isometric (i.e., proportional to biomass increases) (Table 6). Anisometric growth is observed when scaling exponents are either less than or greater than 0.33; as such, organ growth is either less than or greater than proportional increases in total biomass. In the present study, anisometric root growth was observed for the majority of traits in each population. In all three populations, allometric scaling exponents for all crown root length traits were well above 0.33, with the exception of second-order lateral length in the NyH population. This means that as plant growth proceeds, crown roots and their laterals would be longer than would be expected for growth in proportion to plant biomass. The fact that this was observed in three genetically distinct populations emphasizes the importance of the crown root system in the overall function of the maize plant. Over time, preferential allocation of resources to a particular root class would have functional implications due to relatively greater increases in root number or length. Allometric patterns for embryonic root length traits were less consistent across the three populations than results for crown roots. For instance, in the OhW population, allometric relationships did not strongly affect the overall length of the primary and seminal roots (Table 6). In contrast, stronger allometry was observed in the other two populations for primary and seminal root length traits. In the NyH and IBM populations, this would result in deeper embryonic root systems than would be expected for proportional increases in plant biomass. Differences in embryonic root growth influence early plant establishment, and in difficult environments, could affect survival to maturity (Tuberosa and Salvi 2009). It is noteworthy that allometry was consistent across the populations for crown root length traits, but not for primary and seminal roots. In light of this, patterns in phenotypic variation may be more informative for breeders when considered within the context of comparative allometric analysis.

Root system diameter is an indication of the angle of crown root deployment in the soil, and therefore the volume and depth of soil explored. Shallow root angles have been shown to improve phosphorus acquisition in maize (Zhu et al. 2005a), common bean (Bonser et al. 1996; Liao et al. 2001), and soybean (*Glycine max*) (Zhao et al. 2004). In contrast, deep root angles may play a role in obtaining mobile resources concentrated in deeper layers of the soil (Giuliani et al. 2005; Lynch 2013). In common bean, genetic variation is known to exist for this trait (Bonser et al. 1996). Mean root system diameter and the magnitude of variation for this trait were similar in the NyH and IBM populations, and were greater than that observed in the OhW population. Root system diameter was not controlled by allometric relationships in any of the three populations (Table 6). This indicates that shallow or deep soil exploration is independent of plant size in maize, an important point for breeding root systems for more efficient resource acquisition, as well as resistance to lodging.

Table 8 QTL reported in this paper that coincide with meta-QTL from a consensus map of root-related QTL in maize (Hund et al. 2011)

	Maize QTL (this paper)		
	SOLPriLen	NDW	SemNum
Source population	OhW	NyH	OhW
Bin	1.03	1.04	7.03
Meta-QTL	Ax-1	Rt-2	Ax-15
Trait within meta-QTL	Lateral root length on primary root	Nodal root number	Seminal root number
Population	B73 × Mo17	Poly17 × F2	Association Panel UHOH
Growth environment	LP roll ups	pot	paper
Growth stage	V1–2	R6	V1–2
Reference	Zhu et al. (2005b)	Lebreton et al. (1995)	Reimer (2010)

These meta-QTL each included multiple root trait-related QTL from different sources. The traits and sources listed are those within the meta-QTL that correspond with the trait we measured

In the present study, QTL for root or shoot dry weight were found on chromosomes 1 and 9. Mean embryonic root dry weight was greatest in the OhW population, while nodal root dry weight was greatest in the NyH population. Strong allometry ($\alpha > 1.00$) was observed for nodal root dry weight in each family, meaning that as total plant biomass increased, the biomass of the nodal root system increased at more than three times the rate expected for proportional growth. This effect could be the result of greater length, number or branching on nodal roots, or a combination of these factors. However, in all three populations, allometric relationships were weak for root number, and stronger for various root length traits. This indicates that, as they grow larger, plants in these populations preferentially invest in root biomass through root elongation rather than an increase in root number. This pattern reflects favorable resource allocation for improved yields under water-restricted conditions (Lynch 2013).

Certain traits within the same root class had strong correlations, and corresponding vectors in the PCA biplots were closely clustered (Tables 3, 4, 5; Figs. 6, 7, 8). Correlations greater than 0.95 were observed between total or main axis root length in a given class and lateral root length variables in the same class. Vector clustering was also observed for dry weight and root number traits. This pattern offers an opportunity to identify overlapping variables. In the case of root length, total root length traits (PriLen, SemLen and CrnLen) were highly correlated with lengths of the main axis, and first- and second-order lateral roots in each family, and therefore, total root length can be considered a suitable trait to represent root length by class.

There were no QTL of large effect segregating in these populations. Although there is clearly genetic variation, the reason for so few QTL seems to be that the traits are controlled by genes with relatively small effects, coupled with non-genetic variation. QTL for embryonic and nodal root systems did not co-localize among the three populations in the present study (Fig. 2). Genetic control of root traits has previously been shown to differ between the embryonic and secondary root systems in rice (Qu et al. 2008; Cairns et al. 2009). QTL for crown root length were found on chromosomes 3 and 5 in the IBM population, and for nodal dry weight on chromosome 1 in the NyH population. For embryonic root traits, QTL were found for primary root length on chromosome 8 in NyH, seminal length on chromosome 5 in IBM, and length of second-order laterals of the primary root on chromosomes 1, 2, and 9 in OhW. Lack of QTL co-localization across mapping populations could be due to different loci segregating in the populations, the affect of sample size on QTL detection, or the influence of genetic × environmental effects on the expression of root traits (Bernier et al. 2008).

Of the QTL reported here, three coincided with meta-QTL for root traits in consensus maps of QTL for maize root traits in maize (Hund et al. 2011) (Table 8). SolPriLen QTL, measured in this study at V6–V7, overlapped with the meta-QTL Ax-1 designated by Hund et al. (2011) that includes root length and number QTL from several studies, including one for number of lateral roots on the primary root measured in seedlings (Zhu et al. 2005b). Our QTL for NDW coincided with meta-QTL Rt-2, which included QTL for nodal and seminal root number, root pulling force, axile root elongation rate, and others (Hund et al. 2011). Our QTL for SemNum overlapped with meta-QTL Ax-15, which included QTL for number of seminal roots and seminal root dry weight (Hund et al. 2011). These meta-QTL most likely include important regulatory regions for root development across populations.

Conclusions

In this study, phenotypic variation and allometric relationships were described for 21 root architectural traits in

three recombinant inbred populations of maize. In each of the populations, significant and substantial phenotypic variation was observed for all of the traits. Strong correlations were observed among traits within the same axial root class, particularly among length traits. Allometry was important for length and dry weight traits. In addition, 15 QTL were identified for 10 of these traits in the embryonic and nodal root systems. Root traits are central to plant function, but have been underutilized in plant breeding. Root architectural traits, such as those described in this study, could be useful in the development of cultivars tolerant of edaphic stress.

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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 they were performed.

References

Bailey PHJ, Currey JD, Fitter AH (2002) The role of root system architecture and root hairs in promoting anchorage against uprooting forces in *Allium cepa* and root mutants of *Arabidopsis thaliana*. J Exp Bot 53:333–340

Beebe SE, Rojas-Pierce M, Yan XL, Blair MW, Pedraza F, Munoz F, Tohme J, Lynch JP (2006) Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Sci 46:413–423

Bernier J, Atlin GN, Serraj R, Kumar A, Spaner D (2008) Breeding upland rice for drought resistance. J Sci Food Agr 88:927–939

Bonser AM, Lynch J, Snapp S (1996) Effect of phosphorus deficiency on growth angle of basal roots in *Phaseolus vulgaris*. New Phytol 132:281–288

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. Bioinformatics 19:889–890

Burton AL, Brown KM, Lynch JP (2013) Phenotypic diversity of root anatomical and architectural traits in *Zea* species. Crop Sci 53:1042–1055

Cai H, Chen F, Mi G et al (2012) Mapping QTLs for root system architecture of maize (*Zea mays* L.) in the field at different developmental stages. Theor Appl Genet 125:1313–1324. doi:10.1007/s00122-012-1915-6

Cairns JE, Namuco OS, Torres R, Simborio FA, Courtois B, Aquino GA, Johnson DE (2009) Investigating early vigour in upland rice (*Oryza sativa* L.): part II. Identification of QTLs controlling early vigour under greenhouse and field conditions. Field Crop Res 113:207–217

Cattell RB (1966) The scree test for number of factors. Multivar Behav Res 1:245–276. doi:10.1207/s15327906mbr0102_10

Chen J-M, Yu X-P, Cheng J-A, Zheng X-S, Xu H-X, Lu Z-X, Zhang J-F, Chen L-Z (2005) Plant tolerance against insect pests and its mechanisms. Acta Ent Sin 48:262–272

Clark LJ, Price AH, Steele KA, Whalley WR (2008) Evidence from near-isogenic lines that root penetration increases with root diameter and bending stiffness in rice. Fun Plant Biol 35:1163–1171

Collins NC, Tardieu F, Tuberosa R (2008) Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol 147:469–486

de Dorlodot S, Forster B, Pages L, Price A, Tuberosa R, Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. Trend Plant Sci 12:474–481

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) Approach for high diversity species. PLoS One 6(5):e19379

Gamuyao R, Chin JH, Pariasca-Tanaka J et al (2012) The protein kinase Pstoll from traditional rice confers tolerance of phosphorus deficiency. Nature 488:535–539. doi:10.1038/nature11346

Giuliani S, Sanguineti MC, Tuberosa R, Bellotti M, Salvi S, Landi P (2005) Root-ABA1, a major constitutive QTL, affects maize root architecture and leaf ABA concentration at different water regimes. J Exp Bot 56:3061–3070

Gowda VRP, Henry A, Yamauchi A, Shashidar HE, Serrak R (2011) Root biology and genetic improvement for drought avoidance in rice. Field Crop Res 122:1–13

Gregory PJ, Bengough AG, Grinev D, Schmidt S, Thomas WTB, Wojciechowski T, Young IM (2009) Root phenomics of crops: opportunities and challenges. Fun Plant Biol 36:922–929

Guingo E, Hebert Y, Charcosset A (1998) Genetic analysis of root traits in maize. Agronomie 18:225–235

Gutjahr C, Casieri L, Paszkowski U (2009) *Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling. New Phytol 182:829–837

Hebert Y, Guingo E, Argillier O, Barriere Y (1996) The variability of maize root architecture as a key to differences in root lodging. In: Proceedings of the XVIIth conference on genetics, biotechnology and breeding of maize and sorghum, Thessaloniki, Greece

Huang X, Feng Q, Qian Q, Zhao Q, Wang L, Wang A et al (2009) High-throughput genotyping by whole-genome resequencing. Genome Res 19:1068–1076

Hund A, Fracheboud Y, Soldati A, Frascaroli E, Salvi S, Stamp P (2004) QTL controlling root and shoot traits of maize seedlings under cold stress. Theoret Appl Genet 109:618–629

Hund A, Reimer R, Messmer R (2011) A consensus map of QTLs controlling the root length of maize. Plant Soil 344:143–158

Jansen C, Van de Steeg HM, De Kroon H (2005) Investigating a trade-off in root morphological responses to a heterogeneous nutrient supply and to flooding. Fun Ecol 19:952–960

Kaiser HF (1960) The application of electronic computers to factor analysis. Educ Psychol Meas 20:141–151. doi:10.1177/001316446002000116

Kamoshita A, Babu RC, Boopathi NM, Fukai S (2008) Phenotypic and genotypic analysis of drought-resistance traits for development of rice cultivars adapted to rainfed environments. Field Crop Res 109:1–23

Ku LX, Sun ZH, Wang CL, Zhang J, Zhao RF, Liu HY, Tai GQ, Chen YH (2012) QTL mapping and epistasis analysis of brace root traits in maize. Mol Breed 30:697–708

Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10:R25

Lebreton C, Lazić-Jančić V, Steed A, Pekić S, Quarrie SA (1995) Identification of QTL for drought responses in maize and their use in testing causal relationships between traits. J Exp Bot 46:853–865

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009a) The sequence alignment/map format and samtools. Bioinformatics 25(16):2078–2079

Li JZ, Xie Y, Dai AY, Liu LF, Li ZC (2009b) Root and shoot traits responses to phosphorus deficiency and QTL analysis at seedling stage using introgression lines of rice. J Genet Genomic 36:173–183

Liao H, Rubio G, Yan XL, Cao AQ, Brown KM, Lynch JP (2001) Effect of phosphorus availability on basal root shallowness in common bean. Plant Soil 232:69–79

Liao H, Yan X, Rubio G, Pedraza F, Beebe S, Lynch JP (2004) Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. Fun Plant Biol 31:1–12

Liu JC, Li JS, Chen FJ, Zhang FS, Ren TH, Zhuang ZJ, Mi GH (2008a) Mapping QTLs for root traits under different nitrate levels at the seedling stage in maize (*Zea mays* L.). Plant Soil 305:253–265

Liu L, Liao H, Wang X-R, Yan X-L (2008b) Regulation effect of soil P availability on mycorrhizal infection in relation to root architecture and P efficiency of *Glycine max*. Yingyong Shengtai Xuebao (Chin J Appl Eco) 19:564–568

Lynch J (2005) Nutrient acquisition by plants. an ecological perspective. In: BassiriRad H (ed) Root architecture and nutrient acquisition, vol 181. Springer, Heidelberg

Lynch JP (2011) Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiol 156:1041–1049. doi:10.1104/pp.111.175414

Lynch JP (2013) Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357

Lynch JP, Brown KM (2012) New roots for agriculture: exploiting the root phenome. Phil Trans R Soc B 367:1598–1604

Mano Y, Muraki M, Fujimori M, Takamizo T, Kindiger B (2005) Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea mays* ssp. *huehuetenangensis*) seedlings. Euphytica 142:33–42

Mata GH, Sepulveda B, Richards A, Soriano E (2006) The architecture of *Phaseolus vulgaris* root is altered when a defense response is elicited by an oligogalacturointide. Braz J Plant Physiol 18:351–355

McCully M (1999) Roots in soil: unearthing the complexities of roots and their rhizospheres. Annu Rev Plant Physiol Plant Mol Biol 50:695–718

Mickovski SB, Bengough AG, Bransby MF, Davies MCR, Hallett PD, Sonnenberg R (2007) Material stiffness, branching pattern and soil matric potential affect the pullout resistance of model root systems. Eur J Soil Sci 58:1471–1481

Miklas P, Kelly J, Beebe S, Blair M (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. Euphytica 147:105–131

Niklas K (1994) Plant allometry: the scaling of form and process. University of Chicago Press, Chicago, USA

Ochoa IE, Blair MW, Lynch JP (2006) QTL analysis of adventitious root formation in common bean under contrasting phosphorus availability. Crop Sci 46:1609–1621

Postma JA, Dathe A, Lynch JP (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. Plant Physiol. doi:10.1104/pp.113.233916

Qu YY, Mu P, Zhang HL, Chen CY, Gao YM, Tian YX, Wen F, Li ZC (2008) Mapping QTLs of root morphological traits at different growth stages in rice. Genet 133:187–200

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org

Reimer R (2010) Responses of maize (*Zea mays* L.) seedlings to low and high temperature: association mapping of root growth and photosynthesis-related traits. In: AGRL Diss ETH No. 18807 Zurich

Ruta N, Liedgens M, Fracheboud Y, Stamp P, Hund A (2010) QTLs for the elongation of axile and lateral roots of maize in response to low water potential. Theoret Appl Genet 120:621–631

Saengwilai P, Tian X, Lynch JP (2014) Low crown root number enhances nitrogen acquisition from low nitrogen soils in maize (*Zea mays* L.). Plant Physiol. doi:10.1104/pp.113.232603

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. P Natl Acad Sci USA 81(24):8014–8018

Schnable PS, Ware D, Fulton RS, Stein JC, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves TA et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326(5956):1112–1115

Semchenko M, Zobel K (2005) The effect of breeding on allometry and phenotypic plasticity in four varieties of oat (*Avena sativa* L.). Field Crops Res 93:151–168

Trachsel S, Messmer R, Stamp P, Hund A (2009) Mapping of QTLs for lateral and axile root growth of tropical maize. Theor Appl Genet 119:1413–1424

Tuberosa R, Salvi S (2009) QTL for agronomic traits in maize production. In: Bennetzen JL, Hake SC (eds) Handbook of Maize: Its Biology. Springer Science and Business Media, LLC, pp 501–541

Tuberosa R, Sanguineti MC, Landi P, Giuliani MM, Salvi S, Conti S (2002) Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Mol Biol 48:697–712

Tuberosa R, Salvi S, Sanguineti MC, Maccaferri M, Giuliani S, Landi P (2003) Searching for quantitative trait loci controlling root traits in maize: a critical appraisal. Plant Soil 255:35–54

Uga Y, Okuno K, Yano M (2011) Dro1, a major QTL involved in deep rooting of rice under upland field conditions. J Exp Bot 62:2485–2494. doi:10.1093/jxb/erq429

Uga Y, Yamamoto E, Kanno N et al (2013a) A major QTL controlling deep rooting on rice chromosome 4. Sci Rep 3:3040. doi:10.1038/srep03040

Uga Y, Sugimoto K, Ogawa S et al (2013b) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Gen 45(9):1097–1102. doi:10.1038/ng.2725

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genet 136:1457–1468

Zhao J, Fu J, Liao H et al (2004) Characterization of root architecture in an applied core collection for phosphorus efficiency of soybean germplasm. Chin Sci Bull 49:1611–1620

Zhao Q, Huang XH, Lin ZX, Han B (2010) SEG-Map: a novel software for genotype calling and genetic map construction from next-generation sequencing. Rice 3:98–102

Zhu J, Kaeppler S, Lynch J (2005a) Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays* L.). Fun Plant Biol 32:749–762

Zhu J, Kaeppler SM, Lynch JP (2005b) Mapping of QTLs for lateral root branching and length in maize (*Zea mays* L.) under differential phosphorus supply. Theoret Appl Genet 111:688–695

Zhu JM, Mickelson SM, Kaeppler SM, Lynch JP (2006) Detection of quantitative trait loci for seminal root traits in maize (*Zea mays* L.) seedlings grown under differential phosphorus levels. Theoret Appl Genet 113:1–10

Zhu J, Ingram PA, Benfey PN, Elich T (2011) From lab to field, new approaches to phenotyping root system architecture. Curr Opin Plant Bio 14:310–317