Supporting Information

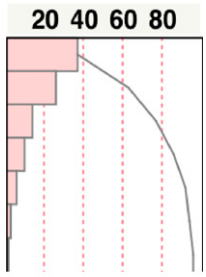
Topp et al. 10.1073/pnas.1304354110

SI Text

A comparison of trait values in this study with relevant data from the literature. Azucena was shown previously to have an average of 44% fewer roots than Bala at 27 d postgermination when grown in cylinders of sand (31.4 versus 55.6; extrapolated from ref. 1). At day 16 in our system, Azucena had a median number roots 3D value of 43.6% compared with Bala (6.2/11.0), suggesting that Bala has a more highly branched root system architecture (RSA) regardless of substrate. Azucena also was estimated to have significantly greater root mass at depth in a sandy loam in several treatments (2, 3). This finding correlated well with the almost twofold (1.96×) larger length distribution 2D value (the percent roots in the lower two-thirds of depth) on day 16 for Azucena.

1. Price AH, Steele KA, Moore BJ, Barraclough PB, Clark LJ (2000) A combined RFLP and AFLP linkage map of upland rice. *Theoretical and Applied Genetics* 100:49–56.
2. MacMillan K, Emrich K, Piepho HP, Mullins CE, Price AH (2006) Assessing the importance of genotype × environment interaction for root traits in rice using a mapping population. I: a soil-filled box screen. *Theoretical and Applied Genetics* 113(6):977–986.
3. MacMillan K, Emrich K, Piepho HP, Mullins CE, Price AH (2006) Assessing the importance of genotype × environment interaction for root traits in rice using a mapping population II: conventional QTL analysis. *Theoretical and Applied Genetics* 113(5): 953–964.

Component	Eigenvalue	Percent	Cumulative %	ChiSquare	DF	Prob>ChiSq
1	9.2672	37.069	37.069	10328.5	296.024	<.0001
2	6.5343	26.137	63.206	8996	288.752	<.0001
3	3.4117	13.647	76.853	7486.02	276.175	<.0001
4	2.432	9.728	86.581	6310.42	258.3	<.0001
5	1.3855	5.542	92.123	4955.17	239.321	<.0001
6	0.5431	2.172	94.295	3727.99	219.724	<.0001
7	0.4244	1.698	95.993	3161.32	199.292	<.0001



Loading matrix

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
MaximumNumberRoots2D	0.80246	0.55111	-0.00688	-0.06147	0.14038	0.07789	0.03995
MaximumNumberRoots3D	0.68353	0.54858	-0.10244	-0.15245	0.31766	-0.20496	0.13043
MedianNumberRoots2D	0.74302	0.49129	0.02755	-0.41386	-0.07317	0.08674	0.04383
MedianNumberRoots3D	0.63234	0.47457	-0.08395	-0.48049	0.09676	-0.2016	0.23703
NetworkBushiness2D	0.08766	0.14127	-0.06362	0.78187	0.51372	-0.0685	0.07603
NetworkBushiness3D	0.23985	0.28272	-0.01884	0.67553	0.53431	-0.06538	-0.1852
NetworkLengthDistribution2D	-0.14341	-0.73909	0.09842	0.26603	0.13899	0.21155	0.5202
NetworkSolidity2D	-0.34955	0.58622	0.52928	-0.37838	0.21365	0.00718	0.00261
NetworkSolidity3D	-0.57982	0.33638	0.5816	-0.07135	0.17474	0.25929	-0.04457
SpecificRootLength2D	0.63592	0.04744	-0.66721	-0.16917	0.14297	0.24727	-0.04684
MajorEllipseAxis2D	0.18651	-0.93885	-0.09853	-0.13135	0.07606	-0.03074	-0.03277
MinorEllipseAxis2D	0.80866	0.17058	0.07237	0.38291	-0.3505	-0.024	0.08062
MaximumRootDepth2D	0.25949	-0.92742	-0.09912	-0.07208	0.14384	-0.0307	-0.02556
MaximumNetworkWidth2D	0.84425	0.18044	0.02856	0.36435	-0.30757	0.02099	-0.01576
NetworkConvexArea2D	0.71591	-0.64016	-0.05835	0.15233	-0.14765	-0.01485	-0.04463
NetworkConvexHullVolume3D	0.74957	-0.49531	-0.0652	0.25236	-0.25432	0.0041	-0.02254
EllipseAspectRatio2D	0.3344	0.82126	0.1375	0.32601	-0.22922	0.01617	0.07313
MaximumWidthDepthRatio2D	0.34246	0.81939	0.11754	0.27076	-0.2862	0.04372	0.00303
AverageRootWidth2D	-0.68105	-0.12951	0.59705	0.12038	-0.15347	-0.28993	0.08848
NetworkPixelArea2D	0.7704	-0.50406	0.33933	-0.12244	0.02443	-0.05871	-0.03379
NetworkSurfaceArea2D	0.8175	-0.38189	0.37133	-0.14209	0.08582	-0.07553	-0.03633
NetworkSurfaceArea3D	0.78434	0.00491	0.51543	-0.10784	0.22145	0.16652	-0.01752
NetworkVolume2D	0.47446	-0.26299	0.77836	-0.02515	0.04001	-0.23508	-0.04273
NetworkVolume3D	0.22319	-0.22948	0.87233	0.10234	-0.03175	0.28788	-0.04519
TotalRootLength2D	0.93139	-0.12291	-0.0879	-0.21629	0.1919	0.01827	-0.04526

Fig. S1. Principal component statistics. Eigenvalues, loadings, and associated statistics for the principal component analysis.

Table S1. Trait names, groupings, descriptions of the features of the RSA they measure, and the method of computing 2D and 3D traits

Trait	Category	Ratio	Measurement	Calculation
Maximum of roots: 2D and 3D	Network distribution	No	Maximum no. of roots exploring a given soil horizon	While sweeping a horizontal plane from top to bottom, compute the number of connected components for each horizontal slice. Sort these numbers. The MaxR is the number at the position 0.85*depth in the sorted array of numbers (and should correspond to the 85-percentile). The computation of the connected components is done for ymin < y < ymax; see definition of Depth.
Median number roots: 2D and 3D	Network distribution	No	Median no. of roots exploring all soil horizons	While sweeping a horizontal plane from top to bottom, compute the number of connected components. Take the median of the distribution of these numbers along all horizontal slices. The computation is done for ymin < y < ymax; see definition of Depth.
Network bushiness 2D and 3D	Network distribution	Yes	High value indicates heterogeneous branching distribution along vertical axis; low value indicates homogenous branching distribution	The ratio of the maximum to the median number of roots
Network length distribution 2D	Network distribution	Yes	Ratio of deep to shallow root exploration relative to total network depth	The fraction of network pixels found in the lower 2/3 of the network. The lower 2/3 of the network is defined based on the network depth.
Network solidity 2D	Network distribution	Yes	2D estimate of soil coverage (similar to root length density)	Network pixel area 2D divided by network convex area 2D
Network solidity 3D	Network distribution	Yes	3D estimate of soil coverage (similar to Root Length Density)	Network volume 3D divided by network convex hull volume 3D
Specific root length 2D	Network distribution	Yes	Network length per unit volume: higher value indicates overall thinner roots (similar to traditional SRL = root length/root mass)	Total root length 2D divided by network volume 2D. Volume is estimated as the sum of cross-sectional areas for all pixels of the medial axis of the root system. The total root length is the number of pixels in the medial axis of the root system
Network convex area 2D	Network extent	No	Overall extent of space the network explores	The area of the convex hull that encompasses the image
Network convex hull volume 3D	Network extent	No	Overall extent of space the network explores	The volume of the smallest convex set that contains the voxels composing the reconstructed 3D root.
Maximum root depth 2D	Network extent	No	Deepest extent of root system (similar to traditional MRD = maximum rooting depth)	The number of pixels in the vertical direction from the uppermost network pixel to the lowermost network pixel
Maximum network width 2D	Network extent	No	Widest extent of root system	The number of pixels in the horizontal direction from the farthest left network pixel to farthest right network pixel
Major ellipse axis 2D	Network extent	No	Long axis of network distribution; correlation to maximum network depth depends on the center of mass	The length of the major axis of the best-fitting ellipse to the network
Minor ellipse axis 2D	Network extent	No	Short axis of network distribution; correlation to maximum network width depends on the center of mass	The length of the minor axis of the best-fitting ellipse to the network
Maximum width: depth ratio 2D	Network shape	Yes	Relative shape of network distribution; increasing values indicate an increase in horizontal relative to vertical exploration	The value of the network width divided by the value of network depth
Ellipse aspect ratio 2D	Network shape	Yes	Relative shape of network distribution; correlation to maximum width depth ratio depends on the center of mass	The ratio of the minor to the major axis of best-fitting ellipse
Average root width 2D	Intrinsic network size	No	Average width of all roots in the network (related to traditional MRT, maximum root thickness)	The mean value of the root width estimation computed for all pixels of the medial axis of the entire root system
Network pixel area 2D	Intrinsic network size	No	Estimate of surface area	Area of network pixels in the image

Table S1. Cont.

Trait	Category	Ratio	Measurement	Calculation
Network surface area 2D	Intrinsic network size	No	Calculation of surface area in 2D	The sum of the local surface area at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image
Network surface area 3D	Intrinsic network size	No	Measurement of surface area in 3D	Area of voxel faces on the surface of the root model
Network volume 2D	Intrinsic network size	No	Calculation of a volume in 2D	The sum of the local volume at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image
Network volume 3D	Intrinsic network size	No	Measurement volume in 3D	Area of voxels comprising the root
Total root length 2D	Intrinsic network size	No	Length of all roots in the network	Length of pixels in the network skeleton

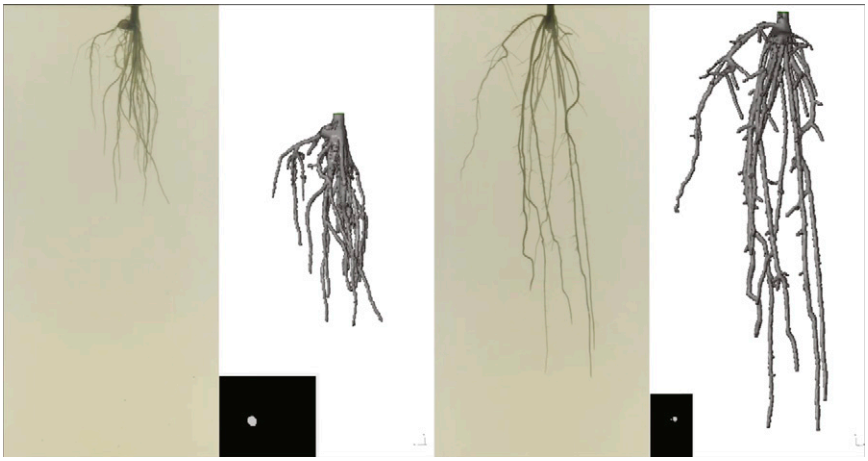
Table S2. Summary of growth rates for all traits between day 12 and day 16

Trait	Four-day average RIL (%)	Daily average RIL (%)	Four-day average Bala (%)	Daily average Bala (%)	Four-day average Azucena (%)	Daily average Azucena (%)
Maximum number roots 2D	14.33	3.58	11.88	2.97	3.51	0.88
Maximum number roots 3D	20.48	5.12	15.52	3.88	2.26	0.57
Median number roots 2D	16.95	4.24	7.82	1.95	4.64	1.16
Median number roots 3D	22.32	5.58	8.18	2.05	0.82	0.21
Network bushiness 2D	−0.65	−0.16	4.53	1.13	3.62	0.91
Network bushiness 3D	3.62	0.91	8.42	2.10	8.40	2.10
Network length distribution 2D	4.37	1.09	6.90	1.73	11.42	2.85
Network solidity 2D	1.81	0.45	0.96	0.24	−2.62	−0.66
Network solidity3 D	0.16	0.04	−2.54	−0.63	−15.32	−3.83
Specific root length 2D	−3.56	−0.89	−9.54	−2.39	−0.93	−0.23
Major ellipse axis 2D	16.13	4.03	13.86	3.46	31.01	7.75
Minor ellipse axis 2D	12.08	3.02	11.58	2.90	6.47	1.62
Maximum root depth 2D	17.86	4.47	15.97	3.99	31.10	7.77
Maximum network width 2D	15.24	3.81	12.98	3.24	10.27	2.57
Network convex area 2D	34.60	8.65	29.57	7.39	43.21	10.80
Network convex hull volume 3D	47.45	11.86	38.74	9.69	57.25	14.31
Ellipse aspect ratio 2D	−2.66	−0.66	−1.80	−0.45	−16.99	−4.25
Maximum width depth ratio 2D	−1.15	−0.29	−2.20	−0.55	−13.32	−3.33
Average root width 2D	1.94	0.48	3.72	0.93	1.59	0.40
Network pixel area 2D	35.88	8.97	30.67	7.67	37.58	9.40
Network surface area 2D	36.36	9.09	30.92	7.73	37.42	9.36
Network surface area 3D	46.83	11.71	33.20	8.30	38.35	9.59
Network volume 2D	40.63	10.16	40.55	10.14	38.11	9.53
Network volume 3D	42.45	10.61	33.67	8.42	28.66	7.16
Total root length 2D	34.22	8.55	27.01	6.75	35.39	8.85

RIL, recombinant inbred line.

Table S3. Projected multivariate phenotypes: Summary of the relative contributions of each univariate trait to each composite quantitative trait locus (QTL)

	Multivariate QTL marker				
Trait	C39 (%)	a12451 (%)	a18438 (%)	C601-OJ1734E02 (%)	C734 (%)
Ellipse aspect ratio 2D	7.7	50.8	9.7	22.1	33.5
Maximum width:depth ratio 2D	31.6	14.0	17.4	7.0	2.0
Maximum network width 2D	28.8	11.4	18.1	21.1	24.0
Maximum root depth 2D	19.4	10.3	10.3	24.5	0.0
Network convex area 2D	0.6	5.5	13.5	1.2	22.1
Maximum number roots 2D	2.2	3.1	15.3	4.1	6.2
Solidity 2D	8.2	4.6	4.2	7.5	2.9
Network length distribution 2D	0.8	0.3	6.9	8.9	3.0
Average root width 2D	0.6	0.0	4.5	3.6	6.3



Movie S1. Parental lines rotational image series and 3D reconstructions. The movie shows 3D raw data and reconstructions. 2D *z*-slices are shown in the black inset boxes and correspond to the green line in the 3D image.

Movie S1

Movie S2. Alternative view of parental lines 3D reconstructions. The movie shows alternate 3D raw data and reconstructions. 2D *z*-slices are shown in the black inset boxes and correspond to the green line in the 3D image.

Movie S2

Dataset S1. Trait correlations: Correlation matrix for all 25 traits using the genetic means

Dataset S1

Dataset S2. Full univariate QTL results: All statistics relevant to the univariate QTL analysis

Dataset S2

Dataset S3. Full multivariate QTL results: All statistics relevant to the multivariate QTL analysis

Dataset S3