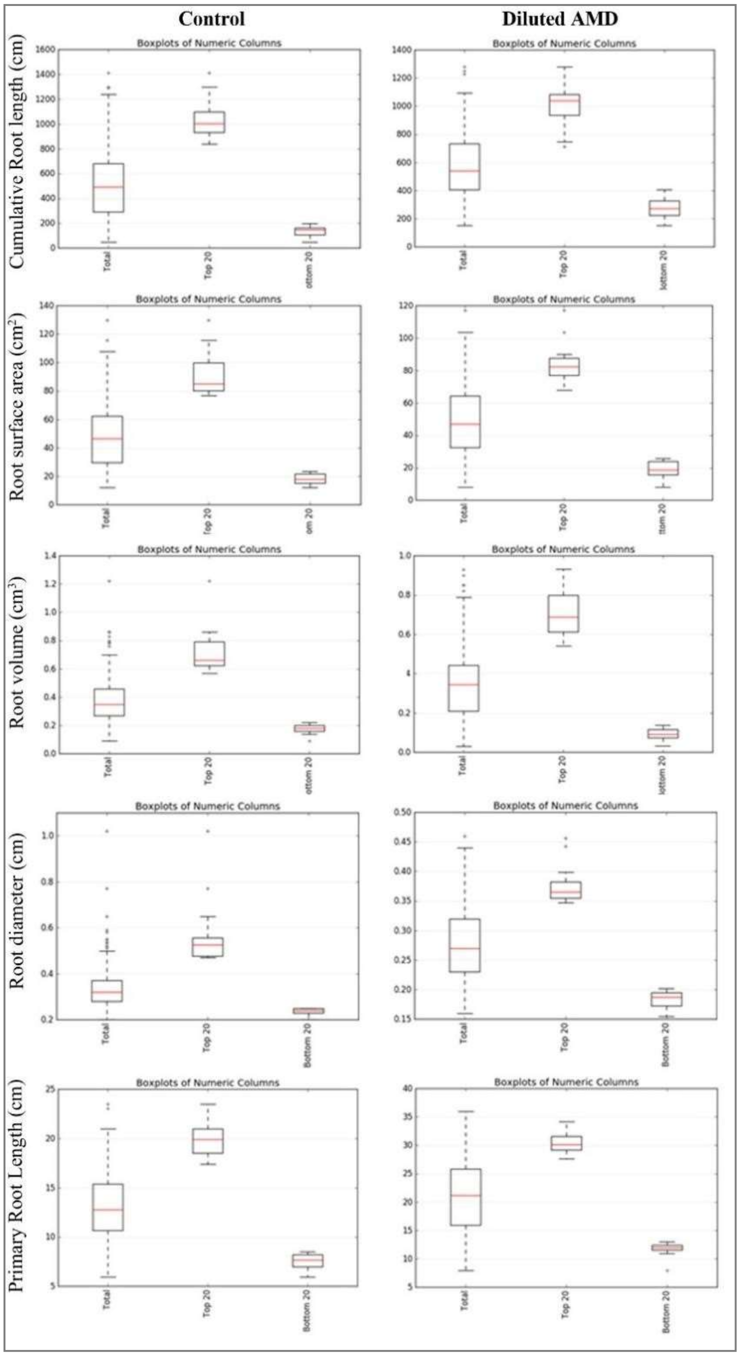


Figure S1. Natural variation was observed for different root traits. Effect sizes for total accessions, top 20 and bottom 20 accessions for cumulative root length, root surface area, root volume, root diameter, and primary root length in control (left panel) and acid mine drainage water (right panel). Yellow and blue indicate a higher and lower reduction in biomass associated with the Col-0 allele, respectively. Box plots represent the median value indicated by a thick red line.

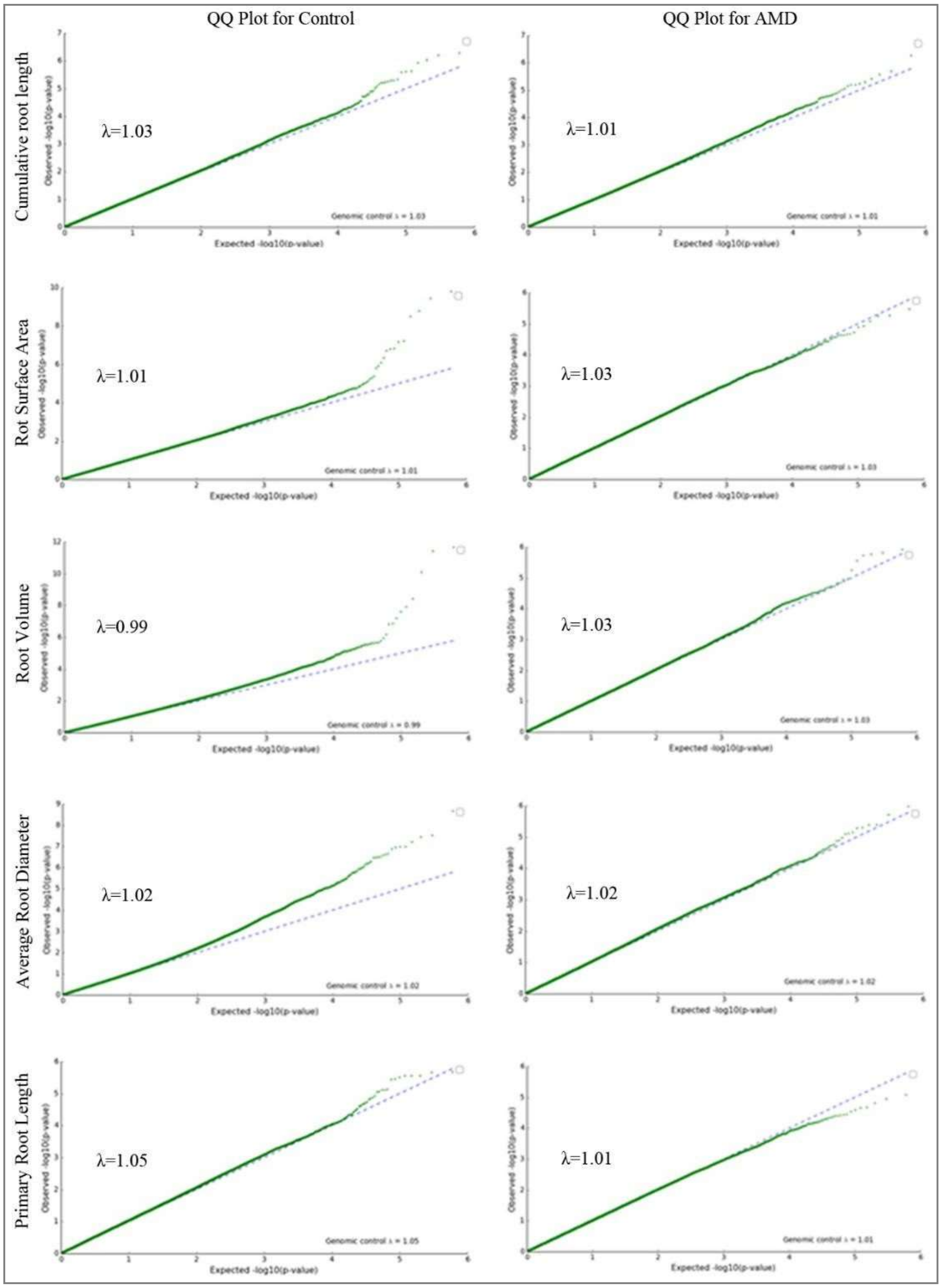


Figure S2. Q-Q (quantile-quantile) Plots of Different Root Traits. The plots were drawn for expected vs. observed -log10 (*p*-values) for cumulative root length, root surface area, root volume, average root diameter, and primary root length both in controlled and AMD conditions.

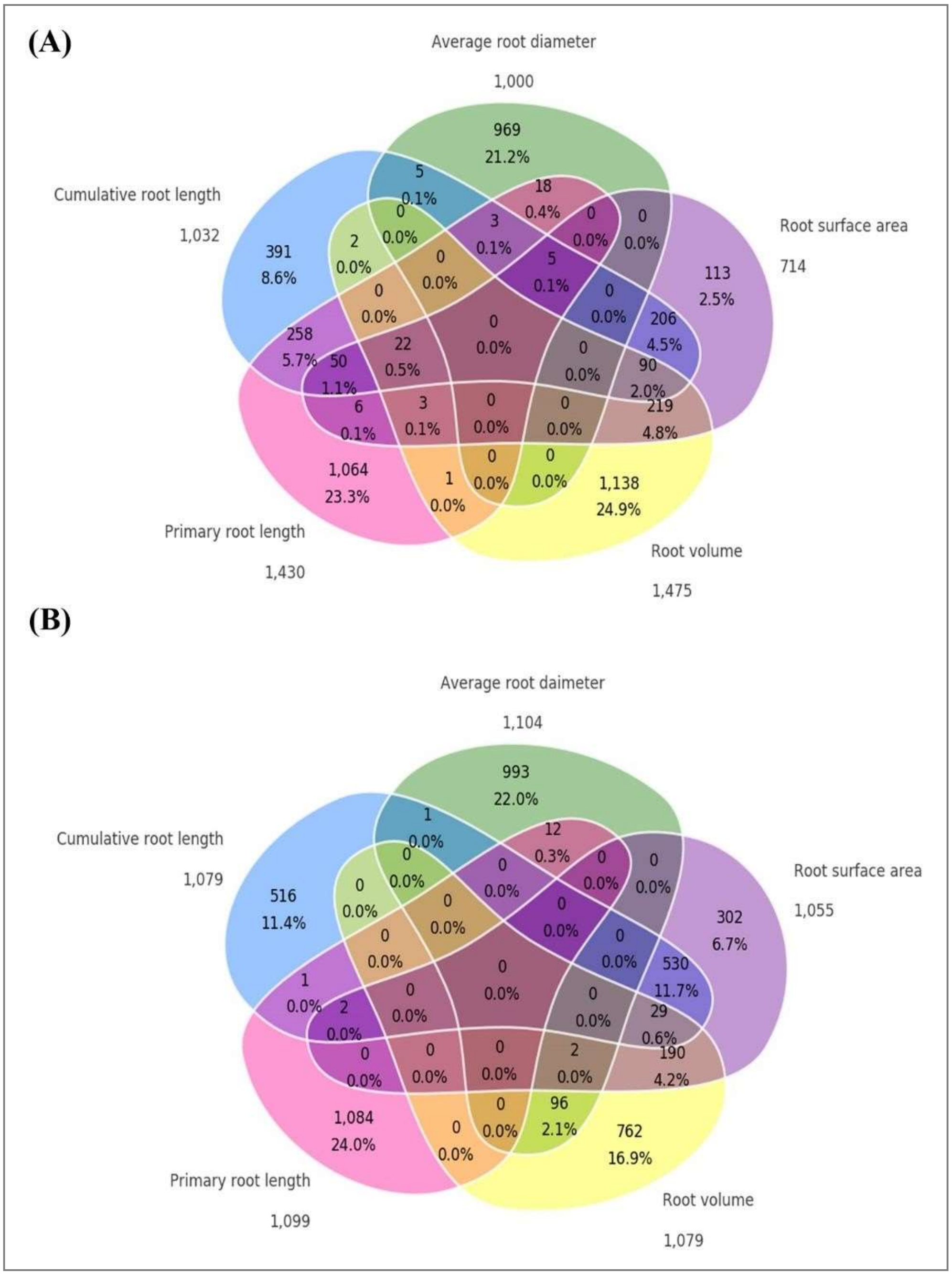


Figure S3. Common SNP markers between and among multiple root traits. Highly significant markers were taken for analysis to identify commonly associated SNPs among multiple traits across different traits for control (**A**) and AMD (**B**).

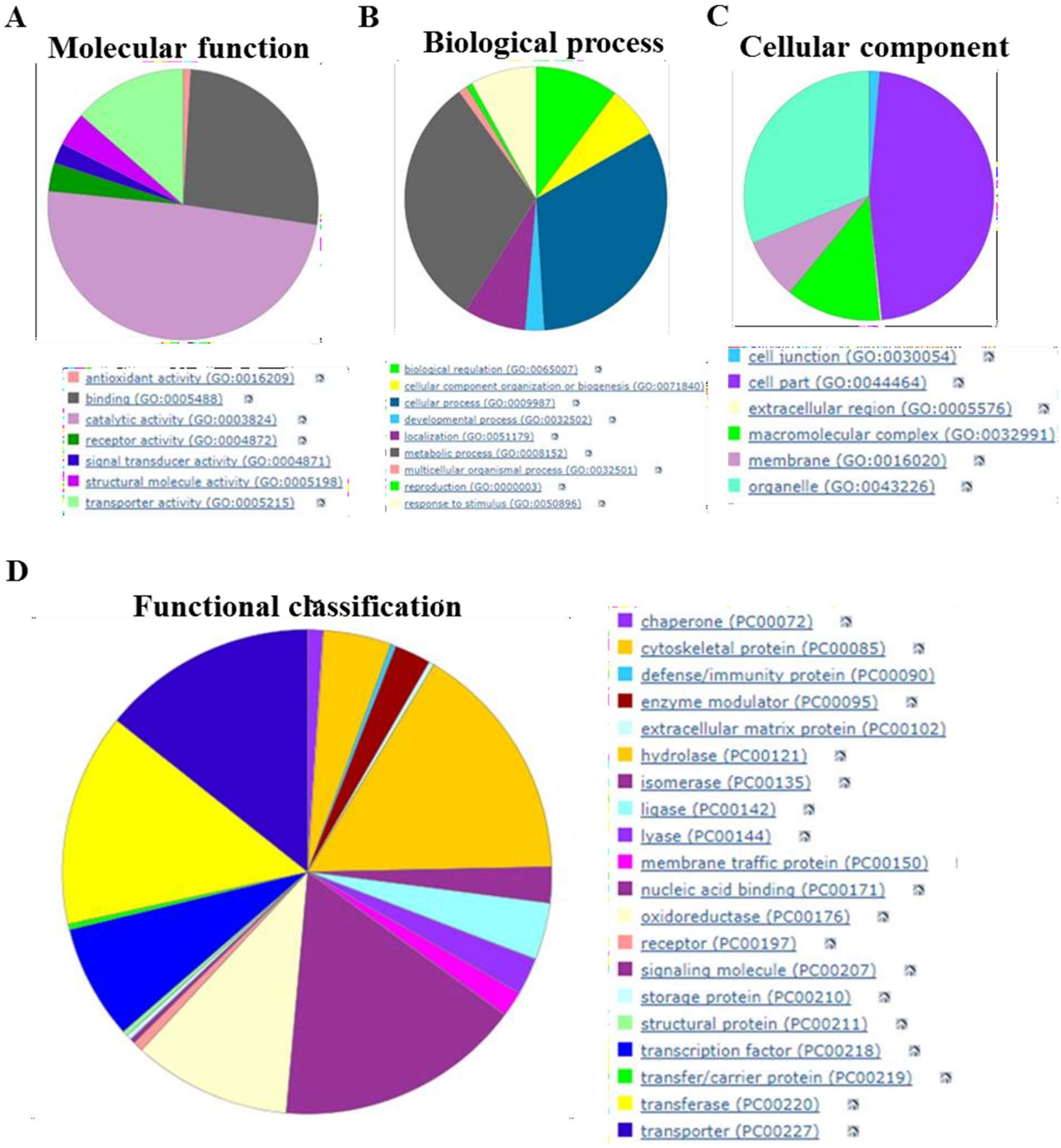
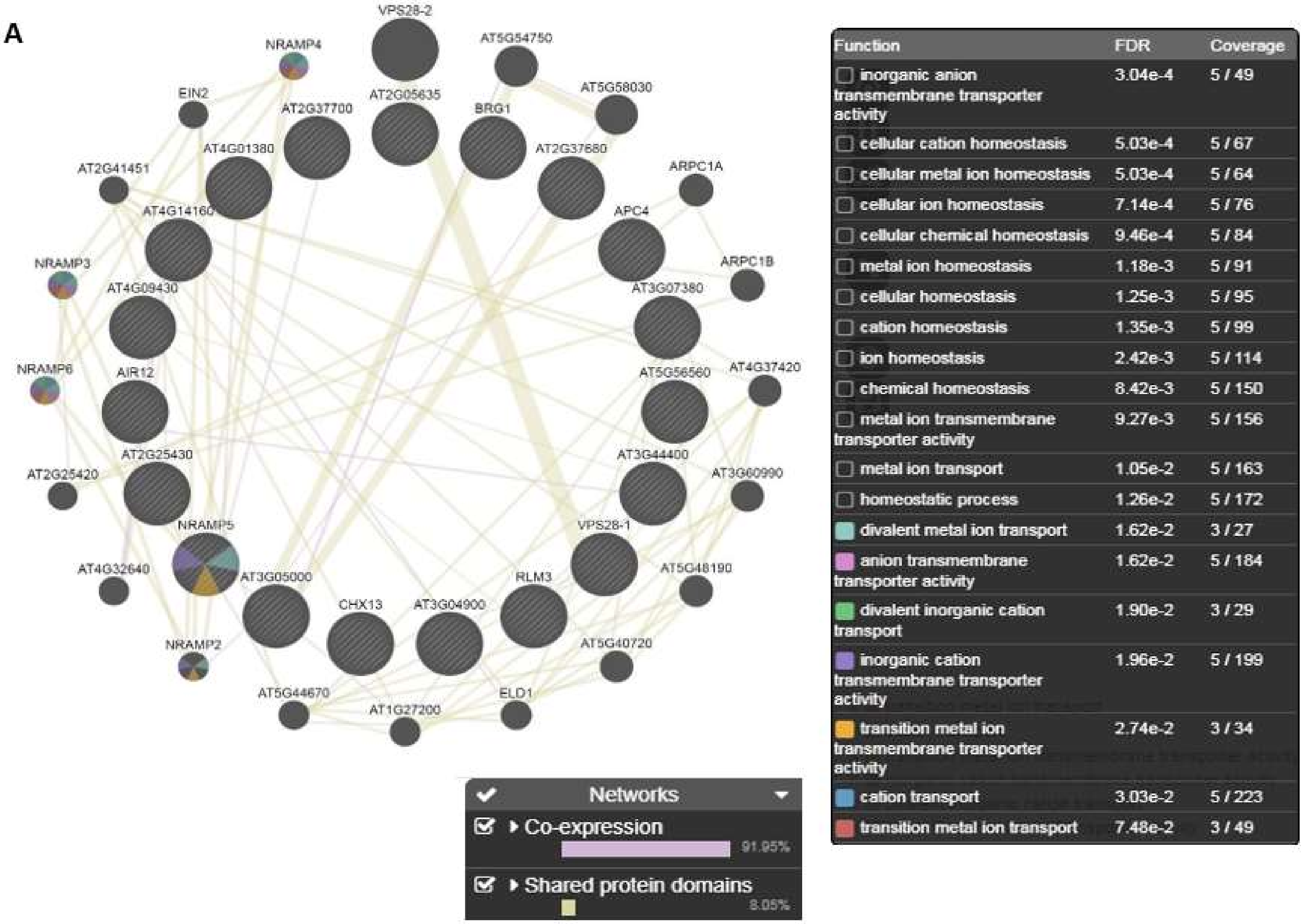


Figure S4. Gene ontology enrichment by Panther classification of the 737 genes representing significant SNPs analyzed for molecular function (**A**), biological process (**B**), cellular components (**C**). Further classification of the molecular functions of the genes (**D**).



Function	FDR	Coverage
☐ inorganic anion transmembrane transporter activity	3.04e-4	5 / 49
☐ cellular cation homeostasis	5.03e-4	5 / 67
☐ cellular metal ion homeostasis	5.03e-4	5 / 64
☐ cellular ion homeostasis	7.14e-4	5 / 76
☐ cellular chemical homeostasis	9.46e-4	5 / 84
☐ metal ion homeostasis	1.18e-3	5 / 91
☐ cellular homeostasis	1.25e-3	5 / 95
☐ cation homeostasis	1.35e-3	5 / 99
☐ ion homeostasis	2.42e-3	5 / 114
☐ chemical homeostasis	8.42e-3	5 / 150
☐ metal ion transmembrane transporter activity	9.27e-3	5 / 156
☐ metal ion transport	1.05e-2	5 / 163
☐ homeostatic process	1.26e-2	5 / 172
🟦 divalent metal ion transport	1.62e-2	3 / 27
🟪 anion transmembrane transporter activity	1.62e-2	5 / 184
🟩 divalent inorganic cation transport	1.90e-2	3 / 29
🟪 inorganic cation transmembrane transporter activity	1.96e-2	5 / 199
🟧 transition metal ion transmembrane transporter activity	2.74e-2	3 / 34
🟦 cation transport	3.03e-2	5 / 223
🟥 transition metal ion transport	7.48e-2	3 / 49

Figure S5. A. Gene network interactions of top 20 candidates associated with primary root length trait.

B

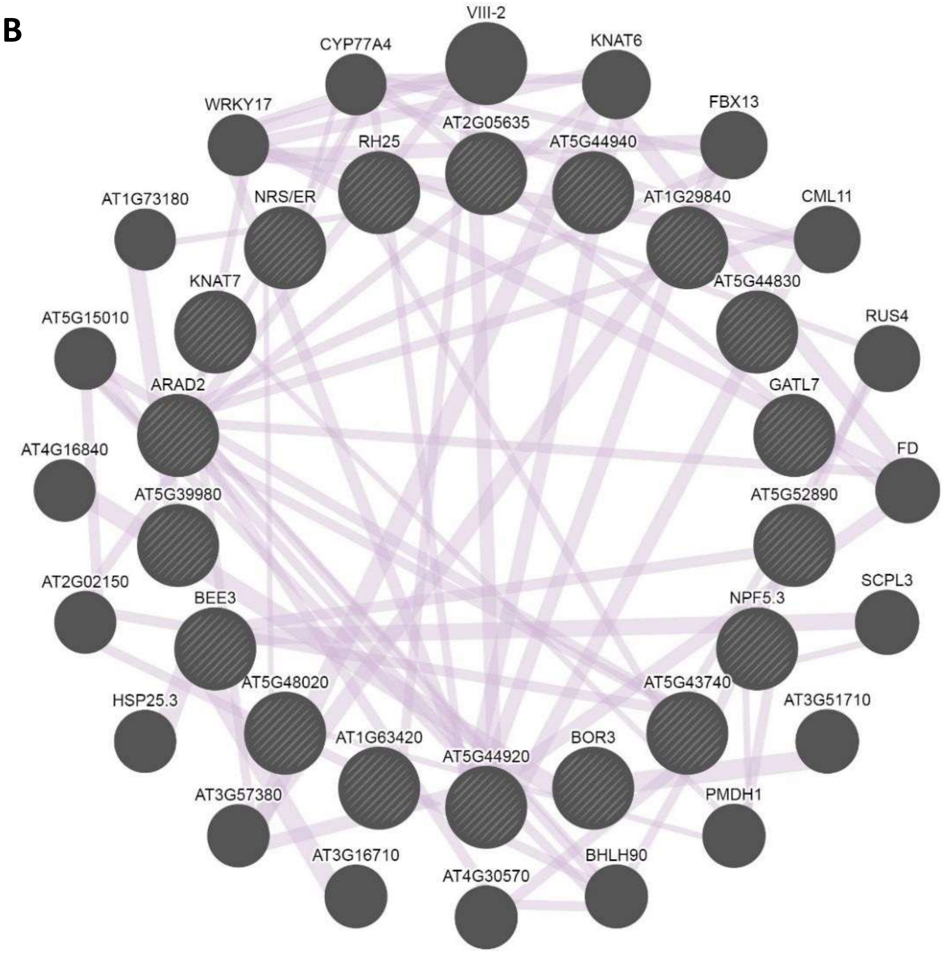


Figure S5. B. Gene network interactions of top 20 candidates associated with cumulative root length trait.

C

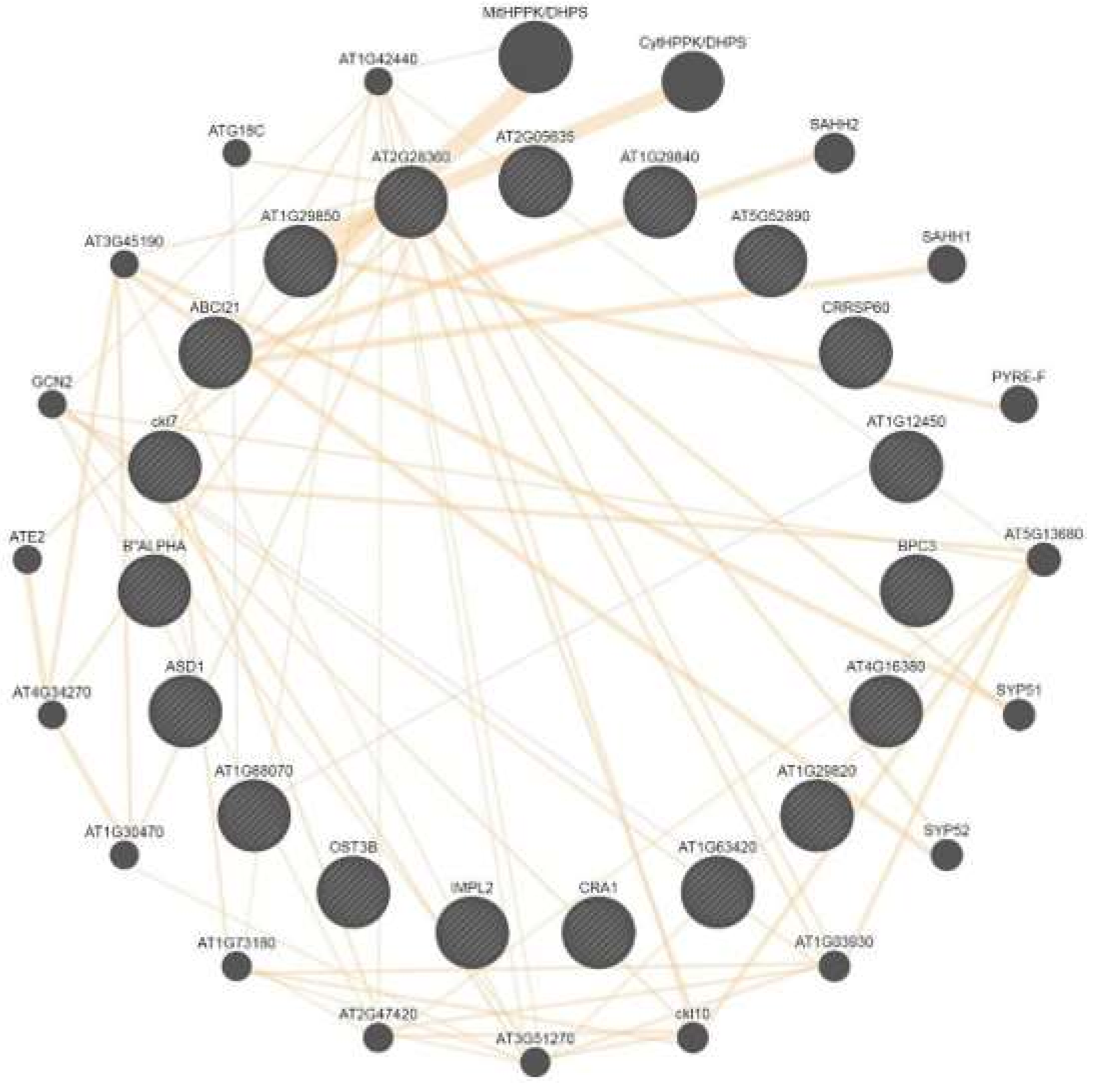
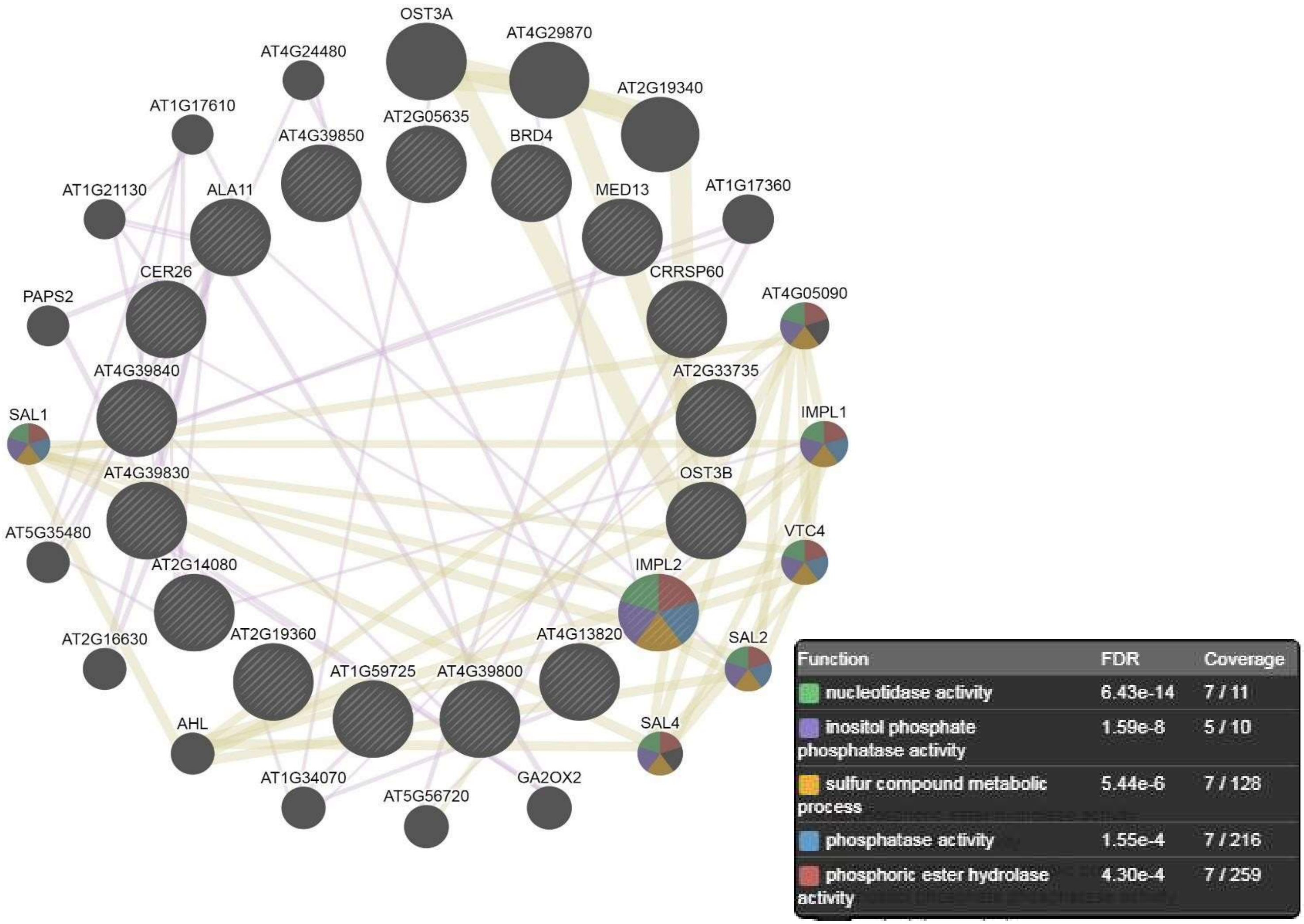


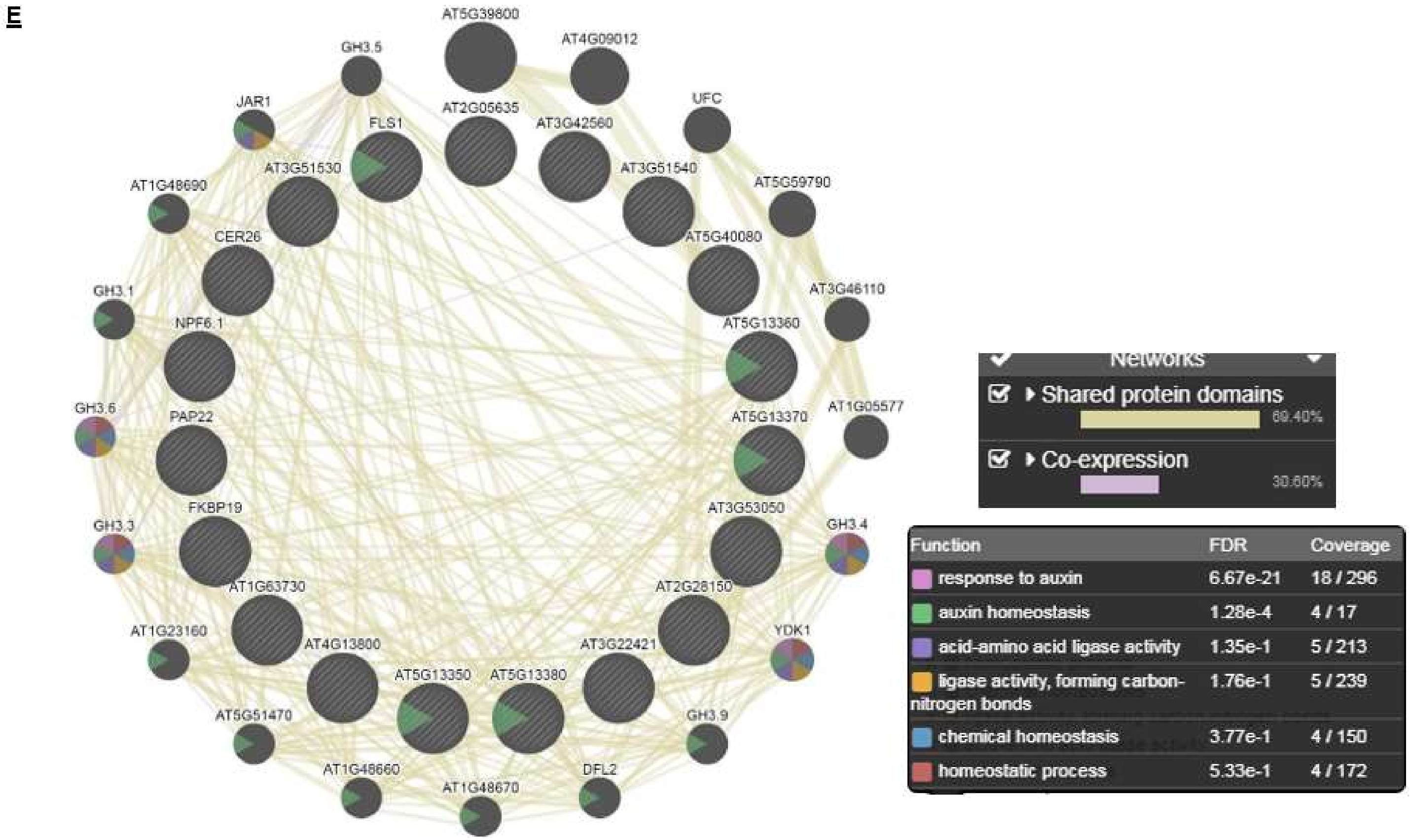
Figure S5. C. Gene network interactions of top 20 candidates associated with root surface area trait.

D



Function	FDR	Coverage
🟩 nucleotidase activity	6.43e-14	7 / 11
🟪 inositol phosphate phosphatase activity	1.59e-8	5 / 10
🟧 sulfur compound metabolic process	5.44e-6	7 / 128
🟦 phosphatase activity	1.55e-4	7 / 216
🟥 phosphoric ester hydrolase activity	4.30e-4	7 / 259

Figure S5. D. Gene network interactions of top 20 candidates associated with root volume trait.



Networks

☑ Shared protein domains 69.40%

☑ Co-expression 30.60%

Function	FDR	Coverage
response to auxin	6.67e-21	18 / 296
auxin homeostasis	1.28e-4	4 / 17
acid-amino acid ligase activity	1.35e-1	5 / 213
ligase activity, forming carbon-nitrogen bonds	1.76e-1	5 / 239
chemical homeostasis	3.77e-1	4 / 150
homeostatic process	5.33e-1	4 / 172

Figure S5. E. Gene network interactions of top 20 candidates associated with average root diameter trait.

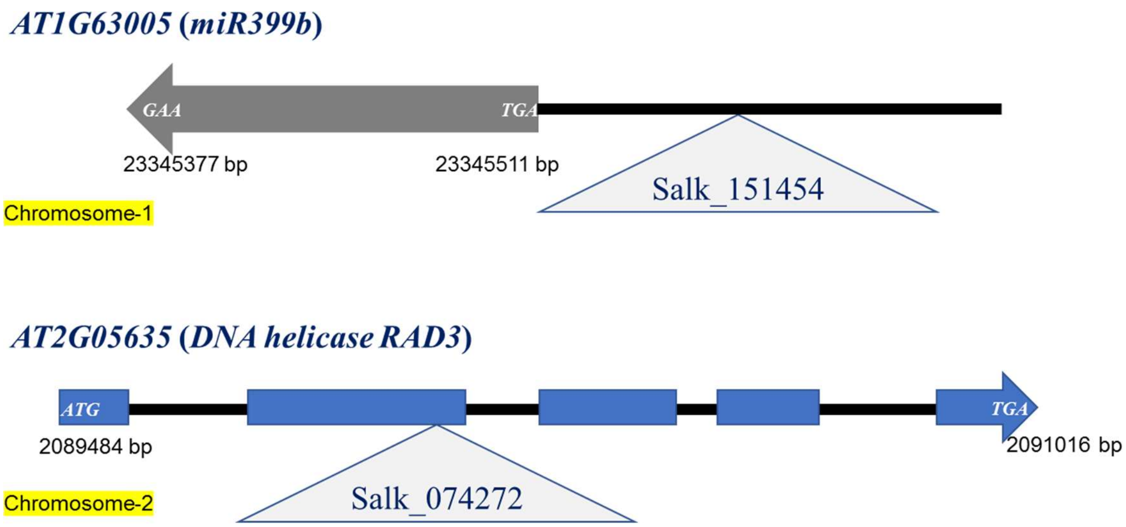


Figure S6. Pictorial diagram showing T-DNA insertion positions on *AT1G63005* and *AT2G05635* genes.

Table S1. Top five ecotypes showed highest and lowest root phenotypic variation.

Ecotypes	**Cumulative Root Length (cm)**	**Genotype**	**Surface area (cm^2)**	**Genotype**	**Ave. Diameter (mm)**	**Genotype**	**Root Volume (cm^3)**	**Genotype**	**Primary Root length (cm)**
Superior Performers									
CS77315	1533	CS77315	133.8	CS77299	0.46	CS77315	0.93	CS77009	36.00
CS76643	1281	CS77320	117.0	CS77136	0.46	CS77320	0.93	CS76861	34.17
CS78772	1250	CS78818	103.5	CS77137	0.44	CS78818	0.90	CS76932	32.50
CS78769	1227	CS78830	99.2	CS77270	0.40	CS77145	0.85	CS76863	32.43
CS77348	1096	CS77146	90.1	CS77060	0.39	CS77146	0.85	CS76986	32.23
Inferior Performers									
CS77359	153	CS76908	14.6	CS76806	0.17	CS76834	0.07	CS77268	11.47
CS77048	162	CS76834	13.8	CS76830	0.17	CS76826	0.07	CS77275	10.93
CS76827	182	CS77359	10.6	CS76826	0.16	CS76908	0.06	CS77304	10.17
CS77050	203	CS77048	10.0	CS76908	0.16	CS77048	0.04	CS77305	9.87
CS77289	206	CS76827	8.1	CS76827	0.16	CS76827	0.03	CS77048	7.93

Table S2. Analysis of variance (ANOVA) of multiple root traits.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Length	Between Groups	30938771.438	164	188651.045	18.155	0.000
	Within Groups	3740785.944	360	10391.072		
	Total	34679557.383	524			
Surfacearea	Between Groups	226174.790	164	1379.115	14.524	0.000
	Within Groups	34184.548	360	94.957		
	Total	260359.338	524			
Avgdia	Between Groups	1.875	164	0.011	7.331	0.000
	Within Groups	0.562	360	0.002		
	Total	2.437	524			
RootVol	Between Groups	16.818	164	0.103	10.266	0.000
	Within Groups	3.596	360	0.010		
	Total	20.414	524			
ShootFW	Between Groups	464.626	164	2.833	11.423	0.000
	Within Groups	89.287	360	0.248		
	Total	553.913	524			
RootL	Between Groups	19377.801	164	118.157	15.874	0.000
	Within Groups	2679.672	360	7.444		
	Total	22057.474	524			

Table S3. Top 20 significant SNPs associated with different root traits.

Primary root length								
Chr	**Position**	***p*-value**	**TestStat**	**Beta0**	**Beta1**	**MAF**	**Gene**	**Gene function**
Chr1	23345414	5.78e-06	10.972	23.014	3.821	0.221	At1g63005	Microrna399b
Chr4	8169552	6.78e-06	10.234	22.213	2.786	0.198	At4g14160	Sec23/Sec24 protein
Chr4	10318421	7.34e-06	9.879	19.987	3.134	0.189	At4g18790	Member of Nramp2 family
Chr4	5974306	8.12e-06	9.825	21.089	2.123	0.179	At4g09430	Disease resistance protein
Chr2	15805079	1.16e-05	9.614	19.435	3.101	0.162	At2g37680	Glucose-induced
Chr2	15810955	1.16e-05	9.614	19.435	3.101	0.162	At2g37700	Fatty acid hydroxylase
Chr4	9565257	1.56e-05	9.332	21.817	-2.503	0.275	At4g16990	Resistance to leptosphaeria
Chr3	16044911	5.28e-05	8.173	19.896	2.827	0.096	At3g44400	Disease resistance protein
Chr3	1349568	5.71e-05	8.099	19.769	2.539	0.132	At3g04900	Heavy metal
Chr3	1388495	6.21e-05	8.019	21.335	-2.996	0.150	At3g05000	Transport protein particle
Chr2	10822583	6.40e-05	7.990	19.629	2.939	0.138	At2g25430	Epsin n-terminal homology
Chr4	11468866	6.55e-05	7.969	19.796	2.682	0.120	At4g21560	Vacuolar protein sorting 28
Chr3	2362006	7.11e-05	7.891	21.156	-2.855	0.126	At3g07380	Glycosyltransferase family
Chr4	569954	7.29e-05	7.867	19.448	2.850	0.174	At4g01380	Plastocyanin-like domain-
Chr5	22901770	8.40e-05	7.733	19.830	2.674	0.114	At5g56560	Fbd, f-box and leucine rich
Chr2	2089769	5.31e-06	7.726	19.946	2.428	0.197	At2g05635	DNA repair DEAD helicase
Chr4	11453934	9.67e-05	7.600	19.832	2.664	0.114	At4g21530	Anaphase promoting
Chr5	18219139	1.02e-04	7.552	21.757	-2.039	0.323	At5g45100	Boi-related gene 1
Chr3	2365769	1.05e-04	7.520	20.901	-3.513	0.066	At3g07390	Auxin-induced in root
Chr2	12902361	1.08e-04	7.494	19.717	3.012	0.120	At2g30240	Atchx13, cation/h+
Cumulative root length								
Chr1	23345414	1.88e-06	11.881	413.01	112.951	0.283	At1g63005	Microrna399b
Chr2	2089769	2.37e-06	11.756	415.94	80.539	0.257	At2g05635	DNA repair DEAD helicase
Chr3	1979512	2.42e-06	11.237	345.67	101.654	0.198	At3g06450	HCO3- transporter family
Chr5	2796339	2.88e-06	10.874	198.78	112.450	0.227	At5g08620	STRESS RESPONSE
Chr3	9878645	3.82e-06	10.678	561.46	211.494	0.054	At3g26819	Microrna169n
Chr5	18141321	9.74e-06	9.781	513.01	92.951	0.383	At5g44930	Arabinan deficient 2
Chr5	18150355	1.37e-05	9.456	515.94	90.539	0.377	At5g44940	F-box/rni-like superfamily
Chr1	10446552	1.46e-05	9.393	401.10	147.970	0.084	At1g29840	Alpha/beta-hydrolases
Chr5	18673226	1.59e-05	9.313	122.91	94.613	0.323	At5g46040	Major facilitator
Chr5	21447074	1.83e-05	9.181	633.49	-93.441	0.263	At5g52890	At hook motif-containing
Chr5	17566694	2.97e-05	8.717	631.64	-92.015	0.257	At5g43740	Disease resistance protein
Chr3	23173163	3.33e-05	8.610	551.31	110.027	0.150	At3g62660	Galacturonosyltransferase-
Chr5	16002119	3.39e-05	8.592	552.60	120.119	0.132	At5g39980	Pigment-defective mutant3
Chr5	19464368	3.59e-05	8.538	541.93	110.434	0.192	At5g48020	2-oxoglutarate and fe
Chr5	18101364	3.64e-05	8.526	536.27	91.060	0.263	At5g44830	Pectin lyase-like
Chr1	23518484	4.60e-05	8.304	542.41	120.475	0.174	At1g63420	O-glucosyltransferase-like
Chr1	27760696	4.71e-05	8.280	566.30	166.553	0.054	At1g73830	Br enhanced expression 3
Chr1	23340165	7.26e-05	7.871	452.51	139.188	0.084	At1g62990	Irregular xylem 11
Chr1	23343168	7.26e-05	7.871	452.516	139.188	0.084	At1g63000	Udp-4-keto-6-deoxy-d-glucose-3,5-epimerase-4-

Chr5	18138946	5.99e-05	8.053	665.02	-81.253	0.497	At5g44920	Tir-kash protein involved in
Root surface area								
Chr1	4244990	1.12e-06	11.971	51.456	12.567	0.179	At1g12450	SNARE associated Golgi
Chr4	9255532	1.33e-06	11.278	43.789	14.122	0.149	At4g16380	Heavy metal
Chr1	10439051	2.18e-06	11.123	47.491	12.556	0.098	At1g29820	Magnesium transporter
Chr3	3362581	2.88e-06	10.998	52.258	13.549	0.132	At3g10740	Encodes a bifunctional alpha-l-
Chr1	10446552	3.32e-06	10.810	46.978	13.508	0.084	At1g29840	Alpha/beta-hydrolases
Chr1	22814875	5.34e-06	10.356	47.221	14.074	0.072	At1g61790	Oligosaccharyltransferase
Chr4	18225877	5.69e-06	10.295	47.315	14.634	0.066	At4g39120	Histidine biosynthesis 7
Chr1	23345414	6.44e-06	9.842	53.009	14.443	0.188	At1g63005	Microrna399b
Chr2	2089769	1.12e-05	9.833	45.213	13.234	0.145	At2g05635	DNA repair DEAD helicase
Chr1	10449946	1.16e-05	9.611	47.247	12.819	0.078	At1g29850	Programmed cell death
Chr5	17748147	2.10e-05	9.048	46.037	12.168	0.132	At5g44090	Calcium-binding ef-hand
Chr5	17749166	2.10e-05	9.048	46.037	12.168	0.132	At5g44100	Casein kinase i-like 7
Chr5	17754825	2.10e-05	9.048	46.037	12.168	0.132	At5g44110	Atp-binding cassette a21
Chr5	17756447	2.10e-05	9.048	46.037	12.168	0.132	At5g44120	Cra1
Chr2	12126944	3.89e-05	8.463	44.243	8.187	0.305	At2g28360	Sit4 phosphatase-associated
Chr5	21447074	4.65e-05	8.292	53.210	-7.528	0.263	At5g52890	At hook motif-containing
Chr1	23518484	6.24e-05	8.014	45.725	10.129	0.174	At1g63420	O-glucosyltransferase-like
Chr1	25516286	6.38e-05	7.994	52.106	-8.853	0.162	At1g68070	Zinc finger, c3hc4 type ring
Chr1	25526656	6.81e-05	7.931	52.194	-8.799	0.168	At1g68120	Basic pentacysteine protein
Chr5	14960677	7.32e-05	7.863	47.673	14.565	0.054	At5g37660	Plasmodesmata-located
Root volume								
Chr2	8378956	2.35e-07	12.342	0.287	0.078	0.213	At2g19360	Protein of Unknown
Chr4	8014109	4.37e-07	12.114	0.332	0.118	0.354	At4g13840	HXXXD-type acyl-
Chr1	4509732	1.34e-06	11.889	0.306	0.079	0.099	At1g13210	autoinhibited Ca2+/ATPase
Chr1	23345414	1.69e-06	11.783	0.354	0.145	0.179	At1g63005	Microrna399b
Chr5	14960677	1.72e-06	11.773	0.336	0.153	0.054	At5g37660	Plasmodesmata-located
Chr4	18225877	1.76e-06	11.423	0.335	0.134	0.066	At4g39120	Histidine biosynthesis 7
Chr4	8008049	2.78e-06	10.982	0.290	0.065	0.479	At4g13820	Leucine-rich repeat lrr
Chr2	2089769	3.45e-06	10.443	0.353	0.123	0.257	At2g05635	DNA repair DEAD helicase
Chr4	8014109	5.58e-06	10.314	0.376	-0.100	0.120	At4g13840	Cer26
Chr1	22814875	1.50e-05	9.367	-0.131	0.118	0.072	At1g61790	Oligosaccharyltransferase
Chr2	5929527	1.93e-05	9.128	0.296	0.110	0.078	At2g14080	Disease resistance protein
Chr2	14269142	3.12e-05	8.672	-0.042	0.102	0.084	At2g33735	Chaperone dnaj-domain
Chr1	22573729	3.23e-05	8.639	0.307	0.066	0.287	At1g61215	Bromodomain 4
Chr1	20648069	3.40e-05	8.590	0.054	0.115	0.072	At1g55325	Mediator of rna polymerase
Chr4	18471932	3.55e-05	8.548	-0.098	0.089	0.156	At4g39800	Inositol 3-phosphate
Chr4	18479378	3.55e-05	8.548	-0.098	0.089	0.156	At4g39830	Cupredoxin superfamily
Chr4	18485513	3.55e-05	8.548	-0.098	0.089	0.156	At4g39838	Potential natural antisense gene, locus overlaps with
Chr4	18486544	3.55e-05	8.548	-0.098	0.089	0.156	At4g39840	Cell wall integrity/stress response component-like

Chr4	18489909	3.55e-05	8.548	-0.098	0.089	0.156	At4g39850	Atp-binding cassette d1
Chr1	21951156	3.67e-05	8.518	0.326	0.093	0.138	At1g59725	Dnaj heat shock family
Average root diameter								
Chr1	23642153	2.33e-07	12.453	0.268	0.045	0.315	At1g63730	Disease resistance protein
Chr5	2800515	2.78e-07	12.233	0.277	0.038	0.230	At5g08640	DDT domain-containing
Chr2	12001039	3.67e-07	12.176	0.297	0.027	0.198	At2g28150	Protein of unknown
Chr5	16040827	4.01e-07	12.089	0.212	0.049	0.130	At5g40080	Mitochondrial ribosomal
Chr2	2089769	4.31e-07	11.994	0.314	0.049	0.369	At2g05635	DNA repair DEAD helicase
Chr4	8014109	1.02e-06	11.941	0.287	-0.036	0.120	At4g13840	Cer26
Chr4	8001922	5.04e-06	10.411	0.089	-0.024	0.293	At4g13800	Magnesium transporter
Chr3	19114519	8.12e-06	9.955	0.272	0.050	0.054	At3g51530	F-box/rni-like/fbd-like
Chr3	19117045	8.12e-06	9.955	0.272	0.050	0.054	At3g51540	Mucin-5ac-like protein
Chr1	23345414	9.32e-06	9.883	0.263	0.043	0.313	At1g63005	Microrna399b
Chr3	19575910	1.05e-05	9.708	0.261	0.025	0.347	At3g52820	Purple acid phosphatase 22
Chr3	19669649	2.02e-05	9.085	0.270	0.034	0.114	At3g53050	D-galactoside/l-rhamnose
Chr5	4283945	2.35e-05	8.940	0.272	0.040	0.078	At5g13360	Auxin-responsive gh3
Chr5	4288362	2.35e-05	8.940	0.272	0.040	0.078	At5g13370	Auxin-responsive gh3
Chr5	4290313	2.35e-05	8.940	0.272	0.040	0.078	At5g13380	Auxin-responsive gh3
Chr5	4299175	2.35e-05	8.940	0.272	0.040	0.078	At5g13400	Major facilitator
Chr5	4299756	2.35e-05	8.940	0.272	0.040	0.078	At5g13410	Fkbp-like peptidyl-prolyl
Chr3	7949384	2.60e-05	8.847	0.296	-0.022	0.413	At3g22421	F-box/associated interaction
Chr5	4281420	2.98e-05	8.716	0.271	0.037	0.090	At5g13350	Auxin-responsive gh3
Chr3	14680488	3.73e-05	8.502	0.272	0.035	0.084	At3g42560	Pentatricopeptide repeat

*TestStatt- calculate F-type test statistics for a general linear hypothesis for each of G genes. * Beta0 and Beta1 are resulting coefficient from a fit and SE *MFA-minor allele frequency

Table S4. Primers used for genotyping and RT-qPCR analyses in this study.

Locus	T-DNA mutants*	Forward	Reverse
At1g63005	Salk_151454	TGGGAGGTCATCCTACCAAG	AGGCGCCCTAAAACTAGTGAG
At2g05635	Salk_074272	CCTGTCATCATCATGCAACTC	TCAGTTTTTATGGTAAAGTTTAATTGG
Border primer	LBb1.3	ATTTTGCCGATTTCGGAAC	
		Primers for gene expression studies	
At1g63005	RT-qPCR	AGGGCGCCTCTCCATTG	TCGATCATCGGAAATTTTCGA
At2g05635	RT-qPCR	TTCGTGAGTATAGGAAAACTGGTTACA	CATGAACACCTAATCCTCCTACCAA
Actin8	RT-qPCR	CCCAAAAGCCAACAGAGAGA	CATCACCAGAGTCCAACACAAT

*-claimed to be homozygous T-DNA mutants from ABRC (http://abrc.osu.edu).