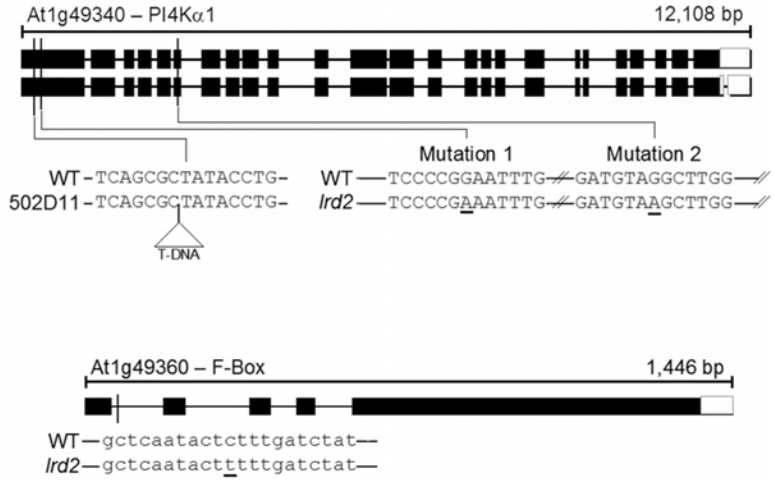
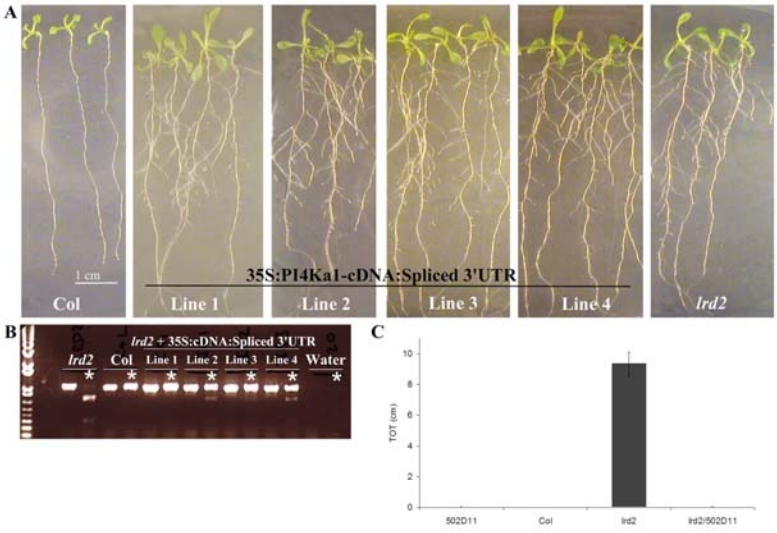
Supplemental Data. MacGregor et al. (2008). Root system architecture in *Arabidopsis* grown in culture is regulated by sucrose uptake in the aerial tissues.



Supplemental Figure 1. *lrd2* plants carry additional EMS-induced mutations. The gene structure of *PI4Kα1* and the F-box protein and the sites of the mutations in *lrd2* are shown. Black boxes represent exons, the black line represents introns, and white boxes represent untranslated regions. Positions of the mutated bases in each allele are indicated by a vertical line. Slashes indicate gaps in the sequence. Mutated bases are underlined and the position of the T-DNA insert in the 502D11 allele is indicated by a labeled triangle. Uppercase letters denote exon sequence while lowercase denote intron sequence. Two 3'UTR splice variants of PI4Kα1 are shown. Length of the genomic coding sequence in base pairs (bp) is indicated. The mutation in At1g49360 is not predicted to alter mRNA splicing or the amino acid sequence of the protein, making it unlikely that this mutation causes the *lrd2* phenotype. In contrast, two point mutations found in At1g49340/PI4Kα1 are predicted to cause glutamic acid-to-lysine and glycine-to-serine substitutions in the first and sixth exons, respectively.



Supplemental Figure 2. The mutations in PI4K 1 do not contribute to the lrd2 phenotype. **A**. Four independent transgenic lines expressing the wildtype PI4Kα1 cDNA with the spliced 3'UTR driven by a CaMV 35S promoter do not rescue the *lrd2* root phenotype. Wildtype (Col), *lrd2*, and T2's from four independently transformed lines were grown for 12 days on mild osmotic stress conditions. Three to four plants from each genotype are shown. Similar data were collected for multiple independent transformants and in transformants in which *PI4Ka1* expression was driven by the endogenous promoter; none showed a rescue of the *lrd2* phenotype (see Supplemental Table 1 online). **B**. RT-PCR shows that the wildtype cDNA is expressed in the transgenic lines shown in **A**. The labels indicate the source of the input cDNA. A water control is also shown. Stars (*) indicate lanes in which HindIII digested amplified DNA was run; remaining lanes show uncut DNA. The piece of cDNA that was amplified contains a HindIII cut site created by the *lrd2* mutation. RNA resulting from the over-expressed transgenic cDNA will not cut with HindIII (upper bands) while RNA resulting from endogenous PI4Kα1 expression will cut with HindIII (faint lower bands). Similar RT-PCR results were seen for other transgenic lines indicating the different constructs were also expressed (data not shown). **C**. Trans-heterozygous plants carrying the *lrd2* and *502D11* alleles of PI4Kα1 (from the GABI-KAT collection, Li *et al*., 2007b) have wildtype levels of lateral root formation. Total lateral root lengths were quantified for the parental lines (502D11, wildtype (Col), and *lrd2*) and F1 plants resulting from a cross between 502D11 heterozygote and *lrd2*. Bars represent average of 25 or more plants per genotype ± SE. Three independent F1 populations showed similar results. Only *lrd2* has a statistically different TOT from wildtype (Col) (Student's T-test, $P \leq 0.00001$).

Supplemental Table 1: Expression of the wild type PI4Kα1 cDNA sequences fail to rescue the *lrd2* phenotype.

Promoter	cDNA Type	3' UTR	Background	Selected T1 lines	Phenotyped T2 lines	In a Representative T2 Line [a]			
						WT-like	lrd2-like	Plants	% \
Untransformed Control			Col	Ø		28	0	28	
Untransformed Control			*lrd2*	Ø		2	30	32	
Vector Control			Col	72	2	30	0	30	
Vector Control			*lrd2*	29	1	0	28	28	
35S	WT	Spliced	*lrd2*	20	4	3	41	44	
35S	WT	Unspliced	*lrd2*	17	8	2	33	35	
Endogenous	WT	None	*lrd2*	7	4	2	47	49	
Endogenous	WT	Spliced	*lrd2*	11	10	0	37	37	
35S:GFP	WT	None	*lrd2*	24	2	3	25	28	
35S:GFP	lrd2	None	*lrd2*	16	8	2	27	29	
35S	lrd2	None	Col	26	10	35	1	36	

a: The cloned cDNA was driven by either the endogenous promoter (defined as the 1.6 Kb from the end of the previous gene to the start of the PI4Kα1) or the CaMV 35S promoter.

b: PI4Kα1 RNAs with spliced and unspliced 3'UTRs were identified by RT-PCR (MacGregor, unpublished). We attempted to rescue *lrd2* plants with cDNAs followed by the unspliced and the spliced 3'UTR variants and a variant with no 3'UTR (see Supplemental Figure 1).

c: Wild type-like was defined as a plant that had three or fewer visible lateral roots.

d: The phenotype of *lrd2* is probably not due to a dominant negative effect because overexpression of the *lrd2* cDNA in the wild type (Col) background does not recapitulate the increase in lateral root formation.

Supplemental Methods

Creation and Verification of the PI4Kα1 Rescue Constructs

The wild-type PI4Kα1 cDNA was PCR amplified in three overlapping pieces from a cDNA population made from 12-day-old Columbia wildtype seedlings. Piece 1 ran from the start codon 1 to 2,618 bp into the cDNA, Piece 2 from 2,359 bp to 4,556, and Piece 3 from 4,342 through the stop codon at 6,088. Each piece was cloned into pBluescript II KS- and sequenced. When amplification-induced mutations were identified as compared to the wildtype sequence from Columbia, these mutated bases were converted to the wild type base using the QuikChange® Site-Directed Mutagenesis Kit (Stratagene cat#200518). Two 3' UTR splice variants were amplified. Each was cloned separately. XbaI and BamHI sites at 2,521 and 4,476 bp respectively were used to anneal the three pieces. The complete wild-type cDNA encoding PI4Kα1 was cloned between NotI and EcoRI in pBluescript II KS- using restriction sites introduced on PCR primers. The primer sequences were as follows: Piece 1 cDNAF: GTCAGCGGCCGCATGGAGGCACTGACGGAGCT; Piece 1 cDNAR: AGATCTCGGCAATTGTGCATAGAGCCTT; Piece 2 cDNAF: TCTAGAGACTTGCTCTTTCAACTGCC; Piece 2 cDNAR: CCTAGGCTGAAGTCACTCAGCTGGTTCA; Piece 3 cDNAF: GGATCCTAATGCTCTGCCAGCATGAAGC; Piece3 cDNAR: GTCAGAATTCCTTACTTCTCGATGCCTTGTTGC.

The endogenous promoter, defined as the 1,319 bp between the start of At1g49340 and the stop of the previous gene, was cloned into pBluescript II KS-. The primers used to amplify this piece of DNA from total isolated wild type genomic DNA were F: GCTGACCGCGGCTCGAGGGCGCGCCTGAgttaggttgattctcc and R: GCTGACCCGGGGCGGCCGCctccgattaaacaaaaaaaacaacgttacggg. The Cauliflower mosaic virus (CaMV) 35S promoter from pCambia1380 was also cloned into the multiple cloning site of both pBluescript II KS-. This was done using F: GCTGACCGCGGCTCGAGGGCGCGCCcccaacatggtggagcacg and R: CGACTGCGGCCGCagagatagatttgtagagagag. The lowercase letters are the sequence of

the promoter and the uppercase letters are sequences that were added in order to facilitate cloning. The wild-type PI4aK cDNA was then moved into these new pBluescript vectors such that the 35S or endogenous promoter was driving the cDNA. Constructs were then moved from pBluescript II KS- into pCambia1380 using the AscI site introduced in the 5' end of the promoter and the SalI site introduced after the 3'UTR. The pCambia1380 vector contains a NOS terminator at the end of its multiple cloning site and therefore all constructs regardless of whether they had an endogenous 3'UTR, were terminated with the NOS.

All constructs were introduced into wild-type Columbia and *lrd2* plants by *Agrobacterium*-mediated transformation using standard methods (Supplemental Table 1 online). The transgenic seeds from both backgrounds were harvested in multiple pools to reduce the likelihood that siblings would be harvested, selected on plates supplemented with Hygromyicin (15mg/ml), and transplanted to soil. T_2 and the resulting T_3 generations were selected by PCR amplification using primers that differentiated between the endogenous copy and the transgenic cDNA copy of PI4Kα1.

Creation and Verification of the lrd2 / PI4αK (GABI-KAT 502D11) Trans-Heterozygote
Homozygous mutants carrying the 502D11 allele could not be obtained. Therefore, homozygous *lrd2* was used to pollinate emasculated flowers from 502D11 heterozygous plants and F1 progeny were analyzed to identify the trans-heterozygotes (expected to represent 50%). To confirm the presence of the chromosome carrying the 502D11 allele, we used a PCR forward primer specific for the GABI-Kat T-DNA (F: 5'ATATTGACCATCATACTCATTGC3') and a reverse primer specific to the PI4Kα1 gene (R: 5'ATCAGACTGCAGAATCGACG3'). To confirm the presence of the *lrd2* chromosome, we used markers created by the two mutations in PI4Kα1 in the *lrd2* mutant. The first leads to the absence of a MspI cut site in the same PCR fragment described above, and a second mutation creates a HindIII cut site in the fragment amplified using F: 5'TGAAAGCTTGGAAGAGGGTG3'; R: 5'AAGGCTCTATGCACAATTGCCG3".