

Supporting Methods

F₂ Analysis of *siz1-2* and *siz1-3* Backcross Progeny. F₂ analyses of *siz1-2* (Col-0 × *siz1-2*, $n = 292$ for $\chi^2 = 0.75$, $P = 0.39$) and *siz1-3* (Col-0 × *siz1-3*, $n = 248$ for $\chi^2 = 1.79$, $P = 0.18$) progeny established a segregation ratio of 3:1 for normal:short primary root length under phosphate (Pi) starvation conditions. This indicates that both *siz1-2* and *siz1-3* segregated as monogenic recessive alleles.

RNA Preparation and Quantitative PCR. Seeds were transferred directly into conical flasks containing basal medium [Murashige and Skoog (MS) micronutrients, 1/5× macronutrients without KH₂PO₄] with 1.25 mM KH₂PO₄ under a 16-h-light (30 μmol m⁻² s⁻¹)/8-h-dark cycle at 24°C with constant shaking (80 rpm) for seven days. Seedlings were washed twice with sterile water, transferred to medium without KH₂PO₄, and harvested after different periods of incubation. Total RNA was isolated by using the TRIzol reagent (Invitrogen). Two micrograms of total RNA was used as a template for first-strand cDNA synthesis with Superscript II (Invitrogen) and an oligo(dT) primer. One microliter of cDNA was used for semiquantitative RT-PCR (Fig. 3). The following primers were used: AtSIZ1-F (5'-GCTGACGTTTCAGGAGGTTTAGTTG-3'), AtSIZ1-R (5'-GCCTTGCTTGTCTACTGTCATTCATAC-3'), Tublin-F (5'CGTGGATCACAGCAATACAGAGCC-3'), and Tublin-R (5'CCTCCTGCACTTCCACTTCGTCTTC-3').

Gene-specific primer pairs for quantitative PCR were designed by using PRIMER3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and purchased from Qiagen (Valencia, CA) (Table 3). Hairpin stability and compatibility of these primers were also estimated by using OLIGO ANALYZER 3.0 (Integrated DNA Technologies, Coralville, IA). The PCR products were ≈150 bp in length and spanned the 3' regions of cDNA sequences. Quantitative PCR was performed in 20-μl reactions containing 5 μl of 50-fold diluted first-strand cDNA (1 μl of sample contains the first-strand cDNA obtained from 2 ng of total RNA), 0.3 μM each primer, and 1× QuantiTect SYBR Green PCR Master Mix (Qiagen). The following PCR program was used in the Applied Biosystems PRISM 7700 Sequence Detection System: 50°C for 2 min, 95°C for 10 min, then 40 cycles of 95°C for 15 s, 60°C for 1 min, and 72°C for 1 min. Primer efficiencies were measured and relative expression level was calculated by using the comparative C_T method (User Bulletin 2 for ABI Prism 7700 Sequence Detection System). 2^{-ΔΔC_T} of wild type without Pi starvation (0 h) was normalized to 1.

Protoplast Preparation and Intracellular Localization. *AtSIZ1* coding region was inserted into the plasmid p326-SGFP (kindly provide by Dr. I. Hwang, Pohang University of Science and Technology, Pohang, Korea) to create a chimeric *AtSIZ1:GFP* fusion under the control of the 35S promoter. Preparation and transformation of *Arabidopsis* protoplasts and analysis were performed as described (1). The red fluorescent protein (RFP) (control) is fused to the consensus nuclear localization signal of the simian virus 40 large T antigen (2).

AtSIZ1-GFP or NLS-RFP fluorescence (Fig. 8A) was monitored by using fluorescence microscopy (Axioplan 2, Zeiss), and the images were processed using an automatic imaging system (FISH, Zeiss). The filter sets, XF116 (exciter, 474AF20; dichroic, 500DRLP; and emitter, 510AF23) and XF33/E (exciter, 535DF35; dichroic, 570DRLP; and emitter, 605DF50) for GFP and RFP, respectively, were used. AtSIZ1-GFP or autofluorescence of chlorophyll (Fig. 8B) was monitored by using confocal laser scanning biological microscope (FV500 configuration with IX71 microscope, Olympus). The filter sets used were U-MGFPHQ mirror unit (exciter, 460~480HQ; dichroic, 485; and emitter 495~540HQ) and U-MWIG2 (exciter, 520~550; dichroic, 565; and emitter, 580IF) for AtSIZ1-GFP and autofluorescence of chlorophyll, respectively.

1. Shin, D., Koo, Y. D., Lee, J., Lee, H., Baek, D., Lee, S., Cheon, C.-I., Kwak, S.-S., Lee, S. Y. & Yun, D.-J. (2004) *Biochem. Biophys. Res. Comm.* **323**, 534–540.
2. Lee, Y. J., Kim, D. H., Kim, Y. W. & Hwang, I. (2001) *Plant Cell* **13**, 2175–2190.