Supplementary Information for
Spatiotemporal analysis identifies ABF2 and ABF3 as key hubs of endodermal response to nitrate.

Orlando Contreras-López, Elena A. Vidal, Eleodoro Riveras, José M. Alvarez, Tomás C. Moyano, Erin E. Sparks, Joaquín Medina, Angelo Pasquino, Philip N. Benfey, Gloria M. Coruzzi, Rodrigo A. Gutiérrez

Corresponding author name: Rodrigo A. Gutiérrez
Email: rgutierrez@bio.puc.cl

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- Datasets S1 to S7
Fig. S1. Cell sorting captures the transcriptomic profile of specific cell types of the Arabidopsis root. A list of enriched genes in epidermis (pWER::GFP (1)), pericycle (E3754 (2)) and stele (pWOL::GFP (3)) was obtained from cell specific transcriptomic data from Bargmann et al., 2013 (4) using the Template Matching Tool available in the MeV software (mev.tm4.org). The expression of this list of enriched genes was then determined in cell-specific data from Gifford et al., (2) and this work. For Gifford et al., 2008, samples were obtained for epidermis-cortex (E1001 (2)), endodermis-pericycle (E470 (2)), pericycle (E3754 (2)) and stele (pWOL::GFP (3)). Samples correspond to 12-day old Arabidopsis seedlings treated for 2 hours with 5 mM KNO₃ or 5 mM KCl as control. For Bargmann et al., 2013, samples correspond to one week Arabidopsis seedlings, treated for 2 hours with 5 µM indole-3-acetic acid (IAA) or solvent alone as control (mock). We show the experimental conditions for each study. In the heatmap, we show the z-score of the mean expression of each gene. EPI: epidermis; END: endodermis; PER: pericycle; STE: stele.
Fig. S2. The whole organ transcriptome profile can be captured at the cellular level. All cell type profiles from roots were combined into a single pseudo-bulk profile and compared with the whole root profile obtained without protoplast generation. Each point represents a unique gene and line showing the Spearman correlation between the whole root and pseudo-bulk expression.
Fig S3. Spatiotemporal approach captures known and novel nitrate-responsive genes. A) Genesect, a tool available in Virtualplant (http://virtualplant.bio.nyu.edu/cgi-bin/vpweb/) (5) was used to determine the gene overlap and the significance of this overlap between our list of nitrate-responsive genes for each cell type and lists of nitrate-responsive genes in roots determined in previous studies (6-11). The p-value of the intersection and the number of genes that overlap are indicated. B) Sungear analysis was used to identify common or unique genes regulated in each experiment (12). Each vertex in the polygon represents a study and the intersection among the sets is represented as circles. Significance of under or over-represented intersections between experiments is estimated using a binomial test.
**Fig. S4.** Protoplast generation control in nitrate response. A) Overlap of nitrate regulated genes over time obtained in this study with temporal, whole root data from Alvarez et al. 2019 (9). GeneSect analysis was used to calculate the significance of the gene intersects, being the Arabidopsis genome the background. Z score was colored in grayscale, with the percentage of captured genes in Alvarez et al. 2019 (9) indicated in each square. B) Real-time qPCR of sentinel nitrate-responsive genes in root protoplasts and whole roots after 2 hours and 3 hours of nitrate treatment. We show three biological replicates with standard error. Different letters indicate statistically different means (One-way ANOVA, p < 0.05).
Fig. S5. Validation of predicted spatiotemporal network. 85,144 TF-target interactions captured by the TARGET system in roots (13) were used to calculate the precision and recall over the spatiotemporal network. A) Histogram of the area under precision recall (AUPR) value for the GRN spatiotemporal predicted (0.39776) compared to a similar network based on randomized edges (mean=0.2429, median=0.2390). B) Comparison of predicted network with the network without filters of TF-target interaction and correlation value. AUPR values were assayed using 10,000 comparisons of the same number of edges; p < 0.05, permutation test.
Fig. S6. Distribution of shared and specific edges in our predicted GRN. A) The pie chart shows the percentage of edges shared between two or more cells and the distribution of specific edges occurring in only one cell type. B) Distribution of the shared edges (15% in panel A) using a Sungear analysis.
Fig. S7. Spatiotemporal response to nitrate of the top 10 connected TFs and their targets. The heatmaps show the nitrate regulation of ABF2 and ABF3 (A) or the other top 8 connected TFs (B) and their targets in each cell type and time point. Genes are induced (value of 1, blue), repressed (value of -1, yellow) or not regulated (0, black).
Fig. S8. ABF2 and ABF3 induction by nitrate treatment depends on NRT1.1. Arabidopsis Col-0, nitrate reductase null mutant (nr), NRT1.1 mutant chl1-5 and TGA1/TGA4 double mutant tga1/tga4 lines were grown hydroponically for 15 days with 0.5 mM ammonium succinate as the sole N source and were treated at the onset of day 15 with 5 mM KNO$_3$ or 5 mM KCl for 20 min. Root transcript levels for indicated genes were analyzed by real-time qPCR. The mean and standard deviation for three biological replicates is shown. Different letters indicate statistically different means (One-way ANOVA, p < 0.05).
Fig. S9. The ABF2 and ABF3 proteins are localized in the endodermis. Confocal microscopy of ABF2pro::GFP:ABF2 and ABF3pro::GFP:ABF3 reporter lines (14). Plants were grown hydroponically for two weeks with ammonium succinate as the only N source and observed on the 15th day. Plants were stained with propidium iodide and imaged by laser scanning confocal microscopy using the 20X objective on an Olympus Fluoview 1000 confocal microscope. (A and C) Longitudinal view of a mature section of the plant root. (B and D) Cross-sectional view of a mature section of the plant root. Scale bars are indicated in the Figure.
Fig. S10. ABF2 and ABF3 binding profile and overlap between ChIP-seq replicates. A) The number of normalized ChIP-seq reads mapping within the 1000-bp upstream region of the TSS to the 1000-bp downstream region of TTS. For each ABF2 or ABF3-bound region mapping to genes, the reads per kb per million sampled reads (RPKM) were calculated using the “bamCoverage” script in deepTools 2.0 (15). The figures were generated using the “plotProfile” functions in the deepTools 2.0 package. Red lines indicate the average, and the shade indicates the standard deviation of normalized sequencing reads in each position of TF-bound genes. B) Overlap between biological replicates of the ChIP-Seq experiments. The number of target genes determined per TF and per library, the number of genes in the overlap, the percentage of total genes in the overlap and p-value of the intersection (hypergeometric test) are indicated.

| ChIP-seq library | Number of genes R1 | Number of genes R2 | Number of genes overlap | % overlap | p-value  |
|------------------|--------------------|--------------------|-------------------------|-----------|---------|
| ABF2 N           | 1769               | 1442               | 798                     | 45.1      | 1.2e-620 |
| ABF2 K           | 2041               | 4175               | 1595                    | 78.1      | 5.5e-994 |
| ABF3 N           | 8709               | 4190               | 3784                    | 43.4      | 1.5e-1654|
| ABF3 K           | 10824              | 8546               | 7465                    | 69.0      | 1.4e-2742|
**Fig. S11.** ABF2 and ABF3 gene expression control in the epidermis and endodermis. A) Venn diagram analysis was used to compare the regulated genes of ABF2 and ABF3 between cells from epidermis and endodermis. B) Sungear analysis (12) was used to identify common or unique biological processes overrepresented between epidermis, endodermis, and shared genes regulated by ABF2 and ABF3. Each vertex in the polygon represents a list of biological processes and the intersection between the lists is represented as circles.
Fig. S12. GO term enrichment analysis of high confidence targets of ABF2 and ABF3 in endodermis. High confidence targets are nitrate-responsive genes in the endodermis that were captured by ChIP-seq and TARGET experiments for ABF2 or ABF3 (Fig. 3C). Overrepresented Biological Process terms were determined using GOstats (FDR<0.01, level 5).
Fig. S13. Spatiotemporal regulation of genes involved in ABA production and transport in response to nitrate. The heatmap shows the nitrate regulation of genes associated with ABA transport, ABA metabolic process, ABA biosynthetic process and ABA catabolic process. We show the genes induced (value of 1, blue), repressed (value of -1, yellow) or not regulated (0, black).
**Dataset S1.** Expression data of cell-specific genes. A list of enriched genes in epidermis (pWER::GFP (1)), pericycle (E3754 (2)) and stele (pWOL::GFP (3)) was obtained from cell specific transcriptomic data from Bargmann et al., 2013 (4) using the Template Matching Tool available in the MeV software (mev.tm4.org). The expression of this list of enriched genes was then determined in cell-specific data from Gifford et al., (2) and this work. For Gifford et al., 2008, samples were obtained for epidermis-cortex (E1001 (2)), endodermis-pericycle (E470 (2)), pericycle (E3754 (2)) and stele (pWOL::GFP (3)). We show the experimental conditions for each study. For Bargmann et al., 2013, samples correspond to one week Arabidopsis seedlings, treated for 2 hours with 5 µM indole-3-acetic acid (IAA) or solvent alone as control (mock). For Gifford et al., samples correspond to 12-day old Arabidopsis seedlings treated for 2 hours with 5 mM KNO₃ or 5 mM KCl as control. We show the z-score of the mean expression of each gene. EPI: epidermis; END: endodermis; PER: pericycle; STE: stele.

**Dataset S2.** List of nitrate-regulated genes in each cell type and time point analyzed in this work. We show for each gene its regulation (1: induced, -1: repressed, 0: no regulation) in each cell type and time point. EPI: epidermis; COR: cortex; END: endodermis; PER: pericycle; STE: stele.

**Dataset S3.** List of GO terms enriched in each cell type at each time point. Enriched GO terms were determined using GOstats with an adjusted p-value cut-off of p< 0.01. We show the list of GO terms and whether they are overrepresented (1) or not (0) in each cell type and time point. We also show the adjusted p-value calculated for each term and the number of genes associated to each term.

**Dataset S4.** Gene regulatory network information for Fig. 2A. We show the node and edge information used to build the gene regulatory network presented in Fig. 2A. For each node we show the number of cells in which the node is regulated, and its regulation in each cell type and time point (1: induced; -1: repressed; 0: no regulation). We also show the degree, indegree, outdegree, whether the node is a TF, and the normalized outdegree for TFs (number of targets for the TF in the network/number of possible targets according to protein-DNA interaction databases used). We show information for each edge in the network, including the TF and target identifiers, source of information (DAP-Seq, CIS-BP, AGRIS), total correlation between the TF-target pair (total correlation or correlation in specific cell types), and cell type in which the interaction is predicted (0: no interaction; 1: interaction). We also show the normalized edge value (total edges/number of TFs) for each cell type and time point. EPI: epidermis; COR: cortex, END: endodermis; PER: pericycle; STE: stele.

**Dataset S5.** TF-target interactions captured by yeast one-hybrid, and transcription factors and promoters cloned. We show the cDNA sequence for ABF2 and ABF3, the primers used to amplify the sequences, the promoter sequence cloned for each target gene and the primers used to clone them. For ABF2, we show the interactions validated by Y1H using the X-Gal assay. We did not find positive interactions for the ABF3 TF.

**Dataset S6.** ABF2 and ABF3 target genes identified by TARGET and/or ChIP-seq assays. For the TARGET results, we show for each ABF2 and ABF3 target the magnitude of TF-regulation (log₂ fold change, TF vector vs. empty vector), and the log₁₀ adjusted p-value, considering 3 independent
biological replicates (NA: gene does not meet the adjusted p-value threshold). For the ChIP results, we show for each ABF2 and ABF3 target whether a peak is detected (YES) or not (NO) in the promoter region (defined as 2 Kb upstream the TSS) in each ChIP-Seq library. We also show for each ChIP-Seq library the genomic regions bound by the TFs (Chromosome_start_end coordinates), the Q-value and Signal (the log2 fold change of enrichment of the peak vs. input).

**Dataset S7.** ABF2 and ABF3 target genes controlled by nitrate in the endodermis. ABF2 and ABF3 regulatory network data in endodermis. Nodes represent genes with differential gene expression in response to nitrate treatment in endodermis and the edges represent regulatory interactions determined by ChIP-Seq, TARGET and ChIP-Seq and TARGET. We also show the high confidence target genes for each TF (detected by ChIP-seq and TARGET).
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