
Figures and figure supplements

Arabidopsis transcriptome responses to low water potential using high-throughput plate assays

Stephen Gonzalez and Joseph Swift et al.

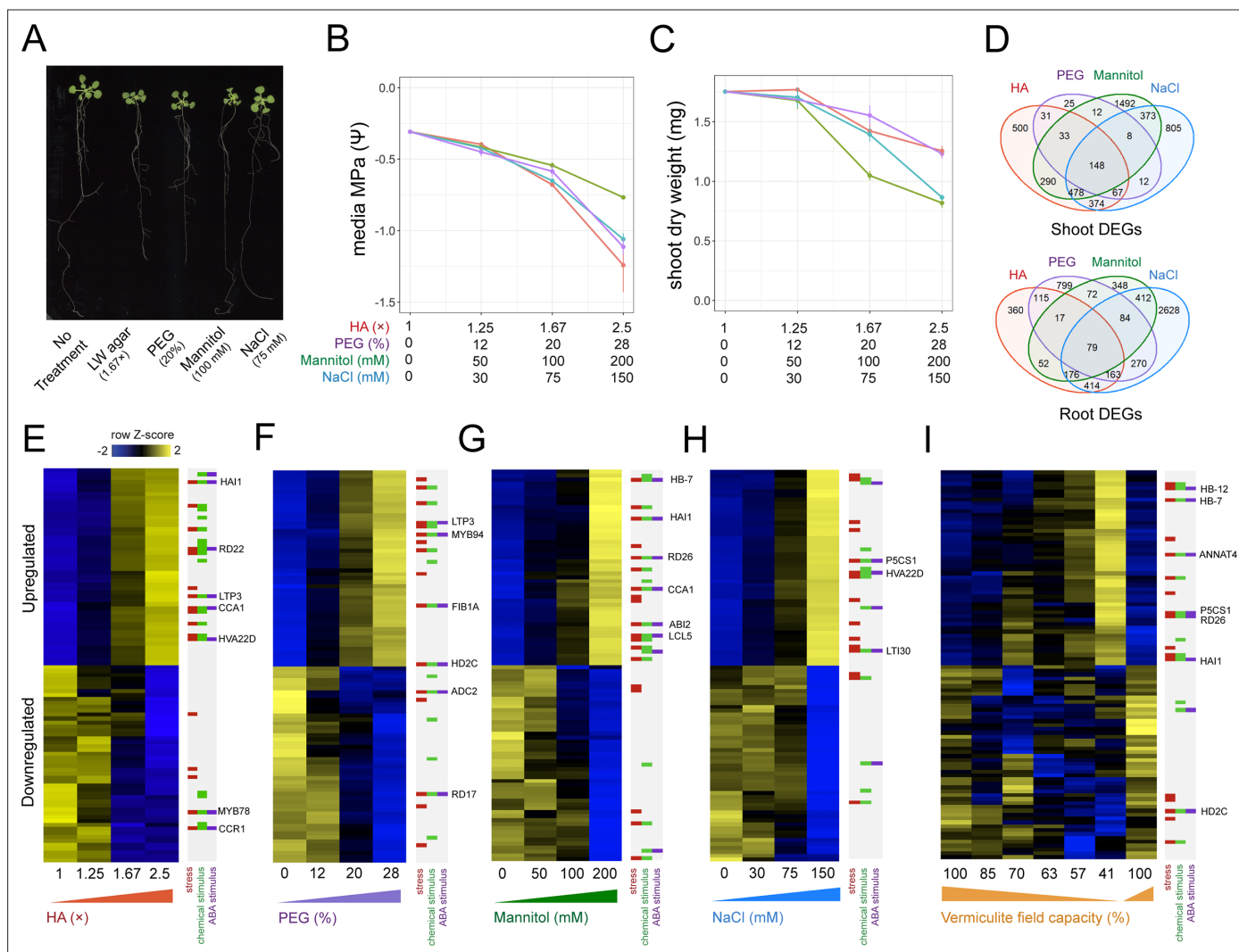


Figure 1. Benchmarking the impact different stress assays have on *Arabidopsis* gene expression. (A) 22-day-old *Arabidopsis* growth on plates under either 1.67× hard agar (HA), 20% polyethylene glycol (PEG), 100 mM mannitol, or 75 mM NaCl treatments. (B) Water potential measurements of treatment media (n=3–4). (C) Dry weight of 22-day-old *Arabidopsis* seedlings under different doses of each stress treatment (n=11–12). (D) Number and intersect of differentially expressed genes (DEGs) that are dose-responsive to each stress treatment within root and shoot tissue. (E–I) Heatmaps displaying the top 50 most significant upregulated or downregulated genes in response to (E) HA, (F) PEG, (G) mannitol, (H) NaCl, and (I) vermiculite drying in the *Arabidopsis* root (n=2–3 biological replicates). Key genes and membership of Gene Ontology (GO) Terms for 'response to stress', 'response to chemical stimulus', or 'response to ABA stimulus' are indicated. ABA, abscisic acid.

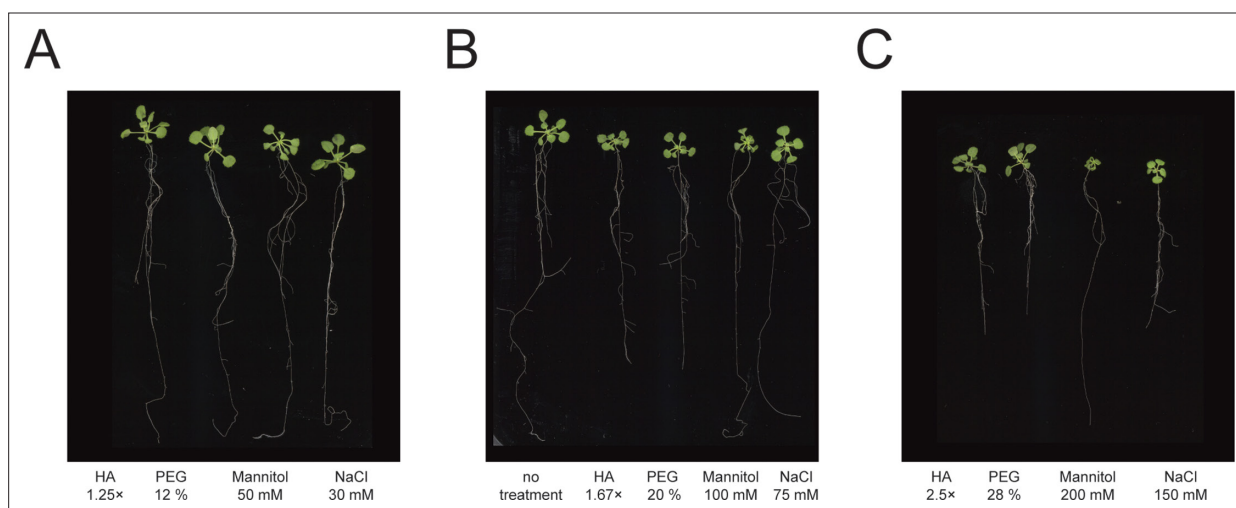
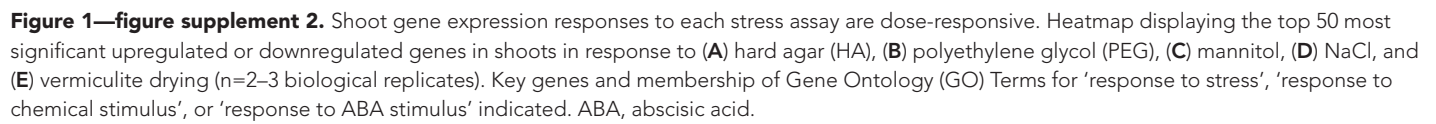
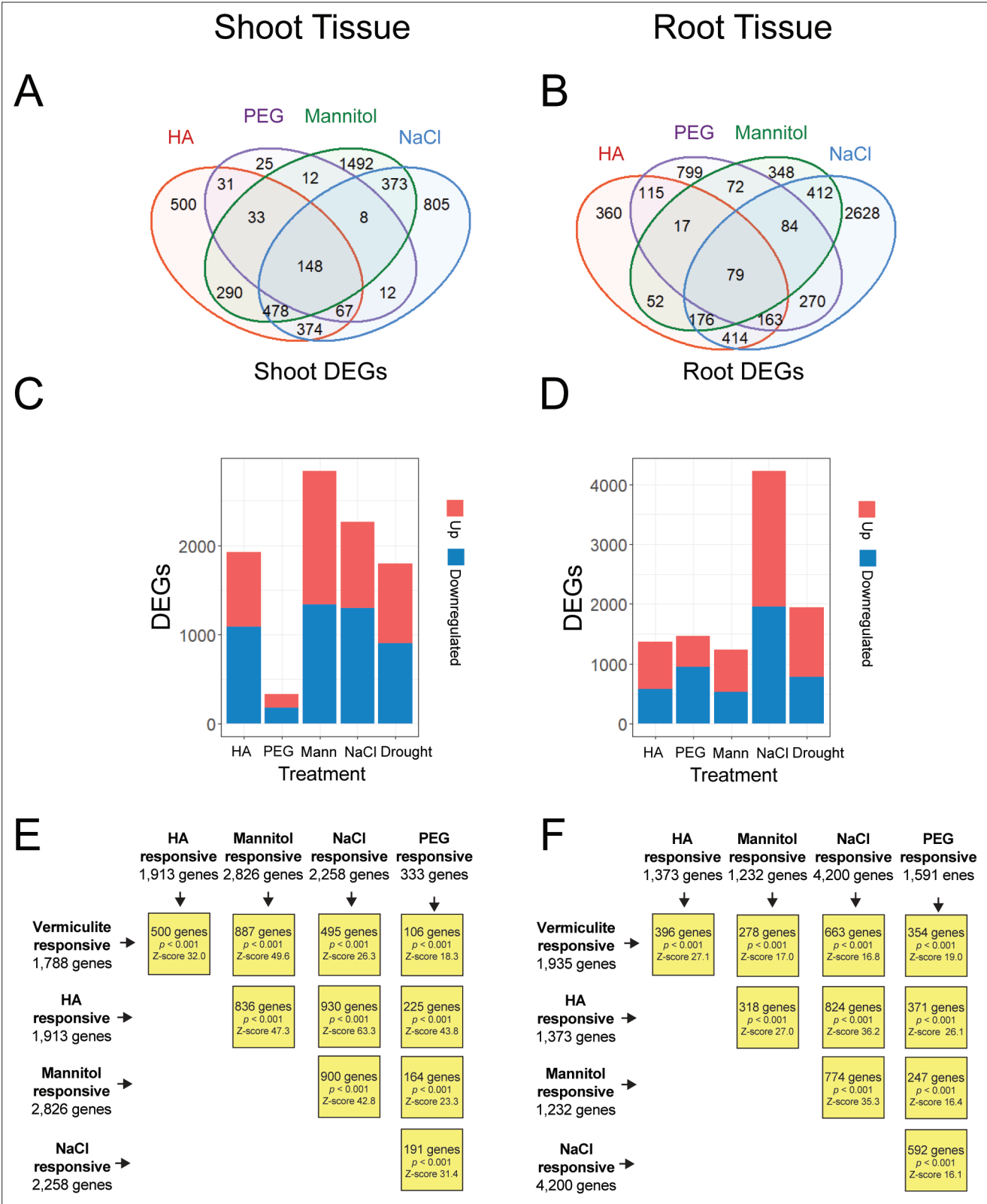


Figure 1—figure supplement 1. Plant growth responses to stress assays. (A–C) Images of 22-day-old *Arabidopsis* seedlings grown under different doses of each agar stress assay.





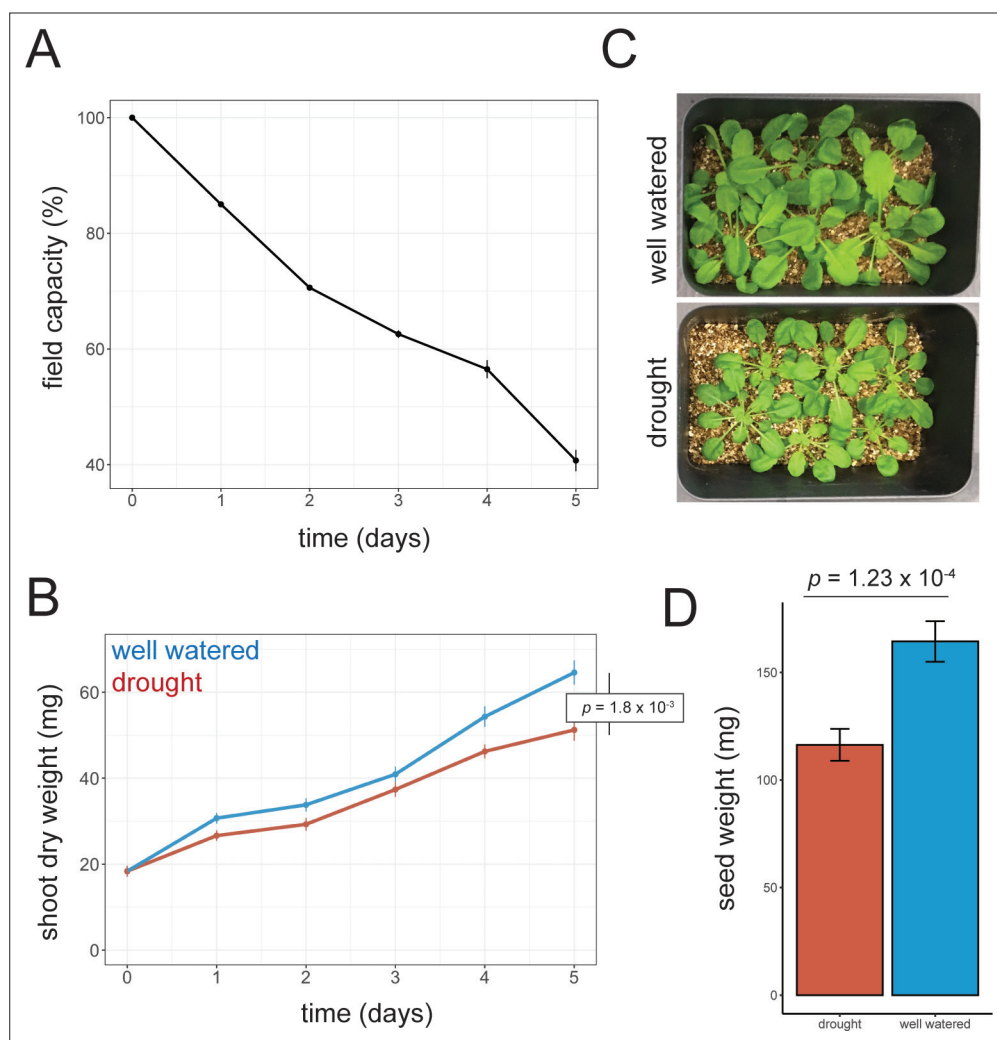


Figure 1—figure supplement 4. Treating vermiculite-grown *Arabidopsis* plants to mild drought stress. **(A)** Field capacity measurements of vermiculite as water evaporated over a 5-day period ($n=6-12$). **(B)** Shoot dry weight of *Arabidopsis* rosettes as they grew either under well-watered conditions or drought conditions over a 5-day period (t-test, $n=12$). **(C)** Images of plants after 5 days of water stress. **(D)** Seed yield resulting from *Arabidopsis* plants after drought recovery (t-test, $n=50$).

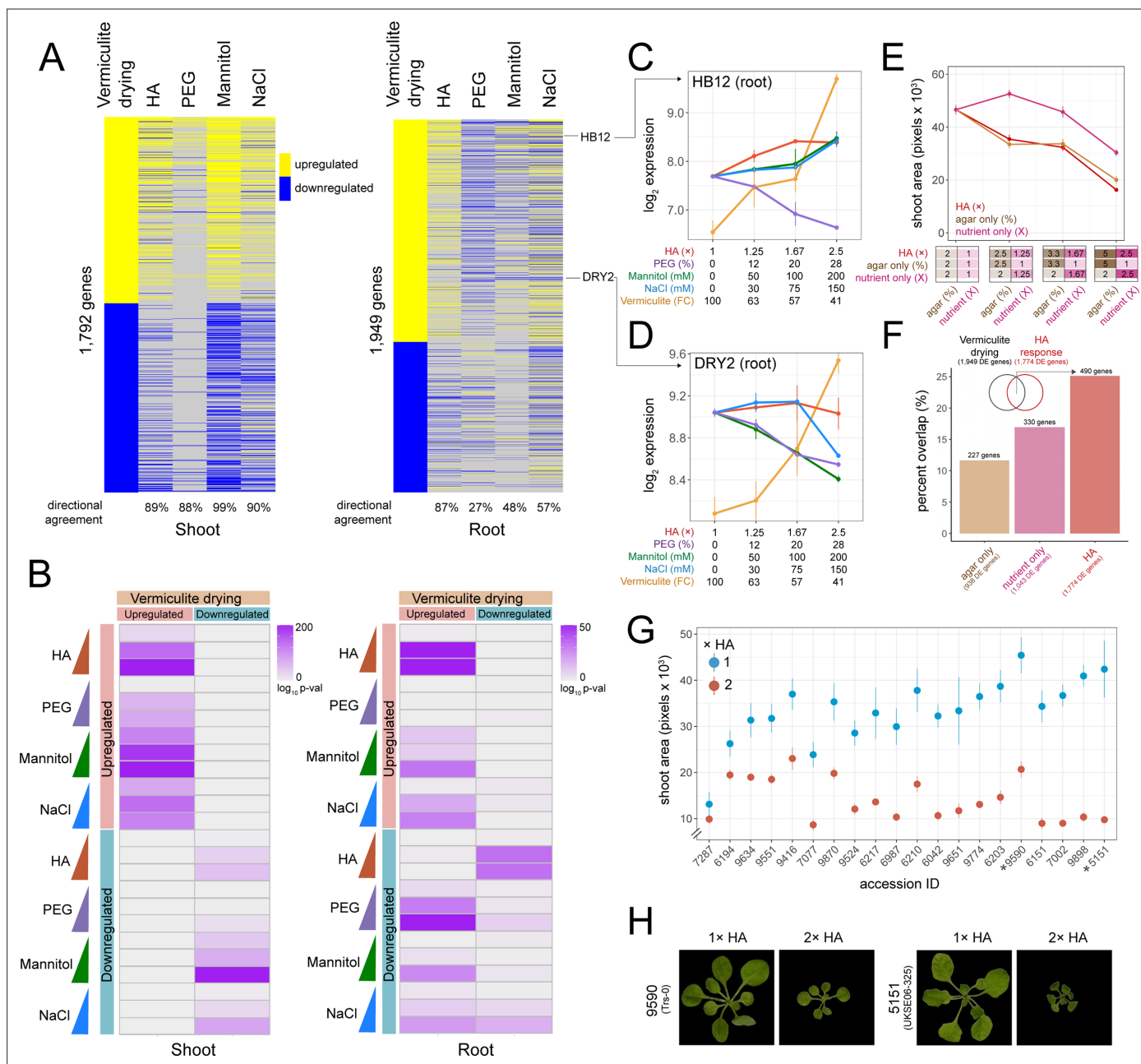


Figure 2. Comparing hard agar (HA), polyethylene glycol (PEG), mannitol, and NaCl gene expression responses to vermiculite drying. **(A)** Heatmap displaying genes differentially expressed in response to vermiculite drying in shoot or root tissue compared to their dose-responsive expression within each plate-based assay. Level of 'directional agreement' (i.e. differentially expressed in the same direction) found within each assay reported. **(B)** Overlap analysis of genes found differentially expressed due to vermiculite drying, compared to those found differentially expressed within each dose of PEG, mannitol, NaCl, or HA assays in both shoot and root (Fisher's exact test, adj. p<0.05). **(C–D)** Expression patterns of *HOMEBOX12* (HB12) and *DROUGHT HYPERSENSITIVE 2* (DRY2) across each assay in root tissue (n=2-3). **(E)** Shoot area of seedlings grown under increasing doses of HA, agar, or nutrient concentrations (n=19). **(F)** Number and percent overlap of genes found differentially expressed in response to increasing doses of HA, agar, or nutrient concentrations with those differentially expressed in response to vermiculite drying. **(G)** Total shoot area of *Arabidopsis* accessions grown under either 1× or 2× HA treatment (n=5-12). **(H)** Images of *Arabidopsis* *Trs-0* or *UKSE06-325* accessions grown on either 1× or 2× HA treatment.

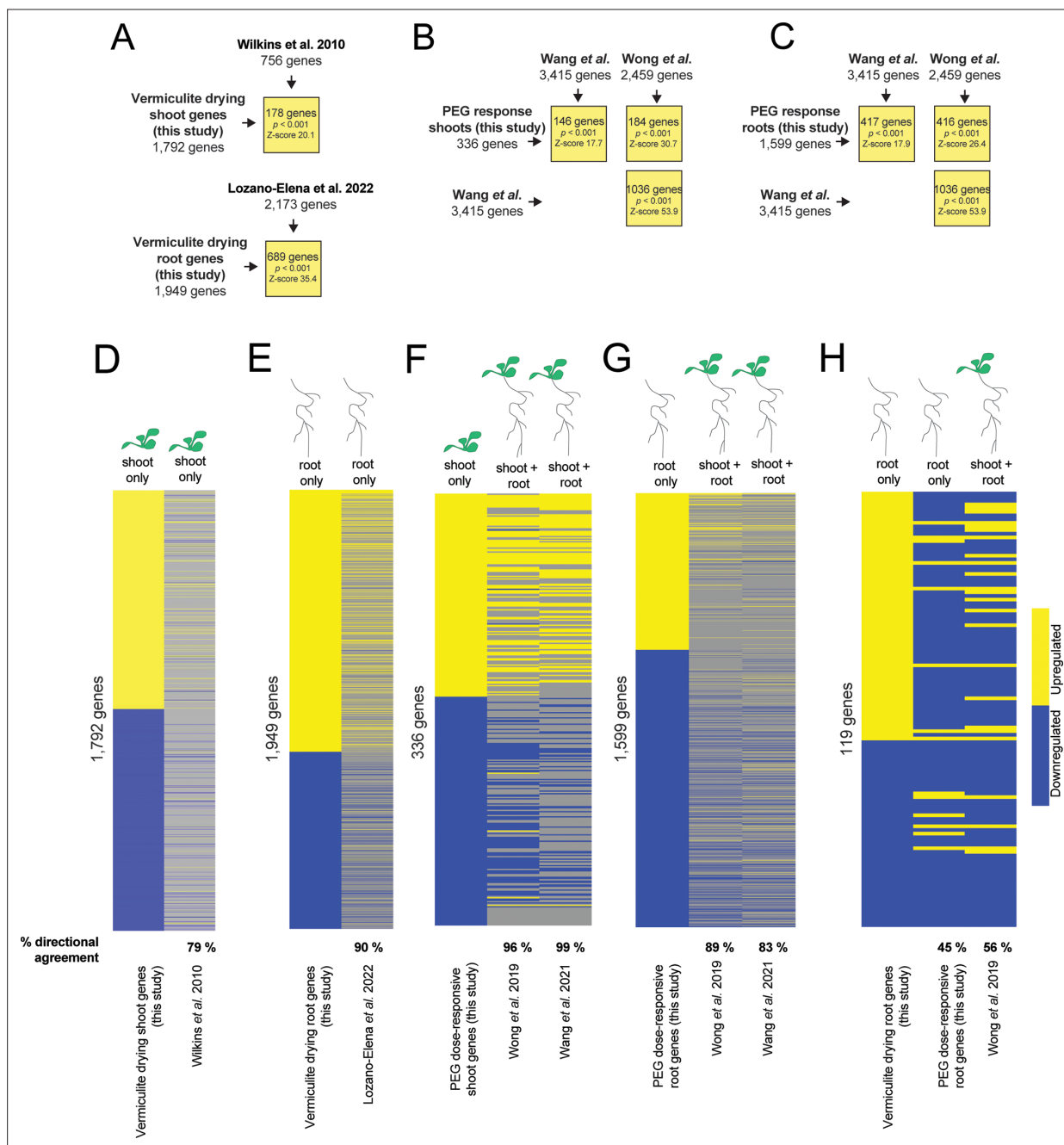


Figure 2—figure supplement 1. Comparing gene expression responses to vermiculite drying and polyethylene glycol (PEG) treatment with previous studies. (A) Intersect analysis of root or shoot genes found differentially expressed in response to vermiculite drying within this study, and genes found differentially expressed in response to soil drying by *Lozano-Elena et al., 2022*, or by *Wilkins et al., 2010* (permutation test, $p < 0.001$). (B–C) Intersect analysis of genes found differentially expressed in response to PEG treatment in shoot (B) or root (C) in this study, with those found differentially expressed in response to PEG treatment by *Wong et al., 2019*, and *Wang et al., 2021* (permutation test, $p < 0.001$). (D–E) Heatmap displaying direction of shoot (D) or root (E) differentially expressed in response to vermiculite drying (this study) and *Wilkins et al., 2010*, or *Lozano-Elena et al., 2022*, respectively. Directional agreement with this study's vermiculite drying response indicated. (F–G) Heatmap displaying direction of genes differentially expressed in response to PEG treatment across each study. We note that both Wong et al. and Wang et al. assess transcriptomic responses of whole seedlings (both root and shoot), and thus we compare our shoot (F) and root (G) data separately. (H) Examining the 119 genes that were differentially expressed in response to drought (this study), PEG treatment (this study), and PEG treatment reported in Wong et al.

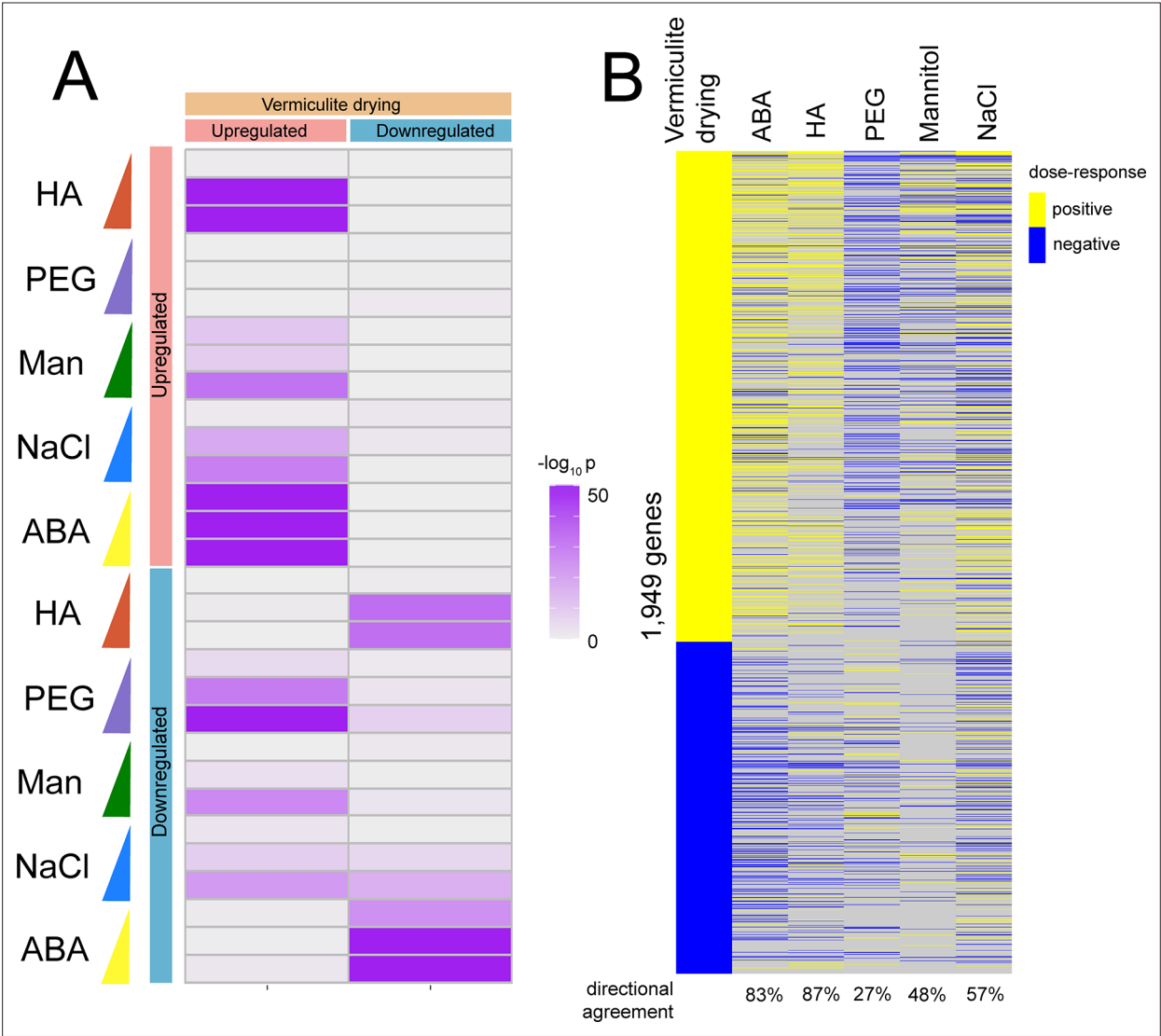


Figure 2—figure supplement 2. Comparing abscisic acid (ABA)-induced differential expression to vermiculite drying and hard agar (HA)-induced gene expression patterns. **(A)** Overlap analysis of genes found differentially expressed in response to vermiculite drying, compared to those within each dose of either transient ABA treatment, polyethylene glycol (PEG), mannitol, NaCl, or HA assays in both root and shoot (Fisher’s exact test, adj. $p < 0.05$). **(B)** Heatmap displaying genes differentially expressed under vermiculite drying in root tissue compared to their dose-responsive expression within each stress assay. Direction of gene expression agreement with vermiculite-drying responsive gene expression (i.e. ‘directional agreement’) indicated.

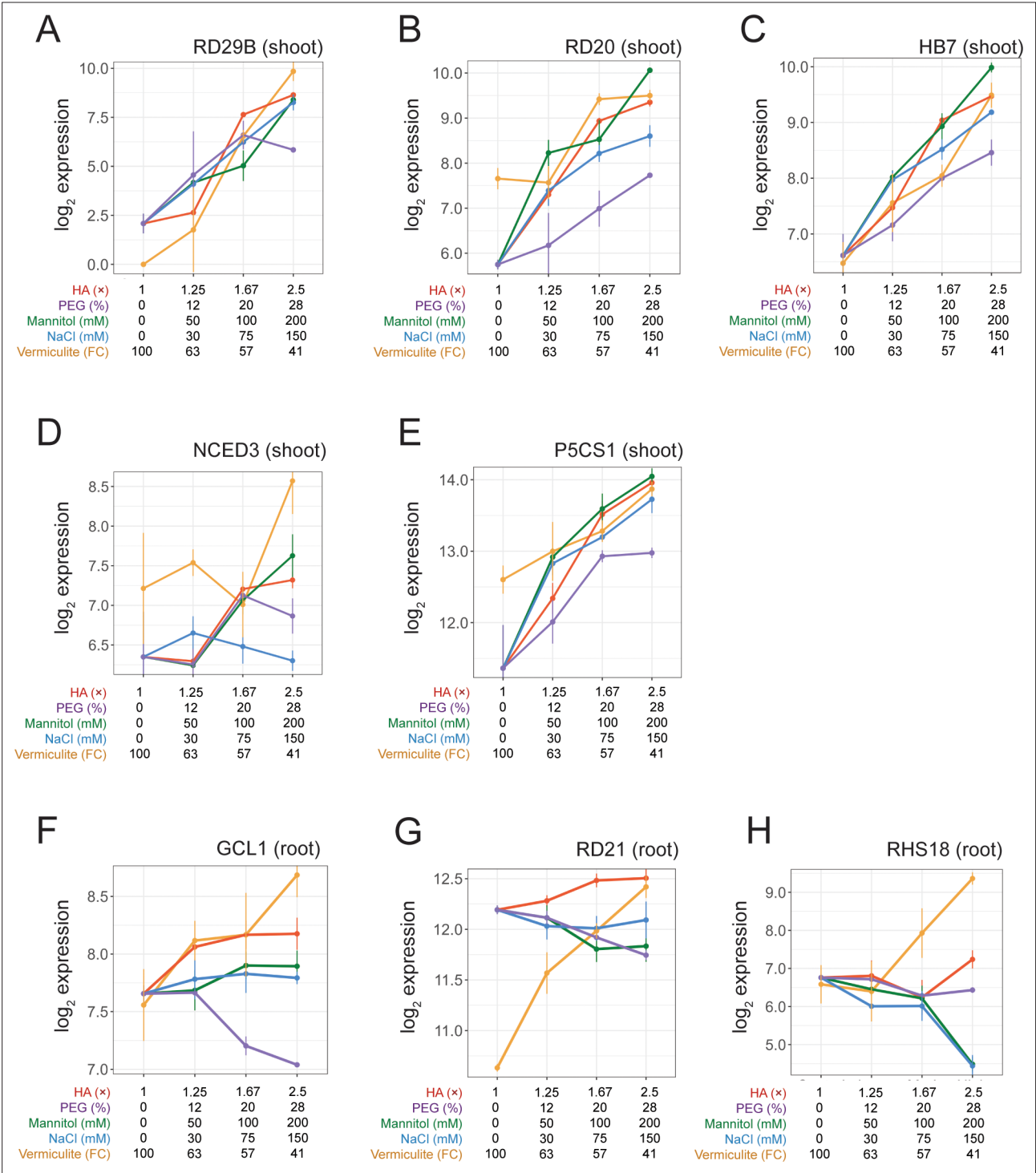


Figure 2—figure supplement 3. Gene expression profiles of individual genes. (A–E) Expression patterns of individual genes under doses of each assay in shoot tissue: (A) RD29B, (B) RD20, (C) HB7, (D) NCED3 and (E) P5CS1. (F–H) Expression patterns of individual genes under doses of each assay in root tissue: (F) GCL1, (G) RD21, and (H) RHS18.

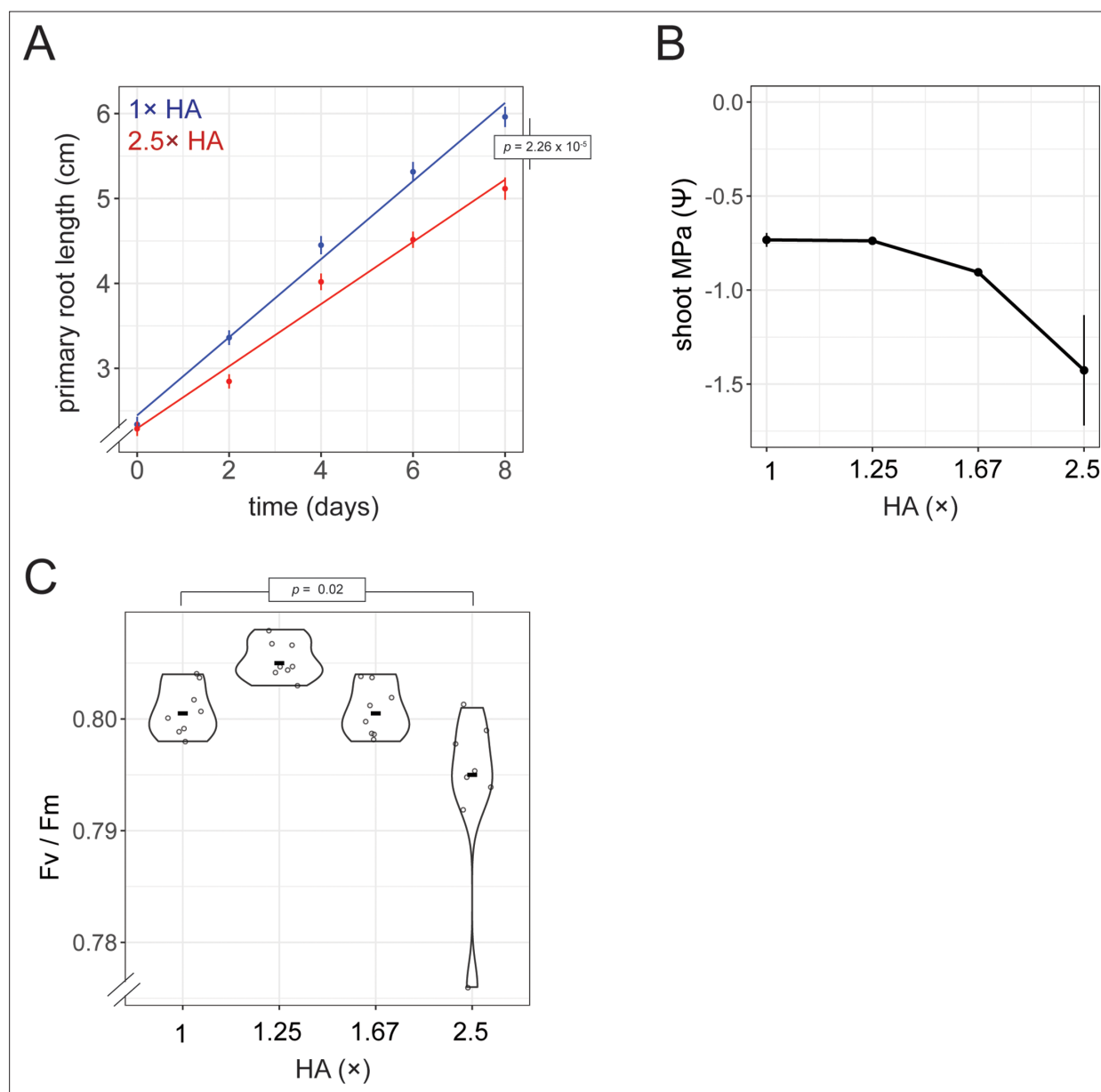


Figure 2—figure supplement 4. Physiological measurements of *Arabidopsis* seedlings in response to hard agar (HA) treatment. **(A)** Measurement of primary root growth rate across 8 days of growth under 1× HA (no treatment) and 2.5× HA conditions ($n=16$, t-test p). **(B)** Shoot water potential measurements of seedlings grown under different HA media doses ($n=3$, Pearson $p=0.009$). **(C)** Measurement of maximum quantum yield of photosystem II (PSII) (F_v/F_m) under different HA media doses ($n=4$, t-test p).

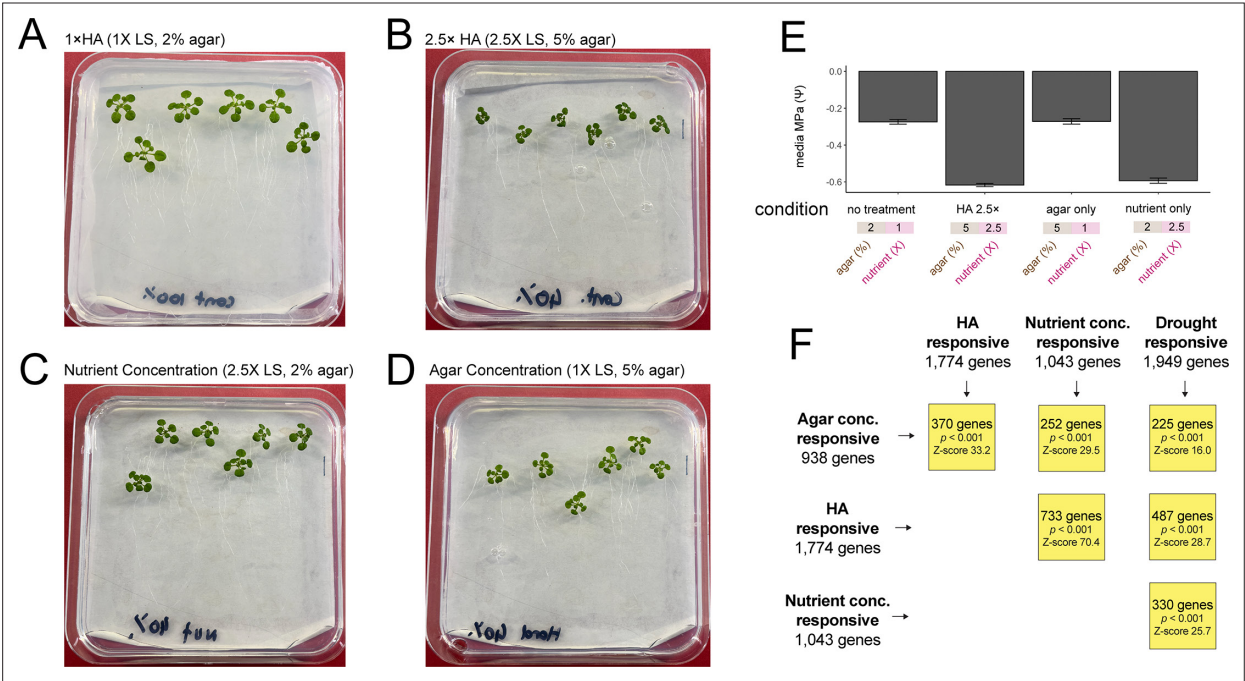


Figure 2—figure supplement 5. Comparing the separate effects of nutrient concentration and agar concentration on seedling growth. Image of *Arabidopsis* seedlings grown on either (A) 1× hard agar (HA) (i.e. 1× Linsmaier & Skoog [LS], 2% agar) or (B) 2.5× HA, which increased both nutrient and agar concentrations to 2.5× and 5%, respectively. (C) Image of seedlings grown on an increased 2.5× nutrient concentration (without a change in agar concentration). (D) Image of seedlings grown on an increased 5% agar concentration (without a change in nutrient concentration). (E) Water potential measurements of media presented in (A–D) (n=3). (F) Intersection of differentially expressed genes responsive to either agar concentration, nutrient concentration, HA treatment, or drought stress (permutation test, $p < 0.001$).

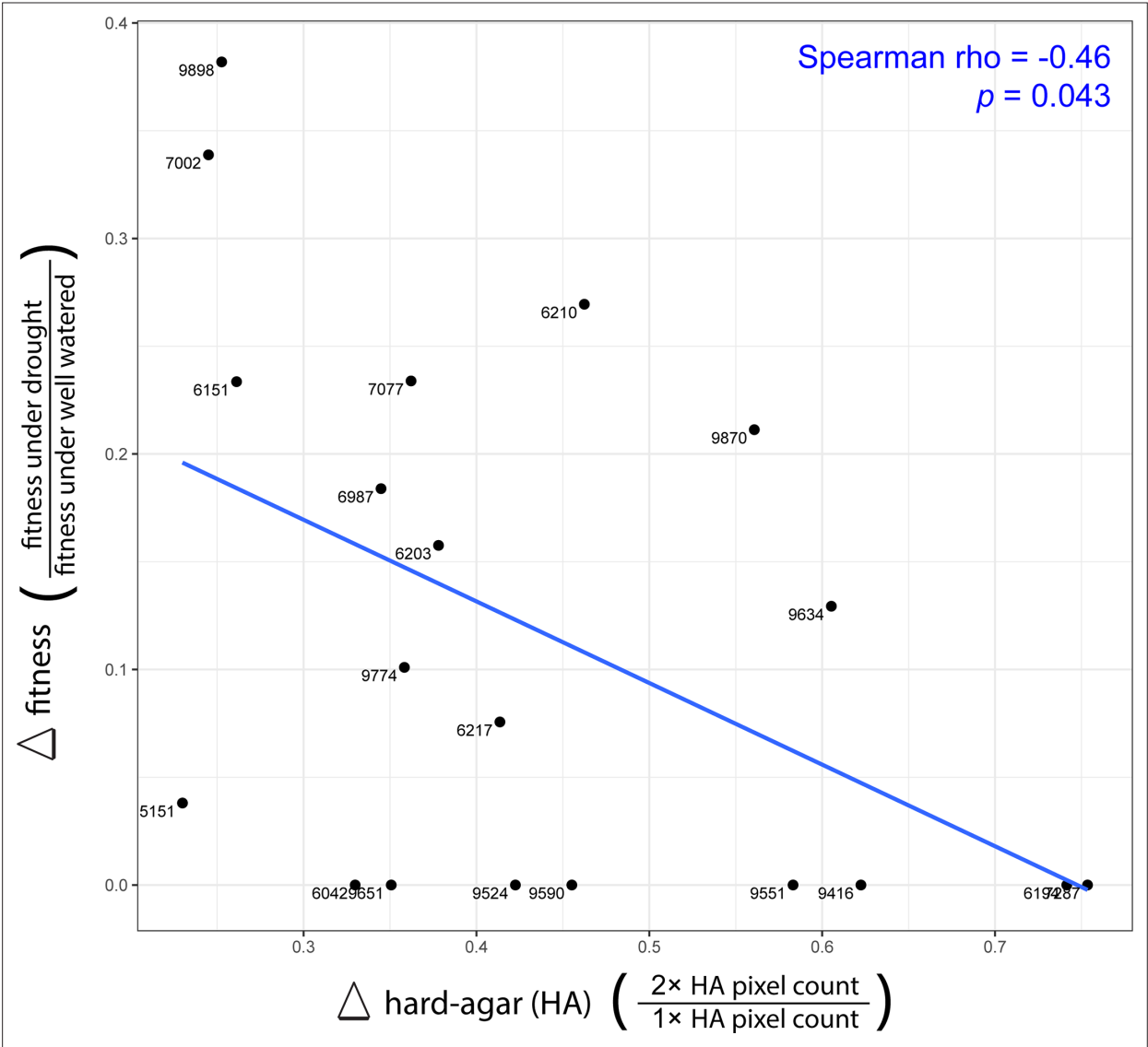


Figure 2—figure supplement 6. Associating hard agar's (HA) impact on shoot size with plant fitness. Comparing the impact HA treatment has on shoot size of 20 different *Arabidopsis* accessions to the change in their fitness found under drought conditions in the field, as reported in **Exposito-Alonso et al., 2019**.

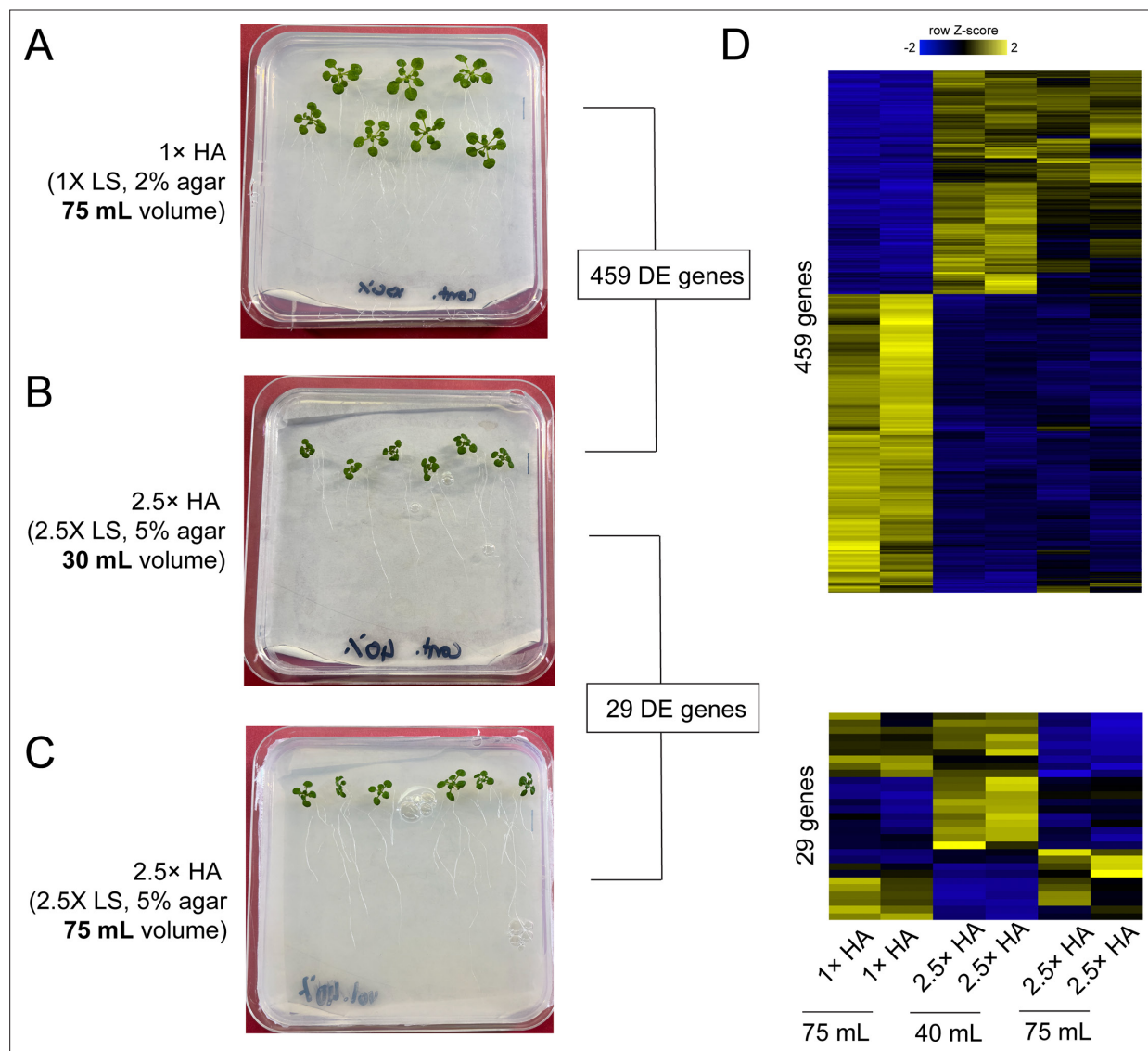


Figure 2—figure supplement 7. The volume of hard agar (HA) has minimal impact on gene expression. Image of *Arabidopsis* seedlings grown on either (A) 75 mL of 1× HA, (B) 30 mL of 2.5× HA, or (C) 75 mL of 2.5× HA. Number of genes found differentially expressed is reported for comparisons (A) and (B), as well as (B) and (C) (DESeq, adj. $p < 0.01$). Of the 29 genes found differentially expressed between (B) and (C), 13 are found in comparison (A) and (B). (E) Heatmap of genes found differentially expressed in either comparison.