# Mimicking drought transcriptional responses using a high throughput plate assay

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## Abstract

Soil-free assays that induce water stress are routinely used for investigating plant responses to drought conditions. Due to their ease of use, the research community often relies on polyethylene glycol (PEG), mannitol and salt (NaCl) treatments to reduce the water potential of growth media, thus mimicking drought conditions in the laboratory. However, while these types of stress can create phenotypes that resemble those of drought, it remains unclear how they compare at the molecular signaling level. Here, using transcriptomics, we demonstrate that while these assays elicit differential expression of root genes involved in drought signaling, many drought-induced genes were repressed by such treatments (and vice versa). In light of this, we designed a new method for simulating drought. By simply adding less water to agar, we found our 'low-water' agar (LW) assay elicits root gene expression responses that hold greater directional agreement with drought signaling responses. Furthermore, we show our approach can be leveraged as a high-throughput assay to investigate natural variation in water stress responses.

## Introduction

As climate change advances, improving crop drought tolerance will be key for ensuring food security (*1, 2*). This has led to intense research at the molecular level to find novel loci and alleles that drive plant responses to drought conditions. Such investigations demand simple assays that can reproduce drought phenotypes at both the physiological and molecular levels. While some researchers use soil-based assays, these are cumbersome. For example, extracting intact root systems from the soil is difficult, and reproducing the rate at which water evaporates from the soil can be challenging (*3*). In light of this, chemical agents such as polyethylene glycol (PEG), mannitol, or salt (NaCl) are often employed to simulate drought stress. When present in aqueous or agar media, they allow precise and dose-dependent control of water potential (*4, 5*). When exposed to these media types, plants exhibit the hallmarks of drought physiology, such as reduced growth rate, reduced stomatal conductance, and increased leaf senescence (*4, 6, 7*). Notably, each type of stress can exert distinct effects (*8*). For example NaCl not only induces osmotic stress, but can cause salt toxicity (*6*). Since it is not metabolized by most plants mannitol is considered less toxic (*3*), however evidence suggests it may act as a signaling molecule (*5, 9*). Since both NaCl and mannitol can enter the pores of plant cell walls (*8, 10*), they can induce plasmolysis, a process that does not typically occur under mild water deficit (*8*). Due to its higher molecular weight, PEG treatment avoids this, and instead causes cytorrhysis (*8*), a physiology more common under drought settings (*10, 11*).

At the molecular signaling level, it remains unclear whether the stress that PEG, mannitol or NaCl elicit is comparable to drought signaling, where we define drought signaling as the transcriptional responses in plant organs in response to water deficit. While some experimental data suggests that they are comparable (*4, 12, 13*), to our knowledge a side-by-side comparison has not been performed. By taking a transcriptomic approach, we show that genes dose-responsive to PEG, mannitol and NaCl treatment in the root display 27 %, 48 % and 57 % directional agreement respectively with genes induced or repressed by drought. Inspired to develop a new method to simulate drought, we present a ‘low-water’ agar (LW) assay, which exhibited a 87 % directional agreement with drought signaling genes.

## PEG, mannitol and NaCl treatments repress drought inducible genes in roots

To benchmark PEG, mannitol and NaCl treatments to one another, we first tested their physiological effect across a range of doses. To this end, we grew *Arabidopsis* seedlings on agar plates supplemented with Linsmaier & Skoog (LS) nutrients for 14 days on three different doses of each stress type. Dose ranges were chosen based on published literature, and ranged from mild to severe stress levels (*3, 4, 11*). As the dose of each stress type increased, the media's water potential significantly decreased in a dose-dependent manner (Spearman, $p < 0.05$). Across the doses tested, we found that each stress type's impact on water potential was not statistically different from one other (ANCOVA post-hoc, $p > 0.17$). In response to these treatments, we found shoot biomass significantly decreased in a dose-dependent manner ($p < 2 \times 10^{-6}$) (**Figure 1A - C**, **Figure 1-figure supplement 1**, **Supplementary File 1**).

Genes that change their expression in response to an environmental signal often do so in a dose-responsive manner (*4, 14*). In light of this, we sought to discover genes whose expression was dose-responsive to the amount of PEG, mannitol, or NaCl applied. Crucially, by identifying genes that were stress responsive across a range of doses, we ensured such genes responded to and were directional with the stress as a whole, and not simply induced or repressed at an individual dose. Taking this approach, we sequenced root and shoot bulk transcriptomes by RNA-seq, and associated each gene's expression with the dose of stress with a linear model. To ensure we captured steady-state differences in gene expression, and avoided those that were transient, we sequenced root and shoot transcriptome profiles after 14 days of stress exposure (*3, 6, 15*). By these means, we found hundreds of genes that were dose-responsive to each treatment within root and shoot tissue (**Figure 1E – 1H, Figure 1-figure supplement 2, Supplementary File 2**) (adj. $p < 0.05$). We note many of these dose-responsive genes were shared across treatments (**Figure 1D**, **Figure 1-supplement Figure 3**).

Next, we wanted to compare these different methods of lowering water potential to an assay that more closely resembled drought. To create this benchmark, we subjected mature *Arabidopsis* plants grown in pots on vermiculite supplemented with LS media to a mild drought stress by withholding water for 5 days. During this period, field capacity reduced from 100 % to 41 %. This treatment led to a reduction in plant biomass ($p = 1.8 \times 10^{-3}$), as well as seed yield ($p = 1.2 \times 10^{-4}$), but did not induce visible signs of senescence or wilting (**Figure 1-figure supplement 4**, **Supplementary File 3**). We assayed root and shoot gene expression responses each day during

water loss by RNA-seq. We observed a dose-dependent relationship between a decrease in field capacity and gene expression responses in both roots and shoots, identifying 1,949 drought-responsive genes in roots and 1,792 in shoots (adj. $p < 0.01$) (**Figure 1I, Figure 1-figure supplement 2, Supplementary File 2**). We ensured these genes' expression patterns recovered upon rewatering (**Figure 1I**). We note the genes we found differentially expressed agreed with a previous report detailing transcriptional responses to water deficit in soil (*16*) (**Figure 2-figure supplement 1**), as well as agreed with genes responsive to transient treatment with Abscisic Acid (ABA), a stress hormone whose levels rise in response to water deficit (*4*) (**Figure 2-figure supplement 2**). Thus, we considered the differentially expressed genes we find through our vermiculite drying approach to be involved in drought signaling responses, but acknowledge that this definition excludes other climatic effects associated with drought such as heat stress or light intensity.

To assess how PEG, mannitol and NaCl treatments compared to the drought signaling response described above, we overlapped genes found differentially expressed in each experiment. For shoot tissue, we found genes that were differentially expressed under drought stress overlapped significantly with genes that were differentially expressed by either PEG, mannitol and NaCl treatments (Fisher test, adj. $p < 0.05$). Additionally, there was 88 – 99 % directional agreement within these overlaps, indicating that genes induced or repressed by drought signaling were similarly induced or repressed by agar stress treatments (**Figure 2A** & **2B**). Along these lines, across all conditions we saw differential expression of stress markers *RESPONSE TO DESSICATION 20;29B (RD20;29B) (17, 18)*, the osmo-protectant gene *DELTA1-PYRROLINE-5-CARBOXYLATE SYNTHASE 1* (*P5CS1*), and ABA signaling and biosynthesis genes *HOMEOBOX 7* (HB7) (*19*) and *NINE-CIS-EPOXYCAROTENOID DIOXYGENASE* (*NCED3)* (*20*) (**Figure 2-figure supplement 3**).

In contrast, we did not find a similar result in the root. Here, we found a reduced directional agreement; a greater number of genes that were upregulated during drought signaling were downregulated by PEG, mannitol and NaCl treatments (and vice versa) (**Figure 2A**). This trend persisted when we assessed genes found differentially expressed at each discrete dose of stress (**Figure 2B**). For example, 27 % of PEG dose-responsive genes shared the same direction of expression seen in drought signaling responses. We note that previously published PEG transcriptome datasets agreed with our own (**Figure 2-figure supplement 1**). Such mis-regulation is exemplified by the expression of stress markers *HOMEOBOX 12* (*HB12*) (*19*)

(**Figure 2C**), *GRC2-LIKE 1* (*GCL1*) (*21*), and *RESPONSE TO DEHYDRATION* 21 (*RD21*) (*22*) (**Figure 2-figure supplement 3**). Mannitol and NaCl held a 48 % and 57 % agreement in gene expression direction respectively. Examples of stress genes that followed a pattern of mis-regulation in mannitol and NaCl treatments were *DROUGHT HYPERSENSITIVE 2* (*DRY2*) (*23*) (**Figure 2D**) and *ROOT HAIR SPECIFIC 18* (RHS18) (*24*).

Why does PEG down-regulate drought-induced genes in the root? We hypothesize that the genes that were repressed by PEG may be the result of hypoxia. We base this on PEG's ability to impede oxygen solubility (*25, 26*). This appears to be reflected at the molecular level, where genes downregulated by PEG are over-represented in the 'monooxygenase activity', and 'oxygen binding' Gene Ontology (GO) Terms ($p < 1 \times 10^{-15}$, **Supplementary File 4**). In contrast, NaCl-responsive GO Terms included a specific downregulation of 'phosphorous metabolic processes' ($p = 5.2 \times 10^{-6}$), suggesting that the roots were changing phosphate levels in response to NaCl, a process known to help maintain ion homeostasis (*27*). For mannitol, we observed a specific downregulation of 'cell wall organization or biogenesis' and 'microtubule-based processes' ($p < 7.8 \times 10^{-3}$), suggesting a unique root developmental response to mannitol, possibly mediated by mannitol acting as a signaling molecule (*9*). For both mannitol and NaCl stress, we saw photosynthesis-related GO Terms enriched among genes upregulated in the roots, which may be due to an interaction between the osmotic stress and exposing the roots to light (*28*) (**Supplementary File 4**).

## The 'low-water agar' assay recapitulates drought signaling responses in the root

In addition to examining PEG, mannitol and NaCl transcriptional responses, we were also motivated to design a new way of simulating drought on an agar plate. We hypothesized that instead of adding a compound to reduce water potential, we could mimic drought by adding less water to agar media (leading to both higher agar and nutrient concentrations). We called this media 'low-water' (LW) agar, and by testing three different doses (80 %, 60 % and 40 % water content), found that it limited plant shoot dry weight and media water potential in a similar way to PEG, mannitol and NaCl (**Figure 1A - C**). Additionally, we found that LW treatment limited primary root growth rate, shoot water potential, and photosynthesis efficiency (**Figure 2-figure supplement 4**). At the molecular level, RNA-seq revealed 1,376 and 1,921 genes that were dose-responsive to the level of LW stress in roots and shoots respectively (**Figure 1E & Figure1-figure**

**supplement 2**). We found that these gene expression responses overlapped significantly with those found involved in drought signaling (Fisher test, $p < 1 \times 10^{-32}$) (**Figure 2A** & **2B**). Compared to PEG, mannitol and NaCl, we found genes differentially expressed in response to LW agar held a greater directional agreement with drought signaling responses (87 % directional agreement). Such directional agreement can be seen in stress marker expression of genes such as *HB12* (**Figure 2C**), *GCL1* and *RD21* (**Figure2-figure supplement 3**).

Adding less water to nutrient agar solution increases both nutrient and agar concentrations. An increase in nutrient concentration can induce salt-like stress while increasing agar concentration will increase tensile stress (*29*). We tested each of these variables separately to understand the role each played in eliciting the gene expression responses found in the LW assay. To do this, we repeated our LW dose experiment, but now increasing only the concentration of LS nutrients (1, 1.25, 1.67 and 2.5 X) or the concentration of agar (2, 2.5, 3.3 and 5 %) (**Figure 2E**). We found a significant decrease in shoot area size in response to an increase in nutrient concentration (Pearson $p = 1.7 \times 10^{-7}$) and agar concentration ($p = 3.9 \times 10^{-13}$), where the latter more closely phenocopied the effect of LW (**Figure 2E**, **Figure 2-figure supplement 5, Supplementary File 5**). Since the increase in nutrient concentration alone was responsible for changing media water potential, the phenotypic response to increased agar concentration was not in response to a lower water potential (**Figure 2-figure supplement 5**). Next, we examined the transcriptional responses underlying nutrient and agar responses by sequencing root tissue across each dose tested. Through linear modeling, we found 1,043 genes and 938 genes that were dose-responsive to nutrient or agar concentration, respectively. Next, we investigated whether these genes agreed with those found in drought signaling responses. We found that genes differentially expressed in response to an increase in agar or nutrient concentration overlapped 12 % and 17 % of drought responsive gene expression respectively (permutation test, $p < 0.05$) (**Figure 2F, Figure 2-figure supplement 5, Supplementary File 6**). However, we found genes differentially expressed in response to LW treatment led to a higher overlap (26 %), suggesting that both nutrient and agar concentration contribute to LW performance.

Finally, we tested if our LW assay was sensitive enough to detect phenotypic variability. To achieve this, we grew 20 different *Arabidopsis* ecotypes on 50 % LW agar, where ecotypes were selected from a previous drought study that assessed fitness in a common garden experiment (*30*). By comparing the total shoot area after three weeks of growth, we found that our assay revealed variability in shoot growth responses (**Figure 2G** & **2H**, **Supplementary File 7**).

Similarly, we found that the relative impact LW agar had on an accession's shoot size was associated with the relative impact drought had on its fitness, as measured under field conditions (Spearman $p$ = 0.04, **Figure 2-figure supplement 6**) (*30*). This suggests that our assay may be useful for screening for novel drought-associated loci among a wider group of accessions or mutants.

In summary, the LW agar assay presents a new approach for simulating root drought signaling responses on an agar plate. We note that our assay cannot mimic many additional effects drought stress can have on gene expression in real environments – such as those that arise from changes in soil structure or the root microbiome (*31*). However, by inducing growth arrest and gene expression responses comparable to drought signaling, LW agar offers a high-throughput method to screen phenotypes and probe gene regulatory responses associated with drought signaling. We describe how to make LW media in the Materials and Methods.

Supplementary

**Supplementary File 1** – Plant physiological measurements
**Supplementary File 2** – Differentially expressed genes and normalized counts in LW, PEG, Mannitol, NaCl or Vermiculite drought stress experiments
**Supplementary File 3** – Vermiculite drought stress assay measurements
**Supplementary File 4** – GO Term enrichment of differentially expressed genes
**Supplementary File 5** – Shoot area of seedlings grown under different agar and nutrient concentrations
**Supplementary File 6** – Differentially expressed genes and normalized counts in response to changes in nutrient or agar concentration
**Supplementary File 7** – Shoot area of different *Arabidopsis* accessions grown on LW media

## Acknowledgements

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## Data Availability

Raw sequencing data is available at the National Center for Biotechnology Information Sequence Read Archive (accession number PRJNA904764). Normalized read counts and raw phenotypic datasets can be found in the Supplementary Material.

## Materials and Methods

Low-Water Agar Assay – *Arabidopsis* seedlings were grown on vertical plates for 8 days under short day conditions (8 h light, 21 C, 150 umoles light) on agar media (1 × Linsmaier & Skoog (LS) (Cassion LSP03) media, 1% sucrose, 2% agar, pH 5.7). After 8 days, plants were transferred to 'low-water' (LW) plates that contained varying water content. The 100% treatment plate, where water is not lacking, contained 75 mL of 2% agar and 1× LS media. Drought was simulated by preparing the same media but reducing the amount of water present. For example, the 80% treatment plate contained 60 mL of 2.5% agar and 1.25× LS media, pH 5.7. Plants were grown upon 3 different treatments (80%, 60%, and 40% water amount) for 14 days. We note that the volume of LW itself does not impact gene expression responses (**Figure 2-figure supplement 7**). On day 14, 2 hours after subjective dawn, shoot and root samples were flash frozen (6 plants per replicate). To test different *Arabidopsis* accessions on LW agar, plants were sown on either 100% or 50% treatments as described above, however supplemented with 0.5% or 1% sucrose respectively to encourage germination. Seedlings were grown for 3 weeks under short day conditions in before imaging plates in duplicate (n = 2 - 5 plants per plate) (**Supplementary File 7**). Shoot area was calculated from images using Plant Growth Tracker (GitHub - https://github.com/jiayinghsu/plant_growth_tracker).

Vermiculite Drought Assay – *Arabidopsis* seedlings were grown on vertical plates for 17 days under short day conditions (8 h light, 21 C, 150 umoles light) on agar media (1×LS, 1% sucrose, 2% agar, pH 5.7), before transfer to vermiculite saturated with 0.75 × LS media. Plants were then grown on vermiculite at 100% field capacity (FC) for 12 days (8 h light, 21 C, 150 umoles light). On the 13$^{th}$ day, the first time point was sampled (4.5 hours after subjective dawn) where tissue was flash frozen in liquid nitrogen. After this, excess aqueous solution was drained from each pot, and then each pot was calibrated to 1 × FC. Plant tissue was harvested each day on subsequent days at the same time of day. Each day, pots were weighed to measure extent of evaporation. By these means, FC was measured (**Figure 1-figure supplement 4**). After the 5$^{th}$ day sample was taken, water was re-added to the remaining pots to an excess of 1× FC. ~ 15 plants were sampled per time point. Plants were then left to grow under long day conditions until flowering. Seeds were harvested, dried, and weighed (n = 50 plants per treatment).

Polyethylene Glycol (PEG) Stress Assay – *Arabidopsis* seedlings were grown on vertical plates for 8 days under short-day conditions (8 h light, 21 C, 150 umoles light) on agar media (1×LS, 1% sucrose, 2% agar, pH 5.7), before transfer to polyethylene glycol (PEG) media of varying concentrations. PEG media plates were prepared by dissolving crystalline 6000 MW PEG into freshly autoclaved 1× LS media pH 5.7 and pouring 50 mL of PEG media solution onto 1× LS, 2% agar, media plates (pH 5.7), letting the PEG solution diffuse into the solid media overnight, then pouring off excess and transferring seedlings to PEG infused media plates as described in (*11*). Plants were grown under 3 different treatments (12%, 20%, and 28% PEG solution w/v) for 14 days. On day 14, 2 hours after subjective dawn, shoot and root samples were flash frozen (6 plants per replicate).

Mannitol and NaCl Osmotic Stress Assays – *Arabidopsis* seedlings were grown on vertical plates for 8 days under short-day conditions (8 h light, 21 C, 150 umoles light) on agar media (1× LS, 1% sucrose, 2% agar, pH 5.7), before transfer to either mannitol or salt (NaCl) media of varying concentrations. Mannitol and NaCl media plates were prepared by adding respective volume of stock solution to 1× LS, 2% agar, pH 5.7 media before autoclaving. Plants were grown under 3 different treatments of mannitol or NaCl (50 mM, 100 mM and 200 mM for mannitol, 30 mM, 75 mM, and 150 mM for NaCl) for 14 days. On day 14, 2 hours after subjective dawn, shoot and root samples were flash frozen (6 plants per replicate).

ABA Exogenous Treatment Assay – *Arabidopsis* seedlings were grown on vertical plates for 8 days under short-day conditions (8 h light, 21 C, 150 umoles light) on agar media (1× LS, 1% sucrose, 2% agar, pH 5.7), before transfer to 1× LS, 2% agar, pH 5.7 control media and grown for 14 days. On day 14, abscisic acid (ABA) solutions of 1 uM, 5 uM and 10 uM were prepared from 10 mM ABA dissolved in ethanol stock, as well as a mock treatment solution containing 0.1% ethanol concentration. 30 min after subjective dawn, 15 mL of each solution was dispersed onto the roots of the seedlings. After 1 min of treatment, the ABA solution was removed from the plates, and the plates returned to the growth chamber. 2 hours after subjective dawn, shoot and root samples were flash frozen (6 plants per replicate).

Osmotic Potential Measurements: The water potential of media was determined considering it equivalent to the osmotic potential (Ψs). Osmotic potential was measured using a vapor pressure osmometer (Model 5600, ELITech Group; Puteaux, France). Readings were taken from melted agar media constituted with the particular stress type. Osmolality readings for each sample obtained were converted to megapascals (MPa) using the equation Ψs = -CRT, where C is the molar concentration, R is the universal gas constant, T is the temperature in Kelvin. Two weeks after transplanting the seedlings to LW agar media, we assessed the osmotic potential of shoot tissue. After immersion in liquid nitrogen 3 shoots were placed into 0.5-ml tubes and centrifuged to extract the tissue sap. The osmotic potential (Ψs) of the extracted sap was determined using a vapor pressure osmometer.

Chlorophyll fluorescence measurements: Chlorophyll fluorescence was assessed in eight seedlings of each plate using the Walz PAM IMAGING PAM M-series IMAG-K7 (MAXI) fluorometer. For every experiment, leaves were pre-conditioned in the dark for 1 h. The maximum quantum yield of PSII ($F_v/F_m$) was calculated using the formula:

$$F_v/F_m=(F_m-F_0)/F_m$$

Where $F_v$ is the variable fluorescence, $F_m$ is the maximal fluorescence following 1 h of dark adaptation and $F_0$ is the minimal fluorescence level of a dark-adapted leaf when all photosystem II (PSII) reaction centers are open.

Root Growth Rate Measurements – *Arabidopsis* seedlings were grown on vertical plates for 8 days under short day conditions (8 h light, 21 C, 150 umoles light) on agar media (1× LS, 1% sucrose, 2% agar), before transfer to 40% LW, 28% PEG, 150 mM mannitol or 150 mM NaCl treatment plates as described above. Root images were acquired every two days for a total of 8

372 days using scanners. Primary root length, defined as the length (scaled to
373 cm) from hypocotyl base to root tip, was quantified using image J. For each treatment we
374 screened 4 plates, with each plate holding 4 individual plants.
375
376 RNA-extraction and Library Preparation – Plant tissue was crushed using the TissueLyser
377 (Agilent) and RNA extracted using RNeasy Mini Kit (Qiagen). RNA quality was assessed using
378 Tape station High Sensitivity RNA assay (Agilent). 0.5 - 1 ug of total RNA proceeded to library
379 preparation, where libraries were prepared using TruSeq stranded mRNA kit (Illumina). Resulting
380 libraries were sequenced on the NovaSeq 6000 (Illumina) with 2x150 bp paired-end read
381 chemistry. Read sequences were aligned to the *Arabidopsis* TAIR10 genome using HISAT2 (*32*),
382 and gene counts called using HT-seq (*33*), by relying on Araport11 annotation (*34*). Normalized
383 counts can be found in **Supplementary File 2**. For each organ, libraries from all experiments
384 were normalized together before calling differential expression.
385
386 Statistical Analysis – To detect differential expression in our drought assay on vermiculite, we
387 called differential expression using a linear model using the DESeq2 LRT function to associate a
388 change in field capacity with change in gene expression. The same statistical approach was used
389 to associate a change in a gene's expression to changes in dose of LW, PEG, mannitol, and
390 NaCl, as well as changes in agar concentration, nutrient concentration and volume of agar used.
391 Resulting model p-values were adjusted to account for false discovery ($p$-value < 0.05). The
392 complete list of differentially expressed genes for each experiment can be found in
393 **Supplementary File 2** and **Supplementary File 6**. Pairwise differential gene expression was
394 called using DESeq2 (*35*). Specifically, for plate based assays, we called differential expression
395 by comparing the control treatment to each treatment dose, using an adjusted $p$-value threshold
396 of 0.05. Overlap analyses were performed using Fisher exact tests, with an adjusted $p$-value
397 threshold of 0.05. The background for these intersects was all expressed genes within the
398 respective organ. Permutation tests and GO Term enrichment analyses was performed in
399 VirtualPlant (*36*), with all expressed genes within the respective organ used as background.
400
401

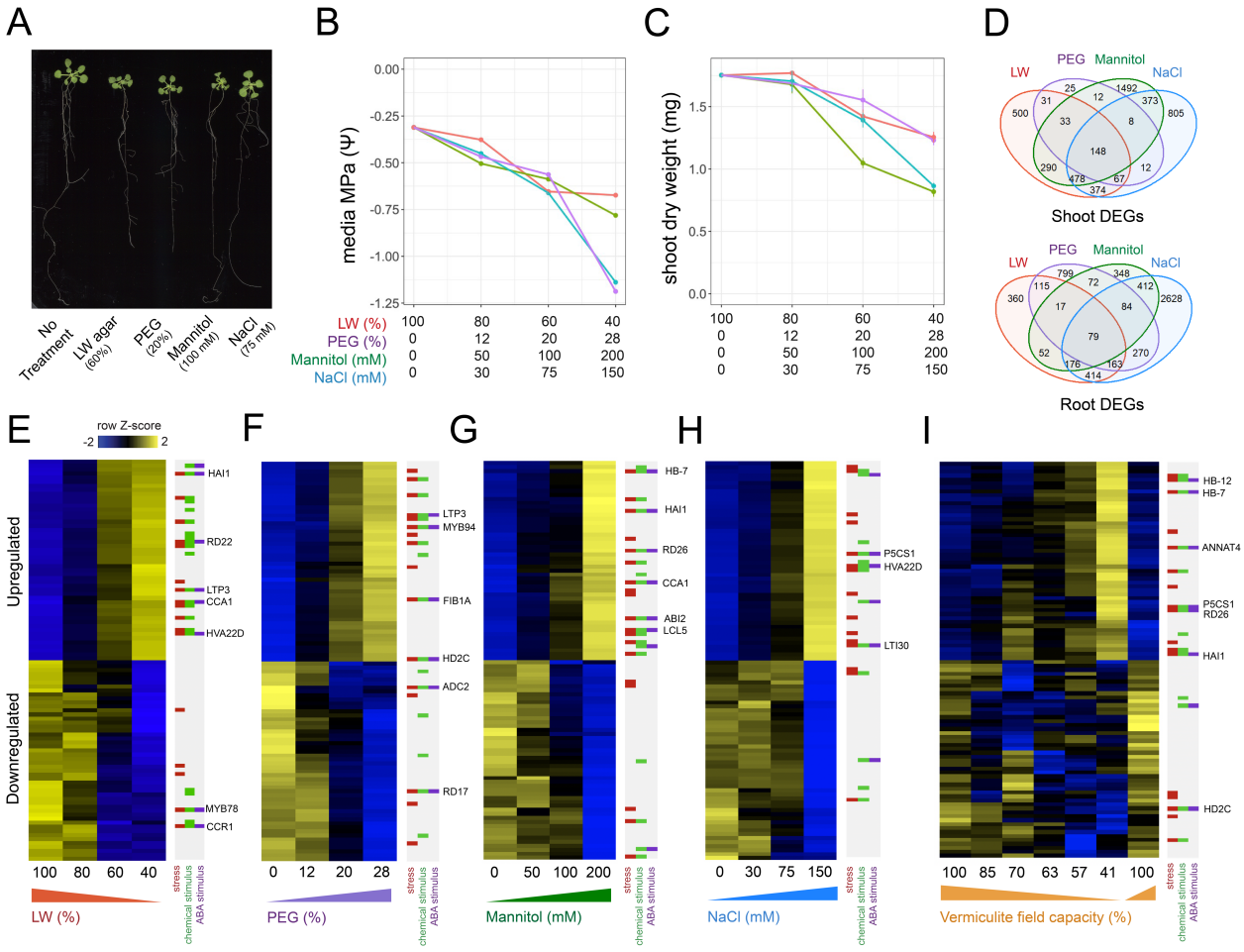


**Figure 1 - Benchmarking the impact different stress assays have on *Arabidopsis* physiology and gene expression. A:** 22-day-old *Arabidopsis* growth on plates under either 60 % low-water (LW) agar, 20 % PEG, 100 mM mannitol, or 75 mM NaCl treatments. **B**: Water potential measurements of treatment media. **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)** LW, **(F)** PEG, **(G)** mannitol, **(H)** NaCl and **(I)** vermiculite-induced drought stress in the *Arabidopsis* root. Membership of Gene Ontology (GO) Terms for 'response to stress', 'response to chemical stimulus' or 'response to ABA stimulus' are indicated.

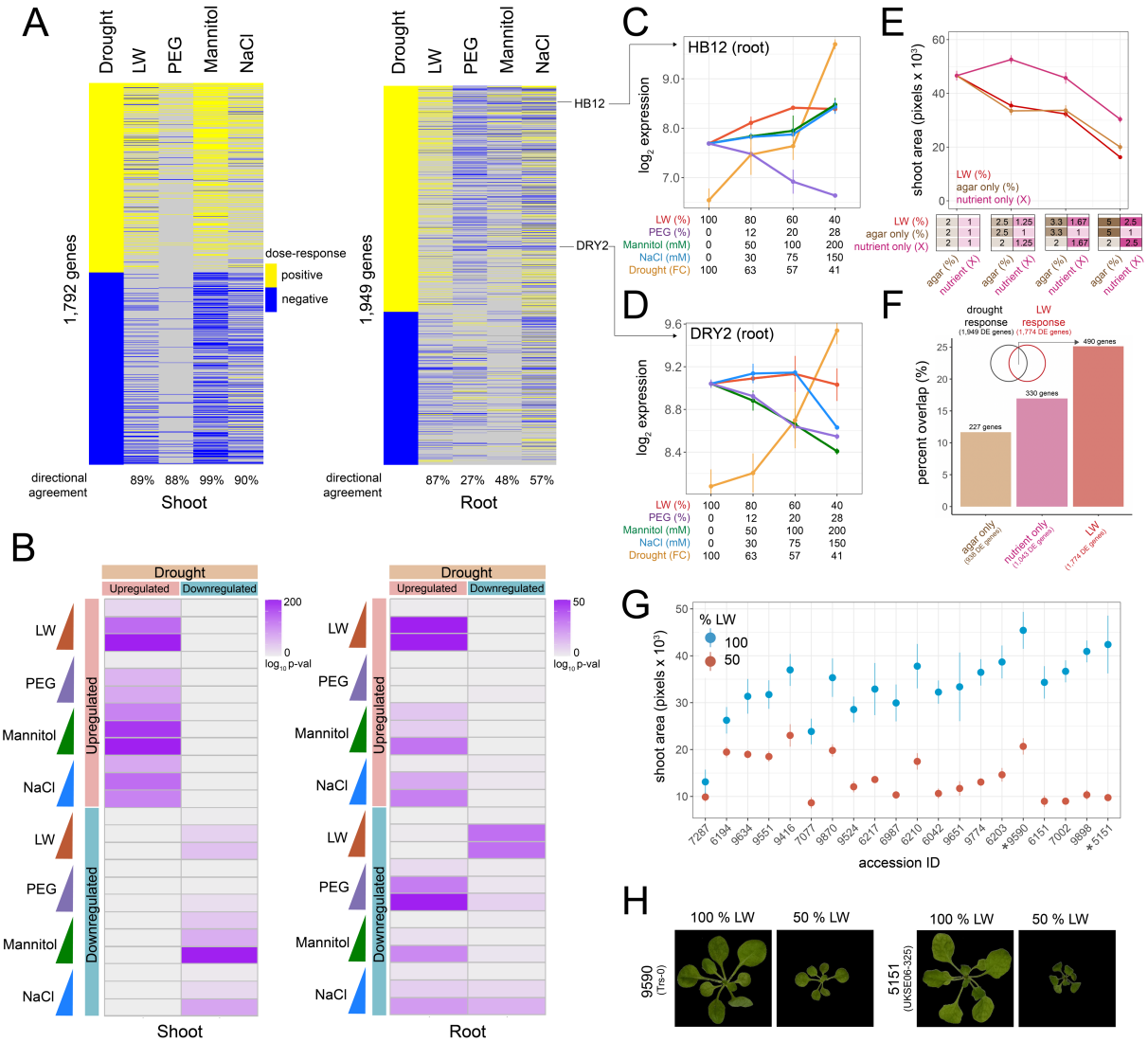


**Figure 2 - Comparative transcriptomic analysis reveals PEG, mannitol, and NaCl downregulate drought-inducible genes in the root. A:** Heatmap displaying genes differentially expressed under drought stress in shoot or root tissue compared to their dose-responsive expression within each agar stress assay. 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 under drought stress, compared to those found differentially expressed within each dose of PEG, mannitol, NaCl or LW agar assays in both shoot and root. **C - D:** Expression patterns of stress marker genes *HOMEOBOX12* (*HB12)* and *DROUGHT HYPERSENSITIVE 2* (*DRY2)* across each assay in root tissue. **E:** Shoot area of seedlings grown under increasing doses of LW, agar or nutrient concentrations (n = 19). **F:** Number and percent overlap of genes found differentially expressed in response to increasing doses of LW, agar or nutrient concentrations with those differentially expressed in response to drought stress. **G:** Total shoot area of *Arabidopsis* accessions grown under either 100 % or 50 % LW agar treatment (n = 5 - 12). **H:** Images of *Arabidopsis Trs-0* or *UKSE06-325* accessions grown on either 100 % or 50 % LW treatment.

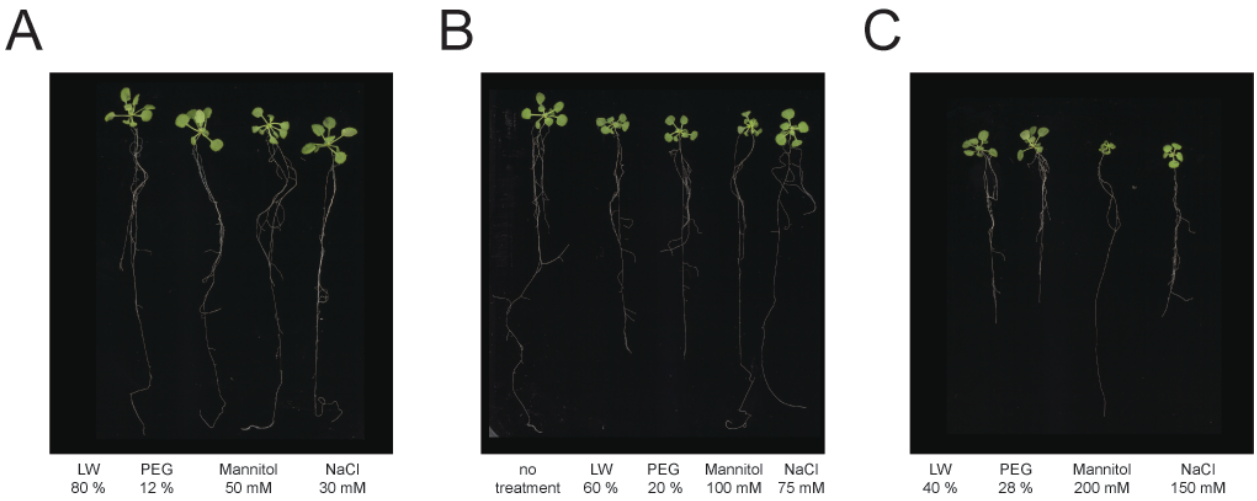


**Figure 1 – figure supplement 1**

**Plant growth responses to stress assays. A – C:** Images of 22-day-old *Arabidopsis* seedlings grown under different doses of each agar stress assay.

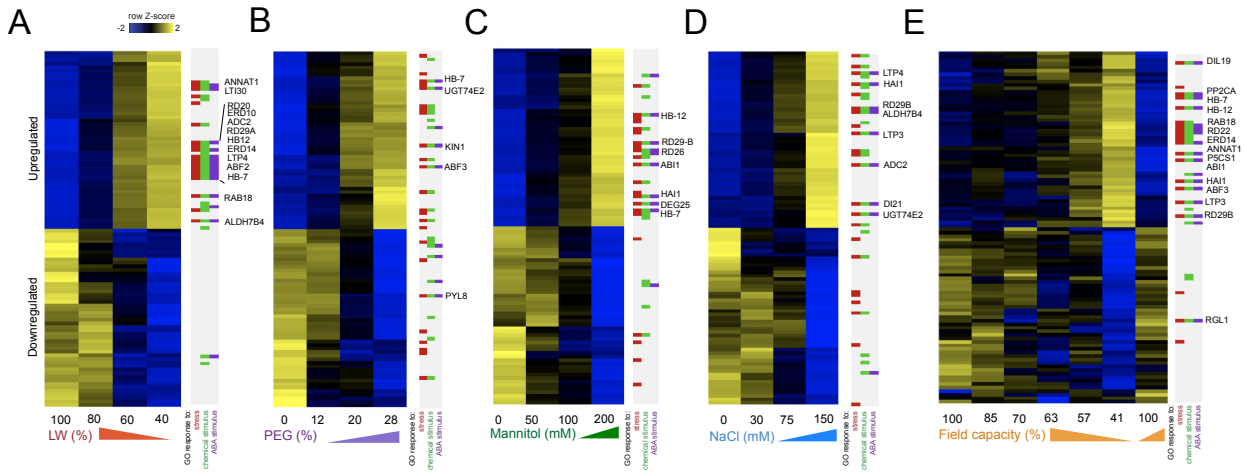


**Figure 1 – figure supplement 2**

**Shoot gene expression responses to each stress assay are dose responsive.** Heatmap displaying the top 50 most significant upregulated or downregulated genes in shoots in response to **(A)** LW, **(B)** PEG, **(C)** mannitol, **(D)** NaCl and **(E)** vermiculite induced drought stress. Membership of Gene Ontology (GO) Terms for 'response to stress', 'response to chemical stimulus' or 'response to ABA stimulus' indicated.

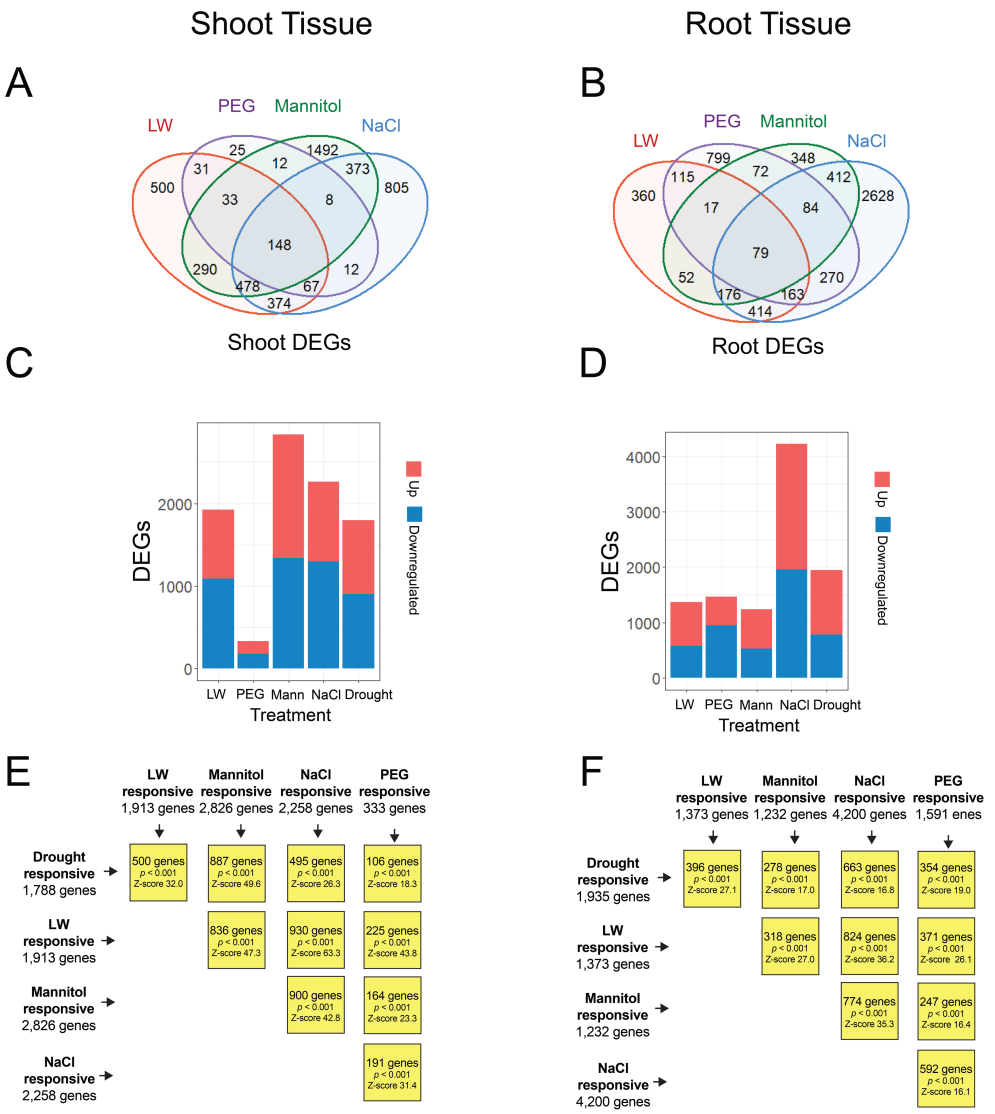


**Figure 1 - figure supplement 3**

**Overlapping differentially expressed genes (DEGs) in response to different assay types.** Overlap of dose-responsive differentially expressed genes in shoot **(A)** and root **(B)** in response to either LW, PEG, mannitol or NaCl (replicated from Figure 1). Number of upregulated or downregulated dose-responsive genes in response to each treatment type in shoot **(C)** and root **(D)**. Overlapping gene sets in **(E)** shoot or **(F)** root tissue (permutation test, $p < 0.001$).

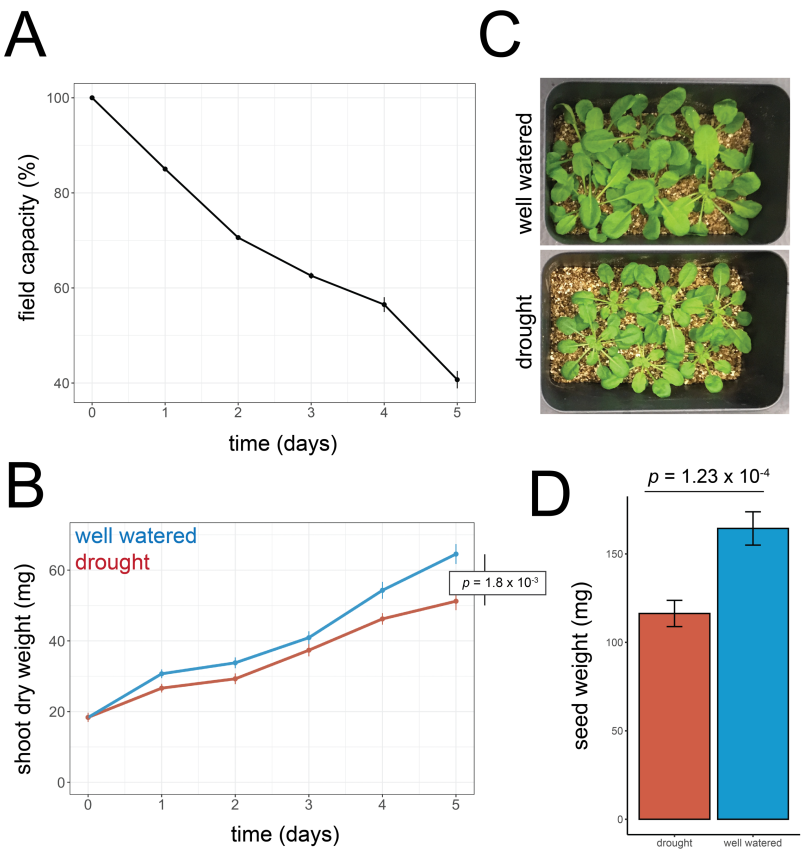


**Figure 1 - figure supplement 4**

**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).

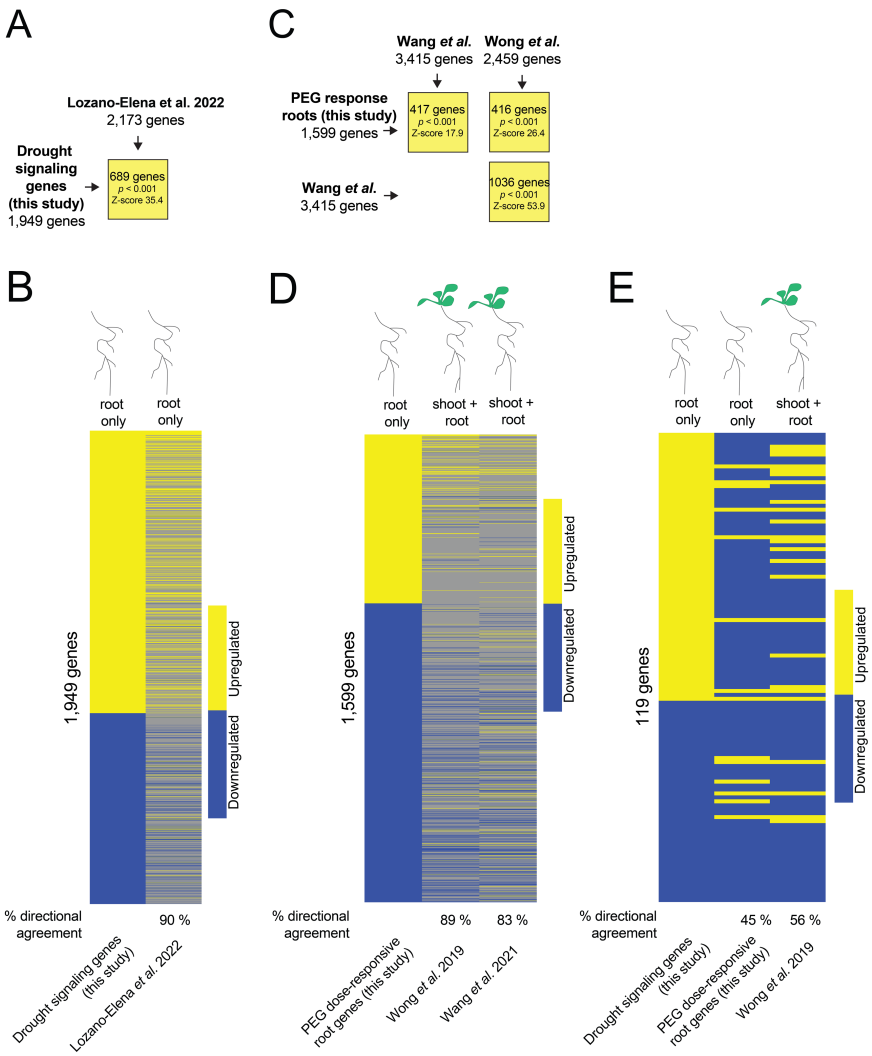


**Figure 2-figure supplement 1**

**Comparative transcriptomic analysis indicates drought signaling genes and PEG dose-responsive genes agree with previous studies. A:** Intersect analysis of root genes found differentially expressed in response to vermiculite drying within this study, and genes found differentially expressed in response to soil drying from Lozano-Elena *et al.* (*16*) (permutation test, $p < 0.001$). **B:** Heatmap displaying direction of root genes differentially expressed in response to either vermiculite drying (this study) or soil drying (*16*). Directional agreement with this study's drought response indicated. **C:** Intersect analysis of genes found differentially expressed in response to PEG treatment in this study, Wong *et al.* (*37*) and Wang *et al.* (*38*) (permutation test, $p < 0.001$). **D:** Heatmap displaying direction of genes differentially expressed in response to PEG treatment across each study. Directional agreement with this study's PEG response indicated. We note that both Wong *et al.* and Wang *et al.* assess transcriptomic responses of whole seedlings (both root and shoot), while our study assesses root tissue only. **E:** 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.* Directional agreement with this study's drought signaling response and each PEG treatment experiment indicated.

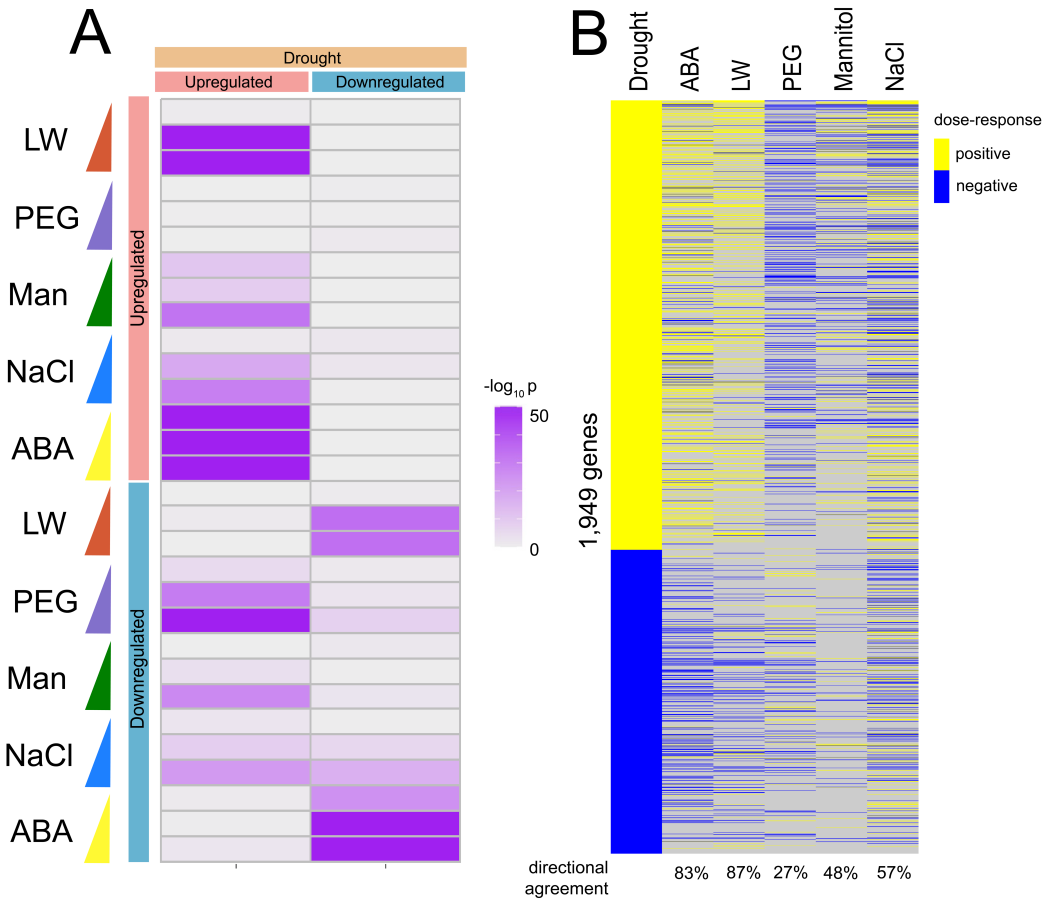


**Figure 2-figure supplement 2**

**Comparative transcriptomic analysis reveals ABA-induced differential expression is comparable to drought and low-water (LW) agar signaling. A:** Overlap analysis of genes found differentially expressed under drought treatment, compared to those within each dose of either PEG, mannitol, NaCl, ABA or LW agar assays in both root and shoot (Fisher exact test, adj. $p < 0.05$). **B:** Heatmap displaying genes differentially expressed under drought stress in root tissue compared to their dose-responsive expression within each stress assay. Direction of gene expression agreement with drought-responsive gene expression (i.e., 'directional agreement') indicated.

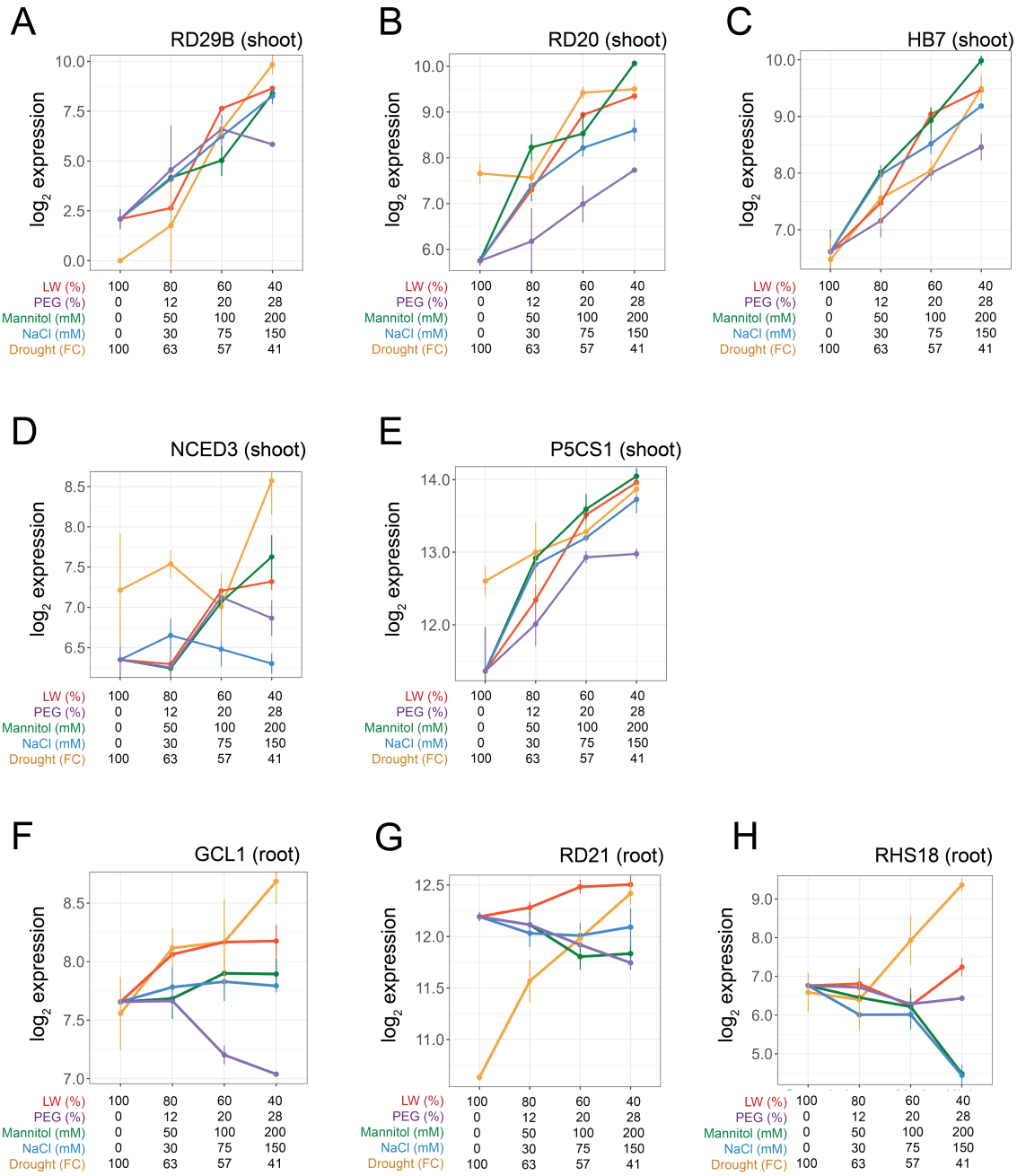


**Figure 2-figure supplement 3**

**Gene expression profiles of stress markers.** **A - E:** Expression patterns of drought marker 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 drought marker genes under doses of each assay in root tissue **(F)** GCL1, **(G)** RD21 and **(H)** RHS18.

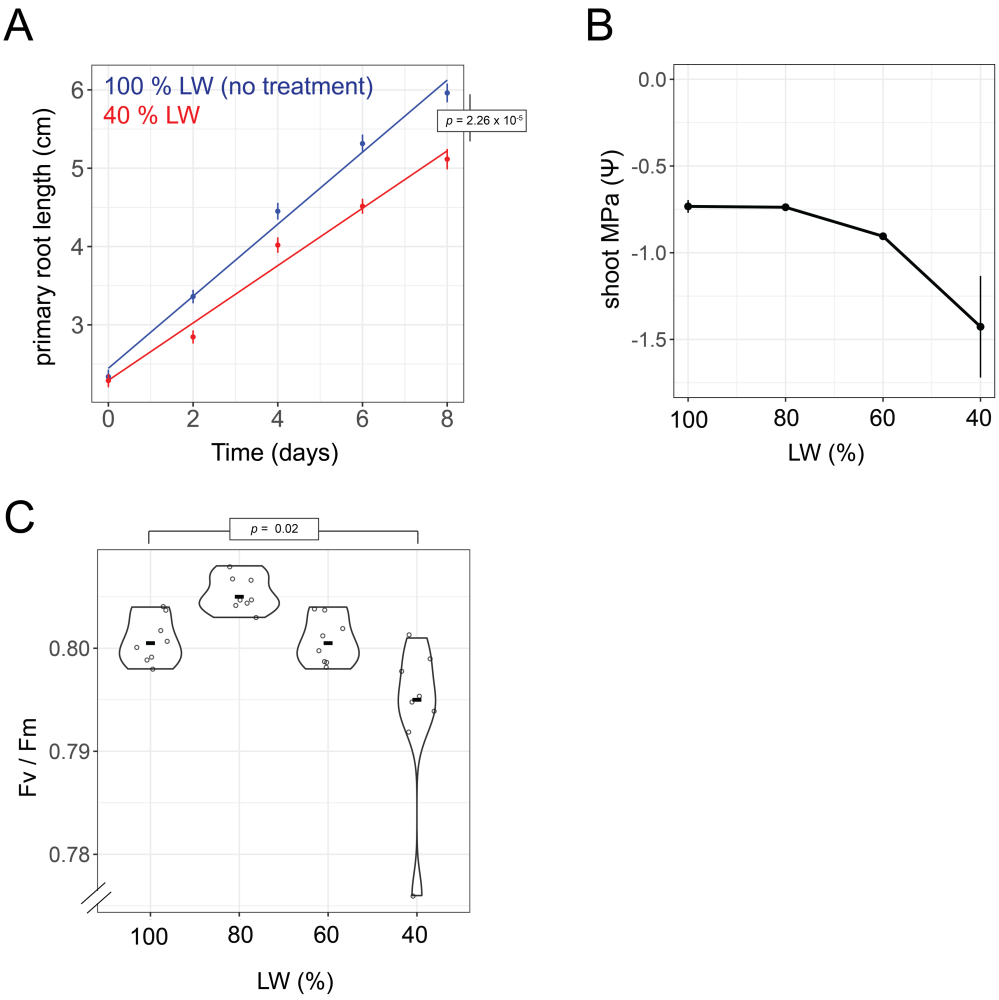


**Figure 2-figure supplement 4**

**Physiological measurements of *Arabidopsis* seedlings in response to LW treatment. A:** Measurement of primary root growth rate across 8 days of growth under no treatment and 40 % LW conditions (n= 16, t-test *p*). **B**: Shoot water potential measurements of seedlings grown under different LW media doses (n = 3, Pearson *p* = 0.009). **C**: Measurement of maximum quantum yield of PSII (Fv / Fm) under different LW media doses (n=4, t-test *p*).

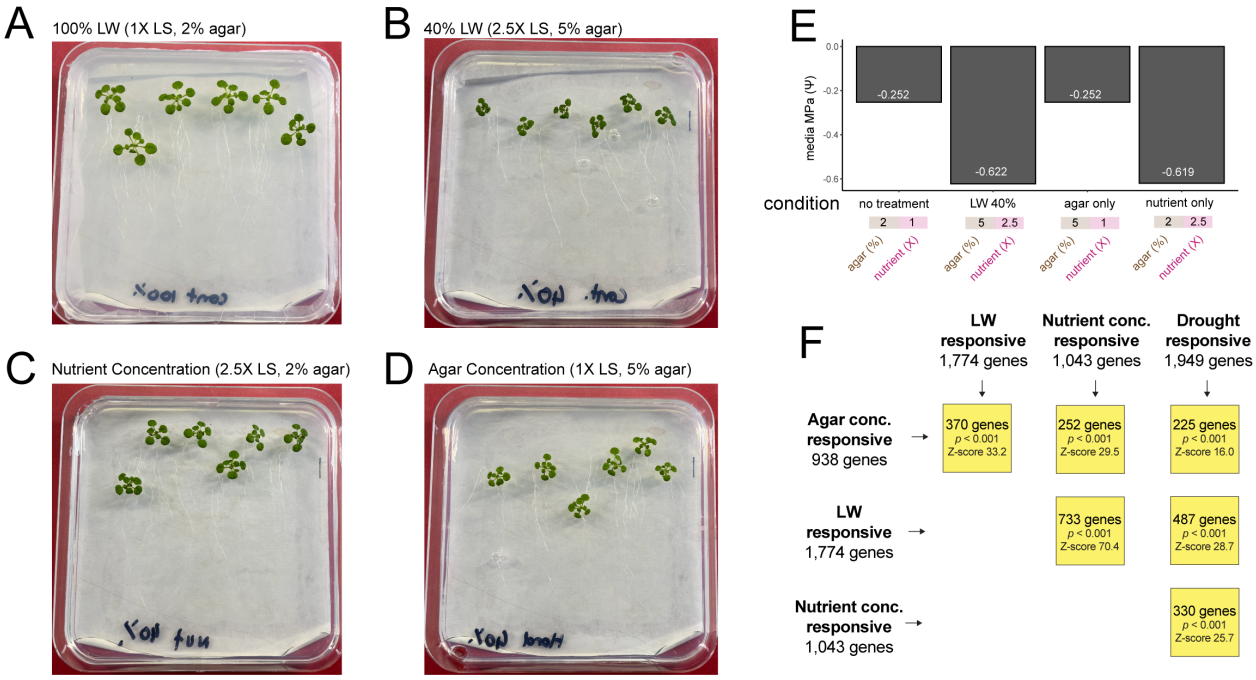


**Figure 2-figure supplement 5**

**Comparing the separate effects of nutrient concentration and agar concentration on seedling growth.** Image of *Arabidopsis* seedlings grown on either **(A)** 100% LW agar (i.e. 1X LS, 2% agar) or **(B)** 40% LW, which increased both nutrient and agar concentrations to 2.5X and 5%, respectively. **C:** Image of seedlings grown on an increased 2.5X 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**. **F:** Intersection of differentially expressed genes responsive to either agar concentration, nutrient concentration, LW treatment or drought stress (permutation test, $p < 0.001$).

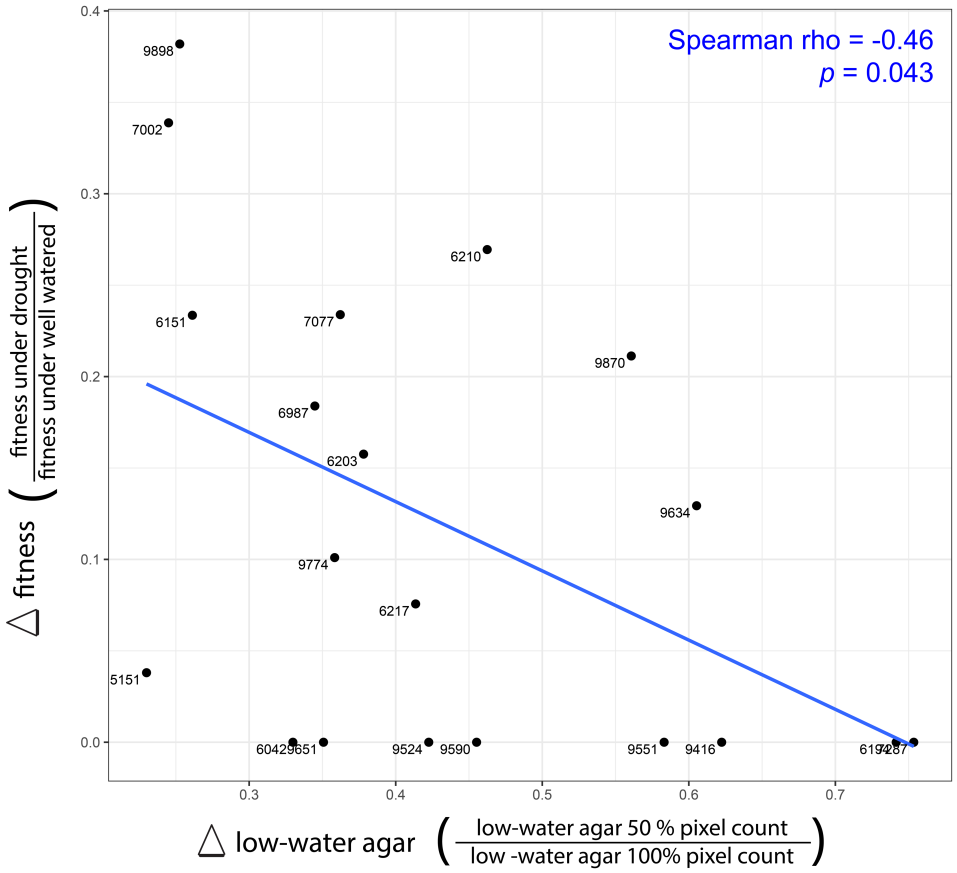


**Figure 2-figure supplement 6**

**Associating low water (LW) agar's impact on shoot size with plant fitness.** Comparing the impact low-water agar 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 (*30*).

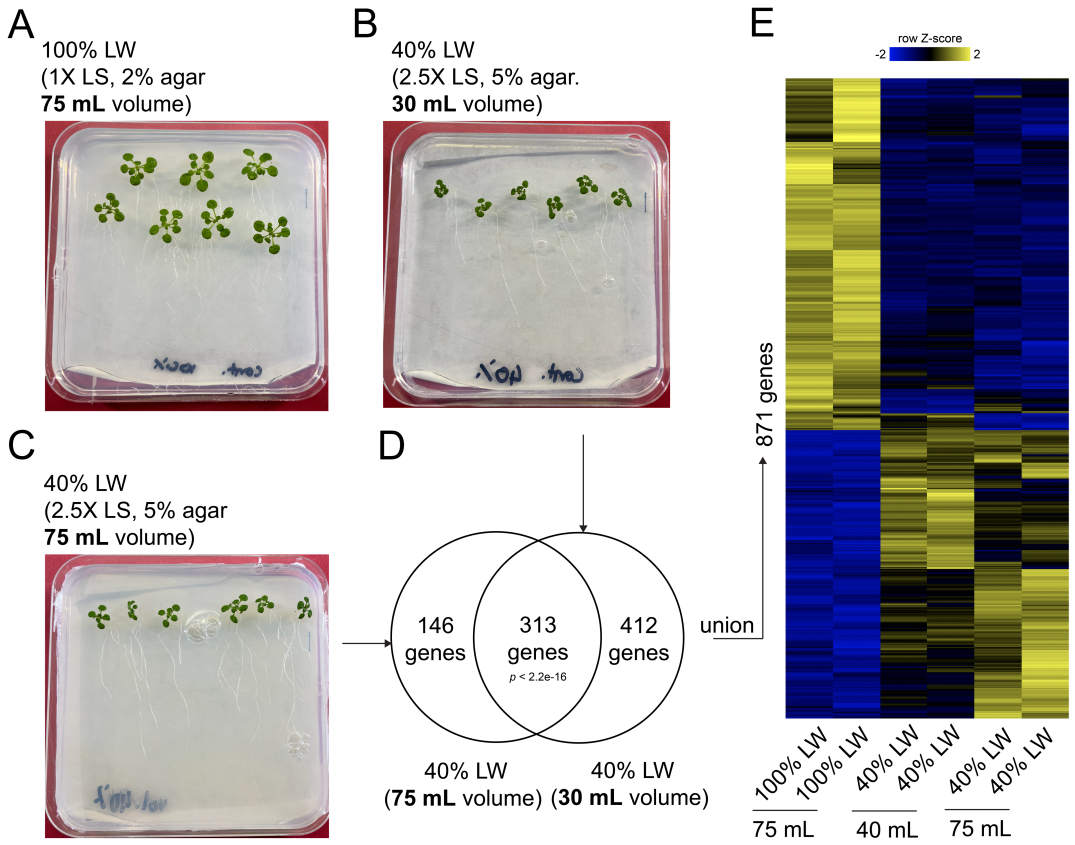


**Figure 2-figure supplement 7**

**The volume of LW agar does not impact gene expression.** Image of *Arabidopsis* seedlings grown on either **(A)** 75 mL of 100% LW agar, **(B)** 30 mL of 40% LW agar, or **(C)** 75 mL of 40% LW agar. **(D)** Overlap genes found differentially expressed on either 75 mL or 30 mL of LW agar is statistically significant (Fisher exact test). **(E)** Heatmap of the union of genes found either differentially expressed on 75 mL or 30 mL of LW agar indicates both volumes elicit similar transcriptional trends.

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