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Seedling response to water stress in valley oak (*Quercus lobata*) is shaped by different gene networks across populations

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Abstract

Drought is a major stress for plants, creating a strong selection pressure for traits that enable plant growth and survival in dry environments. Many drought responses are conserved species-wide responses, while others vary among populations distributed across heterogeneous environments. We tested how six populations of the widely distributed California valley oak (*Quercus lobata*) sampled from contrasting climates would differ in their response to soil drying relative to well-watered controls in a common environment by measuring ecophysiological traits in 93 individuals and gene expression (RNA-seq) in 42 individuals. Populations did not differ in their adjustment of turgor loss point during soil drying, suggesting a generalized species-wide response. Differential expression analysis identified 689 genes with a common response to treatment across populations and 470 genes with population-specific responses. Weighted gene co-expression network analysis (WGCNA) identified groups of genes with similar expression patterns that may be regulated together (gene modules). Several gene modules responded differently to water stress among populations, suggesting regional differences in gene network regulation. Populations from sites with a high mean annual temperature responded to the imposed water stress with significantly greater changes in gene module expression, indicating that these populations may be locally adapted to respond to drought. We propose that this variation among valley oak populations provides a mechanism for differential tolerance to the increasingly frequent and severe droughts in California.

KEYWORDS

drought, gene expression, local adaptation, *Quercus*, turgor loss point, water stress

1 | INTRODUCTION

Drought is a major abiotic stress for plants (Chaves, Maroco, & Pereira, 2003; Chaves et al., 2002; Hsiao, Acevedo, Fereres, & Henderson, 1976), which have a continuous demand to replace water lost through the stomata during photosynthesis (Bray, 1997). In populations with high seedling mortality during drought, selection pressure could favour individuals adapted to the water availability of the local environment. Sites with different climates may favour different phenotypically plastic traits (Nicotra et al., 2010), allowing individual plants to respond to variable conditions at their local site and allowing long-lived species to survive changing conditions throughout their lifespan (Sork, 2017; Sork et al., 2013). However, the plastic adjustments most important to drought tolerance, and variation in those adjustments across plants, are largely unknown. In this study, we quantified the impacts of simulated drought on gene expression in oak seedlings from populations adapted to a range of climatic conditions to determine whether populations from different

climates varied in their drought responses. We also coupled the gene expression analyses with assessments of plant water status and leaf traits to identify the gene expression patterns that drive phenotypic plasticity in key drought tolerance traits.

Plants can exhibit plasticity in many drought responses, including stomatal regulation, xylem anatomy and hydraulic function, and leaf biochemistry (Corcuera, Cochard, Gil-Pelegrin, & Notivol, 2011; Hsiao et al., 1976; Osakabe, Osakabe, Shinozaki, & Tran, 2014; Pinheiro & Chaves, 2011; Seki, Umezawa, Urano, & Shinozaki, 2007; Silim, Nash, Reynard, White, & Schroeder, 2009). Drought may also reduce growth and photosynthesis due to decreased leaf turgor and carbon intake (Chaves et al., 2003; Hsiao et al., 1976; Hummel et al., 2010). These responses can vary among plant taxa depending on how they have evolved to deal with drought; taxa that are adapted to frequent droughts may have responses that optimize their survival during drought and recovery and growth afterwards. We focus here on the osmotic potential at turgor loss point (π_{TLP}), or the wilting point, an ecophysiological trait commonly used to characterize plant drought tolerance (Bartlett, Scoffoni, Ardy, et al., 2012; Lenz, Wright, & Westoby, 2006; Mitchell & O'Grady, 2015). We also measured leaf water potential (Ψ_{leaf}) to characterize the level of osmotic stress the plants were experiencing, with a lower (more negative) value indicating higher solute concentration and less water in the leaves (Lenz et al., 2006). Having a lower (more negative) π_{TLP} means that a plant is able to experience more severe water stress, characterized by more negative leaf water potentials, and resist wilting even at a low Ψ_{leaf}. This ability allows the plant to maintain stomatal conductance and photosynthesis further into drought (Bartlett, Scoffoni, & Sack, 2012; Morgan, 1984; Turner & Jones, 1980) by preventing the stomatal closure and decreased carbon intake that occurs at the wilting point. The π_{TLP} varies across plant species occurring in environments that differ in aridity, and individual plants can lower their π_{TLP} during drought by accumulating solutes; the amount of adjustment possible may vary based on the type of solute and its metabolic cost (Bartlett, Scoffoni, & Sack, 2012; Bartlett et al., 2014; Morgan, 1984; Nilsen & Orcutt, 1996). In this study, we tested whether populations exhibited differences in plasticity of π_{TLP} or differences in water stress, measured as stronger declines in leaf water potential (Ψ_{leaf}) under drought.

Gene expression is one way that individuals can alter phenotypic traits over a short timescale, and can be measured by assessing expression levels of mRNA found in a tissue using RNA-seq (Finotello & Camillo, 2014). Gene expression precedes ecophysiological changes (De Nadal, Ammerer, & Posas, 2011; Jończyk et al., 2017) and can capture a greater diversity of responses beyond measured physiological traits. Differential expression (DE) analyses comparing water-stressed plants to controls have been used to identify candidate drought response genes in a wide range of species, including wild barley (Hübner, Korol, & Schmid, 2015), eucalyptus (Villar et al., 2011), fir (Behringer, Zimmermann, Ziegenhagen, & Liepelt, 2015), pine and spruce (Yeaman et al., 2014), poplar species (Cohen et al., 2010; Street et al., 2006), switchgrass (Meyer et al., 2014) and oaks (Gugger, Peñaloza-Ramírez, Wright, & Sork, 2016; Steele, 2017). Using weighted gene co-expression network analysis (WGCNA, Langfelder & Horvath, 2008), co-expressed genes are grouped into eigengenes, or modules, which can be considered putative functional categories regulated in the same way and can be tested for differences across conditions (Campbell-Staton, Bare, Losos, Edwards, & Cheviron, 2018; Kenkel & Matz, 2016; Passow et al., 2017; Rose, Seneca, & Palumbi, 2016).

Drought responses may be common across a species or may vary among populations. To identify species-wide and population-specific responses to drought, an ANOVA framework is commonly used to identify effects as environmental (E, species-wide responses), genotypic (G, population differences that do not respond) and genotype × environmental (G × E, population differences in response, Des Marais, Hernandez, & Juenger, 2013). Studies comparing drought responses among multiple populations or genotypes have identified variation in ecophysiological traits related to drought response (Corcuera et al., 2011; Kavanagh, Bond, Aitken, Gartner, & Knowe, 1999; Lovisolo et al., 2010; McDowell et al., 2008), as well as in gene and gene network expression (Akman, Carlson, Holsinger, & Latimer, 2015; Akman, Carlson, & Latimer, 2018; Des Marais et al., 2012; Gould, Chen, & Lowry, 2018; Gugger, Peñaloza-Ramírez, et al., 2016; Hübner et al., 2015; Lasky et al., 2014; Shin et al., 2015; Villar et al., 2011; Yates et al., 2014). When investigating whether populations vary in their drought response, it is useful to compare both ecophysiology and gene expression to assess the extent to which the two responses show similar patterns. Population differences in physiological drought responses are likely to be a result of genomic differences (either adaptive or neutral) among the populations, which can be confirmed if populations also differ in their gene expression response. Populations may also have similar physiological responses to drought, indicating either species-wide plasticity due to a similar gene expression response; or that populations have similar physiological responses despite being genetically differentiated, due to neutral processes affecting gene expression or to populations being constrained by their genomic architecture in how they adapt to their site-specific conditions. Population differences in gene expression may also indicate processes occurring that were not directly observed through physiological or trait measurements.

We examined species-wide and population-specific patterns of drought response in valley oak (*Quercus lobata*), a California endemic tree species ideal for testing population-specific responses because it occurs across a range of environments heterogeneous in water availability and temperature. Valley oak populations differ genetically across their range (Gugger, Cokus, & Sork, 2016; Gugger, Ikegami, & Sork, 2013; Sork et al., 2016), and differential gene expression in response to water stress has been found previously in other oak species (Porth, Koch, Berenyi, Burg, & Burg, 2005; Spieß et al., 2012; Steele, 2017). Here, our goals were to test whether (a) California populations of *Q. lobata* have different ecophysiological and gene expression responses to drought simulated by soil drying and (b) whether these responses align with each other, suggesting that the genetic basis of physiological responses is the same for all populations, or do not align, suggesting populations have different genomic mechanisms for

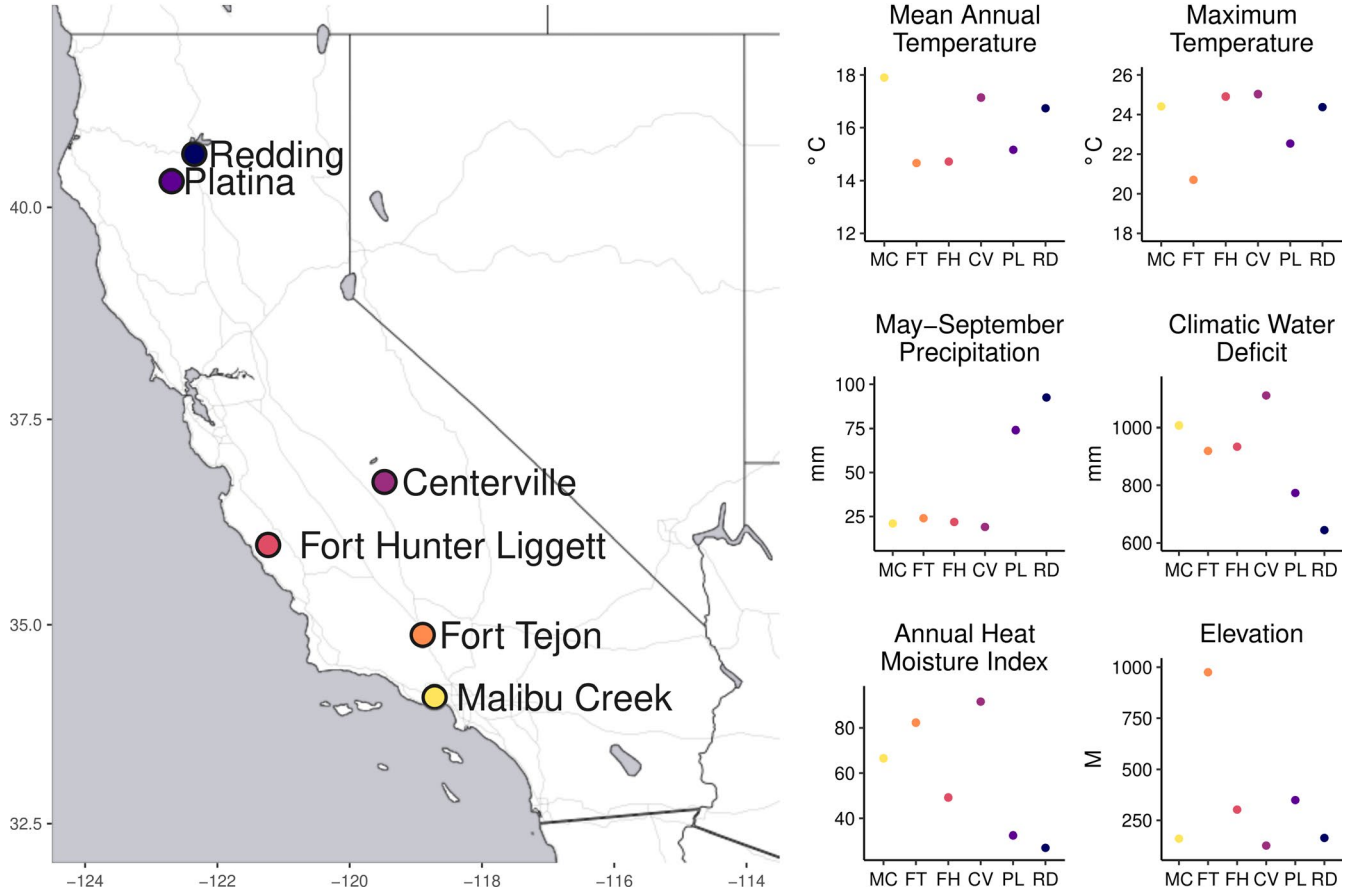

FIGURE 1 Map of sites where acorns were sampled and climate variables for each site. Climatic water deficit and maximum temperature are from the 2014 California Basin Characterization Model's historical data (Flint et al., 2013) for years 1951–1980; mean annual temperature, May–September precipitation and annual heat moisture index are from the AdaptWest project (Wang et al., 2016) for years 1961–1990. Map modified from Stamen Design (CC BY 3.0) and OpenStreetMap (ODbL)

altering their physiology. We compared seven growth and leaf traits, plant drought tolerance, plant water status and gene expression of seedlings originating from six different populations from contrasting climates throughout California under well-watered and soil drying conditions. Additionally, putative regulatory relationships between gene expression and osmotic stress were identified using the maximal information coefficient as a measure of their association. We find that populations of this widespread tree species vary in their gene expression response to water stress, but they do not differ in their physiological drought tolerance response. These results suggest that valley oak populations may be able to respond to stresses in similar ways, but through different genomic mechanisms, providing valuable information in predicting how genetically differentiated populations will respond to future climate changes.

2 | MATERIALS AND METHODS

2.1 | Sampling and experiment design

Acorns for the experiment were collected throughout fall of 2012 as part of a larger range-wide provenance study (Delfino Mix, Wright, Gugger, Liang, & Sork, 2015), and in spring 2013, germinated seedlings were selected from six sites throughout California that vary in temperature, precipitation and seasonality (Figure 1): Malibu Creek State Park (MC), Fort Tejon State Historic Park (FT), Fort Hunter Liggett (FH), Centerville (CV), Platina (PL) and Redding (RD). A total of 93 seedlings were included, with 15–16 individuals from each location (Table 1). Preparation and growth of seedlings is fully described in Delfino Mix et al. (2015). Briefly, acorns were stored at 1.1°C, then sterilized with a 10% bleach solution to kill mold. Acorns were then grown together in a greenhouse at the U.S. Department of Agriculture Forest Service, Institute of Forest Genetics, Placerville, California. In August 2013, 1 year after acorn collection and following spring and summer seedling growth, seedlings were moved to the University of California, Los Angeles campus, where they were transplanted to larger pots and acclimated to the conditions in the UCLA Plant Growth Center.

Seedlings were placed in a well-watered control or a soil drying treatment group, with 3 or 4 individuals from one population in each treatment (Table 1). All seedlings in the water stress group were subjected to a drought-hardening period in which they were not watered for 10 days, then rewatered to allow recovery, to

TABLE 1 Summary of experimental design showing the number of seedlings per treatment per population

	Day 10		Day 20	
Population	Control	Treatment	Control	Treatment
MC	3	4 (3)	4	4
FT	4 (3)	4 (3)	4	4
FH	4	4 (3)	4	3
CV	4	4	4	3
PL	4	4 (3)	4	4
RD	4	4	4	4

Note: Both trait and gene expression data were collected for day 10, and only trait data were collected on day 20. Parenthesis indicate the number of seedlings that were sequenced when that number is different from the number measured for traits due to unsuccessful RNA extraction or sequencing.

partly mimic natural conditions in which seedlings are subject to intermittent periods of drought and acclimation before the onset of severe drought (Vilagrosa, Cortina, Gil-Pelegrín, & Bellot, 2003; Villar-Salvador et al., 2004). After the drought-hardening period, water was withheld again for two time periods, 10 and 20 days after rewatering, to test the effect of water stress at different stages.

2.2 | Ecophysiological measurements and analysis

Seedling traits were measured at 10 days and at 20 days using different sets of seedlings from each population as a consequence of destructive sampling needed for ecophysiological measurements (Table 1). A previous study that measured the gene expression of valley oak seedlings after 15 days of soil drying found that many of the differentially expressed genes were related to death and senescence (Gugger, Peñaloza-Ramírez, et al., 2016), so the 10-day duration was chosen to assess seedling response to an early stage of soil drying and the 20-day duration to assess more severe ecophysiological stress response. We assessed leaf morphology, drought tolerance, plant water status and soil properties. The leaf morphology traits measured were the length, width and thickness of the largest leaf on a seedling; and the leaf thickness, area and dry mass averaged across two leaves. We characterized drought tolerance as the turgor loss point, which was measured using an osmometer (Wescor VAPRO 5600) as described in Bartlett, Scoffoni, Ardy, et al. (2012). Plant water status was measured as the leaf water potential, using a Scholander pressure bomb (Plant Moisture Stress pressure chamber model 1000; PMS Instrument Co). Soil water potential was measured using the water potential of leaves from whole plants that had been bagged for 30 min to 1 hr to prevent transpiration. Leaf and soil water potential were measured at mid-day (1–2 p.m.). We analysed the data by conducting a two-way ANOVA in R (R Core Team, 2019) using the "lm" function with a "trait ~ population × treatment" model. For significant results, post hoc testing was conducted with pairwise *t* tests among all groups. We conducted a power analysis to determine the sample size needed to find a significant difference among populations given the observed among-group and within-group variance using the function "power.anova.stats" in R.

2.3 | RNA extraction and sequencing

Leaf tissue for RNA-seq was collected 10 days after the second dry-down period began in order to assess early response to soil drying. Leaf tissue for RNA-seq and trait measurements were taken from the same seedlings at the same time. After collection, leaves were immediately frozen in liquid nitrogen, then stored in a freezer at −80°C. RNA was extracted in multiple batches. Polyphenolics and polysaccharides were removed from leaves using a lithium chloride/urea-based prewash protocol originally developed for conifers (openwetware.org/wiki/Conifer_RNA_prep). Whole RNA was then extracted using the Qiagen RNeasy Plant Mini Kit protocol. The complete protocol is described in detail at: openwetware.org/wiki/Sork_Lab:Protocols#RNA_Extraction_for_Oak. Briefly, about 50 mg of frozen leaf tissue was ground in grinding tubes using a Retsch mixer mill (model MM 301) at room temperature, with grinding adapters and tubes placed in liquid nitrogen between rounds of grinding. Tissue was then placed in an RNA extraction buffer consisting of (per sample) 0.675 ml LiCl, 0.864 g urea, 0.288 ml 11% PVP K-60 solution and 0.018 ml dithiothreitol. Samples were kept at 4°C overnight. The next morning (after about 15–18 hr), the Qiagen protocol was followed, including the DNase addition step. RNA quality was checked with a Nanodrop, and samples with a low 260/280 (<1.5) or 260/230 (<1.4) ratio were purified using Agilent AMPure beads with a 70% ratio of beads to RNA sample. Final RNA quality was checked using an Agilent TapeStation 2200. As RNA extracted from leaves contains chloroplast rRNAs (Babu & Gassmann, 2016), RINe quality scores are unreliable for leaf tissue; instead, samples were considered acceptable when distinct peaks indicating nondegraded ribosomal RNA were present and primer-dimers were absent.

Library preparation on the extracted RNA samples was done in three batches using an Illumina NeoPrep and a TruSeq Stranded mRNA kit (v1). Library quality was determined on the TapeStation using the presence of a single large peak around 300 bp. Samples were diluted to 10 nM in a solution of 0.1% Tween in Qiagen EB buffer, based on molar concentrations calculated from the cDNA peak and concentration given by the TapeStation. Samples were pooled, and AMPure bead purification was done on pooled libraries using a 1:1 ratio of beads and sample in order to remove primer-dimers. Libraries were sequenced using single-end, 50 bp sequencing on an Illumina HiSeq 4000 machine across four lanes (10 or 11 samples per lane). Samples were assigned to lanes using a balanced design for population, maternal family, treatment and library preparation batch of the sample. A total of 42 individuals were sequenced, 22 from the control treatment and 20 from the soil drying treatment, with 3–4 individuals in the same population and treatment (Table 1).

2.4 | Sequence data preprocessing

Samples were demultiplexed allowing one base mismatch in the barcode sequence. Reads were trimmed using Cutadapt version 1.12 (Martin, 2011) to trim adapters and end regions with a quality score <27, then reads <20 bp long were removed. Sample quality was visualized both before and after trimming using FastQC (Andrews, 2016). Reads were aligned to the *Quercus lobata* transcriptome (Cokus, Gugger, & Sork, 2015) using Bowtie2 end-to-end alignment with default "sensitive" parameters (Langmead & Salzberg, 2012). Reads with a mapping quality (MAPQ) score <20 were removed using samtools version 0.1.19 (Li et al., 2009). Potential exclusion amplification (ExAmp) duplicates, in which a single read forms two clusters on the flow cell, leading to potentially incorrect read counts (Hadfield, 2016), were removed using a custom script that identified identical sequences within a radius of 2,500 pixels, and kept only the sequence with the highest quality score.

Prior to analysis, read counts per sample for a given mRNA feature (hereafter "gene") were filtered to remove lowly expressed genes that did not have at least 10 reads in at least one sample and a total of 15 reads across all 42 samples using limma's "filterByExpr" function. After filtering reads by quality and filtering genes by read count, 19,459 genes remained and were used for further analyses. Read counts were transformed to log2-counts per million (logCPM), a continuous measurement of expression, using the "voom" function in the R package limma (Ritchie et al., 2015).

Because sequencing lane and library preparation batch affected expression (tested using limma's "removeBatchEffect" function), we included lane and batch in the linear models for differential expression analyses. The output of removeBatchEffect, gene expression values corrected by lane and library preparation batch, was used for further analyses of gene expression (WGCNA and association of gene expression with ecophysiology, described below). One sample (069-15, from site CV and treatment group) was a strong outlier based on clustering and PCA plots and also showed quality issues based on the FastQC report, so it was removed from further analyses.

2.5 | Analysis of gene expression

Differential expression analysis was done using the "eBayes" function in limma for the following linear models: (a) expression ~ treatment + library prep + lane + family, to test for differentially expressed (DE) genes between the control and soil drying treatment for all individuals; (b) expression ~ population × treatment + library prep + lane + family, making contrasts of "treatment – control" for each population, to test for DE genes which responded to treatment differently among seedlings from different home sites. Maternal family (i.e., the tree an acorn was collected from) was unbalanced across treatments, so it was included in the model to account for variation in gene expression due to variable relatedness among seedlings. *p*-values were corrected for multiple testing using the Benjamini–Hochberg procedure where the false discovery rate = 0.05 (Benjamini & Hochberg, 1995). *Arabidopsis* orthologs and Pfam categories were identified for each gene using the annotated *Q. lobata* transcriptome (Cokus et al., 2015); of the genes used in the DE analysis, 7,944 had annotation (~41%).

Gene ontology enrichment testing was used to identify GO terms that were overrepresented in each group of upregulated or downregulated DE genes using the R package GOseq, which accounts for bias in gene length (Young, Wakefield, Smyth, & Oshlack, 2010). Only genes that were annotated with TAIR orthologs and had a predicted mRNA length were used in the analysis. The default Wallenius distribution method was used to approximate the null distribution and calculate *p*-values, as it is less computationally intensive. As each GO term is tested for both overrepresentation and underrepresentation, GO terms were designated as more likely to be over- or underrepresented (based on *p*-values), and *p*-values for the two groups were adjusted separately using the Benjamini–Hochberg procedure.

Genes were grouped into "modules," groups of genes that are expressed similarly, using weighted gene co-expression network analysis (WGCNA, Langfelder & Horvath, 2008), an R package. Two outlier samples were removed from the data set prior to analysis based on preliminary clustering (data not shown). The data set included the 19,459 genes remaining after filtering out lowly expressed genes as in the differential expression analysis, and expression values were corrected for lane and library preparation effects using limma's removeBatchEffects function. The analysis used the blockwiseModules function, which constructs gene networks and splits them into eigengenes, or "modules," and all genes were run in a single block. A soft thresholding power of seven was used based on the pickSoftThreshold function, approximating a scale-free topology with an R^2 of .9. The network constructed was unsigned (TOMtype = "unsigned"), meaning that correlated genes were clustered together regardless of whether the correlation was positive or negative, as this will include genes that may be negative regulators of a module. Modules that had a correlation of 0.7 or greater were merged into one module (mergeCutHeight = 0.3). This parameter produced relatively large modules, which tended to have more clear biological functions compared to those produced by smaller mergeCutHeight values. The minimum module size was 50 genes (minModuleSize = 50). Genes were not reassigned based on *p*-values after module construction (reassignThreshold = 0). All other parameters were kept at the default values. Altering these parameters did not substantially change the relationship of module expression with populations and treatments or the overall functions of modules. The module expression, or the first principle component of the module, represents the overall expression of the module in each sample (Langfelder & Horvath, 2008), so it was used in an ANOVA analysis using the "lm" function in R to determine which modules showed expression differences based on population, treatment or population × treatment interactions. Similarly, climate and climate × treatment effects on module expression were identified using the following climate variables for the location of the mother tree of a seedling: mean annual temperature (MAT), maximum yearly temperature (T_{max}), May to September precipitation (MSP), climatic water deficit (CWD), annual

heat moisture index (AHM), elevation, continentality or difference between the mean warmest month temperature and mean coldest month temperature (TD), and the difference between the yearly maximum and minimum temperature (T_{range}). CWD, T_{max} and T_{range} are from the 2014 California Basin Characterization Model's historical data (Flint, Flint, Thorne, & Boynton, 2013) for years 1951–1980; MAT, MSP, TD and AHM are from the AdaptWest project (Wang, Hamann, Spittlehouse, & Carroll, 2016) for years 1961–1990.

The general function of each module was characterized through gene ontology enrichment testing using GOseq as described above, and by identifying "hub genes," or highly connected genes for which expression is strongly correlated with module expression. These genes may have biological significance in regulating expression of other genes in the module. Here "hub genes" will be defined as those with a module membership in the top 10% of all genes in a given module.

2.6 | Relationship between phenotypic drought tolerance and gene expression

To test for relationships between gene expression and π_{TLP} and Ψ_{leaf}, maximal information coefficients (MIC) were calculated using the minerva package in R (Albanese et al., 2013). This test allows both linear and nonlinear relationships to be identified, as gene expression and physiological traits may have a nonlinear relationship (Meyer et al., 2014). Tests were done only on the seedlings in the water stress treatment, because leaf water potential was strongly dependent on treatment, and our goal was to identify genes associated with variation in the degree of water stress experienced by individual seedlings rather than genes that generally respond to water stress, as was previously done using differential expression analysis. For each expression-trait test, a null distribution of MIC values was created using 5,000 bootstrapped MIC tests with randomly associated data points, and *p*-values were calculated by determining the proportion of null MIC values that were higher than the actual MIC value; low values indicate it is unlikely to get an MIC as high as the true value due to random chance. Because the resolution of lower *p*-values is limited by the number of bootstraps, they were not corrected for multiple testing, but values <0.001 were considered statistically significant; out of the 19,459 tests, it would be expected that about 19.5 of them would have $p < .001$ by chance. To test for relationships between module expression and trait variation among individuals, Pearson correlations were performed between WGCNA module expression and trait values for ecophysiological traits, home site and the presence or absence of water stress treatment.

3 | RESULTS

3.1 | Leaf and ecophysiological phenotypic variation

Leaf drought tolerance exhibited significant plasticity under water stress (treatment effect; p = 7.22E-10), but this plasticity was not significantly different among populations (population × treatment effect; $p = .88$). On average across populations, water stress reduced π_{TLP} by 0.18 MPa at 10 days and by 0.43 MPa at 20 days (Figure 2). Each population also experienced a similar degree of water stress. Leaf and soil water potentials were significantly more negative in the drought treatment (treatment effect; p = 4.16E-16 and 1.08E-16, respectively), but the difference across treatments did not vary among populations (population × treatment effect; $p = .83$ and .23, respectively). Ψ_{leaf} values were 0.26 MPa lower after 10 days and 1.63 MPa lower after 20 days in the water stressed relative to the control seedlings, though the difference was only significant at 20 days (Figure 2, Figures S1 and S2). Soil water potential was significantly lower in the water stress treatment at both 10 and 20 days, confirming that soil drying reduced water content in pots and that these plants experienced greater water stress (Figure 2). The power analysis indicated that sample sizes of 196 and 59 would be necessary for the observed among-population and within-population variances to be significant for Ψ_{leaf} and π_{TLP}, respectively; consistent with the small, nonbiologically relevant differences in mean measurements observed among populations (Figure S1 and S2).

In contrast, some growth and leaf morphology traits did vary among populations. Height, largest leaf thickness and average leaf thickness were significantly different among individuals originating from different sites (Table 2, Figure 2), but did not vary significantly between treatments. No traits showed a significant population × treatment interaction.

3.2 | Differential expression analysis

A total of 19,459 *Quercus lobata* transcripts (mRNA features) were identified from the sequenced individuals. Volcano plots show variation in log-fold expression change and number of significantly DE genes for all models tested (Figures S4 and S5). For the treatment model, 689 significant DE genes were identified; 294 downregulated and 395 upregulated. Our analysis identified significantly enriched GO terms among the upregulated genes; these 52 GO terms were generally related to abiotic stress and included "response to abiotic stimulus," "response to temperature stimulus," "response to water deprivation," "response to hormone" and "regulation of gene expression" (Figure 3). No significantly enriched GO terms were identified in the group of downregulated genes.

For the population × treatment model, a total of 470 genes were differentially expressed between treatments for different populations. DE genes were identified in seedlings from four of the six populations: MC (242 upregulated, 121 downregulated), RD (51 upregulated, 37 downregulated), FH (10 upregulated, two downregulated) and PL (two upregulated, five downregulated). FT and CV seedlings did not have any significantly DE genes. Among these DE genes, only five were DE in multiple populations: m01oak00239cT (a phosphoglycerate kinase), m01oak00925cC (a ribosomal protein), m01oak12555CC (TAIR ortholog ATGID1B, a gibberellin receptor), m01oak14960cC (unknown function) and m01oak22045JT (unknown

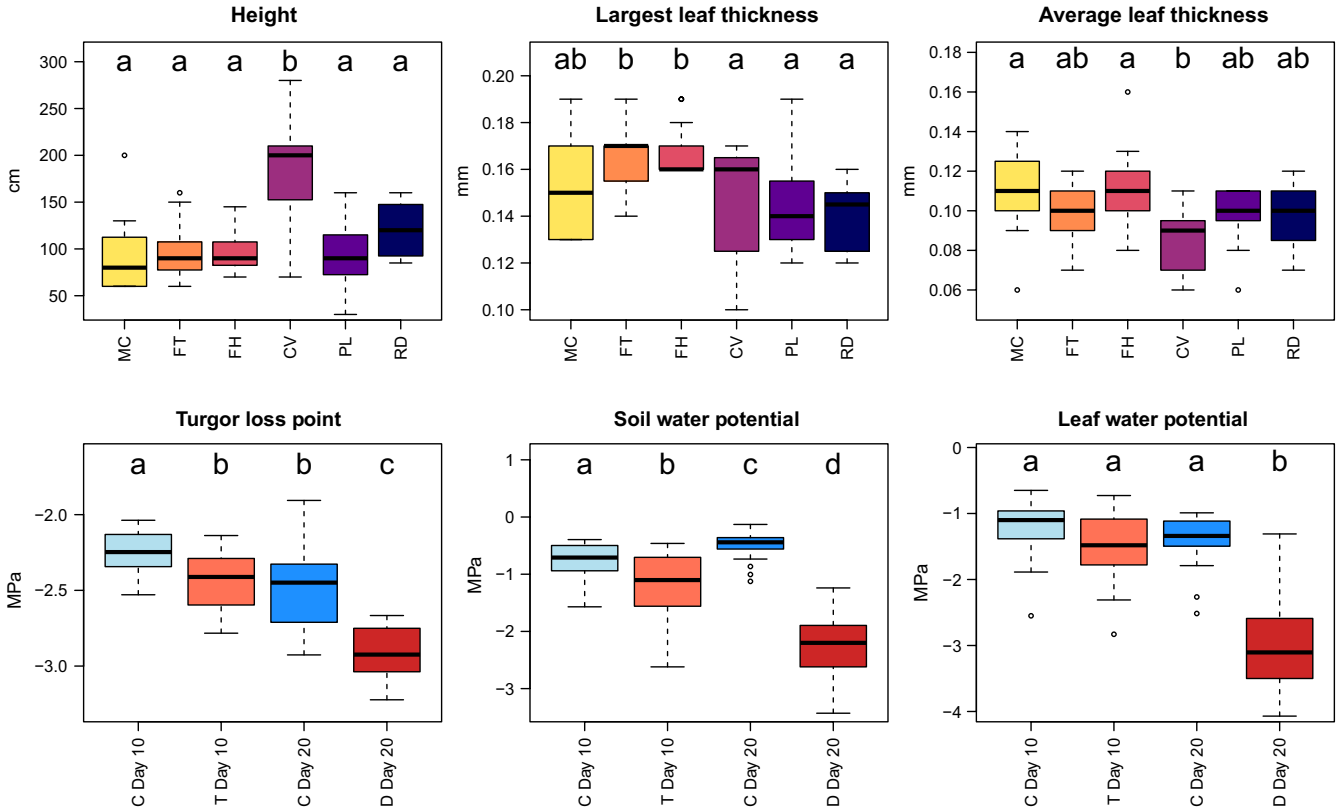

FIGURE 2 Differences among populations or treatments which were significant according to the ANOVA test. Traits (height and leaf thickness) varied among populations. Drought tolerance (turgor loss point) and experimental measurements (soil and leaf water potential) varied among treatment groups (C = control, T = water stress treatment). Because these measurements showed only significant differences for either population or treatment, plots showing population differences pooled together seedlings from different treatments, and plots showing treatment differences pooled together seedlings from all populations. Letters above boxplots indicate groups that are significantly different from each other according to a post hoc two-tailed *t* test ($p < .05$ after Benjamini–Hochberg correction)

function) (Figure S6). Overrepresented GO terms were identified for several sets of genes significantly upregulated or downregulated in a population in response to treatment (Figures S7–S9). MC, the population with the most DE genes, had 95 GO terms overrepresented in the treatment-upregulated genes, including metabolic and biosynthetic processes such as "flavonoid biosynthetic process," "cellular amino acid metabolic process" and "membrane"; responses to signalling such as "response to sucrose"; and "oxidoreductase activity" and related terms (Figure S7). There were also four GO terms enriched in the treatment-downregulated genes in MC seedlings related to chloroplast/plastid relocalization. There were no enriched GO terms among the 12 genes DE in FH seedlings, which included orthologs to the TAIR genes LEA7 (late embryogenesis abundant 7) and GID1B, a gibberellin receptor gene. From the 51 upregulated genes in RD, 37 GO terms were overrepresented (Figure S9), with functions including "cytosolic part," "ribosomal subunit" and "cell wall." Several ribosomal protein genes were upregulated under water stress, as well as ZINC INDUCED FACILITATOR-LIKE 1 protein, which may be involved in regulating stomatal closure (Remy et al., 2013). Although PL seedlings had only one upregulated gene with annotation (GID1B, also upregulated in FH seedlings), this gene resulted in 36 enriched GO terms related to gibberellin and signalling (Figure S8).

3.3 | Relationship of ecophysiological traits and gene expression

Weighted gene co-expression network analysis identified 24 modules of co-expressed genes (Table 3). Of these, four modules showed a population effect on the module expression (the first principle component of the module), six showed a treatment effect, and six showed a population × treatment effect, with four modules showing multiple effects (grey60, darkgreen, pink and yellow; Figure 4). The majority of seedling traits and soil properties were correlated with module expression of at least one module, with the exception of seedling height (Figure S11), suggesting potential functional relationships between traits or treatment conditions and modules. Additionally, one module (red, chloroplast-related) was negatively correlated with π_{TLP} (i.e., seedlings with low π_{TLP} had high expression of the module), but not correlated with treatment, unlike other π_{TLP}-correlated modules. Representative examples of GO terms that are significantly enriched among the genes within each module indicate the processes associated with the modules (Table 3). Based on hierarchical clustering of population means of the treatment-responsive modules (modules with a significant population or population × treatment effect), populations cluster into two main groups: one with MC, CV and RD, and one with

TABLE 2 Results of ANOVA comparing growth, leaf and ecophysiology seedling traits and experimental measurements across treatments and populations of seedlings

	Population					Treatment					Population × Treatment				
Measurement	***df* (num.)**	***df* (denom.)**	***F***	***p***	***p* adj.**	***df* (num.)**	***df* (denom.)**	***F***	***p***	***p* adj.**	***df* (num.)**	***df* (denom.)**	***F***	***p***	***p* adj.**
Height	5	69	13.0793	**5.84E-09**	**5.26E-08**	3	69	1.4931	NS	NS	15	69	1.2936	NS	NS
Largest leaf length	5	69	2.9617	**0.018**	NS	3	69	1.2667	NS	NS	15	69	1.8602	**0.0432**	NS
Largest leaf width	5	69	1.2862	NS	NS	3	69	0.4523	NS	NS	15	69	1.4975	NS	NS
Largest leaf thickness	5	69	6.2929	**7.25E-05**	**0.0005**	3	69	3.5462	**0.0189**	NS	15	69	0.8809	NS	NS
Average leaf thickness	5	69	4.4985	**0.0013**	**0.0085**	3	69	3.4183	**0.0220**	NS	15	69	1.2712	NS	NS
Leaf area	5	68	1.0177	NS	NS	3	68	0.2408	NS	NS	15	68	0.6551	NS	NS
Leaf dry mass	5	61	0.7693	NS	NS	3	61	0.5748	NS	NS	15	61	0.5453	NS	NS
Leaf water potential	5	67	0.7014	NS	NS	3	67	51.0136	**2.78E-17**	**4.16E-16**	15	67	0.7529	NS	NS
Turgor loss point	5	60	1.4514	NS	NS	3	60	26.0665	**6.42E-11**	**7.22E-10**	14	60	0.6509	NS	NS
Soil mass	5	66	0.9125	NS	NS	3	66	146.6538	**3.95E-29**	**1.78E-27**	15	66	0.1781	NS	NS
Soil water potential	5	60	0.9146	NS	NS	3	60	59.9610	**4.80E-18**	**1.08E-16**	15	60	1.6942	NS	NS

Abbreviations: denom., denominator; NS, not significant; Num., numerator.

p-values were adjusted using Benjamini–Hochberg method; when *p*-values were significant before adjustment, both values are shown. Significant ($p < .05$) results are in bold. Degrees of freedom (*df*) varies among traits due to missing measurements for some individuals.

FT, FH and PL (Figure 5). Module expression was also related to the climate of the mother tree (Table S1); in particular, nine modules had a climate × treatment effect for mean annual temperature (MAT), including all six modules showing a population × treatment effect. In addition, populations from higher MAT sites had greater absolute differences in mean module expression between control and treated seedlings for treatment-responsive modules (linear regression, $p = .003$, $R^2 = .15$).

Maximal information coefficients tests identified genes with a strong relationship between their expression and the π_{TLP} and Ψ_{leaf} of each individual seedling under water stress treatment that may be functionally regulated. Overall, Ψ_{leaf}-expression relationships had higher MIC values and lower *p*-values, with 142 genes significant at a $p < .001$ level (Figure 6). Tests for π_{TLP} identified only 24 genes with $p < .001$, close to the 19.5 expected by random chance. The gene modules from WGCNA were identified for these significantly associated genes for Ψ_{leaf}. A high proportion of genes with downregulated expression in the low Ψ_{leaf} seedlings belonged to the black (response to stress) and brown (unknown function) modules, which were also different between treatments, and the red (chloroplast) module, which was correlated with π_{TLP}. Similarly, black, red and grey60 (protein folding) genes were overrepresented among the genes with upregulated expression under low Ψ_{leaf}. Many of these relationships between gene expression and Ψ_{leaf} appear to be nonlinear, particularly for genes downregulated under low Ψ_{leaf}.

4 | DISCUSSION

Valley oak seedlings showed both species-wide and population-specific responses to water stress. Across all populations, turgor loss point shifted in the treated seedlings. Many genes and gene networks differed between treatments in the same way for all populations as well, generally those with functions involved in drought response. However, there was also extensive variation among populations in their gene expression response to treatment. This variation shows that, despite similar phenotypic responses, populations are genetically differentiated and vary in how they respond to water stress. These differences in response may be a result of selection or other evolutionary processes acting differently on each population. The species-wide physiological response may be controlled by the gene expression responses that are species-wide, or it may be a result of different gene networks altering the same traits due to variation in genomic architecture underlying those traits among populations. In either case, it is clear that these populations of valley oaks are genetically differentiated and vary in which genes respond to water stress.

4.1 | Species-wide responses

4.1.1 | Ecophysiology

The seedlings in this study showed a consistent decrease in turgor loss point (π_{TLP}) under water stress for all populations (Figure 3),

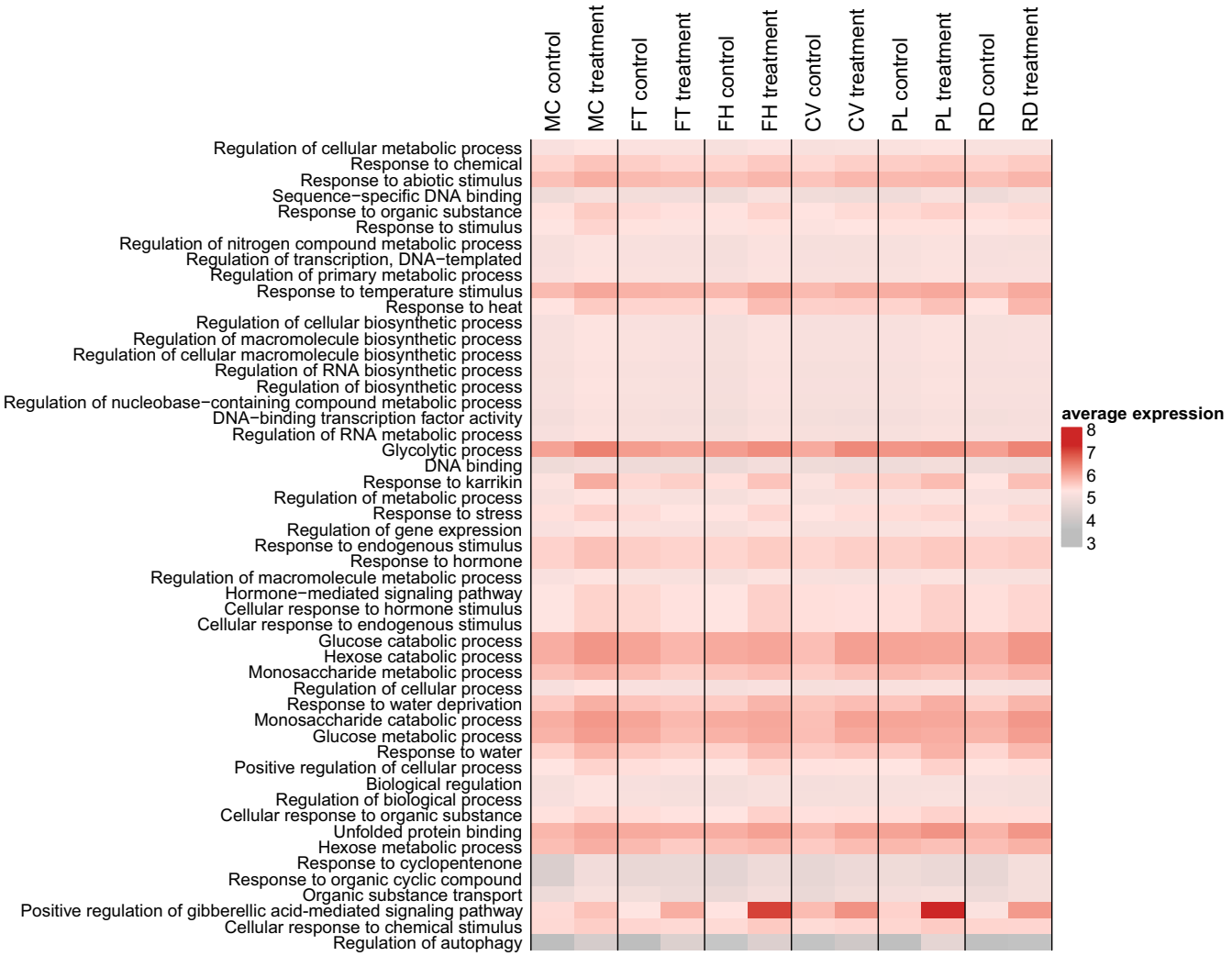

FIGURE 3 Heatmap of average gene expression for GO terms enriched within the genes that were significantly upregulated under treatment. Expression value is the expression of all genes annotated with the given GO term averaged across an individual, then averaged across each population/treatment group

possibly due to a common plastic response. McKown et al. (2014) measured a range of traits in different populations of *Populus trichocarpa* and found that most ecophysiological traits had low heritability and did not correlate with climate, similarly suggesting that these traits varied as a function of their environment rather than genetic background. With our study, we must caution that the lack of population differences in physiological response could be a result of low sample sizes (3–4 individuals in each group), as supported by the power analysis.

4.1.2 | Differential gene expression

This study identified 689 genes that changed expression in response to treatment across all individuals (about 3.5% of all genes included in the analysis), which was fewer than found in a previous study in valley oak, in which 52% of contigs responded to a water stress treatment (Gugger, Peñaloza-Ramírez, et al., 2016). This contrast is likely due to the differences in treatment methods between the studies. The seedlings in the previous study may have been under more severe stress because the period of drought was longer (15 days of water deprivation instead of 10 days) and they were not subject to the pretreatment drought exposure with recovery as was done here. This less stressful treatment resulted in a level of osmotic stress (Ψ_{leaf}) that was not very severe (Figure 2), allowing us to narrow down the genes responding to the stress treatment, which are more likely to be involved in drought response in the environment. If gene expression was measured at the 20-day period when the plants were experiencing much lower Ψ_{leaf} (Figure 2), corresponding with a greater degree of osmotic stress experienced by cells, we may have observed additional gene responses. Nonetheless, our design identifies numerous genes that may be expressed by seedlings experiencing fluctuations in rain and drought in natural settings.

The DE genes that were upregulated in all populations had functions typically associated with response to drought stress, including "response

TABLE 3 Summary information of each WGCNA module

Module name	Number of genes	Proportion of genes (%)	GO terms	Population	Treatment	Population × Treatment
Black	577	2.97	Protein modification process, response to stress, response to water deprivation, abscisic acid-activated signalling pathway		***	
Blue	3,548	18.23	Chloroplast, ribosome, mitochondrial part, gene expression			*
Brown	1,805	9.28	NS		*	
Cyan	218	1.12	Chloroplast, cytoplasmic part			
Darkgreen	97	0.50	Ribosome, gene expression, translation		***	**
Darkred	132	0.68	NS			
Darkturquoise	65	0.33	NS			
Green	647	3.32	ADP binding, DNA replication			
Greenyellow	308	1.58	DNA replication, protein modification by small protein removal			*
Grey60	184	0.95	Protein folding, response to heat, mitochondrion	*	***	
Lightcyan	197	1.01	NS			
Lightgreen	170	0.87	Mitochondrial part, ribosome, oxidation–reduction process			
Lightyellow	145	0.75	Endopeptidase activity, transmembrane transport, proteolysis			
Magenta	512	2.63	NS			
Midnightblue	206	1.06	NS			*
Pink	544	2.80	Anchored component of membrane, protein kinase activity, plasmodesma		**	*
Purple	335	1.72	Thylakoid membrane, photosynthesis, response to light stimulus	*		
Red	632	3.25	Chloroplast, oxidation–reduction process, photosynthesis, mitochondrion	*		
Royalblue	133	0.68	NS			
Salmon	262	1.35	NS			
Tan	297	1.53	Chloroplast, metabolic process, lipid biosynthetic process			
Turquoise	5,038	25.89	Intracellular transport, gene expression, protein transport			
Yellow	1,080	5.55	Catalytic activity, cell wall biogenesis, aromatic compound biosynthetic process, oxidoreductase activity	*		*
Grey (unassigned)	2,327	11.96	Catalytic activity, transferase activity, haem binding		**	
Total	19,459					

Note: Representative examples of significantly enriched GO terms for each module are also shown (NS = none significant). Results of the population × treatment ANOVA are shown for each effect; *p*-values are symbolized as follows: "***" for $p \leq .001$, "**" for $p \leq .01$, and "*" for $p \leq .05$. The "grey" module includes genes that could not be assigned to a module.

to abiotic stimulus," "response to water deprivation" and "response to hormone" (Figure 3). Interestingly, genes with the GO term "response to karrikins" were also significantly enriched in this group of genes. There has been some evidence that karrikins are involved in stress responses, and they may play a role similar to that of ABA (Li & Tran, 2015). Additionally, Wang, Waters, and Smith (2018) found that karrikins enhanced expression of non-ABA-responsive abiotic stress genes.

4.1.3 | Modules responding to water stress

Similar to individual genes, the species-wide treatment response modules (i.e., modules that showed treatment effects but no population × treatment effects) included genes with typical drought response functions. The black module appears to include many genes classically involved in water stress response and was enriched for

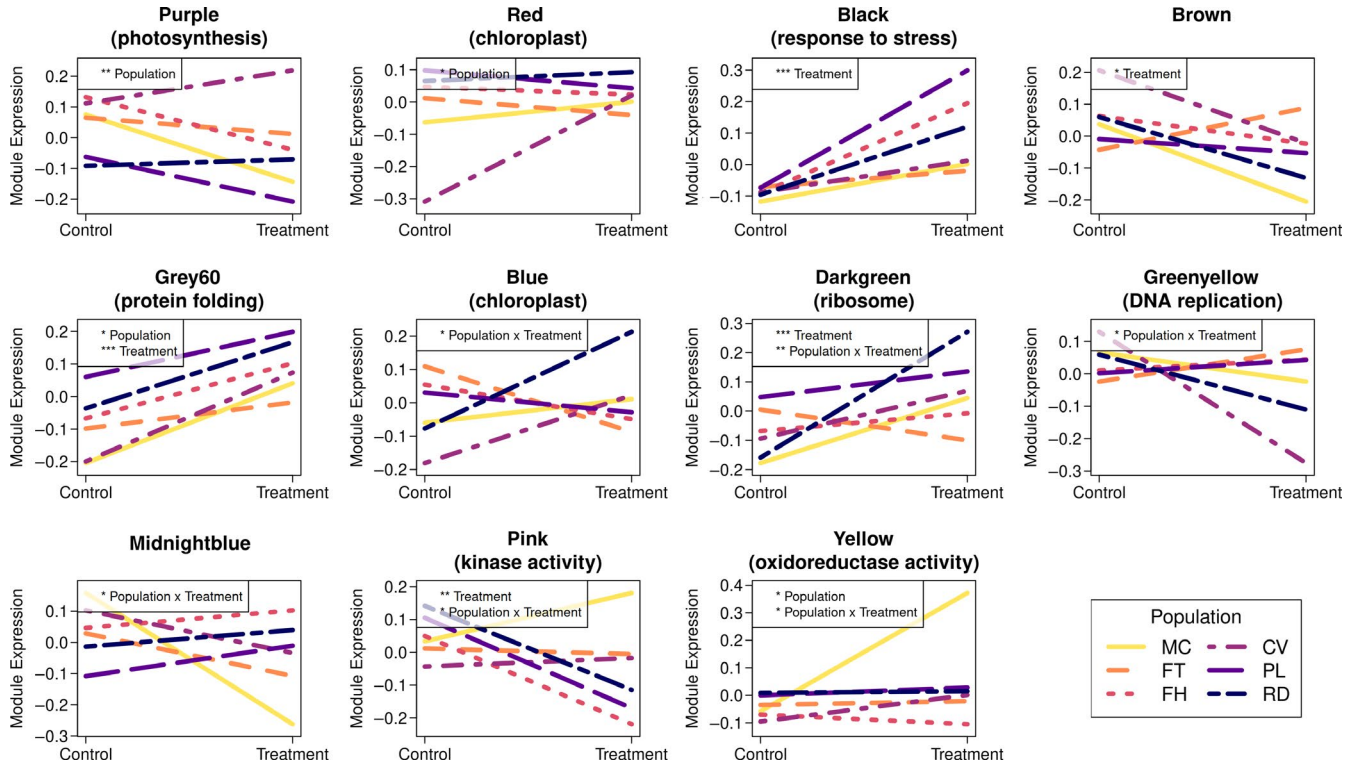

FIGURE 4 Norm of reaction plots for module expression in each population and treatment for modules which showed a significant population, treatment or population × treatment effect. Significance of each is shown on plot; *p*-values are symbolized as follows: "***" for $p \leq .001$, "**" for $p \leq .01$ and "*" for $p \leq .05$. Plot titles give module colours and one of the top enriched GO terms unless there were no significantly enriched GO terms. Scale of *y*-axis varies

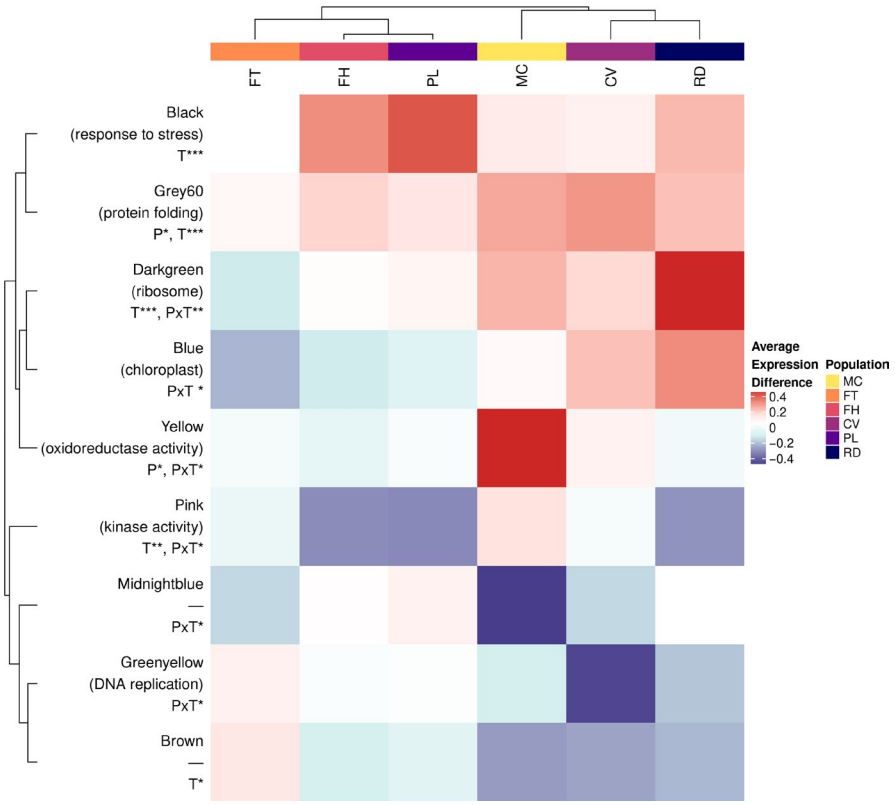

FIGURE 5 Heatmap summarizing differential gene expression and module expression differences among sites. Columns are populations, and each cell for the gene module row shows the average change in module expression between control and treated individuals for each site. Positive (red) values indicate upregulation under water stress treatment, and negative (blue) values indicate downregulation. Both *x*-axis and *y*-axis are clustered by similarity using the complete Euclidean distance. Row names give module name, one of the top enriched GO terms when there were significantly enriched GO terms, and the population (P), treatment (T) or population × treatment (P × T) effect (significance level: "***" for $p \leq .001$, "**" for $p \leq .01$ and "*" for $p \leq .05$)

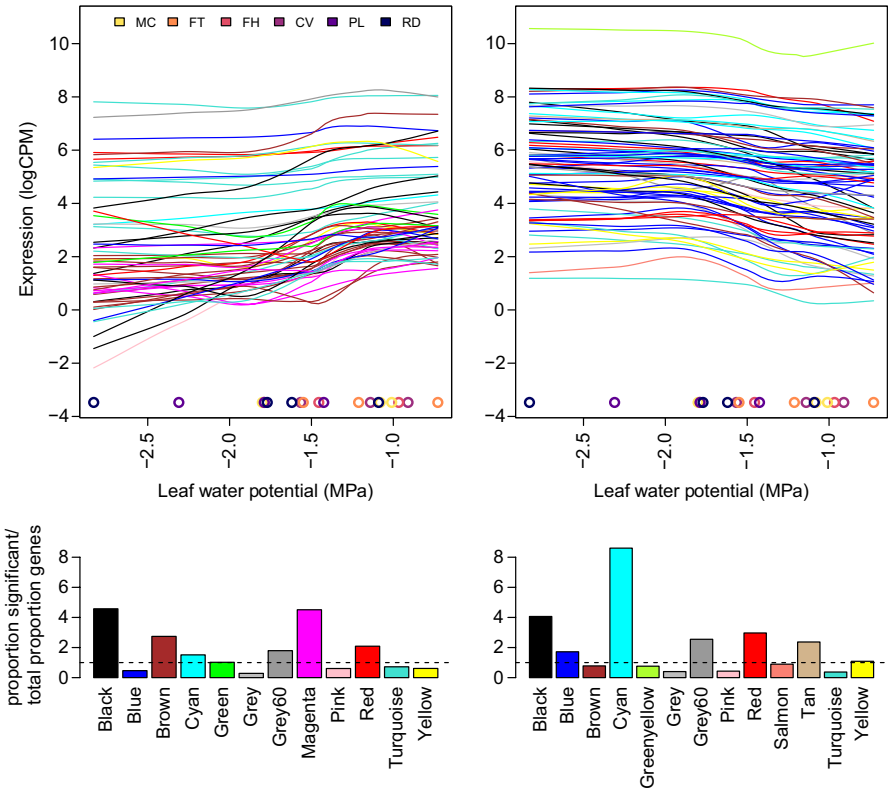

FIGURE 6 Smoothed lines showing the relationship of leaf water potential and gene expression for 20 seedlings in the water stress treatment. Genes shown had MIC *p*-values <0.001 (142 genes total) and are sorted by positive (left) and negative (right) relationships. Line colours indicate the WGCNA module assigned to the gene (Table 3). Points at the bottom show the Ψ_{leaf} for each individual seedling, coloured by home site. Bar plots show the proportion of significant associated genes (left—positive correlation, right—negative correlation) belonging to the module divided by the total proportion of genes in the module, so modules with a value much greater than one have more Ψ_{leaf}-associated genes than expected by chance

stress-responsive functions, particularly those relating to ABA response. Hub genes for this module include ABF2/AREB1 (ABA-responsive elements-binding protein 2), a LEA (late embryogenesis abundant protein) gene and several genes annotated as involved in ABA synthesis and ABA response. AREB genes are transcription factors that respond to ABA (Yoshida, Mogami, & Yamaguchi-Shinozaki, 2014), and LEA proteins are associated with drought tolerance and may protect the cell from damage due to dehydration (Bartels & Sunkar, 2005; Shinozaki & Yamaguchi-Shinozaki, 2007). The grey60 module includes genes related to protein folding and heat shock proteins, which may be a response to the increased leaf temperatures in water-stressed plants as transpiration is reduced and evaporation of water from leaves decreases or stops (Chaves et al., 2016). Together, these modules make up the species-wide response to water stress observed in these valley oak seedlings, many of which are typical of water stress responses in plants.

4.1.4 | Candidate genes identified by relationship with leaf water potential

The measurement of ecophysiological traits provides a method to identify candidate genes that may be involved in stress response by determining which genes alter their expression concurrently with the trait. Because leaf water stress and drought tolerance (Ψ_{leaf} and π_{TLP}) were variable among individuals but not significantly different among populations at 10 days, each seedling can be considered a replicate at a slightly different level of stress (likely due to slight variation in experimental conditions). An association between gene expression and ecophysiology may indicate that the gene is either induced by changes in the trait or involved in causing the phenotype, but, in either case, this association implies that the gene is functionally related to the water stress response. Many genes had expression that was significantly associated with Ψ_{leaf} during water stress. Because Ψ_{leaf} can be considered a measurement of water stress, a gene with increased expression under low Ψ_{leaf} may be a drought-responsive gene controlled by osmotic status or turgor pressure in leaves (Chaves et al., 2003; Osakabe et al., 2014; Reddy, Chaitanya, & Vivekanandan, 2004). The relationship of expression with Ψ_{leaf} for many of these genes was non-linear, similar to the pattern found by Meyer et al. (2014).

Genes with variable expression at different water stress levels may be functionally related to water stress response. This interpretation is further supported by the finding that some gene modules showing treatment or population × treatment effects (black, blue, brown, grey60) were present in this set of genes at a higher proportion than their overall proportion across all genes, which may indicate that Ψ_{leaf} is acting as part of a pathway signalling water stress for these modules. Cyan, red and tan modules, which all include genes related to chloroplasts (Table 3), were also overrepresented, particularly in the genes with higher expression under low Ψ_{leaf}. This finding suggests that photosynthesis-related genes may respond to increased stress levels.

4.2 | Population-specific responses

Analyses of variation in gene expression identified both individual genes and gene networks that varied in their response to water

stress among populations. The functions of individual genes with variable expression among populations generally agreed with those from gene networks. While each population has a unique gene network response, they appear to segregate into two main groups. Seedlings from three populations (MC, CV and RD) cluster together due to more modules having large differences in module expression between treatments; PL and FH seedlings show fewer differences between treatments, followed by FT with little difference in most modules (Figure 5). Interestingly, these differences in expression patterns are not related to local precipitation. The three populations with a stronger response occur at sites with a higher mean annual temperature (MAT) than the other three populations (Figure 1), and all six modules having a significant population × treatment effect also show a MAT × treatment effect. When a decrease in water availability is accompanied by high temperatures, greater stress occurs than under water stress alone because high temperatures increase evapotranspiration when the stomata are open, and increase heat stress in the leaf when the stomata are closed. The combination of drought and heat stresses requires a qualitatively different response than only one stress (Suzuki, Rivero, Shulaev, Blumwald, & Mittler, 2014; Zandalinas, Mittler, Balfagón, Arbona, & Gómez-Cadenas, 2018). Populations with high MAT may have adapted to deal with water stress occurring along with high temperatures, resulting in the response of different gene modules. The response of additional gene modules in certain populations also suggests a selective advantage of greater plasticity in some climates (Nicotra et al., 2010).

It is difficult to tell whether a large difference in gene expression between treatments (as observed in high MAT populations) is indicative of a more adaptive stress response in more tolerant genotypes, or of greater stress in more sensitive genotypes (DeBiasse & Kelly, 2016). The correct interpretation likely depends on variation in the level and duration of the stress as well as the species being studied, so the function of the genes involved is important for interpreting whether a response is adaptive or due to major stress. Because the populations in this study did not significantly differ in turgor loss point, it is difficult to determine which ones were more tolerant.

The GO enrichment analysis of modules with a G × E effect, however, can identify possible functional differences in population responses. For example, the populations from the hotter sites upregulated ribosomal protein genes (darkgreen module) and chloroplast and mitochondrial structure genes (blue module) while downregulating DNA replication genes (greenyellow module). Photosynthesis and growth rates typically decrease under water stress, so upregulation of these chloroplast and ribosomal genes is somewhat unexpected (Hoth et al., 2002; Hsiao et al., 1976; McIntosh & Bonham-Smith, 2006; Zhu, 2016). However, many studies investigating gene expression under drought have used high stress or shock treatments (Verslues, 2017), while the stress in this study was relatively mild, which may result in different gene expression responses that are more similar to the effects of soil drying in the environment. Similar to this study, ribosomal genes were upregulated under soil drying in some accessions of *Arabidopsis thaliana* (Des Marais et al., 2012). Ribosomal proteins were also upregulated in gradually water-stressed potato cells but downregulated under a shock treatment, possibly indicating that maintaining protein synthesis is important for stress acclimation, but simply shuts down in more stressful conditions (Ambrosone et al., 2011).

Similarly, an increase in the expression of photosynthesis-related genes may also indicate acclimation to water stress. An increase in photosynthetic proteins (Bogeat-Triboulot et al., 2007; Des Marais et al., 2012) and a surplus of carbon (Hummel et al., 2010) has been found in other studies and may be an acclimation response to compensate for the negative effects of water stress by minimizing the decrease in carbon assimilation and growth (Flexas, Bota, Galmés, Medrano, & Ribas-Carbó, 2006). *Populus euphratica* seedlings had an increased abundance of photosynthesis proteins under drought stress as internal CO_2 concentration was decreasing, which may have allowed the high photosynthesis rates and increased instantaneous water use efficiency that was observed (Bogeat-Triboulot et al., 2007), and multiple *A. thaliana* accessions increased photosynthesis protein gene expression and root growth under soil drying treatment (Des Marais et al., 2012). Seedlings from warm sites also show greater decreases in DNA replication genes (greenyellow), potentially indicating decreased growth in leaves. Overall, increased expression of photosynthesis and ribosomal protein genes and decreased expression of growth-related genes in leaves could be important in acclimation and survival during water stress; Des Marais et al. (2012) suggested that this response functioned to increase whole-plant carbon status and invest in root growth to allow better water uptake.

The changes in gene expression by seedlings from warmer sites are consistent with an increase in photosynthetic capacity to maintain CO_2 assimilation as stomata close, an increase in ribosomal activity to maintain normal functions and/or restructure cellular components, and a decrease in DNA replication and leaf growth, which may result in a higher root to shoot ratio. If changes in the expression of these genes indicate changes in related physiological processes, some seedlings may be undergoing acclimation during the early stages of water stress, suggesting that seedlings from warmer sites are adapted to respond to water stress in ways that will help them survive long-term. Overall, variance in gene module expression among populations shows that most populations have responses in modules with typical drought-responsive functions, while some populations, particularly those from hotter sites, have additional responses. It is not possible to determine whether these differences are adaptive from this study, but their functions are consistent with those of some other studies and suggest potential differences in acclimation responses in seedlings from warmer locations.

Extreme droughts are projected to become more frequent in California and other regions (Trenberth, 2011). In order to survive climate change, species must either migrate to new regions with a suitable climate, adapt to the new climate or respond to

new conditions through phenotypic plasticity (Aitken, Yeaman, Holliday, Wang, & Curtis-McLane, 2008; Nicotra et al., 2010), but rapid anthropogenic climate change will make it more difficult for species to migrate or adapt quickly enough to survive. Assisted gene flow may allow maintenance of valley oak populations by transporting individuals or gametes between populations to climates where they are better adapted to future conditions than the current population (Aitken & Whitlock, 2013). However, this would require knowledge of the adaptations and range of climatic tolerance of each population.

Our findings show that valley oak populations differ in their genomic response to water stress, suggesting differences in the genetic basis of their drought adaptation. The magnitude of response was not clearly related to precipitation levels of the home site, underscoring the importance of testing a population's response before assuming which populations are more vulnerable and which could be candidates for strategies such as assisted migration to mitigate the impact of climate warming. Further studies are needed to assess the extent to which population differences are due to local adaptation—in particular, whether temperature influences the response to drought in valley oak—or due to genetic variation among populations that leads to the involvement of different gene networks. Collecting a greater range of ecophysiology and growth traits would help determine whether differences in module expression actually result in differences in carbon assimilation or root and shoot growth, for example. Additionally, collecting data related to fitness, such as survival or growth, could determine which populations are better adapted to drought and temperature, and determine which gene expression responses are linked to higher fitness.

5 | CONCLUSIONS

Long-lived plant species such as trees may live through many fluctuations in climate, including periodic droughts as well as seasonal and annual temperature variation. When periodic climate stresses vary throughout the range of a species, populations may become locally adapted to these stresses. This study provides evidence that valley oak populations are genetically differentiated in their response to drought, which may be due to local adaptation to environmental conditions at their site. Valley oak populations have both quantitatively and qualitatively different responses to water stress. Populations varied in the number of genes and gene modules responding to treatment, suggesting differences in their drought response plasticity. The differences in types of genes and gene modules responding to treatment suggest that populations from different climates also have qualitatively different responses to drought stress, possibly related to temperature differences among the climates. This differential response to water stress may determine how populations will be affected by increasingly severe and frequent droughts, especially when accompanied by increased temperatures.

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AUTHOR CONTRIBUTIONS

J.P.R., M.K.B., J.W.W., L.S. and V.L.S. designed the experiment; J.W.W. oversaw growth of the seedlings prior to experiment; J.P.R. and M.K.B. carried out the greenhouse experiment; and A.M. generated sequence data and conducted all statistical analyses. A.M. and V.L.S. wrote the manuscript; all authors contributed to final draft.

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DATA AVAILABILITY STATEMENT

RNA-seq and seedling trait data are available at Dryad (https://doi.org/10.5068/D1HH31; Mead et al., 2019).

Sequence data processing scripts are available at github.com/alaynamead/RNAseq_scripts and archived on Zenodo (http://doi.org/10.5281/zenodo.3458604; Mead, 2019a).

Analysis scripts are available at github.com/alaynamead/valley_oak_water_stress and archived on Zenodo (http://doi.org/10.5281/zenodo.3459705; Mead, 2019b).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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