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ORIGINAL RESEARCH

Differential gene expression in tall fescue tissues in response to water deficit

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Abstract

Tall fescue (*Festuca arundinacea* Schreb.) is a popular pasture and turf grass particularly known for drought resistance, allowing for its persistence in locations that are unfavorable for other cool-season grasses. Also, its seed-borne fungal symbiont (endophyte) *Epichloë coenophiala*, which resides in the crown and pseudostem, can be a contributing factor in its drought tolerance. Because it contains the apical meristems, crown survival under drought stress is critical to plant survival as well as the endophyte. In this study, we subjected tall fescue plants with their endophyte to water-deficit stress or, as controls with normal watering, then compared plant transcriptome responses in four vegetative tissues: leaf blades, pseudostem, crown, and roots. A transcript was designated a differentially expressed gene (DEG) if it exhibited at least a twofold expression difference between stress and control samples with an adjusted *p* value of .001. Pathway analysis of the DEGs across all tissue types included photosynthesis, carbohydrate metabolism, phytohormone biosynthesis and signaling, cellular organization, and a transcriptional regulation. While no specific pathway was observed to be differentially expressed in the crown, genes encoding auxin response factors, nuclear pore anchors, structural maintenance of chromosomes, and class XI myosin proteins were more highly differentially expressed in crown than in the other vegetative tissues, suggesting that regulation in expression of these genes in the crown may aid in survival of the meristems in the crown.

1 | INTRODUCTION

Grasslands occupy nearly 70% of global agricultural land and are the primary source of livestock feed. Hence, grasslands play a crucial role in food security, carbon fixation, ecosystems, and the economy (Falloon & Betts, 2010; Loka et al., 2019). Tall fescue [*Festuca arundinacea* Schreb. = *Lolium arundinaceum* (Schreb.) Darbysh. = *Schedonorus arundinaceus* (Schreb.) Dumort.] is a highly adaptable,

Abbreviations: ABA, abscisic acid; ARF, auxin response factor; BP, biological process; BPGO, biological process gene ontology; CTE, common toxic endophyte; DEG, differentially expressed gene; ERF, ethylene response factors; GO, gene ontology; NCED, 9-*cis*-epoxycarotenoid dehydrogenase; OFP, OVATE family protein; PP2C, protein phosphatase 2C; RFO, raffinose family of oligosaccharide; RNA-seq, RNA sequencing; TPS, trehalose-6-phosphate synthase.

cool-season grass native to temperate and cool climates throughout Europe, North Africa, and west-central Asia and introduced to North America where it is one of the most abundant turf and cultivated pasture grasses in the United States, occupying >15 million ha (Buckner & Bush, 1979). It has good drought and winter tolerance thus allowing for its broad adaptability, and it has high growth rates, is highly competitive with other pasture species, and has a good nutritive value for grazing animals. Tall fescue has a bunch-type growth habit such that, although it can produce short rhizomes, its spread is primarily through individual upright tillers originating at the crown of the plant. The vigorous spring and fall growth and extensive root system help it to withstand drought conditions.

Perennial species, when confronted with stress, are faced with a productivity–persistence trade-off. Depending upon the time of occurrence, magnitude, and duration of drought stress, the negative impact on plant productivity and performance needs to be balanced with survival mechanisms (Hu & Xiong, 2014). Dehydration tolerance ensures plant survival by accumulation of water-soluble carbohydrates and dehydrins in order to maintain cell integrity and membrane stabilization (Volaire et al., 2009). Under progressive soil drying, minimizing water loses and maximizing water uptake by converting biomass allocation to roots, and an extensive root system are commonly observed in drought-avoidance species (Comas et al., 2013). Under severe soil drought conditions, root mortality ensues because of tissue dehydration, and drought tolerant species rely on the stored nutrients and carbohydrates in the belowground tissues and organs for continued survival (Eissenstat & Yanai, 1997; Facette et al., 1999). Perennial species provide interesting plant models for drought studies, as they survive drought through adaptive strategies associated with dehydration avoidance and dehydration tolerance (Zwicke et al., 2015).

A contributing factor in tall fescue persistence and productivity has been shown to be through the beneficial symbiosis with the endophytic fungus, *Epichloë coenophiala* [(Morgan-Jones et W. Gams) C.W. Bacon et Schardl] (Leuchtmann et al., 2014). Many species of grasses (family Poaceae) harbor symbiotic fungal species. Although the mechanisms underlying benefits of the endophyte to the plant in regards to drought responses have not yet been clearly identified, alterations to leaf morphology, osmotic regulation stemming from changes in sugar or phenolic compounds, stomatal conductance, rooting depth and architecture, and photosynthetic rates have been implicated (Arachevaleta et al., 1989; Bacon, 1993; Bush et al., 1993; Elmi & West, 1995; Malinowski & Belesky, 1999, 2000; Nagabhyru et al., 2013; Richardson et al., 1992; West, 1994). In tall fescue, the fungal hyphae absorb nutrients (amino acids and sugars) directly from the host plant through the cell wall where it grows intercellularly in the aboveground portion of the plant. The *Epichloë* species most commonly

Core Ideas

- Gene expression was evaluated in four tissues of stressed and unstressed tall fescue clones.
- Differentially expressed genes (DEGs) were identified using RNA-seq.
- Gene ontology pathways were identified by DEGs in different tissues.
- Crown-specific, stress-responsive DEGs were presented.
- Nuclear pore anchor and class IX myosin genes involved in development were downregulated in crown under stress.

associated with tall fescue, *E. coenophiala*, does not reproduce sexually but relies on the plant for continued survival, as it is transmitted vertically and clonally through seeds (Bacon & Siegel, 1988). The fungus moves from the embryo following germinating and colonizes mainly leaf sheaths, is less abundant in the leaf blade, meristems, and internodes of elongating stems, and is sparse or not found in the roots (Bacon & Siegel, 1988; Hinton & Bacon, 1985; Welty et al., 1986). The highest concentration of the fungus in the aboveground portion of the plant is in the pseudostem (Christensen & Voisey, 2007). It seems likely that metabolic crosstalk between the grass and the fungus results in complex up- and downregulation of both fungal and plant genes, and concentrations of the products they code for, in complex ways that contribute to providing for stress tolerance. However, the present state of knowledge on this aspect of the interaction appears to be somewhat contradictory (Dupont et al., 2015; Dinkins et al., 2017, 2019; Hu et al., 2014; Talukder et al., 2015). Nevertheless, persistence of this beneficial symbiont despite death of the aboveground vegetation brought on by summer droughts or winter conditions, as well as its dispersal via the seed, require the fungus to survive in the meristematic region of the crown.

Transcriptome studies on tall fescue conducted by our groups and others so far have focused on de novo transcriptome assembly (Talukder et al., 2015; Dinkins et al., 2017), comparison between drought-tolerant and susceptible tall fescue plants, comparison between endophyte-infected and endophyte-free tall fescue genotypes under unstressed condition (Dinkins et al., 2017), effects of presence or absence of endophyte on gene expression in pseudostem and reproductive tissues (Nagabhyru et al., 2019), under salinity stress (Amombo et al., 2018), under lead and cadmium stress (Li et al., 2017; Zhu et al., 2018), effects on responses to drought stress in pseudostem tissue in different host–endophyte genotypes comprising combinations of tall fescue plants with

common toxic endophytes (CTEs) or nontoxic endophytes, so designated based on (respectively) whether or not they produce the ergot alkaloids that are toxic to livestock (Dinkins et al., 2019).

Despite its crucial role in water uptake in response to drought conditions, plant growth belowground remains poorly quantified compared with aboveground tissues (Reich & Cornelissen, 2014). Many perennial species have developed belowground organs, such as corms, tubers, bulbs, and rhizomes, that aid in survival during stress periods. Furthermore, since tall fescue does not persist via rhizomes, the crown, which consists of the root and shoot meristematic regions, is the fulcrum for continuity of both the plant and fungus. In this study we sought to monitor the tall fescue molecular stress response by quantifying overall genome-wide expression in the different tissues in response to imposed water withholding, and, in particular, we sought to compare transcriptome responses in the crown (which contains the apical meristems) to those of other plant tissues above- and belowground.

2 | MATERIALS AND METHODS

2.1 | Plant materials and growth conditions

Tall fescue is obligately outcrossing, so each plant represents a unique genotype, and we used two KY31-derived plants that had same endophyte genotype, also known as the CTE strain (Dinkins et al., 2017). Plants were greenhouse grown as previously described (Dinkins et al., 2017; Nagabhyru et al., 2013). Briefly, three tillers from each clone were transplanted in individual pots and grown for 4 wk in 8.5- by 8.5-cm square pots grown in sand and watered twice daily, allowing for accumulation of biomass prior to sampling. At the onset of the experiment, water was withheld from three pots for each day treatment (stress treatment) and three pots for each day were maintained under regular watering regime (control treatment). Harvesting and separating the plant tissues for freezing was done every 24 h for 5 d of water withholding. Three sets of pots were maintained during the experiment: one set of three harvested for metabolite sampling, a second for RNA isolation, and a third set was rewatered following the stress and used to monitor recovery. Each pot represented a biological replicate. Sand was used as the soil medium to apply a rapid stress treatment to the plants and to easily access the belowground tissues with minimal damage. The plants in each pot were cleaned of sand, and the roots, crowns, pseudostem, and leaf blades were immediately frozen in liquid nitrogen for RNA extraction or put on ice for metabolite isolation.

2.2 | Amino acid, carbohydrate, and abscisic acid analysis

Sugars were analysed by high-pH anion–exchange chromatography, and amino acids were quantified using liquid chromatography-mass spectrometry as described in Nagabhyru et al (2013). Abscisic acid (ABA) was extracted and quantified following the method described in Forcat et al. (2008) with minor modifications. Briefly, 25 mg of finely ground lyophilized tissue was extracted in 500 µl extraction solution of 10% methanol with 1% acetic acid containing 50 ng ml^{-1} of internal standards d4-SA and d5-JA (these were also used to estimate ABA amounts because we were unable to procure d6-ABA as internal standard). First, 300 µl of extraction solution was added to each sample, which was vortexed thoroughly, incubated on ice for 30 min, and then centrifuged at full speed for 10−15 min at 4 ˚C. After removing the supernatant, the pellet was re-extracted with 200 µl of extraction solution repeating the same procedure. The supernatants from the two extractions were pooled and used for the analysis of ABA. The calibration curve of standards was developed using the samples containing ABA, salicylic acid, and jasmonic acid each at 1, 5, 10, 50, 100 ng ml^{-1}, along with 50 ng ml^{-1} d4-SA and d5-JA. The column and chromatography conditions were the same as that of Forcat et al. (2008) and extracted samples were analyzed using liquid chromatography mass spectrometry with a dual pump ProStar 210 HPLC and 1200 L quadrupole MS-MS (Varian).

2.3 | RNA isolation and RNA-seq cDNA preparation and procedures

The RNA was extracted from each tissue sample using TRIzol Reagent (Invitrogen Corporation) as per the manufacturer recommendations as described in Dinkins et al (2019). Briefly, RNA was treated using TURBO DNA-free (Ambion, Applied Biosystem) for removal of contaminating DNA and for the removal of DNase after treatment. The RNA integrity number (RIN number) was checked using the Bio-Rad Experion Automated Electrophoresis Station (Bio-Rad Laboratories). High-quality RNA (3−4 µg, RNA integrity number > 8) was used for cDNA library preparation according to the Illumina TruSeq RNA sample preparation guide (Cat #RS 930-2001, Illumina, Inc.). Individual libraries were prepared from three replicates of each tissue for each plant after 2 d of withholding water (stress), along with three replicates of each that had been maintained under normal watering conditions (control) as described previously (Dinkins et al., 2017, 2019). The samples were indexed with different sequence tags (bar-coding) from the Illumina TruSeq RNA preparation kit,

and six samples were sequenced per lane (single read, 100 bp) on an Illumina HiSeq 2500 at the Iowa State DNA Facility (Ames, IA), yielding approximately 30−35 million reads per library sample. The raw RNA sequencing (RNA-seq) data for the experiments presented in this work are available at the National Center for Biotechnology Information under BioProjects PRJNA284541 and PRJNA658975.

2.4 | RNA-seq data analysis

Mapping of the RNA-seq reads and data analysis was done as previously described in Dinkins et al. (2019). Briefly, reads were trimmed to remove low-quality base calls using the CLCbio Trim tool (CLCbio Workbench; v12.0.1; Qiagen). The filtered reads were mapped against the *E. coenophiala* isolate e4163 genome assembly and cDNAs (https://www.ncbi.nlm.nih.gov/bioproject/720666) using the CLCbio RNA-Seq analysis tool with the mapping settings set to minimum length fraction (0.5) and minimum similarity fraction (0.95). The nonmapped reads were then mapped to our in-house tall fescue transcriptome assembly (Dinkins et al., 2017) (available at: https://data.cyverse.org/dav-anon/iplant/home/rdinkins/Tall_Fescue_Assembly/TF_153KSeq.fa) with the mapping settings set to minimum length fraction (0.8) and minimum similarity fraction (0.8). The reads were transformed by adding the value of one to all to eliminate zeros (0) and normalized using the quantile method, genes that contained at least 20 mapped reads over the genotype–tissue–treatment combination were used to calculate differential expression.

Analysis of differential expression was performed in JMP Genomics (v8.0; SAS Institute) using the JMP Basic RNA-Seq Workflow. Briefly, the reads datasets containing genes that had a minimum of 20 normalized average mapped reads was normalized using the trimmed mean of *M* values method (Robinson & Oshlack, 2010) for analysis. Analysis of variance for the differential stress expression within and between tissue comparisons was based on a randomized design where the three replicates consisted of individual library preparations. Based on the error estimated by ANOVA for each gene, contrasts were done between the treated and control means for each tissue. A false discovery rate multiple testing method at $P < .05$ and a twofold difference was used to determine significant differences.

2.5 | Gene ontology pathway annotations

The TF153 tall fescue assembly transcripts were annotated for biological processes (BPs), and molecular function gene ontology (GO) terms were annotated based on the *Arabidopsis thaliana* gene annotations as described in Dinkins et al (2017). The DEGs from the various treatments were identified based on gene lists associated with overrepresented DEGs when comparing with the overall TF153K unigene assembly to identify overrepresented pathways using the custom input tool at the web-based agriGO v2.0 (http://systemsbiology.cau.edu.cn/agriGOv2/) using the Fisher Statistical test method, Bonferroni multitest adjustment method, and a cut-off of 10^{-5} (Tian et al., 2017).

2.6 | Pathway enrichment and clustering analysis

The DEGs between stress-treated and corresponding control samples were used for overrepresentation analysis using the 'PageMan' tool in 'MapMan' (Usadel et al., 2009). Briefly, DEGs were compressed into different 'MapMan' annotation BINs, and over- and underrepresentations of such BINs were tested using the 'ORA-Fisher' test in 'PageMan' with 'Benjamini-Hochberg' multiple testing correction and an ORA cut off value of 1.0 (Usadel et al., 2006).

3 | RESULTS

3.1 | Metabolite and ABA changes of tall fescue plants in response to water deficit

Sampling was done over a 5-d period every 24 h following water withdrawal. Survival data following rewatering recovery has been previously presented (Dinkins et al., 2019; Nagabhyru et al., 2013). Metabolite data for one of the clones (P46 endophyte-free and P46 endophyte-infected [CTE+]) has also been reported previously (Nagabhyru et al., 2013). Since recovery of the P27CTE+ plants appeared to be more sensitive to water-deficit stress (Dinkins et al., 2019), metabolite analysis was done on aboveground tissues of P27CTE+. Similarly to the results observed for P46CTE+ (Nagabhyru et al., 2013), the level of proline increased from Day two under the water-withholding treatment, and glucose and fructose increased at Day 1 after withholding water (Figure 1a; Supplemental Figure S1). We also monitored the change in the level of ABA in clone P27CTE+ and observed a significant increase in Day 2 of water-withholding treatment (Figure 1b). To monitor potential changes in gene expression cause by withholding water, RNA from the four tissues was analyzed by RNA-seq. Because of cost constraints, only Day 2 sampling was analyzed because this was the timepoint when the most significant changes in metabolite and ABA levels occurred (Nagabhyru et al., 2013).

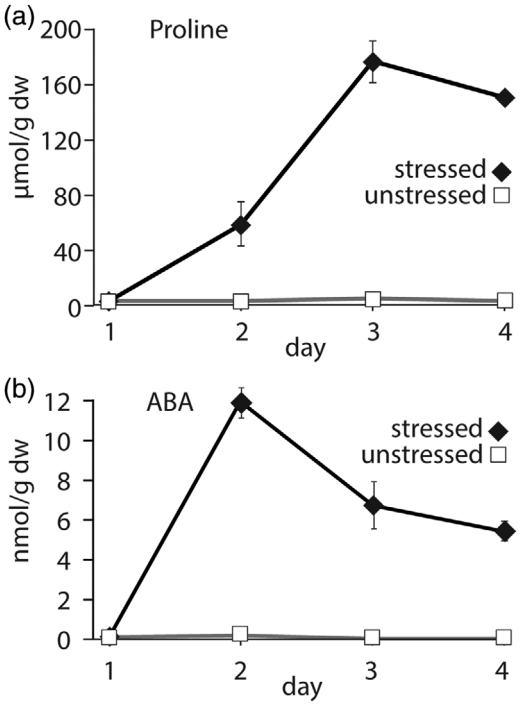

FIGURE 1 Proline and abscisic acid (ABA) level comparison in stressed and unstressed P27CTE+ plants

3.2 | Spatial overview of tall fescue gene expression in response to water deficit

Reads from each library derived from the different tissues of the tall fescue genotypes, P27CTE+ and P46CTE+, were mapped onto the tall fescue TF153K transcriptome and 76−88% of the reads from each library were found to map (Supplemental Table S1). An average correlation of 0.94 (0.80–0.96) was observed between the three replicates within each plant genotype, treatment combination, and tissue type across all sequenced libraries (Supplemental Excel File S1). Following normalization of the reads for each tissue and treatment combination, 76922 unigenes were found to have a minimum of 20 normalized reads in at least one genotype–tissue–treatment combination. Most of the variation was due to differences between tissue types and in response to the treatment, although genotype effects were also seen (Figure 2a). For the present study, tissue and treatment expression comparisons were done across both plant genotypes. Overall, expression in the leaf blades was very different than expression in the other three tissues (Figure 2b). In the control (unstressed) condition, the highest correlations were between pseudostem and crown and between crown and root (Figure 3).

Following the 2-d water withholding treatment, the imposed stress appeared to coalesce the expression in all tissues as the correlations between tissues was higher between the four tissues when compared with the control conditions

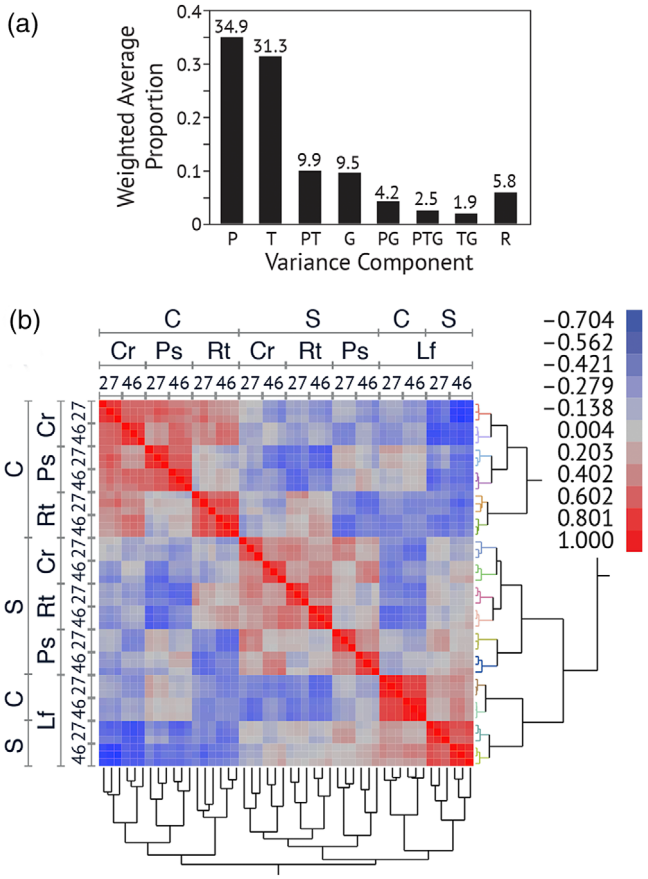

FIGURE 2 (a) Principle component analysis and (b) heat map and dendogram correlation of tall fescue tissues gene expression under water withholding and control conditions. (a) P, T, G, and R denote process treatment, tissue, plant genotype, and residual, respectively, and combined letters (PT, PG, TG, and PTG) denote the variance components in the interactions. (b) S and C denote the stress and control treatments; Lf, PS, Cr, and Rt denote leaf, pseudostem, crown, and root, respectively; the numbers 27 and 46 denote the CTE27 and CTE46 tall fescue genotypes

(Figure 3). Roughly a third of the unigenes where expression was observed were differentially expressed because of the stress treatment in at least one or more tissues, where 10,346 (13.4%) of the unigenes were differentially expressed in all four tissues. Of these, 5,106 were DEGs higher in stress than watered control (stress > control) and 5,240 were control > stress (Figure 4). The correlation between the leaf blades and the other three tissues remained the lowest (Figure 3). The highest correlation in the differential responses was observed between the pseudostem and crown (Figure 3).

The GO categories enriched for DEGs were identified for the common transcripts for all four tissues. Of the 5,240 control > stress DEGs, 2,427 matched *A. thaliana* biological process gene ontology (BPGO) annotations, and among the stress > control DEGs, 1,887 of 5,106 had BPGO annotation matches (Supplemental Excel File S2). Significant BPGO annotations enriched in stress >

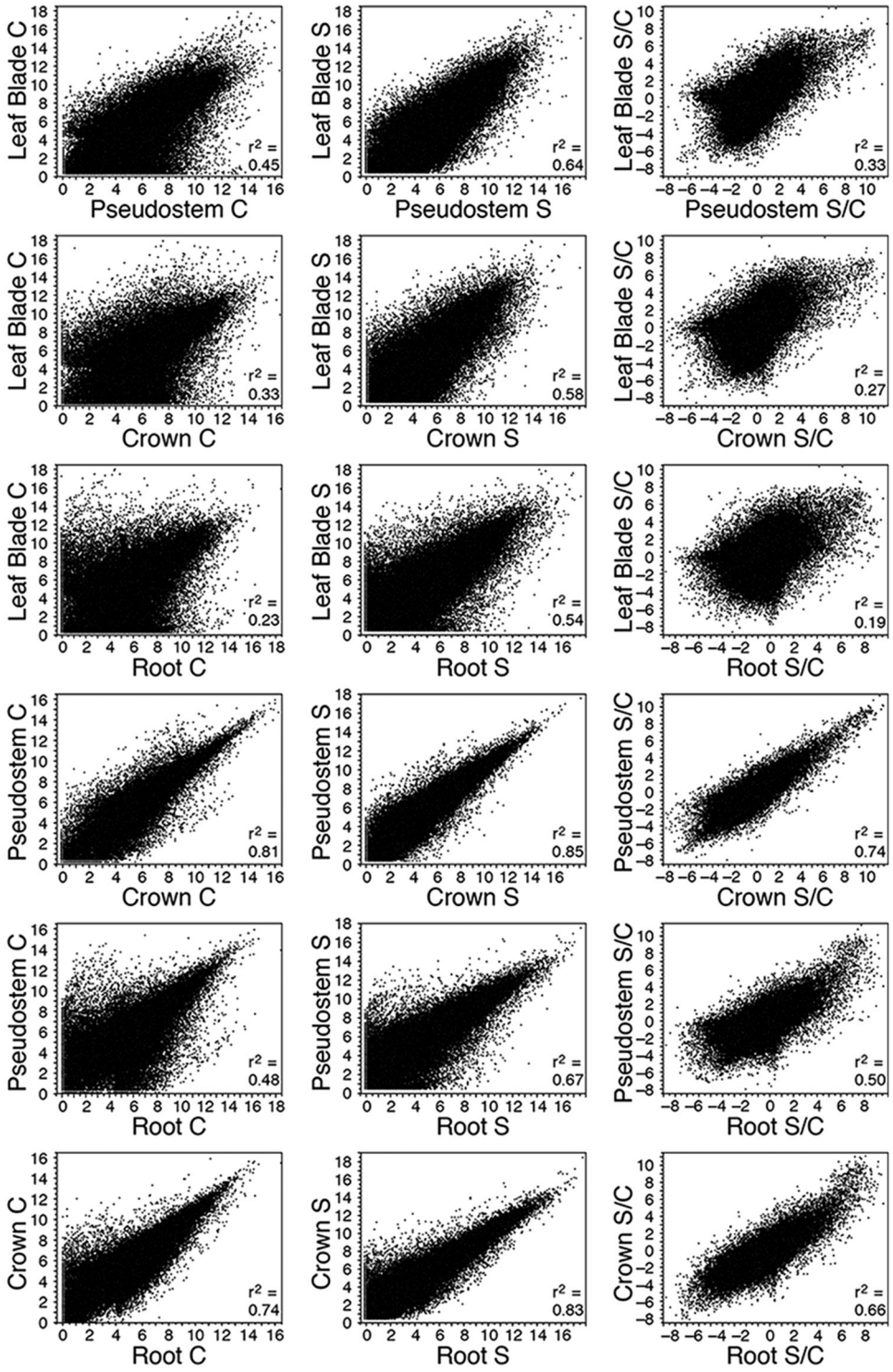

FIGURE 3 Gene expression correlations between the four different tissues: leaf blade, pseudostem, crown, and root. 'C 'denotes under water control conditions; 'S' denotes the water withholding stress conditions; and S/C denotes the differential expression of stress over control within each tissue correlated to the differential expression of the second tissue

control contained 40 significant terms that included processes associated with water stress and deprivation response (GO:0009414; GO:0009415), proline biosynthetic process (GO:0006561), proline metabolic process (GO:006560), ABA-mediated signaling pathway (GO:0009738), oxidative stress (GO:0006979), hyperosmotic salinity response (GO:0042538), response to heat (GO:0009408), and heat acclimation (GO:0010286) (Table 1). Proline biosynthetic process (GO:0006561) genes included those for Δ1-pyrroline-5-carboxylate synthases (Bandurska & Jozwiak,

TABLE 1 Gene ontology (GO) biological process terms associated with stress greater than water control differentially expressed genes (DEGs) in tall fescue across all tissues

GO term	Description	No. in input list	No. in BG/Ref	*p* value	False discovery rate
GO:0042538	Hyperosmotic salinity response	78	367	2.70×10^{-23}	3.40×10^{-20}
GO:1901700	Response to oxygen-containing compound	510	5,407	5.40×10^{-23}	6.80×10^{-20}
GO:0009414	Response to water deprivation	146	1,039	4.60×10^{-22}	5.90×10^{-19}
GO:0009415	Response to water	146	1,045	8.00×10^{-22}	1.00×10^{-18}
GO:0001101	Response to acid chemical	322	3,239	5.20×10^{-19}	6.50×10^{-16}
GO:0009737	Response to abscisic acid	171	1,407	6.80×10^{-19}	8.60×10^{-16}
GO:0097305	Response to alcohol	171	1,407	6.80×10^{-19}	8.60×10^{-16}
GO:0033993	Response to lipid	202	1,800	4.50×10^{-18}	5.70×10^{-15}
GO:0009651	Response to salt stress	219	2,115	1.20×10^{-15}	1.60×10^{-12}
GO:0006972	Hyperosmotic response	95	664	1.20×10^{-15}	1.60×10^{-12}
GO:0010035	Response to inorganic substance	322	3,440	1.30×10^{-15}	1.60×10^{-12}
GO:0006970	Response to osmotic stress	228	2,229	1.30×10^{-15}	1.60×10^{-12}
GO:0044712	Single-organism catabolic process	244	2,531	8.50×10^{-14}	1.10×10^{-10}
GO:0042221	Response to chemical	675	8,492	1.50×10^{-13}	1.90×10^{-10}
GO:0090487	Secondary metabolite catabolic process	78	538	1.70×10^{-13}	2.20×10^{-10}
GO:0009407	Toxin catabolic process	78	538	1.70×10^{-13}	2.20×10^{-10}
GO:0009404	Toxin metabolic process	81	573	2.40×10^{-13}	3.10×10^{-10}
GO:0010029	Regulation of seed germination	35	145	4.00×10^{-13}	5.10×10^{-10}
GO:0006560	Proline metabolic process	14	23	2.20×10^{-12}	2.80×10^{-9}
GO:0009719	Response to endogenous stimulus	351	4,076	3.20×10^{-12}	4.00×10^{-9}
GO:0006561	Proline biosynthetic process	13	20	4.00×10^{-12}	5.10×10^{-9}
GO:0009408	Response to heat	93	747	7.80×10^{-12}	9.80×10^{-9}
GO:1900140	Regulation of seedling development	35	162	1.20×10^{-11}	1.50×10^{-8}
GO:0009620	Response to fungus	136	1,272	1.70×10^{-11}	2.10×10^{-8}
GO:0009725	Response to hormone	310	3,614	5.90×10^{-11}	7.40×10^{-8}
GO:0010286	Heat acclimation	38	198	7.10×10^{-11}	8.90×10^{-8}
GO:0010033	Response to organic substance	486	6,194	4.20×10^{-10}	5.30×10^{-7}
GO:0009266	Response to temperature stimulus	212	2,354	7.60×10^{-10}	9.60×10^{-7}
GO:0009738	Abscisic acid-activated signaling pathway	73	582	7.90×10^{-10}	1.00×10^{-6}
GO:0097306	Cellular response to alcohol	76	627	1.70×10^{-9}	2.20×10^{-6}
GO:0071215	Cellular response to abscisic acid stimulus	76	627	1.70×10^{-9}	2.20×10^{-6}
GO:0009628	Response to abiotic stimulus	511	6,655	2.60×10^{-9}	3.20×10^{-6}
GO:0006950	Response to stress	676	9,098	3.10×10^{-9}	4.00×10^{-6}
GO:0006979	Response to oxidative stress	120	1,182	4.30×10^{-9}	5.40×10^{-6}
GO:0009611	Response to wounding	98	916	7.50×10^{-9}	9.50×10^{-6}
GO:0046482	Para-aminobenzoic acid metabolic process	23	99	8.20×10^{-9}	1.00×10^{-5}
GO:1901565	Organonitrogen compound catabolic process	76	660	1.50×10^{-8}	1.90×10^{-5}
GO:0009753	Response to jasmonic acid	107	1,050	2.20×10^{-8}	2.70×10^{-5}
GO:0071396	Cellular response to lipid	92	872	3.90×10^{-8}	4.90×10^{-5}
GO:0009064	Glutamine family amino acid metabolic process	20	85	6.00×10^{-8}	7.50×10^{-5}

Note. BG/Ref, background reference.

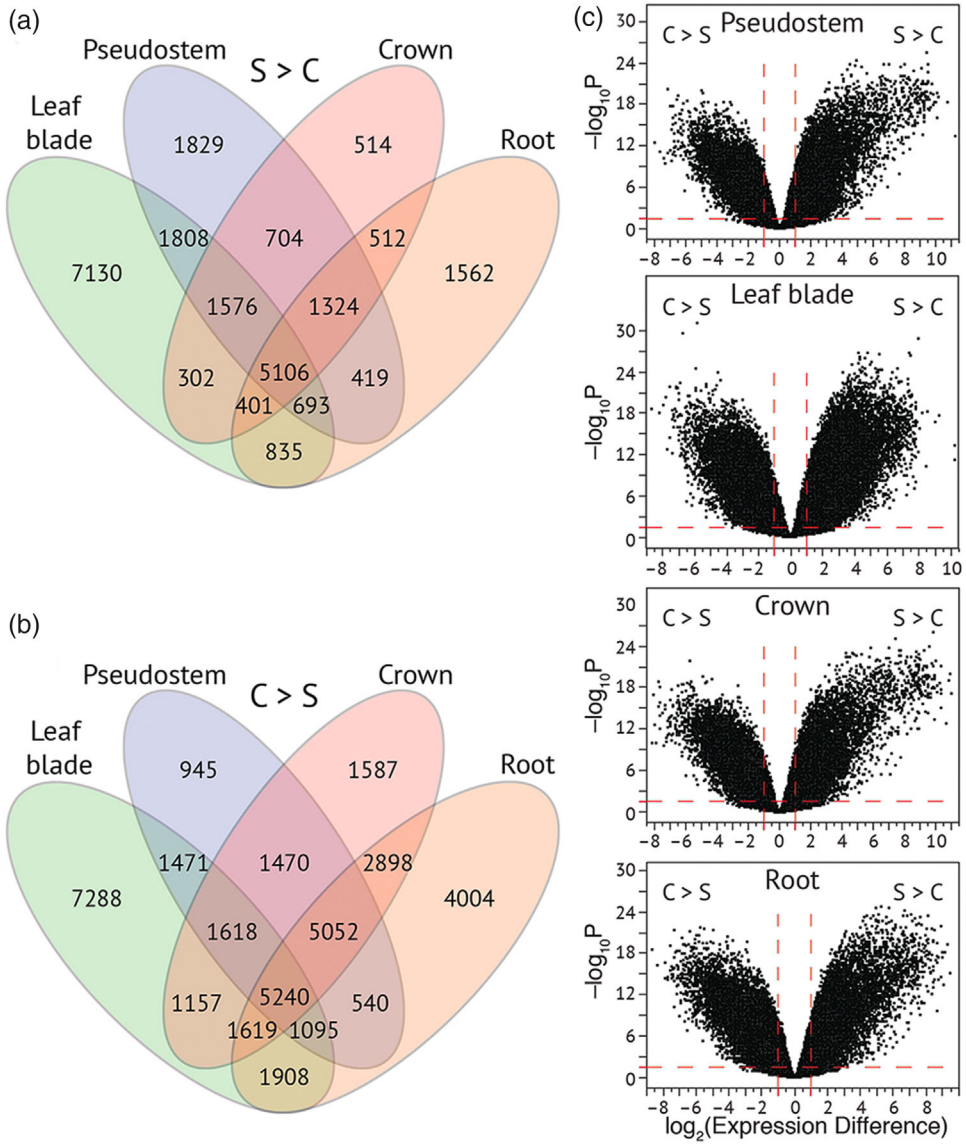

FIGURE 4 Venn diagrams and volcano plot comparing differential gene expression (DEG) comparing water withholding and control condition in different tall fescue tissues. C, control; S, stress treatment

2010), whereby three DEGs were homologous to *P5CS1* genes and two DEGs were homologous to *P5CS2* genes. Six of 22 DEGs homologous to trehalose-6-phosphate synthase genes were stress > control in all tissues, as were another 16 DEGs in one or more tissues.

To identify the most highly differentially expressed genes that responded to the stress treatment across the four tissues, a 10-fold cut-off was used to identify a subset of 789 genes (Supplemental Excel File S2). Some of these stress > control 10 × DEGs encode late-embryogenesis associated proteins, nine unigenes homologous to different 9-*cis*-epoxycarotenoid dehydrogenase (NCED) genes that are part of the ABA biosynthetic pathway and *SWEET*/*MtN3*-like genes for proteins involved in stress responses.

The GO annotations were identified for 2,427 of the 5,240 control > stress DEGs (Supplemental Excel File S2) and included 29 significant BPGO terms including chloroplast organization (GO:0009657), mitochondrion organization (GO:0007005), multidimensional cell growth (GO:0009825), peptide transport (GO:0015833), protein targeting to the nucleus (GO:0044744) and mitochondrion (GO:0006626), regulation of hormone levels (GO:0010817), and others (Table 2). Of the control > stress DEGs, 83 gene models were 10 × DEGs across all four tissues (Supplemental Excel File S2). MapMan analysis indicated pathways that were significantly over- or underrepresented in response to water-deficit stress and are further described below.

TABLE 2 Gene Ontology Biological Process terms associated with water control greater than stress differentially expressed genes (DEGs) in tall fescue across all tissues

GO term	Description	No. in input list	No. in BG/Ref	*p* value	False discovery rate
GO:0009825	Multidimensional cell growth	61	303	1.10×10^{-12}	1.60×10^{-9}
GO:0015833	Peptide transport	66	350	3.20×10^{-12}	4.50×10^{-9}
GO:0006857	Oligopeptide transport	66	350	3.20×10^{-12}	4.50×10^{-9}
GO:0042886	Amide transport	66	361	1.30×10^{-11}	1.80×10^{-8}
GO:0009657	Plastid organization	109	775	2.00×10^{-10}	2.80×10^{-7}
GO:0007005	Mitochondrion organization	45	217	3.20×10^{-10}	4.50×10^{-7}
GO:0008361	Regulation of cell size	38	166	3.80×10^{-10}	5.20×10^{-7}
GO:0006626	Protein targeting to mitochondrion	40	185	8.10×10^{-10}	1.10×10^{-6}
GO:0072655	Establishment of protein localization to mitochondrion	40	185	8.10×10^{-10}	1.10×10^{-6}
GO:0070585	Protein localization to mitochondrion	40	185	8.10×10^{-10}	1.10×10^{-6}
GO:0072528	Pyrimidine-containing compound biosynthetic process	62	366	1.20×10^{-9}	1.70×10^{-6}
GO:0072527	Pyrimidine-containing compound metabolic process	62	375	3.20×10^{-9}	4.50×10^{-6}
GO:0090407	Organophosphate biosynthetic process	179	1,530	3.30×10^{-9}	4.60×10^{-6}
GO:0010817	Regulation of hormone levels	130	1,036	6.70×10^{-9}	9.30×10^{-6}
GO:0009218	Pyrimidine ribonucleotide metabolic process	58	350	9.10×10^{-9}	1.30×10^{-5}
GO:0009220	Pyrimidine ribonucleotide biosynthetic process	58	350	9.10×10^{-9}	1.30×10^{-5}
GO:0006220	Pyrimidine nucleotide metabolic process	58	351	1.00×10^{-8}	1.40×10^{-5}
GO:0006221	Pyrimidine nucleotide biosynthetic process	58	351	1.00×10^{-8}	1.40×10^{-5}
GO:0006796	Phosphate-containing compound metabolic process	480	4,925	1.00×10^{-8}	1.40×10^{-5}
GO:1902593	Single-organism nuclear import	52	300	1.10×10^{-8}	1.50×10^{-5}
GO:0044744	Protein targeting to nucleus	52	300	1.10×10^{-8}	1.50×10^{-5}
GO:0006606	Protein import into nucleus	52	300	1.10×10^{-8}	1.50×10^{-5}
GO:0006793	Phosphorus metabolic process	480	4,940	1.50×10^{-8}	2.00×10^{-5}
GO:0009260	Ribonucleotide biosynthetic process	63	400	1.60×10^{-8}	2.20×10^{-5}
GO:0046390	Ribose phosphate biosynthetic process	63	400	1.60×10^{-8}	2.20×10^{-5}
GO:0051170	Nuclear import	52	305	1.90×10^{-8}	2.70×10^{-5}
GO:0034504	Protein localization to nucleus	52	307	2.40×10^{-8}	3.30×10^{-5}
GO:0007166	Cell surface receptor signaling pathway	98	741	3.30×10^{-8}	4.50×10^{-5}
GO:0016556	mRNA modification	40	212	4.60×10^{-8}	6.50×10^{-5}

Note. BG/Ref, background reference.

3.3 | Stress significantly altered expression of genes implicated in photosynthesis and carbohydrate metabolism

Water withdrawal exerted significant effects on expression of genes involved in photosynthesis and carbohydrate metabolism (Supplemental Figure S2, Supplemental Excel File S2). As expected, genes involved in photosynthesis were among the control > stress DEGs in leaf blade, pseudostem, and crown tissues (Supplemental Figure S2). These included genes encoding components of thylakoid membrane-bound protein complexes photosystem-II (PS-II) and photosystem-I (PS-I), which are involved in mediating the light reaction of photosynthesis (Supplemental Figure S2).

Genes encoding enzymes catalyzing raffinose family of oligosaccharides (RFOs) biosynthesis, such as galactinol synthase and raffinose synthase, were among the stress > control DEGs (Supplemental Figure S2). A heatmap representing expression differences of DEGs implicated in RFO biosynthesis is presented in Figure 5a. Genes implicated in starch synthesis were among the control > stress DEGs in leaf blades but were mostly among the stress > control DEGs in roots (Supplemental Figure S2). Responses of genes involved in starch synthesis are depicted in Figure 5b. This set includes genes that encode enzymes such as starch synthase, starch debranching enzyme, and others. Another set of genes implicated in the metabolism of the disaccharide trehalose were mostly among the stress > control DEGs. This set included genes involved in trehalose biosynthesis, which includes trehalose-6-phosphate

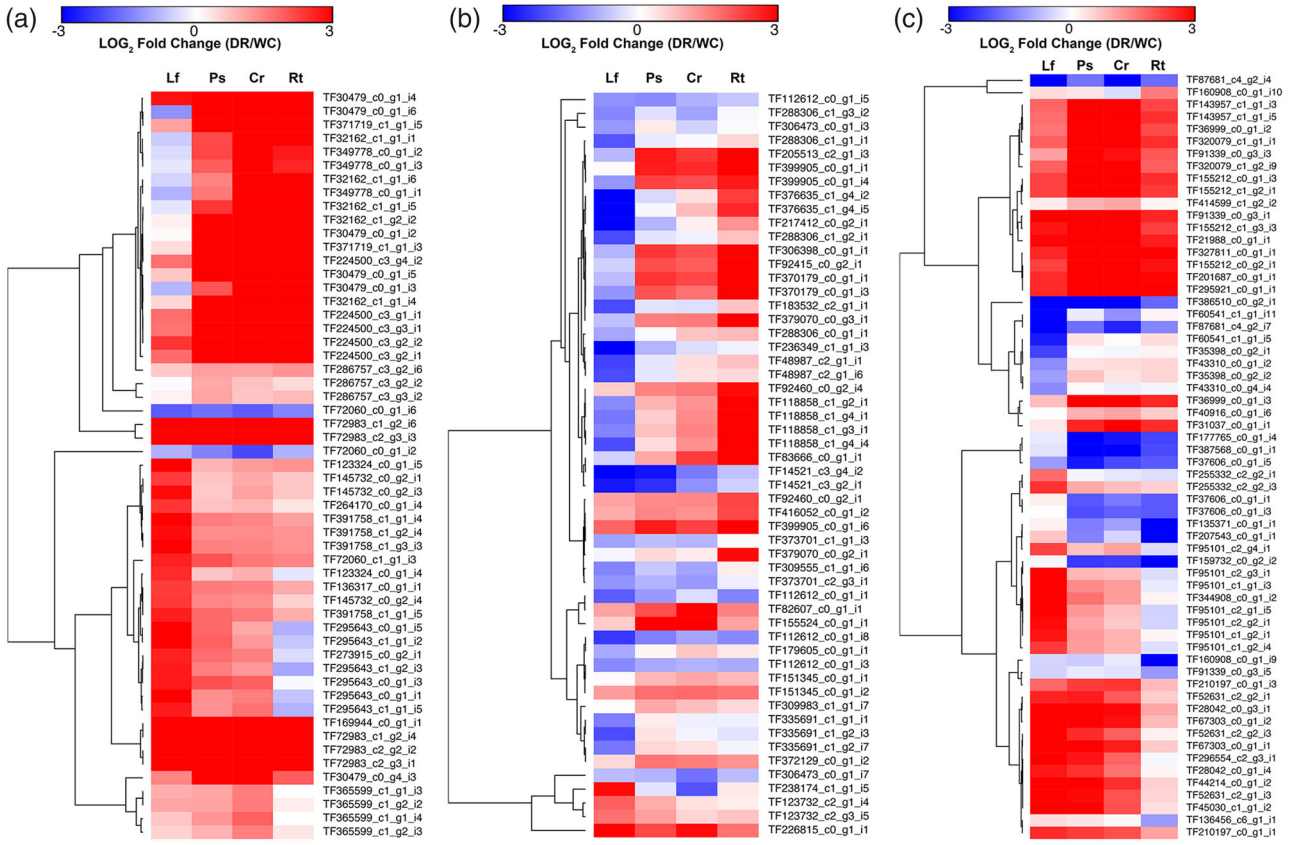

FIGURE 5 Drought treatment alters expression of genes involved in carbohydrate metabolism. Heatmaps represent drought-induced expression changes of genes involved in (a) raffinose family oligosaccharide biosynthesis, (b) starch synthesis, and (c) trehalose metabolism. Log_2 fold change (drought/control [DR/WC]) values were used for the analysis. Only unigenes differentially expressed in at least one comparison were included in the heatmaps. Lf, leaf; Ps, pseudostem; Cr, crown; Rt, root

synthase (TPS) and trehalose-6-phosphate phosphatase. The majority of these genes were stress > control in at least one of the tissues, and many were stress > control in all tissues (Figure 5c).

3.4 | Phytohormone metabolism gene expression was drastically altered in response to stress

Water-deficit stress also significantly altered expression of genes involved in phytohormone metabolism including biosynthesis and signal transduction (Supplemental Figure S3). Phytohormones ABA and ethylene are known to play vital roles in mediating abiotic stress responses. The DEGs implicated in ABA synthesis and signaling were mostly stress > control in all four tissues (Supplemental Figure S3). Stress-induced changes in the expression of genes involved in ABA biosynthesis and signal transduction are represented in a heatmap in Figure 6a. This set includes genes that encode NCED and abscisic aldehyde oxidase, key enzymes implicated in ABA biosynthesis.

Differentially expressed genes involved in ethylene synthesis and signaling were mostly stress > control in leaf blade, pseudostem, and crown (Supplemental Figure S3). A heatmap representing expression of genes implicated in ethylene biosynthesis and signaling is presented in Figure 6b. Differentially expressed genes encoding 1-aminocyclopropane-1-carboxylate (ACC) synthase, which catalyzes synthesis of ACC from *S*-adenosyl methionine, were stress > control in aerial tissues (Supplemental Figure S3). Genes encoding AP2-domain transcription factors named ethylene response factors (ERFs) are intrinsic parts of ethylene signaling cascade. The DEGs encoding ERFs were mostly stress > control especially in the leaf blade, pseudostem, and crown (Supplemental Figure S3; Figure 6b).

In contrast, genes involved in brassinosteroid metabolism, including synthesis and signal transduction, were control > stress (Supplemental Figure S3). Genes, that encode cytochrome P450 51 (CYP51), which catalyzes oxidative removal of 14-α methyl group from sterol precursors in sterol biosynthesis, were significantly control > stress mostly in pseudostem, crown, and root tissues (Supplemental Figure S3). On the other hand, genes encoding

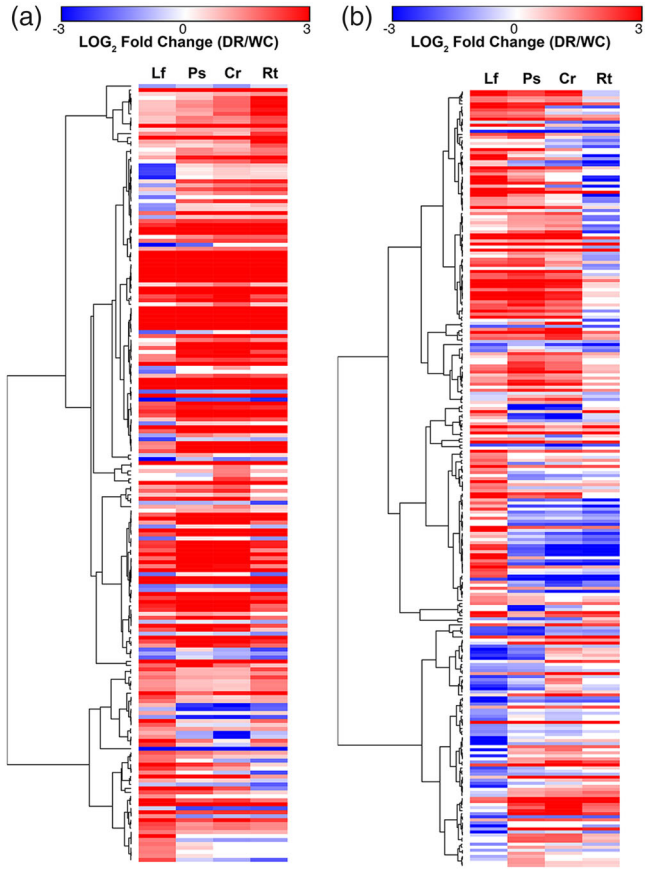

FIGURE 6 Drought leads to changes in expression of genes implicated in phytohormone metabolism. Heatmaps represent drought-induced expression changes of genes involved in (a) abscisic acid (ABA) and (b) ethylene metabolism. Log_2 fold change (drought/control [DR/WC]) values were represented in the heatmaps. Only unigenes differentially expressed in at least one comparison were represented in the heatmaps. Lf, leaf; Ps, pseudostem; Cr, crown; Rt, root

cycloartenol synthase 1 (CAS1), which catalyzes synthesis of cycloartenol from epoxysqualene in the biosynthesis of brassinosteroids, were control > stress mostly in leaf blade and pseudostem (Supplemental Figure S3). Genes encoding CAS1 are represented in the 'brassinosteroid.synthesis-degradation.sterols.other' MapMan BIN category. Additionally, genes encoding brassinosteroid insensitive 1-like (BRI1-like), a leucine-rich repeat receptor kinase involved in brassinosteroid signal perception, were primarily control > stress in all tissues (Supplemental Figure S3).

3.5 | Stress significantly changed expression of genes encoding transcription factors

Genes encoding members of various transcription factor families also displayed common and unique expression patterns in response to water-deficit stress in various tissues. The DEGs encoding heat shock factors were stress > control in all four tissues (Supplemental Figure S4), and DEGs encoding members of the NAC domain transcription factor family were similarly stress > control in all tissues (Supplemental Figure S4). In contrast, DEGs encoding Aux/IAA transcription factors were control > stress in all four tissues (Supplemental Figure S4), and DEGs encoding members of the basic helix-loop-helix (bHLH) transcription factor family were primarily stress > control in leaf blade but control > stress in pseudostem, crown, and root tissues (Supplemental Figure S4).

Interestingly, some transcription factor-encoding genes displayed tissue-specific expression in response to water-deficit. DEGs encoding auxin response factors (ARFs) showed more dramatic control > stress responses in the crown tissue as compared with leaf blade, pseudostem, and root (Supplemental Figure S4; Figure 7a). The DEGs encoding WRKY transcription factor family members were control > stress in pseudostem, crown, and root tissues, and this effect was most striking in root tissue (Supplemental Figure S4; Figure 7b). On the other hand, DEGs encoding WRKY transcription factors were mostly stress > control in leaf blades (Supplemental Figure S4; Figure 7b). The DEGs encoding TCP transcription factor family members were control > stress in pseudostem and crown tissues (Supplemental Figure S4). A heatmap displaying control > stress expression of genes encoding TCP transcription factors in pseudostem and crown tissues is presented in Figure 7c. After stress treatment, DEGs that encode PHOR1 transcription factor family members were mostly control > stress in root tissue. Whereas DEGs encoding PHOR1 transcription factors were predominantly stress > control in leaf blades (Supplemental Figure S4). The DEGs encoding members of OVATE family proteins (OFPs), a group of plant-specific transcription factors, were control > stress in pseudostem, crown, and root tissues (Supplemental Figure S4).

3.6 | Gene expression in response to drought treatment in the crown

MapMan analysis also revealed control > stress expression of DEGs encoding myosin class XI proteins primarily in the crown tissue (Supplemental Figure S5). The actin-myosin class XI system plays a vital role in regulating different stages of plant growth and development. A heatmap displaying stress-induced changes in expression of genes encoding myosin class XI proteins is presented in Figure 8a. Additionally, genes that encode actin proteins, important constituents of cytoskeleton, were mostly control > stress in pseudostem and crown tissues (Supplemental Figure S5).

As presented above (Figure 4), a large number of unigenes (11,035 in the stress > watered control and 13,824 in the watered control > stress) were among the DEGs in individual tissues. Of those, we chose to focus on DEGs that were unique

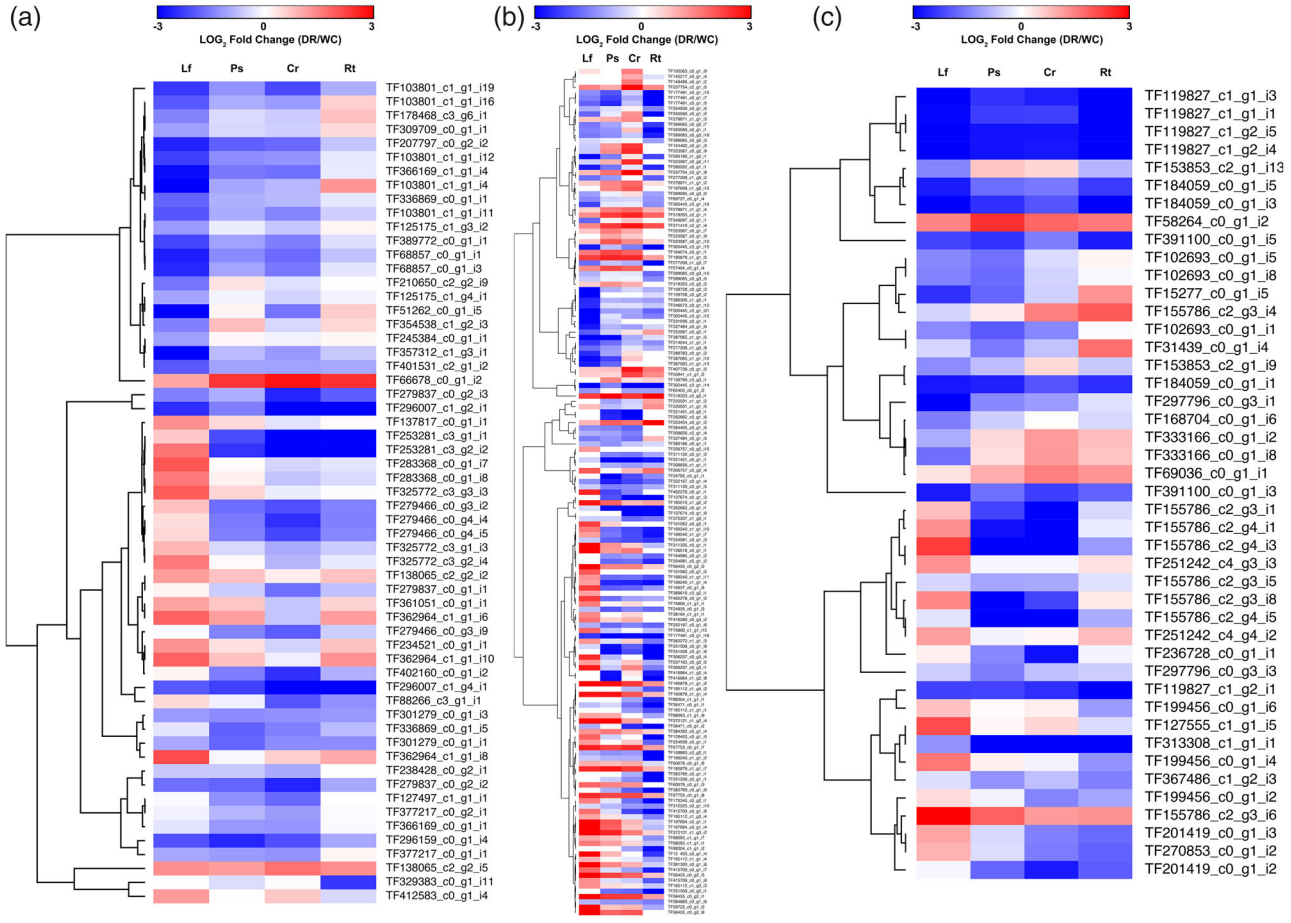

FIGURE 7 Drought stress modulates expression of genes encoding transcription factors. Heatmaps depicting expression changes of genes encoding (a) auxin response factors (ARFs), (b) WRKY, and (c) TCP family transcription factors. All heatmaps represent $\log_2$ fold change (drought/control [DR/WC]) values and only include unigenes that were differentially expressed in at least one comparison. Lf, leaf; Ps, pseudostem; Cr, crown; Rt, root

to the crown. A total of 1,587 genes were specific control > stress DEGs in the crown and 514 genes were stress > control (Figure 4b; Supplemental Excel File S3). However, a number of the unigenes that were declared significant gave <20 mapped reads per gene in the crown tissue but were kept in the dataset because they gave >20 reads in at least one other tissue–treatment combination. Thus, when the genes with <20 mapped reads in the crown data were removed, 1,319 genes were specific control > stress DEGs, and 374 were stress > control DEGs in the crown. Of the 374 crown-specific stress > control DEGs, 150 had associated BPGO terms, but no significant pathways were associated.

Of the 1,319 crown-specific control > stress DEGs, 474 had associated BPGO terms, of which 17 terms were significant (false discovery rate < 0.05) (Supplemental Excel File S3). The most significant BPGO terms were GO:0033233 and GO:0033234, negative regulation of protein sumoylation, and GO:0016973, polyA mRNA export from the nucleus. Eight DEGs that encode nuclear-pore anchor (NUA) proteins are all control > stress DEGs in the crown (Figure 8b). Other control > stress DEGs specifically found in the crown tissues were in GO:0033044, regulation of chromosome organization. The DEGs associated with GO:0033044 included genes homologous to *A. thaliana* At3g54670 (SMC1-structural maintenance chromosome 1). In fact, all unigenes that were homologous to different SMC genes were among control > stress DEGs in the crown.

4 | DISCUSSION

Plants can withstand water-deficit conditions through three mechanisms: drought escape, drought avoidance, and drought tolerance. In drought escape, plants hasten their growth under adequate water availability but enter dormancy under water-limited conditions (Levitt, 1980; Kramer, 1980; Loka et al., 2019). Forage grasses that exhibit drought escape include Kentucky bluegrass (*Poa pratensis* L.) and summer dormant Mediterranean ecotypes of tall fescue (Assuero et al., 2000; Humphreys et al., 2005; Fry & Huang, 2004; Missaoui et al.,

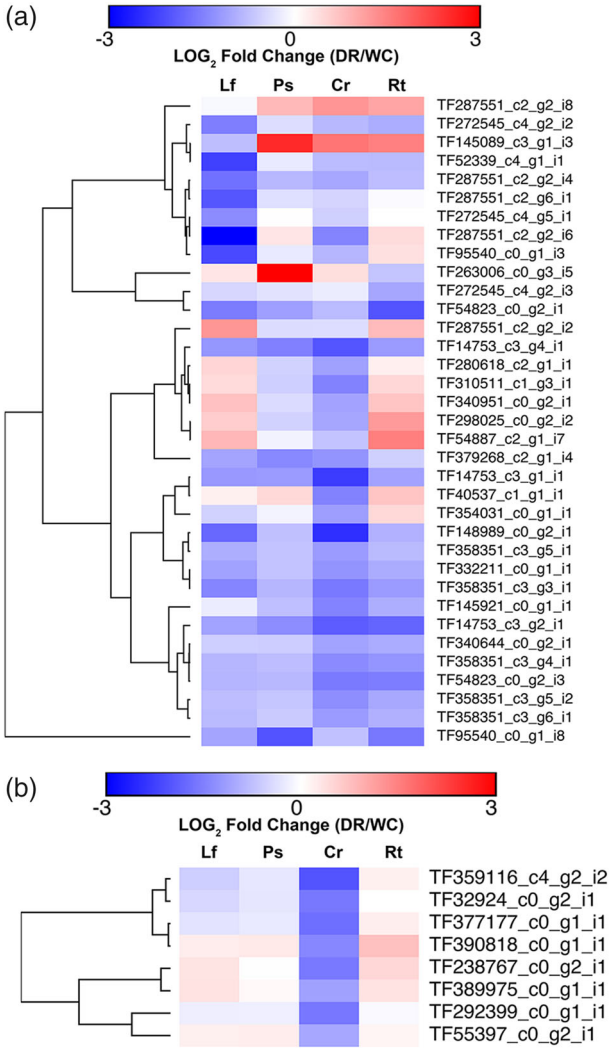

FIGURE 8 Drought stress gives rise to crown-specific changes in gene expression. Heatmaps displaying drought-induced expression changes of genes that encode (a) myosin class XI and (b) nuclear pore anchor (NUA) proteins. Log_2 fold change (drought/control [DR/WC]) values were represented in the heatmaps and only unigenes differentially expressed in at least one comparison were displayed. Lf, leaf; Ps, pseudostem; Cr, crown; Rt, root

2017). In case of drought avoidance, under water-deficit conditions plants reduce water loss and also continue optimum water uptake and thereby maintain a high tissue water potential (Levitt, 1980). Plants displaying drought avoidance are characterized by well-developed root system and reduced numbers of stomata and leaves (Qian et al., 1997). Plants exhibiting drought tolerance, on the other hand, can lead to an increase in the levels of compatible solutes, such as carbohydrates, mineral ions, and amino acids within the plant cells, thus decreasing the solute potential or osmotic potential. This allows plants to maintain turgor in water-limited conditions (Levitt, 1980). Drought tolerance in forage grasses, such as creeping bentgrass (*Agrostis stolonifera* L.), is typically achieved by modifying plants' physiological and metabolic processes (Nilsen & Orcutt, 1996). The mechanisms for drought avoidance or tolerance are sensitive to the environment and also there is considerable spatiotemporal variation (Hu & Xiong, 2014; Mickelbart et al., 2015). Survivability under drought is regulated by multiple loci, where each locus contributes a portion of the overall cumulative effect (Xu et al., 2014; Sandhu et al., 2014; Ravi et al., 2011). Thus, to better understand drought responses in a plant species, it is crucial to elucidate its global transcriptional landscape and its spatiotemporal dynamics.

4.1 | Photosynthesis and carbohydrate metabolism exhibit drought-induced changes in tall fescue

Drought stress exerts significant negative impacts on various plant metabolic processes including photosynthesis. Stomatal aperture and stomatal density are two major attributes affected by drought stress (Nilsen & Orcutt, 1996). Apart from such stomatal features, other factors implicated in the regulation of photosynthetic ability, such as rubisco activity, chlorophyll content, and carotenoid content, are also adversely impacted by drought stress in various forage plant species including tall fescue (Loka et al., 2019; Yu et al., 2012; Fu & Huang, 2001). Our analysis also revealed strong adverse effects of drought stress on expression of photosynthesis-related genes in all aerial tissues. Improving photosynthetic efficiency under water-limited conditions can significantly aid in achieving higher food production. Stomatal aperture plays critical roles in regulation of transpiration of water vapor from the leaves and uptake of CO_2 during photosynthesis. A recent study showed that overexpression of *Photosystem II Subunit S* (*PsbS*) in transgenic tobacco (*Nicotiana tabacum* L.) lines reduced water uptake by 25% per molecule of CO_2 assimilated (Głowacka et al., 2018). This can help increase water use efficiency to maintain crop yield in water-limited conditions. It would be interesting to assess the effects of *PsbS* over-expression to improve water use efficiency in forage grasses, such as tall fescue.

Starch is another important mediator of abiotic stress response. Our results revealed that expression of starch biosynthetic genes was low in leaf blade and higher in root tissue in response to drought treatment in tall fescue. Starch not only acts as a storage molecule, but remobilization of glucose from starch provides energy and carbon during stress conditions including drought (Thalmann & Santelia, 2017). Additionally, sugars released by starch metabolism can act as osmoprotectants and signaling molecules (Rook et al., 2006; Krasensky & Jonak, 2012). Drought stress results in reduced expression of genes involved in starch biosynthesis and

concomitant decreases in starch accumulation in leaves of different plant species (Thalmann & Santelia, 2017).

The biosynthesis of the disaccharides trehalose-6-phosphate and trehalose has been implicated in abiotic stress tolerance (Fernandez et al., 2010; Delorge et al., 2014). Constitutive overexpression of TPS-encoding genes from *Escherichia coli* (*OtsA*) and yeast (*TPS1*) are reported to enhance drought tolerance in transgenic tobacco plants but also result in significantly altered plant phenotypes (Holmstrom et al., 1996; Romero et al., 1997). Overexpression of endogenous TPS-encoding genes enhances drought tolerance in *A. thaliana* and rice (*Oryza sativa* L.) without significant adverse effects to plant growth and development (Avonce et al., 2004; Li et al., 2011). Trehalose may play a role in abiotic stress tolerance by acting as a compatible solute, sugar sensor, or through its role in carbohydrate metabolism (Fernandez et al., 2010). Our study also indicated alterations in the expression of genes for trehalose biosynthetic enzymes TPS and trehalose-6-phosphate phosphatase in response to water withholding, suggesting involvement of trehalose and trehalose-6-phosphate in mediating response to drought stress in tall fescue.

Raffinose family oligosaccharides are known to accumulate in response to various osmotic stresses including drought and are also considered to impart stress tolerance possibly as osmoprotectants, antioxidants, and signaling molecules (ElSayed et al., 2014). Expression of genes involved in RFO biosynthesis, such as galactinol synthase and raffinose synthase, are reported to be higher in response to different abiotic stresses including drought (Taji et al., 2002; Egert et al., 2013). In line with the previously reported findings, our analyses also revealed strong stress > control expression of RFO biosynthetic genes encoding galactinol synthase and raffinose synthase.

4.2 | Biosynthesis and signaling of phytohormones constitute critical components of drought response

Among different phytohormones, ABA plays a crucial role in mediating drought stress responses. Abscisic acid was implicated in regulation of stomatal closure to reduce transpiration during drought stress (Kollist et al., 2014). Drought stress leads to spikes in ABA levels through upregulation of ABA biosynthetic genes including those that encode NCEDs and abscisic aldehyde oxidase (Cheng et al., 2002; Daszkowska-Golec & Szarejko, 2013). Cleavage of carotenoid precursors to xanthoxin catalyzed by NCED is a major regulatory step in the ABA biosynthesis. On the other hand, abscisic aldehyde oxidase oxidizes abscisic aldehyde to ABA. Additionally, this set of genes also encode enzymes known to participate in ABA signaling, such as ABA-induced protein phosphatase 2C (PP2C). Abscisic acid, upon reaching guard cells via ABA transporters, binds to ABA receptors such as PYRABACTIN-RESISTANCE 1 (PYR1) and PYR1-Like (PYL). Binding of ABA to ABA receptors inhibits PP2Cs, including ABA Insensitive 1 (ABI1) and ABI2, which are negative regulators of ABA signaling (Park et al., 2009). Inactivation of PP2Cs leads to phosphorylation and activation of downstream targets such as SNF1-related kinase (SnRK2s) (Nishimura et al., 2010). SNF1-related kinase activates anion channels leading to depolarization of membrane, which activates K^+ efflux channel GORK guard cell outward rectifying K^+. The effluxes of anion and K^+ from the guard cells accompany efflux of water, which altogether decrease turgor of guard cells and leads to stomatal closure under drought stress (Daszkowska-Golec & Szarejko, 2013). Current study also revealed significant stress > control expression of genes involved in ABA biosynthesis and signaling in all tissue types in response to drought stress, suggesting that modifications of genes encoding ABA receptors and other players in the ABA signaling cascade may be useful in conferring drought tolerance to forage grasses including tall fescue.

Ethylene synthesis and signaling are also major regulators of responses to abiotic stresses in various plant species. For instance, downregulation of ethylene biosynthetic gene ACC synthase resulted in improved drought tolerance in maize (*Zea mays* L.) (Habben et al., 2014). Members of AP2/ERF (APETALA2/ETHYLENE RESPONSE FACTOR) superfamily are important constituents of ethylene signal transduction and play crucial roles in regulating responses to abiotic stresses including drought (Mizoi et al., 2012). Overexpression of AP2/ERFs enhances drought tolerance in several plant species (Müller & Munné-Bosch, 2015). For example, overexpression of TaERF3 and JERF1 improve drought tolerance in wheat (*Triticum aestivum* L.) and rice, respectively (Rong et al., 2014; Zhang et al., 2010). Our analyses also revealed drought-induced changes in the expression of genes encoding ACC synthase and AP2/ERFs mostly in leaf blade, pseudostem, and crown tissue, which suggest a general role of ethylene in mediating drought response across different tissue types.

Brassinosteroids are another important class of phytohormones that play a vital role in mediating responses to drought stress (Nolan et al., 2020). Drought stress elicits tissue-specific effects on brassinosteroid signaling. Overexpression of vascular-enriched brassinosteroid receptor BRL3 is reported to impart drought tolerance without hampering plant growth and development, whereas mutations in the ubiquitously expressed leucine-rich repeat containing brassinosteroid receptor BRI1 also enhances drought tolerance but at the cost of reduced plant growth (Fàbregas et al., 2018). Our study also displayed drought-induced control > stress expression of genes encoding BRI1-like receptors in all four tissues, indicating a role of brassinosteroid-mediating

signaling in regulating responses to drought stress in tall fescue.

4.3 | Drought stress results in dynamic expression of genes encoding transcription factors across different tissues

Our results have revealed general stress responses across all tissue types involving changes in expression of genes that encode some classes of transcription factors that have been implicated in drought response in earlier studies. This includes stress > control expression of genes encoding heat shock factors and NAC domain transcription factors and control > stress expression of Aux/IAA transcription factors in all tissue types (Wu et al., 2016; Guo et al., 2016; Salehin et al., 2019). Our study also identified unique tissue-wide expression of several classes of transcription-factor-encoding genes. The current study revealed control > stress expression of genes encoding ARFs predominantly in crown tissue. Auxin response factors are transcriptional regulators that bind to AuxRE (auxin responsive elements) in the promoters of auxin responsive genes (Ulmasov et al., 1999). Expression of genes that encode ARFs are regulated by miRNA and transacting siRNAs (tasiRNAs) (Curaba et al., 2014; Matsui et al., 2014). Auxin response factors were reported to be involved in regulating drought responses in rice and sorghum [*Sorghum bicolor* (L.) Moench] (Zhou et al., 2007; Wang et al., 2010). Additionally, a recent study revealed the role of an ARF, ARF7, in regulating root branching toward water (Orosa-Puente et al., 2018). In light of these previous discoveries, our findings suggest that ARFs may play vital roles in regulating drought response in the tall fescue crown tissue.

Our analyses also showed differential tissue-wide expression changes in the genes that encode WRKY transcription factors in response to drought treatment. Genes encoding WRKY transcription factors were mostly stress > control in leaf blades, while they were mostly control > stress in pseudostem, crown, and root tissues. The WRKY transcription factors play crucial roles in regulation of growth, development, and abiotic stress responses in various plant species (Tripathi et al., 2014). The WRKY transcription factors have been implicated in mediating drought responses in several members of the grass family. For example, overexpression in *A. thaliana* of two WRKY transcription factors from wheat, TaWRKY1 and TaWRKY33, enhances drought tolerance in the transgenic lines (He et al., 2016). Similarly, overexpression of TaWRKY2 and OsWRKY11 bestows greater drought tolerance to transgenic wheat and rice lines, respectively (Gao et al., 2018; Wu et al., 2009). A WRKY transcription factor, HvWRKY38 was reported to be implicated in regulation of the drought response in barley (*Hordeum vulgare* L.) (Marè et al., 2004). Our previous study has revealed differential expression of genes encoding WRKY transcription factors between endophyte-plus and endophyte-minus tall fescue plants under control condition (Dinkins et al., 2017). Considering possible involvement of WRKY transcription factors in mediating drought response in tall fescue, it would be interesting in future to assess the role of WRKY transcription factors in conferring drought tolerance to endophyte-plus tall fescue plant genotypes as compared with their E- counterparts.

Our study also revealed control > stress expression of genes encoding TCP transcription factors mostly in the pseudostem and crown. The TCPs are plant-specific transcription factors that play important roles in regulating different aspects of plant growth and development, ranging from leaf blade development to senescence (Danisman, 2016). However, recent studies have shown implications of TCP transcription factors in mediating drought tolerance in several plant species including maize, rice, and moso bamboo [*Phyllostachys edulis* (Carrière) J. Houz.) (Ding et al., 2019; Liu et al., 2020; Mukhopadhyay & Tyagi, 2015). Thus, it is reasonable to hypothesize that changes in expression of genes encoding TCP transcription factors may help regulate drought tolerance in the crown and pseudostem tissues.

Our analyses also revealed changes in expression of genes encoding plant-specific OFPs in pseudostem, crown, and root tissue where control > stress expression was observed. Some OFPs play vital roles in regulating growth and development in several plant species and have also been shown to mediate brassinosteroid signaling (Wang et al., 2016). Functions of OFPs include control of organ shape in multiple species via regulation of pattern of cell division through their interactions with TONNEAU1 recruiting motif (TRM) proteins (Wu et al., 2018). Our findings indicate that members of the OFPs may also play a role in regulation of cell division patterns in the crown and pseudostem during drought responses.

4.4 | Drought stress induces changes in crown-specific gene expression

Our analysis revealed crown-specific control > stress expression of genes associated with protein sumoylation as well as polyA mRNA export from the nucleus. Eight transcripts that encode the nuclear pore anchor (NUA) proteins and six additional putative components of the nuclear pore complex (NPC) scaffold were identified in the genes that were expressed sufficiently to be identified in our tall fescue assembly. Two unigenes, TF284971_c0_g2_i2 and TF73211_c1_g1_i1, encoding putative homologues of the *Arabidopsis* Nup96/SAR3/MOS3 and Nup155, respectively, were also found to be DEGs (control > stress) in the crown, as well as other tissues. However, the other putative genes

that would encode components of the NPC, encoding the putative Nup50, Nup75, Nup93, and Ndc1-Nup protein homologues were not differentially expressed in any tissue (data not shown). Although a number of the components of the plant NPCs are still unresolved (Parry, 2014; Tamura et al., 2010), it is the gateway in mediating exchange of proteins and RNAs between the nucleus and cytoplasm and critical in the regulation of developmental, hormonal, biotic, and abiotic responses (Yang et al., 2017). The NUA proteins as part of the NPC have been shown to be involved in a number developmental and stress associated pathways including flowering, RNA homeostasis, sumoylation, and auxin signaling (Jacob et al., 2007; Xu et al., 2007).

Another set of control > stress genes were those encoding class XI myosin. This set of genes displayed control > stress expression preferentially in the crown tissue. The present study also indicated control > stress expression of genes encoding actin filament proteins in the crown as well as in the pseudostem. Members of class XI myosins act as actin-dependent molecular motors involved in cytoplasmic streaming and trafficking of organelles such as Golgi, ER, peroxisome, mitochondria, and chloroplasts (Tominaga & Ito, 2015). The speed of cytoplasmic streaming has been identified as a regulator of overall plant size in *A. thaliana* (Tominaga et al., 2013). Class XI myosins are also implicated in actin organization, cell expansion, pollen tube growth, and root hair elongation (Madison et al., 2015; Peremyslov et al., 2008; Prokhnevsky et al., 2008). Recently, members of class XI myosins have also been implicated in mediating auxin response and senescence-induced cell death in *A. thaliana* (Ojangu et al., 2018). In light of our findings and results from these previous studies, we suggest that organellar movements regulated by the myosin XI-actin system may play a role in mediating drought stress response in tall fescue especially in the crown tissue.

5 | CONCLUSIONS

In summary, we have presented a detailed account of drought response in the forage grass, tall fescue, in the four major vegetative tissues. First, we have demonstrated general drought response across tissue types and also tissue-specific dynamics of drought response. Our results have implicated genes involved in photosynthesis, carbohydrate metabolism, phytohormone biosynthesis and signaling, cellular organization, and transcriptional regulation in mediating plant-wide drought responses in tall fescue. Secondly, we have identified a set of candidate genes that might be modified or manipulated in an effort to enhance drought tolerance in tall fescue such as genes that encode ARF, WRKY, and TCP family transcription factors. Third, we have also shown a set of genes possibly involved in regulating crown-specific drought response in tall fescue such as nuclear pore anchor, structural maintenance of chromosomes, and class XI myosin proteins. We note that the fungal endophyte *E. coenophiala* resides primarily in the pseudostem and crown, and hence, survivability of crown under stress condition may play a vital role in maintaining a successful host–endophyte symbiosis, which leads to improved performance in endophyte-infected plants under stressed conditions (Nagabhyru et al., 2013; Dinkins et al., 2019; Malinowski & Belesky, 2019). In the future, it would be worthwhile to compare the findings of the current study on drought-induced dynamic spatial gene expression in endophyte-infected with endophyte-free plants to further delineate the contribution of the endophyte in the crown in conferring drought tolerance to tall fescue.

DATA AVAILABILITY STATEMENT

Illumina RNA-seq data for the experiments are available at NCBI under BioProjects PRJNA284541, PRJNA658975. The TF153K transcriptome assembly is available at: https://data.cyverse.org/dav-anon/iplant/home/rdinkins/Tall_Fescue_Assembly/TF_153KSeq.fa

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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