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ORIGINAL PAPER

Drought response in the spikes of barley: gene expression in the lemma, palea, awn, and seed

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Abstract The photosynthetic organs of the barley spike (lemma, palea, and awn) are considered resistant to drought. However, there is little information about gene expression in the spike organs under drought conditions. We compared response of the transcriptome of the lemma, palea, awn, and seed to drought stress using the Barley1 Genome Array. Barley plants were exposed to drought treatment for 4 days at the grain-filling stage by withholding water. At the end of the stress, relative water content of the lemma, palea, and awn dropped from 85% to 60%. Nevertheless, the water content of the seed only decreased from 89% to 81%. Transcript abundance followed the water status of the spike organs; the awn had more drought-regulated genes followed by lemma and palea, and the seed showed very little change in gene expression. Despite expressing more drought-associated genes, many genes for amino acid, amino acid derivative, and carbohydrate metabolism, as well as for photosynthesis, respiration, and stress response, were down-regulated in the awn compared with the lemma, palea, and seed. This suggests that the lemma and the palea are more resistant to drought stress compared with the awn.

Keywords Awn · Drought · Lemma · Palea · Seed · Transcriptome

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Introduction

Drought is the single most important environmental stress that reduces crop yield (Boyer 1982). The first symptom of drought stress is a rapid inhibition of growth due to reduced cell expansion (Fan et al. 2006). This is closely followed by inhibition of photosynthesis from partial or complete closure of stomata and reduction in the amount and activity of photosynthetic enzymes (Flexas and Medrano 2002; Chaves et al. 2007). Drought affects all stages of plant development, but the reproductive stage is the most sensitive. Shortage of water early in the reproductive development (pollination) increases sterility and senescence of flowers thereby reducing the number of seeds per plant (Saini and Westgate 2000; Zinselmeier et al. 2002). Post-anthesis drought disrupts the supply of carbohydrate from the source organs and reduces seed size (Setter et al. 2001; Boyer and Westgate 2004). Understanding how drought affects reproductive development is crucial for improving yield in environments characterized by terminal drought.

In cereals, the photosynthetic organs of the spike (lemma, palea, awn, and glumes) are vital sources of carbohydrate for the developing seed. In barley, carbohydrates derived from the lemma, palea, and awn account for as much as 76% of the final seed dry weight (Duffus and Cochrane 1993). The contribution of spike photosynthesis to grain-filling is greater during drought. Under drought, the flag leaves wilt and most of the lower leaves senesce. However, the spike is more resilient and could sustain grain-filling during drought. The spike has many features for adaptation to drought stress including xeromorphic anatomy (Araus et al. 1993), better osmotic adjustment (Tambussi et al. 2007), high water use efficiency (Blum 1985), and delayed senescence (Tambussi et al. 2007).

Most genome-wide transcriptional profiling studies on drought stress in barley and other cereals have focused on the response of vegetative organs (Walia et al. 2005; Talame et al. 2006; Tommasini et al. 2008; Guo et al. 2009). Response of the spike transcriptome to drought stress has been largely overlooked even though drought at the reproductive stage causes the greatest yield loss and the photosynthetic organs of the spike are the major suppliers of carbon to the developing seed (Duffus and Cochrane 1993). Barley varieties with drought-resistant spikes have a better chance of achieving normal seed size when available water is reduced during grain-filling. Earlier, we showed that the lemma and the palea of barley preferentially express genes for photosynthesis compared with the flag leaf (Abebe et al. 2004). Recently, we also demonstrated that, under normal growth conditions, the awn is the major photosynthetic organ of the spike (Abebe et al. 2009, in press). In this study, we compared the transcriptome of lemma, palea, awn and seed of barley to determine the spike organ more resistant to drought stress.

Materials and methods

Plant material and drought stress

Barley plants (*Hordeum vulgare* L. cv. Morex) were grown in a controlled environment chamber at 22°C day/18°C night temperatures, 60% relative humidity and 16 h light with an intensity of 700 μmoles $m^{-2}s^{-1}$. Six plants per pot were grown in 20 cm×25 cm pots filled with a mixture of 17% topsoil, 50% Canadian peat moss, 25% vermiculite, and 8% rice hull. Plants were fertilized with Osmocote® slow release fertilizer (NPK, 19-6-12; Scotts Company LLC, Marysville, OH). Drought stress was initiated at Zadok stage 71 (kernel watery-ripe; Zadoks et al. 1974) by withholding water for 4 days. Control (non-stressed) plants received 500 ml water every day.

Experimental design

We evaluated the response of the transcriptome of the lemma, palea, awn, and seed to drought stress using a randomized complete block design (RCBD; Nettleton 2006). Drought stress and organ type were the main treatment factors. For each stress level by organ combination, triplicate samples were collected at the grain-filling stage (Zadok scale 83; Zadoks et al. 1974) from plants stressed on three different dates (blocks). On the fourth day of stress, each organ was collected from four plants (per pot) from pots assigned to the respective treatment. For four organs with two drought stress levels and three biological replications, 24 pots were used. RNA from each replicate sample was hybridized to a single Barley1 GeneChip (Affymetrix, Santa Clara, CA).

Determination of relative water content

Relative water content (RWC) of the lemma, palea, awn, and seed was measured on the 4^{th} day of stress according to Rachmilevitch et al. (2006). Organs from stressed and control plants were collected and fresh weight (FW) was measured immediately. Then, organs were fully hydrated in de-ionized water overnight at 4°C. After blotting dry, the turgid weight (TW) of each sample was measured. Plant materials were dried at 70°C for 48 h and the dry weight (DW) was recorded. Percent RWC was computed using the equation: $\mathrm{RWC}\ (\%) = (\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW}) \times 100$.

Sample collection and RNA extraction

Lemmas, paleas, awns, and seeds were collected on the fourth day of drought stress between 10:00 a.m. and 12:00 p.m. when plants were at the early grain-filling stage (Zadok scale 83). Young spikelets at the tip and the base of each spike (three to four nodes) were discarded. Also, 1 cm of the bottom and 2 cm of the tip of the awn was discarded. Samples were immediately frozen in liquid nitrogen and stored at −80°C. Total RNA from the lemma, the palea, and the awn was extracted with guanidinium thiocyanate (Chirgwin et al. 1979). RNA from the seed was extracted using Tri Reagent (Molecular Research Center Inc., Cincinnati, OH) followed by precipitation of starch with 0.8 M sodium citrate and 1.2 M sodium chloride according to the manufacturer's direction.

RNA labeling, array hybridization, and normalization

Labeling of RNA samples, hybridization, and data acquisition were performed at the Iowa State University GeneChip Facility according to the Affymetrix One-Cycle Target Labeling protocol (http://www.affymetrix.com/support/technical/index.affx). Labeled cRNA was hybridized

to the Barley1 Genome Array (Close et al. 2004) for 16 h at 45°C. Background correction, normalization and summarization were performed using the Microarray Analysis Suite v 5.0 (MAS5) algorithm (Affymetrix). Normalization was achieved by scaling mean signal intensities for each GeneChip to 500.

Quality of GeneChip data

Quality of probe labeling and hybridization to the Barley1 GeneChips assessed by Expression Console v 1.1.0 (Affymetrix) were within the values recommended in the Affymetrix GeneChip® Data Analysis Fundamentals. Pearson's correlation coefficients computed on normalized signal intensities for each independent biological replicate had values between 0.93 and 0.96, suggesting low variability between the replicates. In addition, visual inspection of images of GeneChips showed no serious spatial variation.

Analysis of GeneChip data

Expression values were $\log_2$-transformed and imported into JMP Genomics 3.2 (SAS Institute) for statistical analysis. Differential gene expression among drought-stressed spike organs was analyzed using a mixed linear model analysis (Nettleton 2006). The $\log_2$ expression values were the response variables, drought stress and organ type were fixed treatment effects, and date of sample collection (block) and residuals (error) were random effects. The model was represented by the equation: $y_{ijk} = \mu + \text{stress}_i + \text{organ}_j + \text{block}_k + \text{stress} \times \text{organ}_{ij} + \text{error}$, where y_{ijk} was the normalized $\log_2$ expression level for stress i, organ j, and block k for each gene, μ was overall mean of $\log_2$ signal intensities common to all observations, and error was the deviation for ijth subject. The model assumes no interaction between treatment and block effects and a constant variance from block to block. The significance of differential gene expression in stressed and non-stressed organs was determined using the F statistic. To control false discoveries due to multiple hypotheses testing, we used the q value method described by Storey and Tibshirani (2003). Probe sets with a q value <0.01 and a fold change of ≥2 were declared significant. The importance of blocking was tested indirectly using block as a fixed (rather than a random) effect. The block effect was significant or closer to significant for many probe sets, suggesting that RCBD was an appropriate design.

Functional classification of stress-responsive genes

Drought-regulated genes with known functions were categorized according to gene ontology (GO) terms (biological process, molecular function, and cellular component) to determine their biological roles. The Barley1 probe set annotations from Blast2GO Functional Annotation Repository (FAR; http://www.blast2go.org/b2gfar) were imported into Blast2GO v 2.3.5 (Götz et al. 2008; http://www.blast2go.de/) for GO analysis. At the time of the analysis, B2G-FAR contained GO annotations for 42% of the Barley1 probe sets. Metabolic pathways associated with selected drought-regulated genes were obtained from the MetaCyc metabolic pathways and enzymes database (Caspi et al. 2008; http://MetaCyc.org).

Validation of gene expression by real-time PCR

Six differentially-expressed genes were assayed by real-time polymerase chain reaction (PCR) to verify the GeneChip hybridization data. Total RNA from the original samples used for GeneChip hybridization was treated with DNAse I (Promega) and reverse transcribed with oligo(dT) primer using AMV reverse transcriptase (Promega). Then, 150 ng of cDNA was used for real-time PCR analysis using 2× Maxima™ SYBR Green qPCR Master Mix (Fermentas) and 200 nM of gene-specific primers (Table S1). Assays were performed in triplicate and analyzed on the 7300 Real-time PCR system (Applied Biosystems). Relative mRNA abundance (in fold change) was determined using the delta delta C_t ($\Delta\Delta C_t$) method (Schmittgen and Livak 2008) after normalizing the C_t value for each gene against cytosolic glyceraldehyde-3-phosphate (GAPDH) as the endogenous control. Fold change was calculated using the equation $2^{-\Delta\Delta Ct}$, where $\Delta\Delta C_t = \left[\left(C_{t,\text{ target gene}} - C_{t,\text{ GAPDH}}\right)_{\text{drought}}\right] - \left[\left(C_{t,\text{ target gene}} - C_{t,\text{ GAPDH}}\right)_{\text{control}}\right]$.

Data access

All detailed data from this study have been deposited at the National Center for Biotechnology Information's Gene Expression Omnibus (www.ncbi.nlm.nih.gov/projects/geo) as Accession GSE17669. Data files have also been deposited at PLEXdb (http://www.plexdb.org/), a MIAME-compliant public resource for gene expression in plants and plant pathogens (Shen et al. 2005). PLEXdb files are categorized under Accession BB89.

Results

Data analysis strategy

To determine differences in the response of the transcriptome of the spike organs to drought stress, we first performed a mixed model analysis using the $\log_2$-transformed signal

intensities of stressed organs vs. the corresponding controls (well-watered). We then used differentially expressed genes in each organ and performed a pair-wise comparison to identify genes with organ-specific expression. Finally, drought-responsive genes were categorized according to gene ontology (GO) terms to establish biological processes affected by drought in the lemma, palea, awn, and seed. For this study, we focused on GO biological process categories that contribute to osmolyte (organic solutes) accumulation (amino acid, amino acid derivative, and carbohydrate metabolism), gas exchange (respiration and photosynthesis), and stress response.

RWC of drought-stressed and non-stressed organs of the barley spike

Among the spike organs, the water content of the seed remained unchanged during drought stress; RWC decreased from 89% in the control to 81% under drought stress. In contrast, RWC of the lemma, palea, and awn was significantly reduced. RWC of the lemma decreased from 86% to 60%, the palea from 86% to 63%, and the awn from 85% to 62% (Table S2). At this stress level, the flag leaf had an RWC of 50%, was tightly rolled, and was wilted. All other leaves also wilted and turned yellow (data not shown). Furthermore, stressed awns showed partial chlorosis, especially on the distal half of the awn.

Abundance of drought-regulated genes in the spike organs

The awn had the largest number of drought-regulated genes, followed by the lemma and the palea. The seed had the fewest drought-responsive genes (Fig. S1). There were only 16 up-regulated and 15 down-regulated genes common to all stressed organs (Fig. S1). The remarkably fewer drought-regulated transcripts common to all organs is attributable to the smaller number of genes differentially expressed in the seed. When the seed was excluded from the pair-wise comparison, the photosynthetic organs had 430 up-regulated and 536 down-regulated genes in common (Fig. S1).

Genes up-regulated and down-regulated by drought affect similar biological processes

Classification of drought-regulated genes according to GO biological process terms revealed that cytoskeleton organization, osmolyte biosynthesis, photosynthesis, pigment biosynthesis, protein folding, proteolysis, respiration, signal transduction, transcription, translation, transport, and response to stress were regulated by drought stress. For this study, GO biological process categories associated with osmolyte biosynthesis (amino acids, amino acid derivatives, and carbohydrates), gas exchange (photosynthesis and respiration), pigment biosynthesis, and response to stress

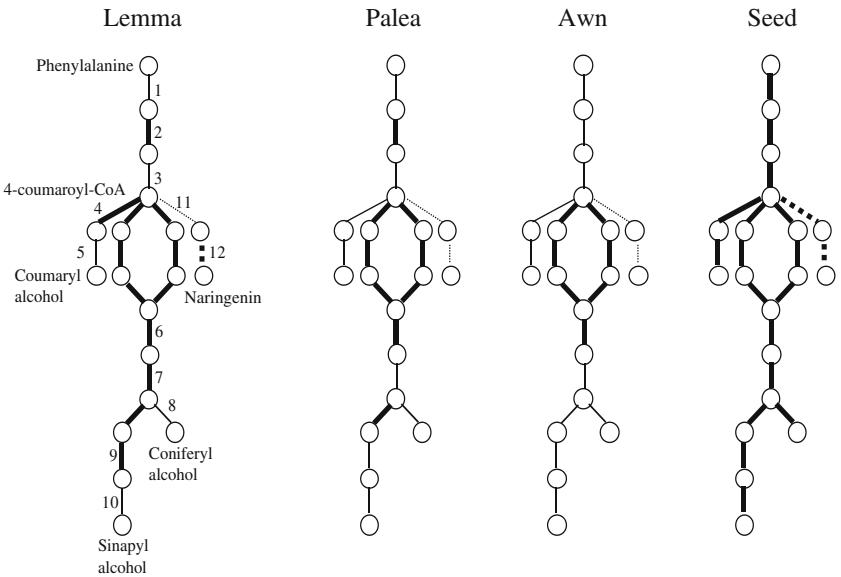

Fig. 1 Differential expression of genes in the phenylpropanoid pathway for the biosynthesis of lignin and chalcones. Reactions in the pathway are depicted by lines, substrates by circles and enzymes catalyzing the reactions by numbers. Thickness of the lines depicts differential regulation of the genes encoding enzymes during drought stress: thicker, non-differentially regulated; thinner, down-regulated. *Broken lines* depict the pathway leading to the biosynthesis of chalcones. Key for enzymes: *1* phenylalanine ammonia-lyase, *2* *trans*-cinnamate 4-hydroxylase, 4-coumarate-CoA ligase, *5* cinnamoyl CoA-reductase, *4* cinnamyl alcohol dehydrogenase, *5* caffeoyl CoA-3-O-methyltransferase, *6* cinnamoyl CoA-reductase, *7* cinnamyl alcohol dehydrogenase, *8* caffeic acid *O*-methyltransferase, *9* cinnamyl alcohol dehydrogenase, *10* chalcone synthase, *12* chalcone isomerase

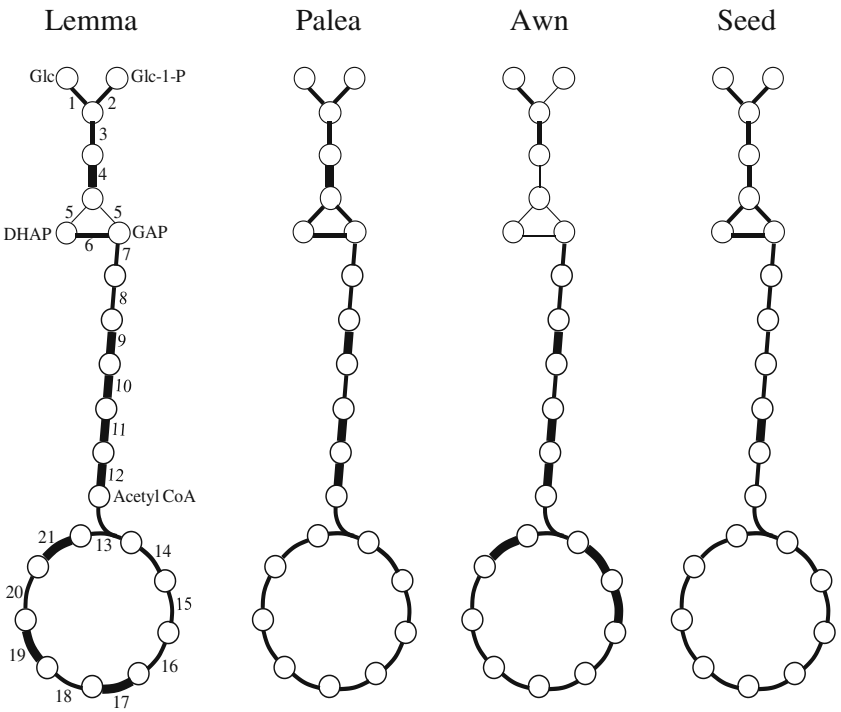

Fig. 2 Expression profile of genes in glycolysis and the TCA cycle in drought-stressed organs of the spike. Reactions in the pathway are represented by lines, substrates by circles and enzymes catalyzing the reactions by numbers. Thickness of the lines depicts differential regulation of the genes encoding enzymes during drought stress: thicker, up-regulated; medium, non-differentially expressed; thinner, down-regulated. Key for enzymes: *1* hexokinase, *2* phosphoglucomutase, *3* phosphoglucoisomerase, *4* phosphofructokinase, *5* fructose-bisphosphate aldolase, *6* triosephosphate isomerase, *7* glyceraldehydes-3-phosphate dehydrogenase, *8* phosphoglycerate kinase, *9* phosphoglycerate mutase, *10* enolase, *11* pyruvate kinase, *12* pyruvate dehydrogenase. Abbreviations: *DHAP* dihydroxyacetone phosphate; *GAP* glyceraldehyde-3- phosphate; *Glc* glucose; *Glc-1-P* glucose-1-phosphate

were chosen (Figs. 1, 2, and 3 and Tables 1, 2, 3, and 4). As described below, each of these biological process categories contained both up-regulated and down-regulated genes. We also found that for multigene families, some members were up-regulated while others were down-regulated during drought stress.

Amino acid metabolism

The effect of drought on gene expression for amino acid metabolism in the lemma and palea was very small compared with the awn. The seed did not show changes in the expression of genes for amino acid metabolism (Tables 1

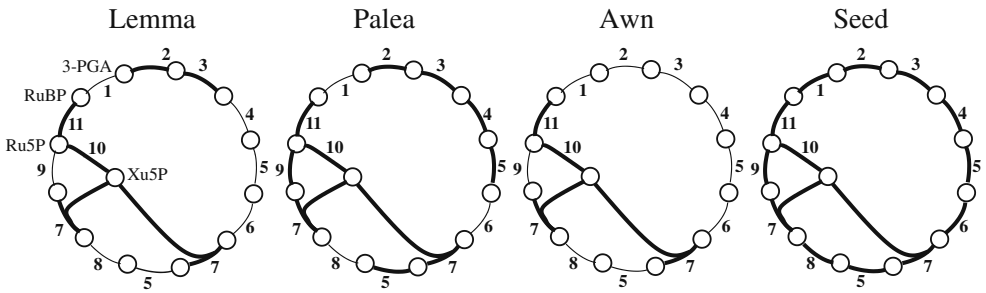

Fig. 3 Differential expression of genes in the Calvin cycle in the lemma, palea, awn, and seed of barley during drought stress. Reactions in the pathway are shown by lines, substrates by circles and enzymes catalyzing the reactions by numbers. Thickness of the lines depicts differential regulation of the genes encoding enzymes during drought stress: thicker, non-differentially regulated; thinner, down-regulated. Key for enzymes: *1* Ribulose-1,5-bisphosphate carboxylase/oxygenase, *2* phosphoglycerate kinase, *3* glyceraldehyde-3-phosphate dehydrogenase, *4* triose-phosphate isomerase, *5* fructose-bisphosphate aldolase, *6* fructose-1,6-bisphosphatase, *7* transketolase, *8* sedoheptulose-1,7-bisphosphatase, *9* ribose 5-phosphate isomerase, *10* ribulosephosphate 3-epimerase, *11* phosphoribulokinase. Abbreviations: *3-PGA* 3-phosphoglycerate; *Ru5P* ribulose -5 phosphate; *RuBP* ribulose -1,5-bisphosphate; *Xu5P* xylulose-5-phosphate

Table 1 Differentially expressed genes associated with amino acid and amino acid derivative metabolism in drought-stressed organs of the spike

Probe set ID		Lemma	Palea	Awn	Seed
Amino acid metabolism					
Glutamine family amino acids					
Contig21017_at	Pyrroline-5-carboxylate reductase (P5CR)	+	0	+	0
Contig5654_at	Arginase	+	+	+	0
HA18O08r_s_at	Ornithine aminotransferase	+	+	+	0
Contig5661_at	Peptidase d	+	+	+	0
Contig1648_at	Glutamine synthetase	0	0	+	0
Aspartate family amino acids					
Contig10079_at	Diaminopimelate epimerase	0	−	−	0
Contig12265_at	Dihydrodipicolinate reductase family protein	0	0	−	0
Contig10838_at	Threonine synthase	0	0	−	0
Contig15900_at	Homoserine dehydrogenase	0	0	−	0
Contig10043_at	Aspartate kinase	0	0	−	0
Serine family amino acids					
Contig909_s_at	Aminomethyltransferase, mitochondrial precursor	−	−	−	0
Contig2993_at	Glycine decarboxylase complex H subunit	−	−	−	0
Contig14767_at	Dihydrofolate reductase-thymidylate synthase	0	0	−	
Contig16415_at	Tha1 (threonine aldolase 1)/aldehyde-lyase	−	−	−	0
Contig1824_s_at	Glycine hydroxymethyltransferase	−	−	−	0
Contig10681_at	Phosphoglycerate dehydrogenase	−	−	−	0
Contig5879_at	Phosphoserine aminotransferase	0	0	−	0
HW07D08u_s_at	Cysteine synthase	−	−	−	0
Branched-chain amino acids					
Contig8247_at	Branched-chain amino-acid aminotransferase, chloroplast	+	+	+	0
Contig16535_at	3-methylcrotonoyl-coa carboxylase alpha subunit	0	+	+	0
Contig16403_at	3-hydroxyisobutyrate dehydrogenase	+	+	+	0
HY09E16u_s_at	3-hydroxyisobutyryl-coa hydrolase	0	0	+	0
Histidine family					
Contig13741_at	Histidinol dehydrogenase	0	0	−	0
Contig5640_at	Imidazoleglycerol-phosphate dehydratase	0	0	−	0
Contig9226_at	ATP phosphoribosyl transferase	−	−	−	0
Aromatic amino acids					
Contig11515_at	Shikimate kinase	−	−	−	0
Contig3173_s_at	3-deoxy-D-arabino-heptulosonate 7-phosphate synthase	0	0	−	0
Contig17591_at	Dehydroquinate dehydratase	0	0	−	0
Contig6733_at	Prephenate dehydratase	0	0	−	0
Contig20987_at	Indole synthase	0	0	−	0
Contig11179_at	Anthranilate synthase alpha subunit	−	0	0	0
Contig5883_s_at	Anthranilate phosphoribosyltransferase	−	−	0	0
Contig6407_s_at	Indole-3-glycerol phosphate synthase	0	0	−	0
Contig8636_at	Arogenate dehydrogenase isoform 2	0	−	−	0
Contig1797_at	Phenylalanine ammonia-lyase	−	−	−	0
Amino acid derivative metabolism					
Spermidine					
Contig1203_at	Spermidine synthase	+	0	+	0
HI05A17u_x_at	S-adenosylmethionine decarboxylase	+	+	0	0
Contig5654_at	Arginase	+	+	+	0
Contig7023_at	Acetylornithine aminotransferase	+	0	+	0

Table 1 (continued)

Probe set ID		Lemma	Palea	Awn	Seed
GABA shunt					
Contig1385_at	Glutamate decarboxylase	+	0	+	0
Contig4230_at	Gamma-aminobutyrate transaminase	+	0	+	0
Contig4991_s_at	Succinic semialdehyde dehydrogenase (SSADH)	+	+	+	0
Glycine betaine					
Contig7094_at	Betaine aldehyde dehydrogenase (BADH)	+	+	+	0

+ up-regulated genes with significant changes in expression ($q<0.01$ and ≥2-fold change)

− down-regulated

0 no significant change in expression

and S3). Among the drought-regulated genes, only those involved in the metabolism of Gln (Arg, Gln, Glu, and Pro) and branched-chain amino acid (Leu, Ile, and Val) families were up-regulated. In the Gln family amino acids, genes for the biosynthesis of Pro were highly expressed in drought-stressed lemma, palea, and awn. Unlike Pro biosynthesis, genes up-regulated in drought-stressed organs for branched-chain amino acid metabolism were primarily for degradation. We also found that genes for the biosynthesis of aromatic amino acids (Phe, Trp, and Tyr) were down-regulated in the lemma, palea, and awn (Tables 1 and S3), most of them in the awn.

Amino acid derivative metabolism

Among the compounds derived from amino acids, genes in the metabolism of spermidine, gamma-aminobutyric acid (GABA), glycine betaine, and phenylpropanoids were differentially regulated during drought stress in the lemma, palea, and awn (Table 1). Even though there were differences in the expression of individual genes among the stressed organs, genes for the biosynthesis of spermidine and the GABA shunt were highly expressed in the lemma, palea, and awn (Table 1). In addition, transcripts for betaine aldehyde dehydrogenase, an important enzyme in the biosynthesis of glycine betaine, were accumulated in drought-stressed lemma, palea, and awn (Table 1). The phenylpropanoid pathway channels aromatic amino acids toward the synthesis of precursors for secondary metabolism. Expression of genes for the biosynthesis of lignin and flavonoid precursors from Phe was down-regulated in the lemma, palea, and awn during drought stress (Fig. 1, Table S3).

Carbohydrate metabolism

In addition to their use as a source of energy and carbon skeleton for various metabolic processes, low molecular weight carbohydrates accumulate during drought as osmolytes. We were also interested to determine genes differentially regulated for carbohydrate metabolism. We found that fructan biosynthesis genes were down-regulated in stressed awn (Table 2). Expression of sucrose biosynthesis genes was relatively unaffected; only sucrose-phosphate synthase was up-regulated in stressed lemma and palea. For sucrose degradation, transcripts for invertases accumulated in stressed-lemma and palea, while fructokinase 2 and sucrose synthase were down-regulated in the lemma, palea, and awn (Table 2). Furthermore, genes for raffinose biosynthesis were up-regulated only in drought-stressed awn and transcripts for trehalse-6-phosphate synthase, a trehalose biosynthesis enzyme, accumulated in stressed lemma and palea (Table 2).

Investigation of genes for polysaccharide metabolism revealed that drought had little effect on expression of genes for starch metabolism in the lemma, palea, and seed. Only transcripts for the large subunit of ADP-glucose pyrophosphorylase were accumulated in stressed lemma and palea, and alpha-amylase and β-amylase were up-regulated in the seed. On the other hand, genes for the biosynthesis and degradation of starch were down-regulated in stressed awn (Table 2). Despite differences in expression of individual cell wall metabolism genes, it was apparent that genes for cell wall degradation were up-regulated while cell wall biosynthesis genes were down-regulated in stressed lemma, palea, and awn (Table 2).

Respiration

Figure 2 shows expression patterns of genes in glycolysis and the TCA cycle. Starting with glucose and glucose-1-phosphate as substrates, four of the 12 genes in glycolysis were down-regulated in the awn and three were up-regulated. Only one gene was down-regulated and five were up-regulated in the lemma. None of the glycolysis genes were down-regulated in the palea and the seed. To the contrary, expression of four genes in the palea and pyruvate kinase in the seed was up-regulated. Furthermore, drought stress did not reduce expression of genes in the

Table 2 Changes in expression of selected genes in the carbohydrate metabolism in drought-stressed organs of the barley spike

Probe set ID	Description	Lemma	Palea	Awn	Seed
Trehalose					
Contig11099_at	Trehalose-6-phosphate synthase	+	+	0	0
Fructan					
Contig13653_at	Sucrose/sucrose 1-fructosyltransferase	0	0	−	0
HA11P12u_s_at	Sucrose/fructan 6-fructosyltransferase	0	−	−	−
Raffinose					
HVSMEc0004G11r2_s_at	Galactose kinase	+	0	+	0
Contig3812_at	Galactinol synthase	0	0	+	0
Contig26196_at	Raffinose synthase	0	0	+	0
Sucrose					
Contig6787_at	Apoplastic acid invertase (fructan 6-exohydrolase)	0	+	0	0
Contig6787_at	Cell wall invertase	+	+	0	0
Contig6787_at	Fructokinase 2	−	−	−	0
Contig6787_at	Sucrose synthase	−	−	−	0
Contig19734_at	Sucrose-phosphate synthase	0	+	0	0
Starch					
Contig3390_at	ADP-glucose pyrophosphorylase large subunit	+	+	+	0
Contig10765_at	ADP-glucose pyrophospharylase small subunit	−	−	−	0
Contig10722_at	Starch synthase III	0	0	−	0
Contig11522_at	Beta-amylase	0	0	0	+
Contig14542_at	Alpha amylase	0	0	0	+
Contig6517_at	Starch phosphorylase	0	0	−	0
Contig6654_at	4-alpha-glucanotransferase	−	−	−	0
Cell wall					
Contig15116_at	Cellulose synthase	−	−	−	0
Contig20165_at	Cellulose synthase catalytic subunit	0	0	−	0
Contig1633_at	Alpha-1,4-glucan-protein synthase (UDP-forming)	−	−	−	0
Contig11769_at	Beta-glucosidase isozyme 2	0	0	+	0
Contig12692_at	Xyloglucan endotransglycosylase	+	+	+	0
Contig2670_x_at	Xyloglucan endotransglycosylase	−	−	0	0
Contig19112_at	UDP-xylosyltransferase, transferring glycosyl groups	−	−	−	0
Contig15389_at	UDP-xylosyltransferase, transferring glycosyl groups	−	−	0	0
Contig4632_s_at	Xylanase inhibitor	+	+	+	0
Contig2346_at	Glycosyl hydrolase family 1 protein	−	−	−	0
Contig13013_at	Polygalacturonase	0	+	+	0
Contig13838_at	Endo-beta-1,4-glucanase	0	0	+	0
Contig14522_at	Glucan endo-1,3-beta-glucosidase	+	+	+	0
Contig3596_at	Alpha-L-arabinofuranosidase C-terminus family	0	0	+	0
Contig4970_at	Beta-D-glucan exohydrolase isoenzyme exo1	+	+	+	0
Contig8722_at	Glycoside hydrolase family 28 protein	+	0	+	0
Contig13838_at	Endo-beta-1,4-glucanase precursor	−	−	0	0
Contig17526_at	1,4-beta-xylan endohydrolase	0	0	−	0
Contig2672_at	Xyloglucan endotransglycosylase	0	0	−	0

+ up-regulated genes with significant changes in expression ($q<0.01$ and ≥2-fold change)

− down-regulated

0 no significant change in expression

Table 3 Expression profile of genes in the mitochondrial electron transport, the light reaction of photosynthesis and pigment biosynthesis in drought-stressed spike organs

Probe set ID	Description	Lemma	Palea	Awn	Seed
Mitochondrial electron transport chain					
Contig21843_at	ATP synthase subunit 9 protein	0	0	−	0
Contig2722_at	Mitochondrial ATPase F1-gamma subunit	0	0	−	0
Contig5246_at	Cytochrome *c* oxidase subunit 6b	−	−	−	0
Contig5887_at	Alternative oxidase	+	+	+	0
Contig5888_at	Alternative oxidase	+	+	0	0
Light reaction of photosynthesis					
Contig15111_at	Oxygen-evolving enhancer protein 3	−	−	−	0
Contig1604_at	23 kDa oxygen evolving complex protein	−	0	−	0
Contig1523_at	LHCb6 protein	0	0	−	0
Contig1529_at	LHCa2 protein	0	0	−	0
Contig7863_at	LHCA5	−	−	−	0
Contig2859_at	Photosystem I subunit O	−	−	−	0
Contig6002_s_at	Photosystem II reaction center family protein	0	−	−	0
Contig7238_at	Photosystem II protein W	−	−	−	0
Contig996_s_at	Psbr, photosystem II 10 kDa protein	−	−	−	0
HA28E09r_at	Photosystem II protein K	0	0	−	0
baak26h09_x_at	Chlorophyll a/b-binding protein	−	0	−	0
Contig1888_at	Chlorophyll a/b-binding protein cp29	−	0	−	0
Contig12833_at	Cytochrome c6 (soluble cytochrome f)	0	0	−	0
Contig2080_at	Chloroplast ATP synthase gamma-subunit	−	−	−	0
Contig2142_s_at	Plastocyanin precursor	0	0	−	0
Contig3221_at	PSI reaction center PSI-N calmodulin-binding protein	−	0	−	0
Contig3657_s_at	Cytochrome *b6f* complex subunit	−	0	−	0
Contig4022_at	Thioredoxin f	0	0	−	0
Contig9479_s_at	Ferredoxin	0	0	−	0
Contig2278_at	Ferredoxin-NADP+ reductase	−	0	−	0
Pigment metabolism					
Contig11892_at	4-diphosphocytidyl-2-*c*-methyl-D-erythritol kinase	0	0	−	0
Contig9801_at	2-*c*-methyl-D-erythritol-2,4-cyclodiphosphate synthase	0	0	−	0
Contig3822_at	Hydroxymethylbutenyl 4-diphosphate synthase	0	0	−	0
Contig13305_at	Phytoene synthase	+	0	0	0
Contig14290_at	Lycopene epsilon cyclase	−	0	−	0
Contig3148_at	Glutamyl-tRNA reductase	−	0	−	0
Contig5956_at	Porphobilinogen deaminase/hydroxymethylbilane synthase	−	−	−	0
Contig5401_s_at	Coproporphyrinogen oxidase	−	0	−	0
Contig5341_at	Mg chelatase subunit of protochlorophyllide reductase	−	0	−	0
Contig2262_at	Mg-protoporphyrin IX monomethyl ester (oxidative) cyclase	0	0	−	0
Contig5998_s_at	Chlorophyll *a* oxygenase	0	0	−	0
HV_CEa0011A01r2_at	Chlorophyllase	0	0	+	0
Contig7359_at	Lethal leaf-spot 1(pheophorbide *a* oxygenase)	+	+	+	0
Contig7139_at	Tocopherol cyclase	0	+	+	0

+ up-regulated genes with significant changes in expression ($q<0.01$ and ≥2-fold change)

− down-regulated

0 no significant change in transcript abundance

Table 4 Expression profile of selected genes associated with stress response in drought-stressed organs of the barley spike at the grain-filling stage

Probe set ID	Description	Lemma	Palea	Awn	Seed
Defense response					
AF108010_at	Hv1LRR2, NB-ARC domain containing protein	0	0	+	0
AF427791_CDS-25_at	Maize protease inhibitor (mpi)	0	+	+	0
Contig12367_at	NBS-LRR disease resistance protein homologue	0	0	–	0
Contig223_s_at	Chymotrypsin inhibitor 2	+	+	0	+
Contig2787_s_at	Pathogenesis-related protein 5 (PR5, thaumatin)	0	0	+	0
Contig3059_at	Serpin	0	0	0	+
Contig3381_s_at	Maize proteinase inhibitor (mpi) gene	+	+	0	0
Contig3947_s_at	Pathogenesis-related protein 5 (PR5, thaumatin)	0	0	+	0
Contig4174_at	Class II chitinase	0	+	0	0
Contig514_s_at	Ribosome-inactivating protein II	0	+	0	0
Contig5368_at	Pathogenesis-related protein 1 (PR1)	+	+	0	0
Contig6354_s_at	NADPH oxidase	0	0	+	0
Contig6933_s_at	Amylase inhibitor-like protein	+	+	0	0
HD07M22r_s_at	Wound-induced protease inhibitor	0	0	+	0
HF19N13r_s_at	Monomeric alpha-amylase inhibitor	0	+	0	0
HT12I14u_x_at	Gamma thionin	0	0	0	+
HZ58F11r_at	Ribosome-inactivating protein II	0	+	0	0
Mla12DH_orf_3pri12_s_at	CC-NBS-LRR resistance protein mla13	0	0	0	+
Contig845_s_at	Lipid transfer protein (LTP)	+	+	0	0
Contig6042_at	Lipid transfer protein (LTP)	+	+	+	0
Contig6043_at	Lipid transfer protein (LTP)	+	+	+	0
LEA genes					
Contig1832_at	Group 1 Lea (Em) gene	0	+	0	0
Contig1868_s_at	Group 1 Lea (Em) gene	0	+	0	0
Contig1701_s_at	Dehydrin 1	+	+	+	0
Contig1709_at	Dehydrin 7	+	+	+	0
Dhn10(Morex)_s_at	Dehydrin 7	+	+	+	0
Contig1718_s_at	Dehydrin 9	0	0	+	0
Heat shock proteins					
Contig1205_s_at	Heat shock protein 82	+	+	+	0
Contig12708_at	Dnak (70 kDa heat shock) protein	+	+	+	0
Contig12854_at	Heat shock protein chloroplast GrpE protein	0	0	+	0
Contig13151_at	DnaJ family protein $p58^{ipk}$	0	0	+	0
Contig8280_at	Mitochondrial chaperonin-60	+	0	+	0
EBem05_SQ003_L06_at	Small chloroplast heat shock protein Hsp26	+	+	+	0
ROS scavenging					
Contig2730_s_at	Catalase	+	+	+	0
Contig4337_at	Peroxidase 8, class III peroxidase	+	0	0	0
Contig11509_at	Peroxidase 12, class III peroxidase	0	0	+	0
Contig8515_s_at	APX, thylakoid lumen 29 kDa protein	0	0	–	0
Contig1864_at	Peroxidase 16, class III peroxidase	–	–	0	0
Contig1868_at	Peroxidase BP, class III peroxidase	+	0	0	0
Contig1871_at	Peroxidase 52, class III peroxidase	–	–	–	0

+ up-regulated genes with significant changes in expression ($q<0.01$ and ≥2-fold change)

− down-regulated

0 no significant change in expression

TCA cycle. In fact, transcripts for α-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase accumulated in the lemma while aconitase and α-ketoglutarate dehydrogenase accumulated in the awn.

Cytochrome *c* oxidase of the mitochondrial electron transport chain was sensitive to drought stress. Expression of the cytochrome *c* oxidase gene was down-regulated in drought-stressed lemma, palea, and awn. In addition, expression of the mitochondrial ATP synthase gene was inhibited in the awn. However, genes for alternative oxidase were up-regulated in the lemma, palea, and awn in response to drought stress (Table 3).

Photosynthesis and pigment biosynthesis

Many genes in the light reaction of photosynthesis were down-regulated in drought-stressed awn compared with the lemma and palea (Table 3). In addition, drought stress reduced expression of more Calvin cycle genes in the awn than in the lemma and palea (Fig. 3). Expression of eight of the eleven Calvin cycle genes was down-regulated in stressed awn compared with six genes in the lemma and only three in the palea (Fig. 3). Drought stress also suppressed expression of genes for the biosynthesis of isoprenoids (precursors of carotenoids and the phytol tail) in the awn. For carotenoid biosynthesis, only the phytoene synthase gene (for biosynthesis of carotenoids) was up-regulated in stressed lemma. Accumulation of transcripts for the biosynthesis of the porphyrin ring was down-regulated in stressed lemma and awn (Table 3). We also found that genes for chlorophyll degradation were up-regulated in response to drought stress. Chlorophyllase and pheophorbide *a* oxygenase (lethal leaf-spot 1) were highly expressed in drought-stressed awn. Stressed lemma and palea expressed only the pheophorbide *a* oxygenase gene. Tocopherol cyclase (for biosynthesis of tocopherol) was up-regulated in the lemma, palea, and awn.

Response to stress

Several biotic and abiotic stress response genes were up-regulated by drought in the lemma, palea, and awn. The major stress response genes up-regulated were defense response, late embryogenesis abundant (LEA) proteins, heat shock proteins, and ROS-scavenging proteins (Table 4). The defense response genes include lipid transfer proteins (LTPs), pathogenesis-related (PR) proteins, proteases, protease inhibitors, and NADPH oxidase. Two groups of *LEA* genes were highly expressed during drought in the spike: two early-methionine-labeled (*Em*) genes (group1 *LEA*) in the palea and three dehydrins (group2 *LEA*) in the lemma, palea, and awn. More heat shock protein genes were up-regulated in drought-stressed awn compared with the lemma and palea. The heat shock protein genes up-regulated in the lemma, palea, and awn include HSP82, HSP70 and a small heat shock protein HSP26 (smHSP26). Among ROS-scavenging genes, catalase and class III peroxidase genes, which remove H_2O_2, were up-regulated by drought in the spike organs. A catalase gene was up-regulated in stressed lemma, palea, and awn. Also, stressed lemma and awn each expressed one class III peroxidase gene. Some class III peroxidase genes were down-regulated in the lemma, palea, and awn (Table 4).

Replication of GeneChip data by real-time PCR

Six genes with contrasting expression patterns among organs were selected to replicate the GeneChip data by quantitative real-time PCR (Table S4). The PCR data were normalized against cytosolic GAPDH, whose expression was not altered by drought. Analysis of the real-time PCR data indicated expression patterns similar to the GeneChip data and also confirmed organ-specific expression for the selected genes.

Discussion

In this study, our objective was to compare gene expression among the spike organs of barley during drought. We analyzed both up-regulated and down-regulated genes to gain insight into how expression of genes for osmolyte accumulation (metabolism of amino acids, amino acid derivatives and carbohydrates), gas exchange (photosynthesis and respiration) and stress response are affected by drought in the lemma, palea, awn, and seed.

Drought stress and transcript abundance among the spike organs

The pattern of gene expression reflects the severity of drought stress the spike organs experienced. In contrast with the photosynthetic organs, RWC of the seed (Table S2) declined only slightly during the 4 day stress. In parallel with this, very little change in gene expression was observed in the seed (Fig. S1). How did the seed maintain higher cellular water content compared with the other organs? Accumulation of osmolytes to lower osmotic potential to withdraw more water from the parent plant does not seem a possibility. If accumulation of osmolytes had occurred, expression of genes associated with the metabolism of these molecules would have increased in the seed. As shown in Tables 2 and 3, expression of genes for amino acid or carbohydrate biosynthesis did not change in the seed during drought stress.

The most plausible explanation for the maintenance of high tissue water content may have to do with the anatomy of the vascular tissue in the developing seed. The seed is

insensitive to drought stress during grain-filling because it is hydraulically separated from the maternal tissue. In maize (and possibly in the seeds of other species), before pollination, vascular strands in ovules traverse the integument, and supply water to the style. After the seed is formed, most of its water is supplied through the placental/chalazal region. This creates a hydraulic resistance that allows the seed to retain more water during drought (Ober et al. 1991; Zinselmeier et al. 2002). However, it is important to note that even though the water content of the seed is largely independent of changes in the water status of the maternal plant, the seed does not maintain high water content indefinitely. Replacement of water with starch granules gradually decreases the water content of the seed. If drought continues for an extended period, the availability of more water and translocation of carbohydrate from the maternal plant will be limited, eventually leading to premature desiccation and shortening of the grain-filling period (Westgate 1994).

Biosynthesis of cell wall, lignin, and chalcone was down-regulated by drought

Drought stress suppresses expansion growth via reduced turgor pressure (Fan et al. 2006). Maintenance of growth is mediated by enzymes that continually modify cellulose, hemicellulose, and pectin in the primary cell wall. Increased activity of the xyloglucan biosynthesis enzymes, xyloglucan endotransglycosylases (XETs), in drought-stressed young tissues is well documented (Ober and Sharp 2007). XET breaks the β-(1→4) bond of xyloglucans, allowing the primary cell wall to expand more easily (Ober and Sharp 2007). At the grain-filling stage, cells in the lemma, palea, and awn are mature, and expansion growth does not occur. Consequently, some XET and cell wall biosynthesis genes were down-regulated during drought stress (Table 2). In addition, biosynthesis of lignin and flavonoids was suppressed (Table 1 and Fig. 1). Down-regulation of genes for the biosynthesis of cell wall and secondary metabolites would allow the lemma, palea, and awn to divert carbon and other resources to processes essential for survival. This may include biosynthesis of osmolytes and carbohydrates for grain-filling.

Expression of genes for osmolyte biosynthesis

One of the most common responses to drought stress is accumulation of osmolytes (organic solutes), including non-polar amino acids, amino acid derivatives, and sugars. Although most genes for the biosynthesis of these molecules were down-regulated in drought-stressed organs of the spike, genes for Pro, glycine betaine, spermidine, GABA, raffinose, and trehalose were up-regulated, depending on the organ (Tables 1 and 2). This is consistent with previous studies that showed accumulation of these molecules in response to soil water depletion and osmotic stress (Yang et al. 2007; Danuta et al. 2008; Paul et al. 2008; Tassonia et al. 2008).

Pro is synthesized from Glu or Orn (Roosens et al. 1998; Chen et al. 2004). In the pathway from Orn, Arg is first hydrolyzed to Orn by arginase. Pyrroline-5-carboxylate synthetase (P5CS) is a rate limiting enzyme for Pro biosynthesis from Glu, and its expression increases during drought or osmotic stress (Kishor et al. 1995). The *P5CS* gene on the Barley1 Genome Array did not show altered expression in the spike in response to drought stress. However, genes for the biosynthesis of Pro from Orn (arginase and ornithine aminotransferase) were highly expressed in the lemma, palea, and awn. This suggests the pathway via Orn might be the preferred route for the biosynthesis of Pro in the barley spike during drought stress.

Pro, GABA, spermidine, glycine betaine, raffinose, and trehalose can enhance resistance of the spike organs to drought stress in various ways. First, accumulation of osmolytes lowers the osmotic potential of drought-stressed tissues, allowing them to maintain more water. Secondly, osmolytes are strong water structure formers. They can substitute for water by forming hydrogen bonds with polar residues on the surface of proteins and maintain the three-dimensional structure (Bray et al. 2000; Hoekstra et al. 2001). Furthermore, osmolytes protect macromolecules from oxidative damage during stress by scavenging ROS (Smirnoff 1998; Fait et al. 2004). It has also been suggested that spermidine and GABA may play a regulatory role in signal transduction that leads to enhanced resistance to stress (Bouche and Fromm 2004; Kasukabe et al. 2004).

Effect of drought on expression of genes for respiration

Compared with its effect on photosynthesis (described below), drought had very little influence on respiration. Still, there was variation among the spike organs in the expression pattern of genes involved in respiration. For example, four of the 12 genes in glycolysis were down-regulated and three were up-regulated in the awn. On the other hand, only one gene was down-regulated and five were up-regulated in the lemma and the palea. Only the pyruvate kinase gene was up-regulated in the seed (Fig. 2). This suggests glycolysis in the awn is relatively sensitive to drought stress compared with the other organs. However, drought stress did not reduce gene expression in the TCA cycle in any of the organs. In fact, α-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase in the lemma and aconitase and α-ketoglutarate dehydrogenase in the awn were up-regulated by drought.

Cytochrome *c* oxidase was down-regulated in the lemma, palea, and awn (Table 3). In addition, ATP synthase was

down-regulated in stressed awns. Interestingly, alternative oxidase (AOX) was up-regulated in the lemma, palea, and awn (Table 3). This finding is in agreement with previous studies that showed increased activity of the alternative pathway during drought (Giraud et al. 2008). During drought, metabolic processes are slower and the small amount of ATP produced during glycolysis and the alternative pathway may be sufficient to sustain maintenance and repair processes. More importantly, AOX reduces accumulation of ROS due to inhibition of cytochrome *c* oxidase during stress (Umbach et al. 2005; Ho et al. 2008).

Effect of drought stress on expression of photosynthesis genes

Consistent with previous studies (Castrillo et al. 2001; Seki et al. 2002; Chaves et al. 2007), expression of genes involved in photosynthesis were primarily down-regulated in drought-stressed spike organs. However, the response was not uniform. Expression of many genes for the light reaction of photosynthesis and the Calvin cycle was down-regulated in drought-stressed awn compared with the lemma, palea, and seed (Table 3 and Fig. 3). Moreover, expression of genes for the biosynthesis of isoprenoids (precursors for the biosynthesis of carotenoids and the phytol tail) and the porphyrin ring of chlorophyll was suppressed by drought in the awn and to a lesser extent in the lemma. This suggests that even though awn is the major photosynthetic organ of the spike under normal conditions (Abebe et al. 2009, in press), photosynthesis in the awn is more sensitive to drought than in the lemma and palea. Since the effect of loss of water on expression of photosynthesis genes in the lemma and palea is small, it is possible that these organs may sustain grain-filling during drought at the reproductive stage.

Inhibition of photosynthesis during drought interferes with the conversion of light energy to chemical energy (ATP) and a reducing power (NADPH). The consequence of reduced photosynthesis is oxidative injury due to over-reduction of the reaction centers by the excited chlorophyll, producing harmful ROS (Foyer and Noctor 2003). One strategy to limit oxidative damage is degradation of chlorophyll itself. Consistent with this, the awn showed partial chlorosis during drought stress, mainly on the distal half (data not shown). Furthermore, genes for chlorophyllase and pheophorbide *a* oxygenase (key enzymes in chlorophyll degradation) were up-regulated in drought-stressed awns. The lemma and palea expressed only pheophorbide *a* oxygenase (Table 3). During senescence, chlorophyllase catalyzes the initial step of chlorophyll degradation (hydrolysis of the phytol tail from chlorophyll *a*) producing chlorophyllide *a* (Hörtensteiner 2006). Pheophorbide *a* oxygenase degrades the ring structure of chlorophyll, which through a multistep pathway is broken down to non-photosensitizing colorless tetrapyrrolic compounds, known as non-fluorescent catabolites. The non-fluorescent catabolites are stored in the vacuole (Hörtensteiner 2006). Ultimately, reduction of the amount of chlorophyll molecules through inhibition of synthesis and degradation would prevent accumulation of ROS during drought stress.

Regulation of genes for stress response

Often, drought damages cells in a manner similar to that of pathogens and insects. Consequently, there is an overlap in the responses to drought and biotic stresses. Drought responses that mimic defense response are usually triggered by accumulation of ROS, which in addition to the damage they cause to macromolecules, also serve as signals to activate expression of defense-related genes. Defense-related genes up-regulated during drought in the spike organs include NADPH oxidase, ribosome inactivating proteins, LTPs, chitinases, protease inhibitors, and amylases (Table 4). Up-regulation of defense response genes in the spike organs is in agreement with other studies that showed increased expression of genes in this group in response to drought (Bass et al. 2004; Cameron et al. 2006). LTPs may deliver wax for the biosynthesis of cuticle to reduce non-stomatal water loss (Cameron et al. 2006; Yeats and Rose 2008). Apart from a simple response to cellular damage that is similar to those caused by disease and insect attack, the direct benefit of expressing protease inhibitors and pathogenesis-related protein genes in drought stressed tissues is not very clear. It is possible that plants are more susceptible to disease and insect attack when available water is limited. Expression of defense-related genes may ward off pathogens and insects during drought stress.

Two groups of *LEA* genes were up-regulated by drought stress in the spike organs. Two members of group 1 *LEA* (*Em*) were up-regulated in drought-stressed palea and three members of group 2 *LEA* (dehydrins) in the lemma, palea, and awn (Table 4). Even though LEA proteins accumulate during drought in barley (Tommasini et al. 2008), the exact mechanism by which they improve resistance to a shortage of water is not fully understood. Proposed roles of LEA proteins include retention of water, sequestration of ions, stabilization of proteins (either by sharing their hydration shell or by using their hydroxylated amino acids as a replacement for water), and as chaperons for protein folding (Bray et al. 2000; Goyal et al. 2005).

The greatest risk of drought stress is unfolding or misfolding of proteins. Plants, including barley, express heat shock protein genes during drought (Tommasini et al. 2008) to maintain proper folding of polypeptides. Heat shock proteins bind unfolded proteins, prevent intermolecular

interactions, and stabilize the three dimensional structure. Refolding certain proteins cannot be accomplished by chaperons alone. Chaperonins, such as chn60, facilitate folding of these proteins by assisting chaperones. Likewise, the heat shock protein genes HSP82, HSP70, smHSP26 and the chaperonin gene chn60 and GrpE were up-regulated in drought-stressed lemma, palea, and awn (Table 4). Despite similar RWC among lemma, palea, and awn (Table S2), heat shock protein genes were predominantly up-regulated in drought-stressed awn. This may indicate that the awn experienced more damage during drought than the lemma and palea.

Oxidative stress increases during drought (Foyer and Noctor 2003). Plants have non-enzymatic and enzymatic mechanisms to prevent the buildup of ROS. As described above, osmolytes (spermidine, GABA, glycine betaine, trehalose, and raffinose) as well as tocopherol cyclase and alternative oxidase (Tables 1 and 3) could play a major role in reducing accumulation of ROS in drought-stressed spike organs. For enzymatic removal of H_2O_2, a catalase gene was up-regulated in the lemma, palea, and awn during drought stress. Some members of class III peroxidase genes were up-regulated in the stressed lemma and awn while others were down-regulated in the lemma, palea, and awn (Table 4). Class III peroxidases play a role in cross-linking cell wall components and lignification in response to accumulation of ROS during defense response (Almagro et al. 2009).

In conclusion, although the awn had the largest number of differentially regulated genes, many biological processes were negatively affected in the awn compared with the lemma, palea, and seed. Expression of several genes in the awn might be a response to severe cellular damage and a need for repair. Thus, the lemma and palea may be the major sources of carbon for grain-filling in barley during drought.

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