Plant Physiology and Biochemistry 132 (2018) 475–489

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Transcriptomic view of survival during early seedling growth of the extremophyte *Haloxylon ammodendron*

Ligang Fan[a,1], Guannan Wang[b,1], Wei Hu[a], Pramod Pantha[b], Kieu-Nga Tran[b], Hua Zhang[a], Lizhe An[a], Maheshi Dassanayake[b,**], Quan-Sheng Qiu[a,*]

[a] MOE Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, 222 South Tianshui Road, Lanzhou, Gansu, 730000, China
[b] Department of Biological Sciences, Louisiana State University, 202 Life Sciences Building, Baton Rouge, LA, 70803, USA

ARTICLE INFO

Keywords:
Abiotic stress
Desert
Drought
Extremophyte
Psammophyte
Stress tolerance
Transcriptome

ABSTRACT

Seedling establishment in an extreme environment requires an integrated genomic and physiological response to survive multiple abiotic stresses. The extremophyte, *Haloxylon ammodendron* is a pioneer species capable of colonizing temperate desert sand dunes. We investigated the induced and basal transcriptomes in *H. ammodendron* under water-deficit stress during early seedling establishment. We find that not only drought-responsive genes, but multiple genes in pathways associated with salt, osmotic, cold, UV, and high-light stresses were induced, suggesting an altered regulatory stress response system. Additionally, *H. ammodendron* exhibited enhanced biotic stress tolerance by down-regulation of genes that were generally up-regulated during pathogen entry in susceptible plants. By comparing the *H. ammodendron* basal transcriptome to six closely related transcriptomes in Amaranthaceae, we detected enriched basal level transcripts in *H. ammodendron* that shows preadaptation to abiotic stress and pathogens. We found transcripts that were generally maintained at low levels and some induced only under abiotic stress in the stress-sensitive model, *Arabidopsis thaliana* to be highly expressed under basal conditions in the Amaranthaceae transcriptomes including *H. ammodendron*. *H. ammodendron* shows coordinated expression of genes that regulate stress tolerance and seedling development resource allocation to support survival against multiple stresses in a sand dune dominated temperate desert environment.

1. Introduction

How plants establish and survive in extreme environments is a question best explored using extremophile plants (extremophytes) naturally adapted to such environments than stress-sensitive model plants. The need to understand how plants survive in desert environments is imperative at a time desertification is increasingly threatening human lifestyles (IPCC, 2014; FSIN, 2017). Psammophytes are extremophytes able to colonize sand dunes and present a genetic repository for understanding naturally selected mechanisms for plant survival in deserts. They play a vital ecosystem service by preventing and reversing the process of desertification (Li et al., 2011; Liu et al., 2016). Therefore, understanding the genetic mechanisms underlying plant survival during water-deficit stress in extremophytes will have far-reaching outcomes from improving crops adapted to drought stress to developing effective land management practices that would limit the expansion of deserts (Lai, 1985; Li et al., 2014).

Haloxylon ammodendron (black saxaul), is a pioneer tree psammophyte widely distributed in temperate Afro-Asian deserts (Sheng et al., 2005; Song et al., 2005, 2006; Zheng and Wang, 2015). It acts as windbreakers, arrest sand movement, maintain microclimates, and facilitate growth of other plants (NRC, 1980; Aronson, 1985). *H. ammodendron* serves a critical role in maintaining the structure and function in its native ecosystem (Tang and Gavin, 2010; Shamsutdinov et al., 2016). *H. ammodendron* seedlings are known to tolerate higher drought conditions compared to xerophytes found in comparable environments (Tobe et al., 2000a; Song et al., 2005; Xue et al., 2012). However, *H. ammodendron* presents an underexplored genetic resource in our efforts to understand genetic mechanisms governing complex abiotic stress responses.

Exploring how *H. ammodendron* is adapted to drought stress in its early development can provide insight into biological processes prioritized by plants naturally selected to tolerate drought. In this study, we present a curated reference transcriptome for *H. ammodendron* early

* Corresponding author.
** Corresponding author.
E-mail addresses: maheshid@lsu.edu (M. Dassanayake), qiuqsh@lzu.edu.cn (Q.-S. Qiu).
[1] These authors contributed equally and should be considered joint first authors.

https://doi.org/10.1016/j.plaphy.2018.09.024
Received 2 August 2018; Received in revised form 8 September 2018; Accepted 18 September 2018
Available online 28 September 2018

development, quantified transcript responses to water-deficit stress with physiological data that support the inferences made at the transcriptome-level, and the significance of the global transcriptomic response to its survival in a temperate desert. We compared the *H. ammodendron* basal transcriptome to six other closely related transcriptomes in Amaranthaceae, to identify biological processes that may exemplify shared responses in successful adaptations to abiotic stresses and provide a set of transcripts highly enriched in the extremophytes that have orthologs uncharacterized and underrepresented in model plants.

2. Materials and methods

2.1. Plant growth

H. ammodendron seeds collected from Minqin, China, were surface-sterilized, stratified at 4 °C, and germinated for 3 days at 22 °C with a 16 h-light/8 h-dark photoperiod. Seedlings were transferred to 0.5 × Hoagland's solution for control samples and to 5% PEG-6000 added to 0.5 × Hoagland's solution for drought-induced samples. Seedling growth was assessed for all samples collected on day 0, 1, 3, 5 and 7. The seven-day-old seedlings were used for RNA-seq. Three independent biological replicates were performed for the RNA-seq assay.

2.2. Physiological measurements

Root length, hypocotyl length and relative water content (RWC) were measured every two days until seven days. RWC was determined as $(m_a\text{-}m_d)/m_a$, where m_a is seedling fresh weight, and m_d is the dry weight for the same sample. The anthocyanin, and chlorophyll content in shoots and the H_2O_2 content in whole seedlings were measured as described by Rabino and Mancinelli (1986), Porra (2002), and Brennan and Frenkel (1977), respectively. Six independent biological replicates were used in the assay.

2.3. Sequencing, assembly, and annotation of the reference transcriptome

Total RNA was extracted using the Plant RNA Extraction Kit (TaKaRa) from 100 mg seedlings (entire seedlings including roots and hypocotyls) for three biological replicates were processed into sequencing libraries using NEB Next® Ultra™ RNA Library PrepKit, followed by paired-end sequencing on Illumina HiSeq2500 (sequencing conducted by Novogene, China). 56–59 million 125nt long reads were obtained for control and 5% PEG-treated samples, and were deposited in the NCBI-SRA database with the following accessions: SRX1594867, SRX1594866, SRX1594865, SRX1594864, SRX1594863, SRX1594851.

RNA-seq reads following quality checks were assembled using Trinity v2.2.0 (Grabherr et al., 2011) with modified settings for –min_kmer_cov 5 and –group_pairs_distance 300. After removing contaminants, artefacts, contigs with low read support, and redundancy from each control and drought-treated assembly using a custom pipeline (Oh et al., 2015), the two assemblies were merged to get a reference transcriptome. All assembled contigs in the merged assembly showing > 95% sequence identity over > 70% of the total length were clustered using CD-HIT-ESTv4.6 (Li and Godzik, 2006). We selected a representative transcript containing the longest continuous open reading frame (ORF), when present predicted by TransDecoderv2.0.1 (https://transdecoder.github.io/) for each cluster to generate a non-redundant transcriptome.

Annotation was based on a series of BLAST searches using Araport11 (Cheng et al., 2017), NCBI-plant-refseq-rna, and the NCBI-nr databases, with 10^{-5} as the e-value cut-off. Non-plant hits were removed using NCBI non-plant-refseqRNA. The transcriptome was subdivided into coding (with ORFs) and non-coding transcriptomes. Assembly completeness of the coding transcriptome was assessed using CEGMA and BUSCO databases (Parra et al., 2007; Simão et al., 2015).

2.4. RNA-seq analysis

RNA-seq reads from each sample was separately aligned to the coding and non-coding transcriptomes using Bowtie (Langmead et al., 2009). Transcripts with uniquely mapped reads between control and drought-induced samples differently at a p-value < 0.01 were annotated as significantly differently expressed transcripts (DETs) using NOISeq (Tarazona et al., 2015). We also calculated reads mapped per kilobase per million reads (RPKM) for each transcript. Gene ontology (GO) terms enriched in DETs were detected using a custom background (Oh et al., 2015) via BINGO (Maere et al., 2005) and visualized using Cytoscape v3.4.0 (Shannon et al., 2003). qRT-PCR was conducted for 11 randomly selected DETs to validate RNA-seq results, using 2 × SYBR Premix Ex Taq™ II (TaKaRa) on a BIO-RAD CFX96 Real-time Detection System. The primers used are given in Table S1. The expression of actin was used as an internal control to normalize the expression of selected DETs. Three independent biological replicates were used in the assay.

2.5. Comparison of Amaranthaceae transcriptomes

Reference transcriptomes/genomes and RNA-seq data for *Beta vulgaris*, *Amaranthus hypochondriacus*, *Salicornia europeae*¸ *Spinachia oleraceae* and *Chenopodium quinoa* were obtained for the comparison with *H. ammodendron* (Table S11). The qualitative assessments for these transcriptomes/genomes, annotation, and RNA-seq alignments were performed as described earlier for *H. ammodendron*. Homologous clusters were identified using OrthoMCL (Li et al., 2003). Principal component analysis (PCA) for these species was performed based on RPKM values using prcomp (https://stat.ethz.ch/R-manual/R-devel/library/stats/html/prcomp.html) in R and visualized by ggfortify (https://github.com/sinhrks/ggfortify). For the clustering of similarity in abundance of expressed genes in different species, sum RPKM was used to represent the total gene function for transcripts that shared the same *Arabidopsis* homolog in a given species. Transcripts without an *Arabidopsis* homolog was removed before hierarchical clustering using hclust (https://stat.ethz.ch/R-manual/R-devel/library/stats/html/hclust.html) in R. Each remaining gene model was ranked based on transcript RPKM and divided into bins of 20 percentile values with a 5% interval. Missing data for certain gene models in a given species was assigned a zero rank. The one-to-one ortholog comparison excluding *C. quinoa*, was performed using prcomp in R.

3. Results

3.1. Early seedling growth of H. ammodendron in response to water-deficit stress

Our aim is to detect drought response in the transcriptome at a critical life history stage of survival, as early as one-week-old seedlings. Therefore, we examined the effect of drought stress on *H. ammodendron* seedlings subjected to 5% PEG-6000 treated for 7 days after germination. PEG-6000 has been successfully used to study plant growth in response to water-deficit stress under laboratory conditions specially in seedlings (Chazen et al., 1995; Siefritz et al., 2002; Caruso et al., 2008; Yang et al., 2011; Feller et al., 2015; Han et al., 2017; Meunier et al., 2017). The drought-induced seedlings had reduced growth compared to the control (Fig. 1). The difference in root growth was more apparent than in the hypocotyl and the lateral root emergence was delayed in the drought-induced seedlings. Additionally, RWC was significantly less in the drought-induced seedlings compared to the control (Fig. 1D). These results confirmed that the growth of *H. ammodendron* seedlings was significantly affected by the induced drought treatment.

We selected 5% PEG for subsequent analysis, based on a growth response comparison between 2.5 and 10% PEG treatments given for a week to seedlings (Fig. S1). Stress induced by 2.5% PEG had significant growth effects on the shoots, but was not statistically significant for

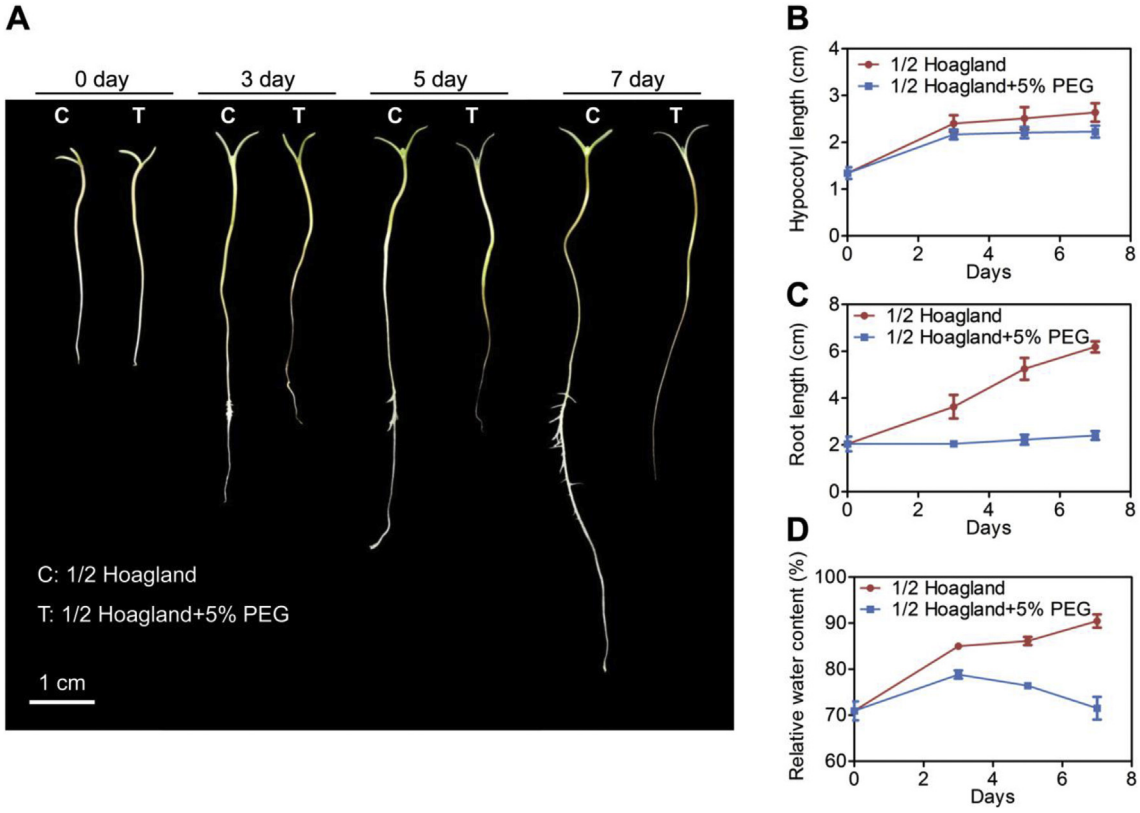

Fig. 1. Growth response of *H. ammodendron* seedlings in control and PEG treated samples. (A) plant growth; (B) hypocotyl length; (C) Root length; and (D) relative water content at 0, 3, 5, and 7 days after germination. Six independent biological replicates were used in the assay.

roots (Fig. S1). A more severe effect induced by 10% PEG, had visibly dried out roots that led to higher seedling mortality in subsequent days. Therefore, selecting a 5% PEG treatment was optimal to investigate the transcriptomic response to drought without overwhelming signals of cell death.

3.2. De novo assembly and annotation of the H. ammodendron seedling transcriptome

The assembled transcriptome was curated to yield coding and non-coding reference transcriptomes. The protein coding transcriptome of 36,099 transcripts with a mean length of 1,388nt was used as a reference for the downstream comparative transcriptome analyses. Fifty percent of the transcriptome was composed of transcripts longer than 1889nt and included 15,894 complete transcript models (i.e. ORFs with start and end) (Table 1; Fig. S2).

We used previously published RNA-seq datasets to examine whether additional RNA-seq sampling from slightly different developmental stages could further improve our current assembly. Long et al., had generated RNA-seq data from soil grown four-week-old seedlings drought stressed for a week and compared to control samples with two replicates for each condition (Long et al., 2014). Li et al., generated RNA-seq from 10-day-old seedling-leaves and 2-day-old seedling-cotyledons without replicates nor a reference assembly (Li et al., 2015) (Table S3). Reads from our current study and previously published datasets mapped at a higher percentage to our *de novo* assembly than to the assembly published by Long et al. (Table S3), and the previous work did not add novel contigs. This implied that we had sampled the *H. ammodendron* seedling transcriptome to a depth for which previously generated reads could not add novel transcripts to our reference transcriptome. The reference transcriptome created for the current study is available at NCBI Bioproject PRJNA312463.

We annotated 90% of our reference transcriptome using Araport11 (58%), NCBI plant-RefSeq (26%), or NCBI-nr (6%) databases (Fig. S3). The completeness of our reference assembly is illustrated by the presence of 97.6% of core eukaryotic genes from CEGMA and 96.5% from BUSCO (Fig. 2).

A total of 78,705 putative non-coding transcripts were identified in our assembly with a mean length of 439 nt (Table S2; Fig. S3). Many of these assembled sequences may represent true non-coding RNA. For example, 42% of these could be matched with non-coding RNAs in other plants. However, in the absence of additional functional information, we have separated all non-coding sequences from coding sequences when assessing the significance of differential expression of protein-coding transcripts, due to the possibility of some of them being non-coding regions of mRNA that were not assembled together with the coding regions of the respective transcripts.

Table 1
Summary statistics of the *H. ammodendron* reference protein coding *de novo* transcriptome.

Putative protein coding transcripts	36,099
Total size of contigs (Mb)	50.1
Shortest contig (nt)	297
Longest contig (nt)	16,746
Number of contigs > 1 K	19,893
Mean contig size (nt)	1388
Median contig size (nt)	1130
N50 contig length (nt)	1889
Mean ORF length (nt)	933
Median ORF length (nt)	660
Minimum ORF length (nt)	300
Maximum ORF length (nt)	16,014

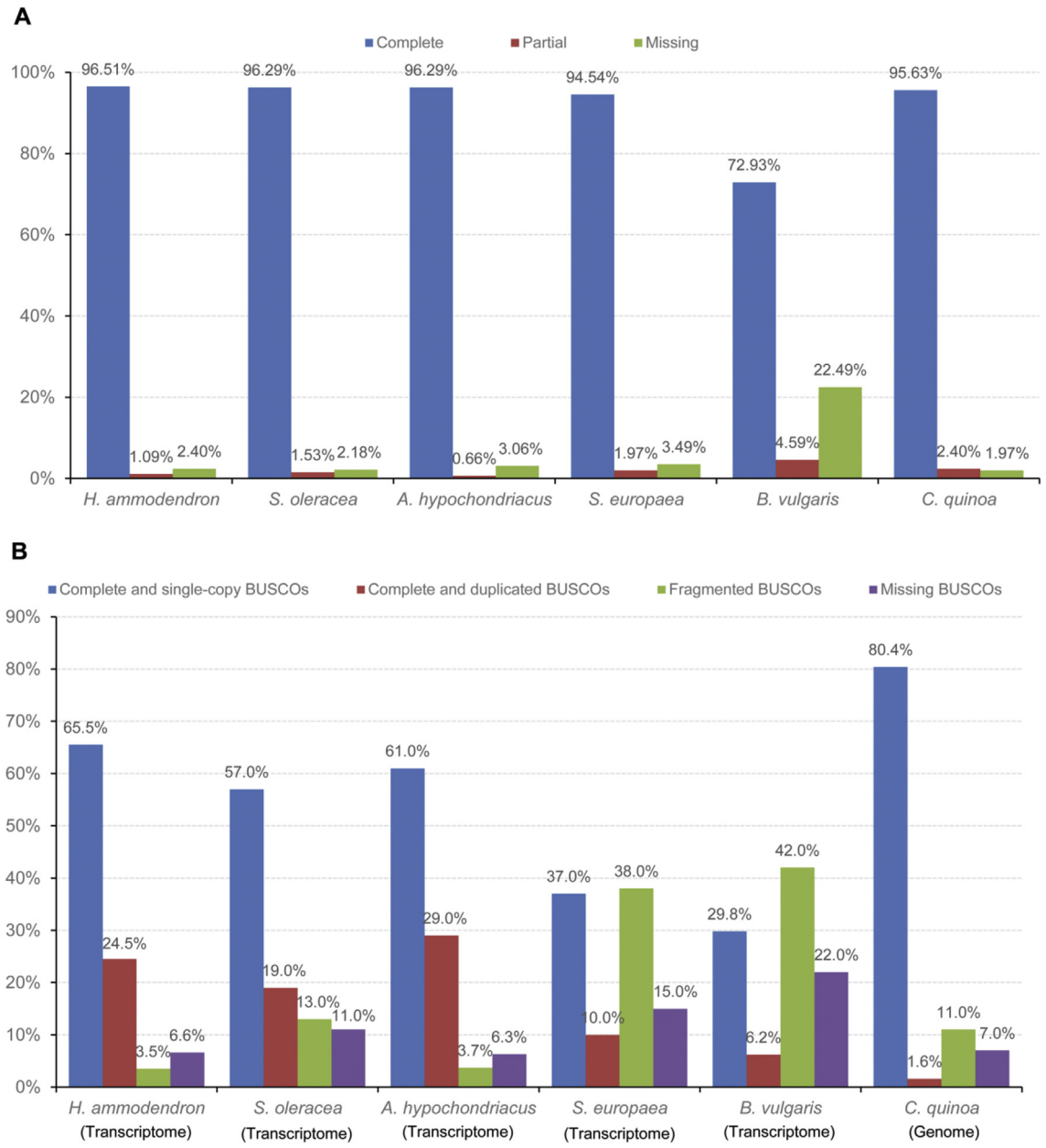

Fig. 2. Assessment of completeness of the transcriptome of *H. ammodendron* compared to the transcriptomes and genomes from Amaranthaceae using the Core Eukaryotic Genes Mapping Approach (CEGMA) (A) and Benchmarking Universal Single-Copy Orthologs (BUSCO) (B).

3.3. Global transcriptional response to water-deficit stress in early H. ammodendron seedlings

The drought-responsive transcriptome compared to the control was spatially well separated in a PCA (Fig. S4). We identified 319 up-regulated and 198 down-regulated significantly differently expressed transcripts (DETs) (Table S4; Fig. S5A), of which 19 were unknown transcripts without any matches in public databases. Additionally, we found 151 DETs among the putative non-coding transcripts (Table S5; Fig. S5B). Randomly selected eleven DETs associated with diverse functions confirmed the expression patterns established by RNA-seq when tested independently with qRT-PCR (Fig. S6; Table S1).

Fig. 3 illustrates the enriched biological processes prioritized by *H. ammodendron* early seedlings in response to water-deficit stress. Among the coding DETs, 73% could be associated with a GO term (Tables S6 and S7). The *H. ammodendron* transcriptome gives a strong signal in response to abiotic stresses, stress signaling via reactive oxygen species (ROS), and mitigating ROS damage resulting from abiotic stresses by increasing molecular chaperone activity, flavonoid, and glutathione metabolism (Fig. 3A). Genes associated in response to water deprivation, cold, heat, UV, osmotic, and heavy metal stresses were upregulated synergistically, although, drought was the only applied stress in our experiment. Contrasting to the up-regulated functions, the *H. ammodendron* transcriptome shows down-regulation in energy metabolism to match the reduced growth rate as well as other functions not directly associated with abiotic stress tolerance. These down-regulated genes were associated with photosynthesis, hormone-mediated pathogen responses, and nutrient uptake (Fig. 3B).

GO functional enrichment gives us a bird's eye view of enriched functions but fail to highlight genes that are DETs that do not conform to an enriched GO function despite their significant roles in stress response mechanisms. Therefore, we developed an overview with DETs functionally annotated and are likely to play major roles in transforming the cellular environment from a stress neutral condition to a

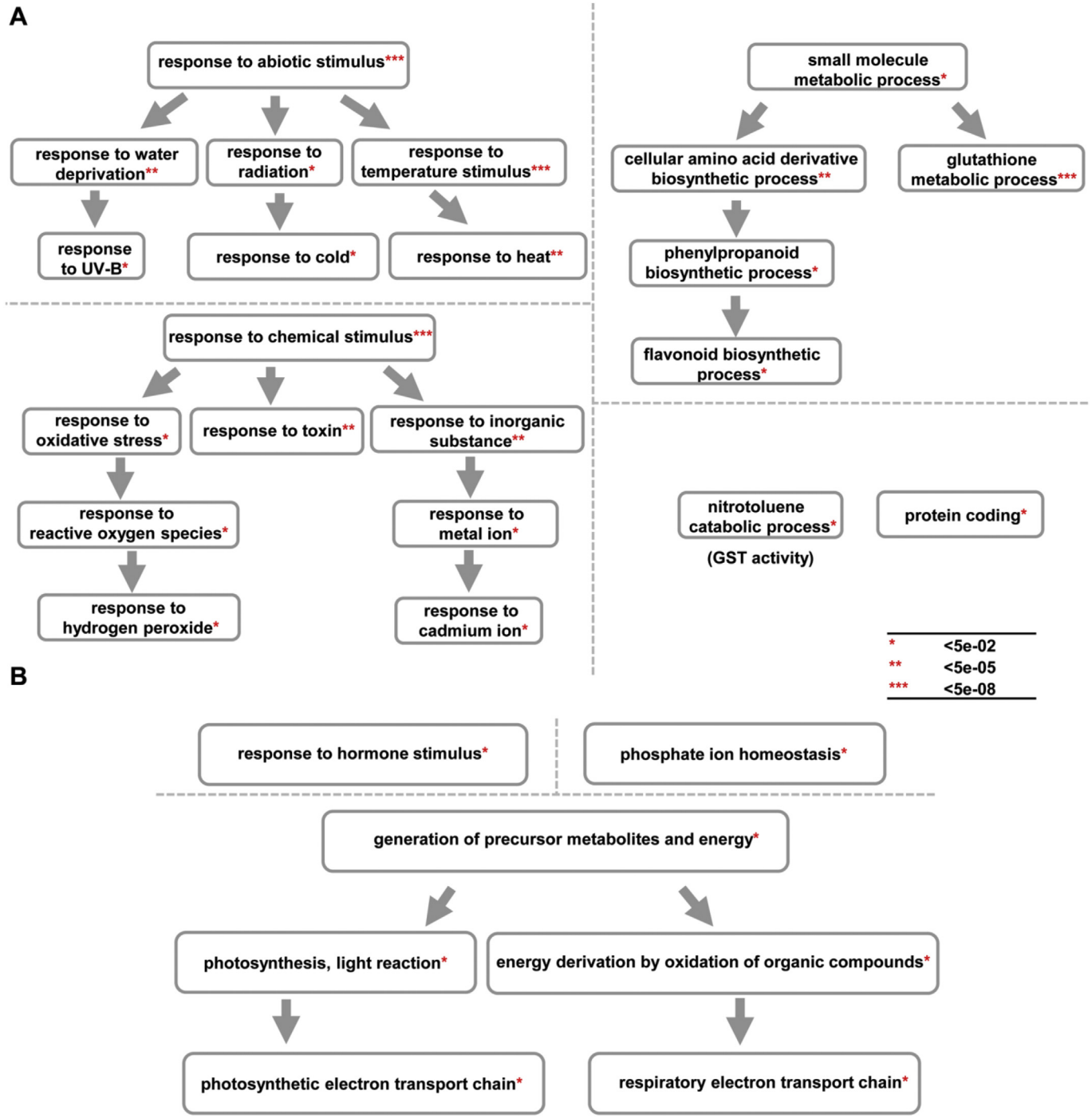

Fig. 3. Directed acyclic graphs representing gene ontology (GO) terms enriched among genes up-regulated (A) and down-regulated (B) in response to 5% PEG in H. ammodendron early seedlings.

stress responsive condition better adapted to abiotic stresses in *H. ammodendron* early seedlings (Fig. 4; Table S8). This allows us to see the overall responses of DETs in pathways that respond to stress synergistically in the early seedlings.

3.4. Physiological response in H. ammodendron seedlings to water-deficit stress corroborate transcriptomic signals

Anthocyanins and hydrogen peroxide accumulation are among the hallmarks of plant stress (Neill et al., 2002; Winkel-Shirley, 2002). We measured the total anthocyanin and H_2O_2 levels in the *H. ammodendron* seedlings. Both metabolites increased significantly in the drought-induced samples throughout the test period compared to the levels observed in the control (Fig. 5). Mirroring the upregulated functions in the transcriptome profile, *H. ammodendron* seedlings accumulate H_2O_2 and anthocyanins in drought-stressed seedlings more than the control (Figs. 4 and 5). The significantly reduced chlorophyll content in stressed seedlings (Fig. 5D) was in line with the down-regulation of chlorophyll biosynthesis genes (Fig. 4), reduced photosynthesis (Fig. 3B), and reduced growth (Fig. 1).

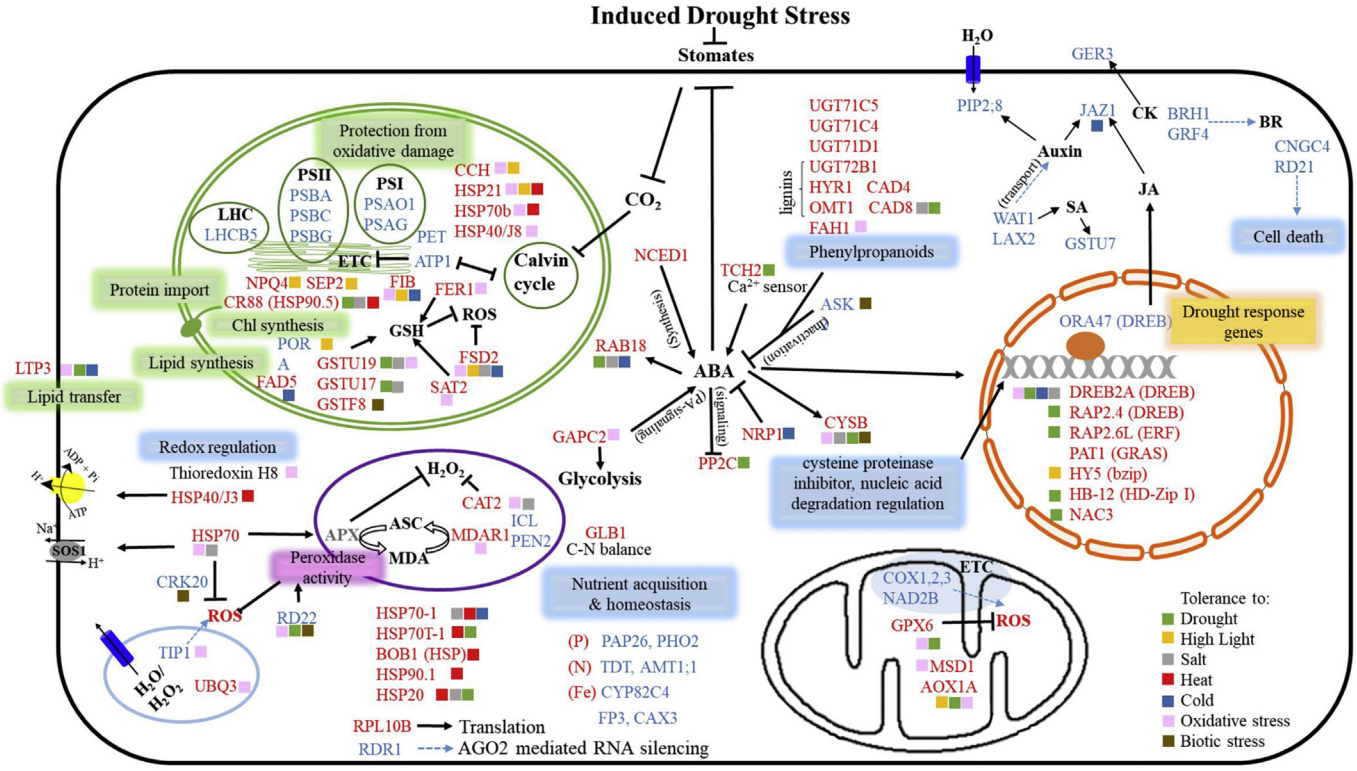

Fig. 4. Overview of differently expressed protein coding genes synergistically involved in cellular processes under drought stress in early *H. ammodendron* seedlings. Genes denoted in red text (up-regulated responses) and blue text (down-regulated responses) are localized to the cellular components and processes based on supportive literature given in Supplementary Table 5. Solid arrows indicate positive regulation, blocked arrows indicate negative regulation, and dashed arrows represent indirect associations. The summarized sketch represent each gene product localized to only one location even though, some proteins are localized to multiple cellular components. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.5. Coordinated induction of abiotic stress response pathways in early seedlings

Transcription factors regulating abiotic stress. An array of transcription factors associated with drought, salt, cold, heat, high-light, and oxidative stress (Fig. 4; Table S8) were concurrently upregulated in *H. ammodendron* drought-stressed samples. Specially, notable is the induction of several *DREB* (Dehydration Responsive Element Binding) family transcription factors (Yamaguchi-Shinozaki, 1994; Lata and Prasad, 2011). *DREB2A*, a positive marker for drought tolerance (Liu, 1998; Sakuma et al., 2006), was up-regulated in *H. ammodendron* drought-stressed seedlings (Fig. 4).

Stress signaling and antioxidant defense. Several groups of up-regulated co-expressed genes are noticeable in their putative role in increasing the antioxidant capacity of the *H. ammodendron* drought-stressed seedlings. (a) Transcripts involved in H_2O_2 metabolism were induced (Figs. 3A and 4; Tables S6 and S8). H_2O_2 is an effective stress priming metabolite (Savvides et al., 2016) that prepares tissues for severe abiotic stress (Mittler, 2002), and confer drought and salt stress tolerance in seedlings (Wahid et al., 2007; He et al., 2009). As predicted by the transcriptomic profile, the H_2O_2 content increased in drought-induced seedlings compared to the control (Fig. 5B). (b) *Glutathione S-transferase* (*GST*) transcripts were up-regulated (Fig. 4). GSTs are transcriptionally induced under drought and salt stress to reduce the level of hydroperoxides that damage DNA and lipids, and participate in stress signaling and flavonoid biosynthesis (Dixon et al., 2002; Thatcher et al., 2007; Chen et al., 2012; Xu et al., 2015). (c) Transcripts coding for phenylpropanoid biosynthesis (including flavonoids) were up-regulated in the drought-stressed *H. ammodendron* seedlings. Among them is the transcription factor HY5 (Brown and Jenkins, 2008) known to induce anthocyanin levels (Figs. 3A and 4). In line with the transcriptomic profile, the drought-induced seedlings showed significantly higher levels of anthocyanins compared to the control (Fig. 5). (d) Other ROS dissipating systems were also induced in the drought-stressed seedlings as seen with transcripts encoding for mitochondrial manganese superoxide dismutase (Morgan et al., 2008), chloroplast Fe superoxide dismutase (Attia et al., 2008), monodehydroascorbate reductase in ascorbate recycling (Urzica et al., 2012), catalase (Savouré et al., 2002), peroxidase (Passaia et al., 2014), alternative oxidase (Giraud et al., 2008), and SAT1 which leads to increased levels of thiol metabolites that buildup the cellular reduction potential (Park et al., 2013) (Fig. 4).

Heat shock proteins and molecular chaperones. In concert with the increased antioxidant defenses, a suite of transcripts coding for cytoplasmic and chloroplast localized heat shock proteins known to facilitate basic metabolic functioning during oxidative stress were up-regulated in the *H. ammodendron* drought-stressed seedlings (Figs. 3A and 4). Among these were, *HSP70*, *HSP90,* and *HSP20* family members that serve as molecular chaperones (Sung et al., 2001; Cazalé et al., 2009; Samakovli et al., 2014), HSP40/J3 which regulates plasma membrane H^+-ATPase under oxidative stress (Yang et al., 2010), *BOB1* involved in protein folding required during seedling development and also increase thermal stress tolerance (Perez et al., 2009).

Several chloroplast heat shock proteins upregulated in the *H. ammodendron* drought-stressed seedlings are known for their functions in minimizing damage to chloroplasts during abiotic stress (Fig. 4). These include, *HSP21* required for Chloroplast transcription and development under heat stress (Zhong et al., 2013), *CR88* required for chloroplast protein import (Oh et al., 2014b; Feng et al., 2014), and *HSP40/J8* required for stabilization of PSII under oxidative stress (Chen et al., 2010). Additionally, transcripts coding for proteins like the Cu chaperone CCH which protects the PSII system from oxidative stress (Shin et al., 2012), *FIB* involved in protection from photoinhibition (Youssef et al., 2010), NPQ4 required for non-photochemical quenching (NPQ)

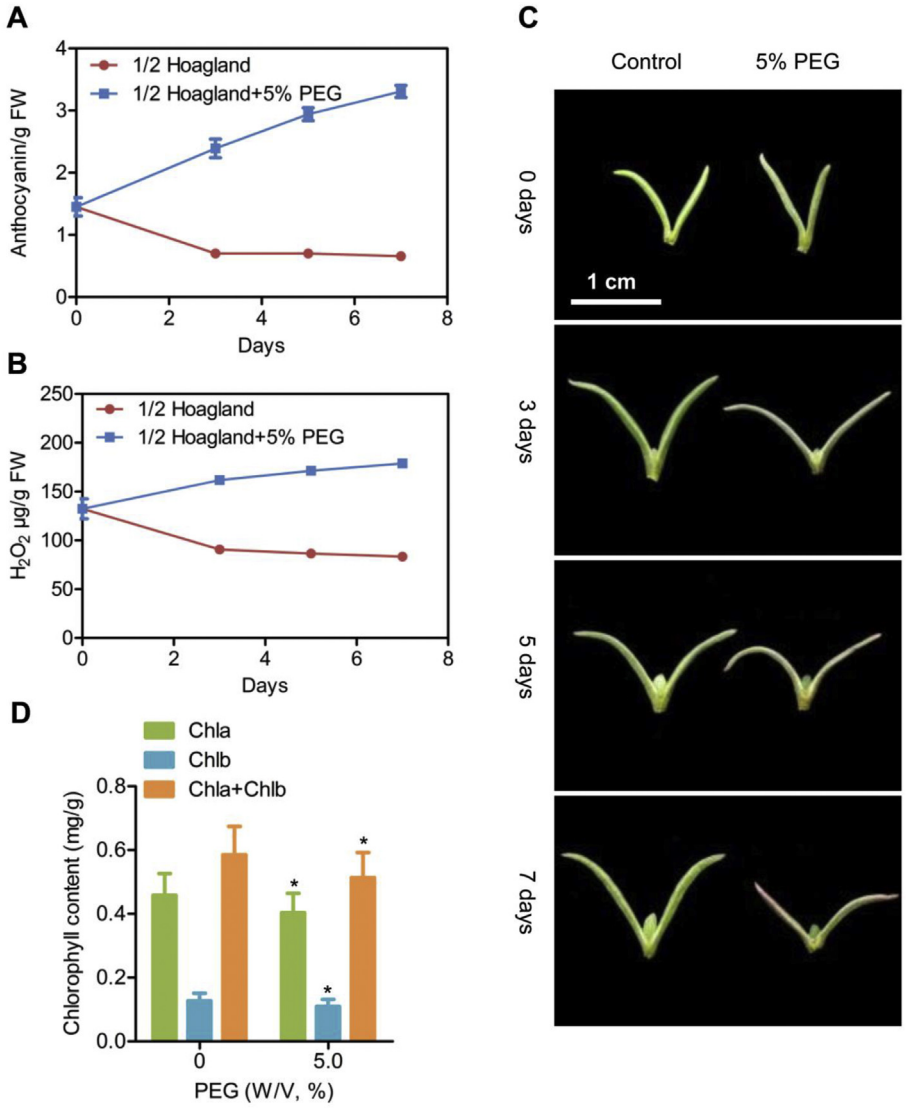

Fig. 5. Selected metabolites differently regulated under drought stress. Stress response markers, anthocyanins (A) and H_2O_2 (B) increase in water stressed seedlings of *H. ammodendron* compared to control samples at 0, 3, 5, and 7 days after treatment. Anthocyanin accumulation is visible at 7 days after treatment (C). Total chlorophyll content is significantly decreased in PEG treated one week old seedlings compared to the control seedlings (D), * indicates a p-value in *t*-test < 0.05. Six independent biological replicates were used in the assay.

to protect PSII from excess excitation energy (Frenkel et al., 2009; Kereïche et al., 2010), and SEP2 (Heddad and Adamska, 2000) that interacts with thylakoid membranes showed up-regulated transcripts in the *H. ammodendron* drought-stressed seedlings.

Transcripts associated with maintaining optimal primary metabolism. The drought-stressed seedlings showed induced transcripts that are involved in sustaining growth during abiotic stress (Fig. 4; Table S8). These include, *GLB1* known to regulate the C-N balance (Chellamuthu et al., 2016), *GAPC2* (Guo et al., 2014), CYSB, a cysteine proteinase inhibitor induced under multiple stresses (Zhang et al., 2008), and *LTP3* (Guo et al., 2013) and *FAD5* (Heilmann et al., 2004) involved in lipid biosynthesis and transport.

Transcripts associated with maintaining optimal seedling development. Lastly, several transcripts with known functions related to seedling development minimizing adverse effects of irreversible growth defects under abiotic stress were upregulated in the drought-stressed samples (Fig. 4; Table S8). These include *HYR1* that prevents etiolation induced cell expansion (Zhao et al., 2007); *FER1* (Ravet et al., 2012; Reyt et al., 2015) and *TH8* (Schnaubelt et al., 2015) that regulate lateral root density resulting in higher plant fitness.

3.6. Coordinated down-regulation of abiotic stress response pathways in early seedlings

Photosynthesis and associated processes. The immediate drought response for all terrestrial plants is to close stomates upon water stress resulting in limited intracellular CO_2 availability followed by reduced photosynthesis although the reduction in the rate of photosynthesis can vary between species (Chaves et al., 2009). Consistent with the expected trend, we observed significant down-regulation of multiple *H. ammodendron* transcripts associated with PSI, PSII, light harvesting system, and chlorophyll biosynthesis (Figs. 3B, 4 and 5D). The down-regulation of transcripts in the photosynthetic electron transport chain (ETC) was in accord with the down-regulation of the respiratory ETC (Figs. 3B and 4). Transcripts coding for Cox1, Cox2 and Cox3, the catalytic subunits of Cytochrome *c* oxidase (Soto et al., 2012) were significantly down-regulated confirming the previous findings, that limiting the activity of cytochrome *c* oxidase would allow more alternative oxidase activity leading to reduced ROS generation by preventing over reduction of the ubiquinone pool (Millar et al., 2011) (Fig. 4). Down regulated *RD22* serves a similar function by allowing

upregulation of peroxidase genes, reduced chlorophyll content, and inducing transcription of defense responses to make the plant more primed for both drought and pathogen stress (Harshavardhan et al., 2014). Transcripts involved in multiple nutrient acquisition pathways were also down-regulated in line with the expectation that the incorporation of nutrients without toxic buildup cannot proceed in the stressed seedlings at the same rate as in the control upon stress induced reduction of photosynthates and ATP (Figs. 3B and 4).

Response to pathogen defense. We observed a reciprocal down-regulation in multiple biotic defense pathways parallel to the upregulation of abiotic stress responses in *H. ammodendron* drought-stressed seedlings (Fig. 4). Two main strategies were apparent from the overall transcriptome response in achieving optimum stress management related to pathogen stress. One set of down-regulated transcripts is required at elevated levels for successful pathogen invasion. Therefore, down-regulation of such transcripts would provide direct protection from pathogen attacks during a vulnerable developmental stage already experiencing abiotic stress. Examples in *H. ammodendron* seedlings include *CRK20* (Ederli et al., 2011), *RDR1* (Cao et al., 2014), and *ASK2* (Schrammeijer et al., 2001; Anand et al., 2012). The other set of down-regulated transcripts associated with pathogen responses represent proteins in resisting pathogens during pathogenesis. Since the primary stress condition experienced by the seedlings in our experimental conditions was abiotic stress, the allocation of resources for biotic stress responses will be limited as a tradeoff necessitated by the current condition. We saw significant down-regulation for *RD21* (Shindo et al., 2012), *CNGC4* (Chin et al., 2013), *ORA47* (Kerchev et al., 2011), *WAT1* (Denancé et al., 2013), *PEN2* (Lipka et al., 2005), *BRH1* (Molnar et al., 2002), *GER3* (Membre et al., 2000), and *GSTU7* (Liao et al., 2014).

3.7. Lineage specific H. ammodendron stress responsive and primed transcripts

Most transcripts that were consistently drought responsive in early and late seedlings (Table 2; Fig. 6B) are of unknown function without any detectable homolog in *Arabidopsis*. These likely represent lineage specific genes in *H. ammodendron* or Amaranthaceae species.

Previous transcriptomic studies on extremophytes have illustrated that the basal transcriptome carries signatures of stress adaptations than the induced transcriptome (Dassanayake et al., 2011; Oh et al., 2014a; Zou et al., 2017). Many stress responsive genes are constitutively highly expressed in the extremophytes (Talke et al., 2006; Gao et al., 2013; Oh et al., 2014a; Velasco et al., 2016; Krämer, 2018). Thus, an induced-drought stress may not change the abundance of transcripts that were highly expressed at the basal level and were primed to respond to common stresses naturally experienced by *H. ammodendron*. To detect transcripts of interests in *H. ammodendron* that may be critical for its survival in an environment defined by multiple abiotic stresses, we compared its basal transcriptome to six other Amaranthaceae basal transcriptomes which included *Beta vulgaris*, *Amaranthus hypochondriacus*, *Salicornia europaea*¸ *Spinacia oleraceae, and Spinacia turkestanica* (Fig. 7; Table 3 and Table S11). Amaranthaceae species are adapted to higher abiotic stress levels than most plants (Russell et al., 1998; Heusser et al., 2015; Santos et al., 2016; Liu et al., 2016). This also allowed us to identify transcripts that were generally highly expressed in Amaranthaceae species.

We tested all target transcriptomes with CEGMA and BUSCO databases to check whether the additional transcriptomes had a comparable level of completeness that would facilitate our comparative transcriptome analysis. The highest number of complete and the lowest number of missing transcripts were observed for the *H. ammodendron* transcriptome and the *Chenopodium quinoa* genome (Fig. 2).

Amaranthaceae basal transcriptomes clustered using an Arabidopsis ortholog. The orthologous relationships among the target Amaranthaceae transcriptomes were first deduced from similarity to an *Arabidopsis thaliana* ortholog to maximize the functionally annotated transcripts included in the analysis. At the same time, it would minimize inclusion of lineage-specific genes only present in these non-model systems from obscuring the identification of candidate genes generally present in most plants. This approach allowed the identification of genes with a potential for broader applications in developing crop systems better adapted to extreme climates.

We used 17,588 ortholog clusters based on 230,716 transcripts from seven species. The separation of *Amaranthus hypochondriacus* from all the other transcriptomes on PC1 (Fig. 7A) may be indicative of it being a sister group to all other species included in the test (Fig. 7B). We then conducted hierarchical clustering based on the normalized expression values used for the PCA to identify transcript clusters that showed similar abundance levels in two or more species (Fig. 7C; Table S12). We included only one spinach species (*Spinachia oleraceae*), in our hierarchical clustering to avoid a bias introduced by two species in a single genus as our goal was to identify similarities of basal level transcripts shared among diverse species.

We selected the C3 (1568 transcripts), C7 (1224 transcripts), and C4 (3423 transcripts) clusters for further analysis (Fig. 7C). Cluster C3 was enriched in transcripts shared between *Salicornia europaea* and *H.*

Table 2
List of shared significantly differently regulated transcripts between current study and Long et al., 2014. Transcript annotations are based on Araport 11 (Krishnakumar et al., 2015) annotations linked to the putative *Arabidopsis* orthologs of the *H. ammodendron* transcripts.

	Transcript ID	*Arabidopsis* putative ortholog	Gene short ID	Putative function
Up Regulated	HA61447	AT1G43710	SDC1	A serine decarboxylase involved in ethanolamine metabolism and is critical for plant growth
	HA61280	–	–	
	HA51341	AT1G26390	–	FAD-binding Berberine family protein
	HA97782	–	–	
	HA114453	–	–	
	HA90445, HA73460	AT5G66400	RAB18	ABA- and drought-induced dehydrin protein.
	HA92013	–	–	
	HA109186	AT4G29890	–	A choline monooxygenase
	HA26132	–	–	
	HA78051	AT3G15850	FAD5	Chloroplast fatty acid desaturase and contribute to cold stress tolerance
	HA86030	–		
Down Regulated	HA96706	–	–	
	HA3468	AT2G44490	PEN2	A glycosyl hydrolase in peroxisomes that acts in inducible preinvasion resistance against bacterial pathogens
	HA106465	–	–	
	HA97855	–	–	
	HA9747	AT4G31940	CYP824	A cytochrome P450 enzyme, CYP82C involved in the early Fe deficiency response

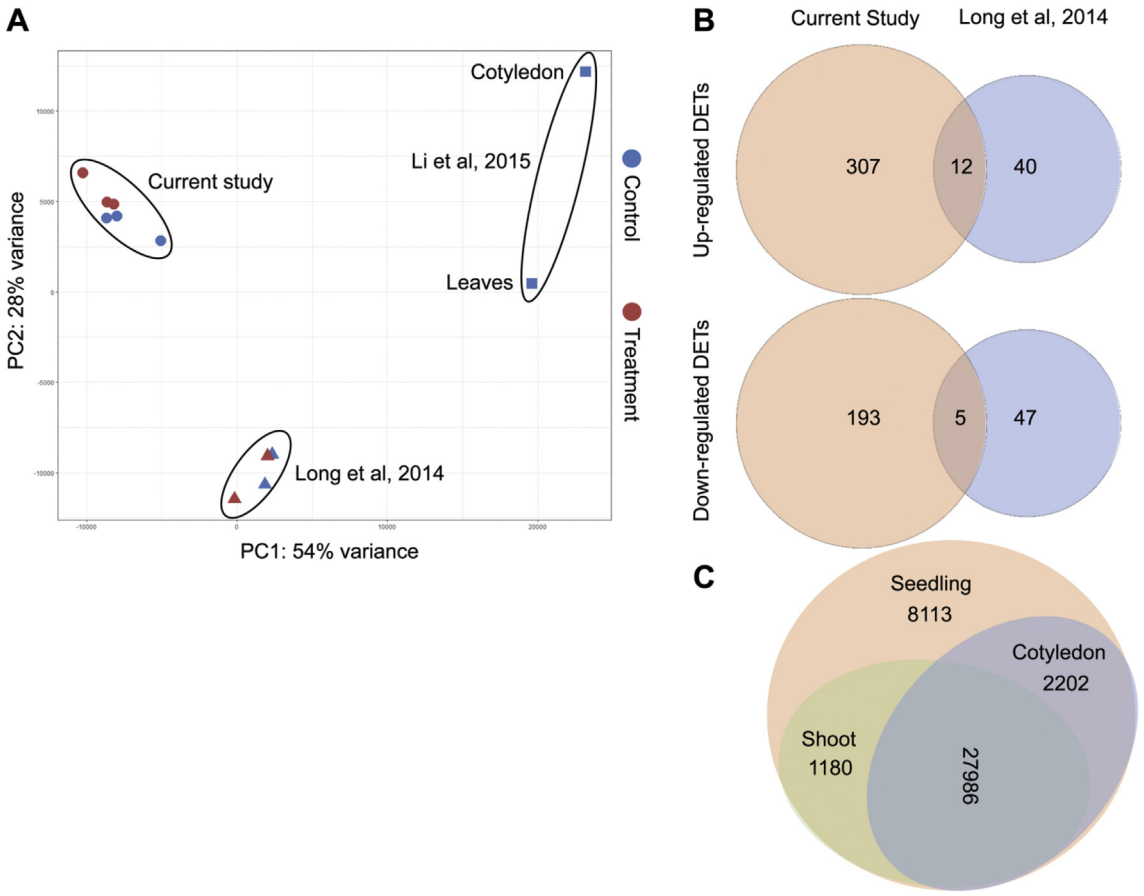

Fig. 6. Transcriptome profiles of *H. ammodendron* at different developmental stages and growth conditions. Principal component analysis for *H. ammodendron* RNAseq data used in the current study compared to Long et al., 2014 and Li et al., 2015 (A). Shared and unique differently expressed transcripts between PEG treated one week old early seedlings and soil grown drought stressed five-week-old seedlings (B). Overlapping transcripts found in leaves and cotyledons from Li et al. (2015) with the total transcripts found in whole seedlings (C).

ammodendron, the two most salt and drought tolerant species in the group. Cluster C3 was representative of functions mostly associated with stress responses. This cluster captures candidate genes abundant in the extremophytes but not merely due to their lineage specificity in Amaranthaceae or their functions as housekeeping genes common to most plants.

Cluster C4 was enriched in transcripts generally highly expressed in all six species. These mostly represented known genes in primary metabolism. Cluster C4 is enriched for genes that may have got selected repeatedly or maintained by purifying selection under abiotic stress in multiple Amaranthaceae species.

Lastly, we looked into Cluster C7 to search for *H. ammodendron* specific genes that may include uniquely evolved genes due to its psammophytic lifestyle. This list largely included functionally uncharacterized genes. Even with the limited information, it was clear that most of these genes have stress response roles and some were associated with biotic stress defense (Fig. 7C; Tables S3 and S13).

Amaranthaceae basal transcriptomes clustered independent from Arabidopsis orthologs. Amaranthaceae species are very likely to carry lineage-specific transcripts that would be absent in *Arabidopsis*. Therefore, additionally, we searched for one-to-one orthologous relationships among the six transcriptomes independent of their similarity to *A. thaliana* genes. We excluded *Chenopodium quinoa* from this search due to a large number of transposable elements annotated in the genome as hypothetical proteins and also because of *C. quinoa* being a tetraploid.

A PCA based on 1160 one-to-one ortholog clusters among the target group places *H. ammodendron* closest to *Salicornia europaea* than to any other species we included in the analysis (Fig. S7). This corresponds to their shared adaptations to high salinity and drought than observed for any other pair in the target group. The abiotic stress tolerance capacity expected for *H. ammodendron* to survive in its native habitat would place it at the extreme tolerant group even among Amaranthaceae (Sheng et al., 2005). *Salicornia europeae* is one of the most salt tolerant terrestrial species among all plants (Zhu, 2001).

3.8. Transcriptome signals consistent during H. ammodendron seedling development

We compared the two-day, ten-day (Li et al., 2015), one-week (current study), and five-week old seedling (Long et al., 2014) transcriptomes to assess transcripts constitutively expressed throughout these developmental stages. The RNA-seq datasets clustered to highlight their different plant growth conditions further separated by stress treatments and tissue conditions within each cluster (Fig. 6A). Early seedlings were separated from the late seedlings on PC2. The drought treatment effect on the transcriptome was spatially distinct for early seedlings, but not for late seedlings (Fig. S4 and Fig. 6). Nevertheless, we found 17 DETs shared between the two seedling stages of which ten of those were not identified in other plants (Fig. 6B; Table 2). Up-regulation of drought responsive genes and down-regulation of chlorophyll biosynthesis were consistent between the early and late seedlings despite the differences in their drought stress treatment and the development stage (Table 2). Table S9 lists DETs in the late seedlings in response to drought.

The early-seedling transcriptome compared to the RNA-seq data

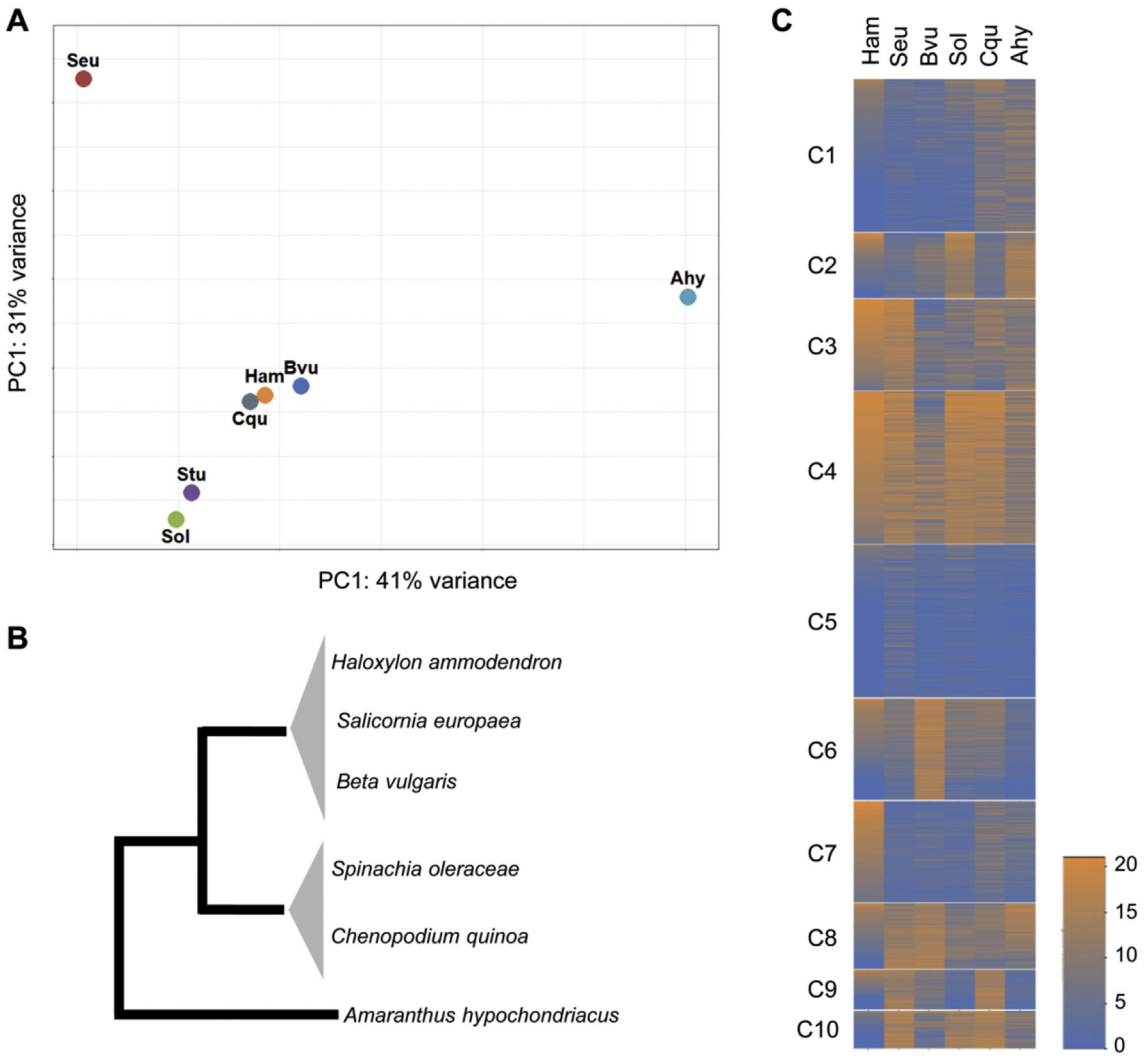

Fig. 7. Basal transcriptomes of Amaranthaceae species compared to the *H. ammodendron* basal transcriptome. PCA plot for normalized basal expression of all annotated transcripts with an ortholog identified in *Arabidopsis thaliana* (A). Heatmap based on hierarchical clustering of normalized orthologous basal expression among the six Amaranthaceae species (B). Species tree of target species based on the APG IV classification system (Byng et al., 2016) (C). Ham: *Haloxylon ammodendron*; Seu: *Salicornia europaea*; Sol: *Spinachia oleraceae* (cultivated spinach); Stu: *Spinachia turkestanica* (wild spinach); Bvu: *Beta vulgaris*; Ahy: *Amaranthus hypochondriacus*; Cqu: *Chenopodium quinoa*. The scale for the heat map represents percentile ranking within each species of FPKM values of orthologs.

from early seedling-leaves and cotyledons (Li et al., 2015) identified 8113 unique transcripts, most likely expressed specifically in seedling-roots, of which 26 were drought-responsive DETs (Figs. 4 and 6C; Tables S4 and S8). The shared 27,986 transcripts among whole seedlings, leaf, and cotyledon largely represents the shoot specific transcripts, of which 467 were DETs (Tables S4 and S8; Fig. 4).

4. Discussion

4.1. *H. ammodendron* seedling transcriptome recounts its lifestyle defined by multiple abiotic stresses

H. ammodendron has evolved to endure drought, high salinity, extreme temperatures, and high light (Lai, 1985; Shamsutdinov et al., 2016). Exemplifying its role as a pioneer keystone species, the total organic carbon, nitrogen, and phosphorus were significantly enriched creating a fertile zone ~40 cm around the *H. ammodendron* roots while pH and salinity were decreased (Li et al., 2011). This effect probably cascades into larger fertile islands in the desert where plant community structure is determined by sites that had the successful seedling establishment of *H. ammodendron* (Kebin, 1989; Wang et al., 2014). Therefore, the establishment of an entire plant community in a fragile ecosystem is dependent on the survival of *H. ammodendron* seedlings.

Understanding the transcriptome of *H. ammodendron* early seedlings in response to drought adds perception to the genetic basis for plant survival amid environmental stress. PEG-6000 has been widely used to study drought stress in many plant systems (Chazen et al., 1995; Siefritz et al., 2002; Caruso et al., 2008; Filichkin et al., 2010; Yang et al., 2011; Li et al., 2013; Feller et al., 2015; Han et al., 2017; Meunier et al., 2017) including psammophytes (Zhu et al., 2006; Gong et al., 2009; Taghvaei and Ghaedi, 2014). Additionally, it allows minimum handling that could be implemented at a very early seedling stage where soil grown samples would not be practical to study without damaging the young roots for our current study.

The early seedlings represent the most vulnerable development stage in *H. ammodendron* survival (Huang et al., 2003; Tobe et al., 2004, 2005; Zou et al., 2010; Li et al., 2011) and drought is the main determinant of seedling survival (Tobe et al., 2005; Yu et al., 2012; Tian et al., 2014). In agreement with previous field studies, we observed a clear growth setback in response to drought compared to control conditions in *H. ammodendron* seedlings (Fig. 1 and Fig. S1). As a consequence of persistent drought in its native habitat, most *H.*

Table 3
Representative transcripts highly expressed in both *H. ammodendron* and *S. europaea* (Cluster 3)[a], transcripts generally highly expressed in all selected Amaranthaceae (Cluster 4)[b], and transcripts highly expressed only in *H. ammodendron* compared to the other species (Cluster 7)[c].

CLUSTER 3		CLUSTER 4		CLUSTER 7	
Transcript ID (*A. thaliana* homolog)	Associated function	Transcript ID (*A. thaliana* homolog)	Associated function	Transcript ID (*A. thaliana* homolog)	Associated function
HA95461 (AT1G67910)	Unknown	HA6760 (AT5G19820)	Unknown	HA104108 (DI21)	Unknown
HA43121 (AT2G18420)	Unknown	HA24745 (SKS5)	Unknown	HA73143 (AT1G10588)	Unknown
HA51776 (MPK19)	Unknown	HA23507 (RAB6A)	Intracellular trafficking	HA89635 (AT1G58420)	Unknown
HA42950 (eIF-3)	Translation regulation	HA41033 (UXS2)	Cell wall biosynthesis	HA113751 (AT4G10265)	Unknown
HA7208 (AT3G56060)	Oxidoreductase	HA19064 (GAE6)	Pectin biosynthesis	HA33367 (RIN4)	Plant defense regulation
HA71089 (TRAPα)	ER membrane protein	HA68798 (SCE1)	Respond to stress	HA57220 (UPF0497)	Casparian strip formation
HA60059 (STH)	Seedling photomorphogenesis	HA40168 (AT4G34450)	Coatomer ϒ2, golgi transport	HA112998 (chitIV)	Plant defense
HA56711 (NRAMP3)	Fe & Mn transport from vacuoles	HA59654 (NIR1)	Nitrate assimilation	HA18310 (AT3G29410)	Terpenoid biosynthesis
HA18160 (CKA2α2)	Posttranslational regulation	HA48436 (CAC3)	Fatty acid biosynthesis	HA111069 (LAC14)	Oxidation of phenolics
HA86902 (GPX1)	Detoxify H2O2 in chloroplasts	HA103647 (AT4G19860)	α/β-hydrolase protein	HA113099 (AT1G06030)	Fructose phosphorylation
HA46720 (VSR3)	Stomatal closure via ABA signaling	HA35611 (PEX13)	Peroxisome biogenesis & transport	HA83036 (MIRO2)	ABA and salt stress response
HA16417 (AT5G47110)	Chlorophyll and tocopherol biosynthesis	HA59615 (PGD2)	Oxidative pentose phosphate pathway	HA44203 (AOX1B)	Mitochondrial alternative respiration
		HA107723 (WDR26)	Cross talk between light acclimation and stress	HA97836 (OZI1)	Response to oxidative, pathogen & ozone stress
		HA62187 (SMAX1)	Karrikin and strigolactone responses	HA107576 (AT5G21940)	UV, heavy metal & oxidative stress
				HA46590 (ACP5)	Fatty acid biosynthesis
				HA73460 (RAB18)	Response to drought, cold & ABA
				HA90377 (AT1G33030)	Lignin biosynthesis

[a] Expression level above 85th percentile in both *H. ammodendron* and *S. europaea*, and below 20th percentile or not detected in other species used in this study.
[b] Expression level above 75th percentile in all species used in this study.
[c] Expression level above 90th percentile in *H. ammodendron*, and below 15th percentile or not detected in other species used in this study.

ammodendron seeds germinate during spring when melting snow is common (Tobe et al., 2005). It is also commonly found in highly saline habitats (Tobe et al., 2000b). These environmental conditions combined, create drought, cold, salt, and osmotic stresses. Being sessile in a desert, *H. ammodendron* needs to tolerate high temperature, light, and UV stress. The above stresses would lead to oxidative stress caused by the generation of intracellular ROS above background levels. Therefore, plants in these environments need basal or induced transcriptional responses to minimize the damage caused by multiple abiotic stresses to sustain growth. The basal transcriptional levels as well as the induced responses we observed for *H. ammodendron* seedlings indeed exemplify adaptations to drought, salt, osmotic, cold, high temperature, UV, and oxidative stresses (Figs. 3 and 4; Table 3).

Notably, only a few transcripts were identified as differentially expressed in response to drought stress in *H. ammodendron.* This is likely because the basal transcriptome already had many of the drought responsive genes at a high expression level that reduced the necessity to upregulate these stress responsive genes from a preadapted expression level (Figs. 6 and 7). However, this is contrasting to drought sensitive plants that show more than 10% of their transcriptome responding significantly to drought stress (Clauw et al., 2015; Zong et al., 2013).

4.2. Modified stress response pathways in H. ammodendron compared to Arabidopsis

Fig. 4 illustrates the overall response to water deficit stress in *H. ammodendron* early seedlings in reference to genes with functional attributes, but excludes species specific unknown genes. The individual components in Fig. 4 are often associated with known stress related functions, but the combination of these components creates a unique transcriptomic signature previously not recognized in *H. ammodendron* or in other psammophytes. This would allow us to hypothesize strategies leading to coordinated gene expression in multiple biological processes to achieve optimal growth under stress, exemplified by extremophytes differently than in stress sensitive plants. For example, *SEP2,* stress-enhanced protein2 localized to the thylakoid membrane, was up-regulated in the drought-induced *H. ammodendron* seedlings (Fig. 4). However, it was previously thought to be induced only during light stress and specifically reported as a gene where drought had no influence on its induction in *Arabidopsis*. None of the stresses, cold, heat, desiccation, salt, or oxidative stress showed any effect on *SEP2* expression in *Arabidopsis* (Heddad and Adamska, 2000). Therefore, *H. ammodendron SEP2* presents an example of a stress responsive gene that may have a modified regulatory circuit from a single-to a multi-stress response that includes drought.

The existence of co-regulated multiple stress response pathways upon a single abiotic stress implied by the induction of stress responsive transcripts in *H. ammodendron* seedlings corroborate with physiological studies previously published. For example, *NPQ4* required for non-photochemical quenching (NPQ) to protect PSII from excess excitation energy is induced by light stress (Frenkel et al., 2009; Kereïche et al., 2010). A previous study has shown that *H. ammodendron* seedlings irrigated with salt had increased NPQ capacity (Han et al., 2010). In our current study, the drought-induced *H. ammodendron* seedlings also showed significantly up-regulated *NPQ4* expression.

Multiple genes associated with single stresses not reported to be affected by drought in previous studies have shown up-regulation in the drought-induced *H. ammodendron* seedlings. It is possible that some of these genes when functionally annotated in model plants were not tested for their response to drought stress. Alternatively, it could be indicative of co-regulated networks in *H. ammodendron* with up-stream regulators governing the induction of such genes in response to any one abiotic stress among multiple stresses that are persistent in the native habitat of *H. ammodendron*. For example, *FAD5* involved in plastidial lipid biosynthesis (Heilmann et al., 2004) known to be up-regulated under cold stress was induced in the drought-stressed *H. ammodendron*

seedlings. The role of *FAD5* in *H. ammodendron* seedlings may be consistent with the known function of chloroplast membrane structural integrity during cold stress, but how such a gene is induced in *H. ammodendron* compared to stress-sensitive plants could be different. *H. ammodendron* seedlings are found in the temperate deserts during early spring when melting snow is a common feature in the environment (Tobe et al., 2005). Therefore, shared stress signaling pathways that trigger a response to cold or drought would minimize the need to evolve or allocate energy on separately induced pathways.

4.3. Quality of transcriptome assemblies determine the extent of informative data that can be mined

Harnessing candidate genes from naturally adapted species to environmental stresses is a popular concept for crop development (Bressan et al., 2011; Dassanayake et al., 2012; Kissoudis et al., 2016; Eshel et al., 2017; Krämer, 2018). However, even with the unprecedented rate of increase in transcriptome resources for non-model species, the promise of effectively mining such data has not been fully realized (Hajjar and Hodgkin, 2007; Ford-Lloyd et al., 2011; Ambrosino et al., 2017). A major bottleneck limiting our ability to search for informative genetic variation is caused by releasing low-quality highly fragmented and redundant transcriptome assemblies, sometimes, without annotations. Many transcriptome analyses that stem from weak assemblies also lack biological replicates, adequate statistical analyses to identify convincing trends, and a reasonable effort to understand the biological significance of the transcriptomic signals. Therefore, in the current transcriptome study, we attempted to use all sequence data generated for *H. ammodendron* to maximize discovery of meaningful biological trends and identify promising candidate genes for future functional studies.

Understanding the overall transcriptomic signature in *H. ammodendron* seedlings first required a high-quality curated reference transcriptome. After a series of quality checks, we separated the relatively high-confidence coding transcriptome from the non-coding transcriptome likely to be more fragmented as well as underrepresented even in model systems. Our attempt to understand adaptations of a psammophyte by reading its transcriptome had two main goals. One was to understand the biological responses beyond GO functional enrichment, as integrated biological responses as much as possible by investigating all significantly regulated genes. We were able to detect coordinated stress responses including tradeoffs in biotic stresses to allow gene expression associated with multiple abiotic stresses (Fig. 4). Results discussed earlier suggest the presence of novel transcriptional regulation and co-option of homologs into general stress responsive pathways in *H. ammodendron* absent in stress specific response pathways described for model plants. A future reference genome for *H. ammodendron* would allow testing this further.

The second approach was to understand the uniqueness of the *H. ammodendron* transcriptome in comparison to other related species in a generally stress tolerant group. Transcriptome analyses of non-model plants are challenged by lack of functional genetic research that allows direct inferences of gene functions. Yet, we were able to identify candidate genes that may have evolved different regulatory mechanisms in extremophytes, of which many have homologs in the model plant. The presence of homologs in *A. thaliana* has a broader significance than being able to delete or overexpress these candidate genes in *Arabidopsis* to check for stress adaptive phenotypes. These candidates represent genes shared by most plants and therefore the genetic variation in regulation of such genes will provide evolutionary and mechanistic insight on how these genes have been recruited differently in naturally stress-adapted plants (Krämer, 2018). Because these are likely to be found in most crops, engineering crops for stress adaptation using such genes may be less daunting than attempting to introduce orphan genes into crops.

The functionally unknown genes found in *H. ammodendron* at high basal levels or induced under stress may be contributing to a cellular environment not only optimized to respond to extreme multiple abiotic stresses but is also optimized to protect the plant from pathogens. We see a significant number of these genes in all three clusters with an ortholog in *Arabidopsis* (Fig. 7). However, the *Arabidopsis* ortholog has not been functionally characterized yet or have escaped attention from researchers; perhaps because *Arabidopsis* does not carry the allele that would result in a significant phenotype, or the regulatory network to evaluate such homologs is absent in *Arabidopsis*. Future studies for functional annotations and transcriptome comparisons with psammophytes could give more insight into the functional value of such transcripts, their regulation upon sensing stress, and their evolutionary significance.

5. Conclusion

Drought is an inevitable stress typical to terrestrial plants. Desert plants show extreme adaptations to drought but their genomes are largely unexplored compared to drought sensitive models studied to understand drought tolerance. Few attempts have been made to understand the coordinated expression of transcripts for growth optimized survival using psammophytes. We have investigated the global transcriptomes to identify prevailing basal and induced pathways in *H. ammodendron* seedlings in response to drought stress. We found a stress prepared transcriptome for multiple abiotic stresses during seedling establishment. Additionally, transcripts that are generally maintained at low levels and some induced only under abiotic stress in *Arabidopsis* were highly expressed under basal conditions in the Amaranthaceae transcriptomes including *H. ammodendron*. These provide novel candidates to expand or initiate discovery of new stress adaptive gene networks and altered mechanisms naturally selected in extremophytes that allow survival under multiple environmental stresses.

Contributions

MD and QSQ developed the experimental design; LF and WH prepared the samples for RNA-seq, conducted physiological experiments and RT-qPCR; GW, PP, and KT performed bioinformatics analyses; QSQ and LA supervised physiological and molecular experiments; MD supervised computational analysis; MD and QSQ wrote the article.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (31571464/31371438/31070222), the National Basic Research Program, China (973-project-2013CB429904), the Research Fund for the Doctoral Program of Higher Education, China (20130211110001) to QSQ, and the National Science Foundation, USA (MCB#1616827) and the Next-Generation BioGreen21 Program (PJ01317301) of the Rural Development Administration, Republic of Korea to MD. The HPC services at LSU provided computational resources for data analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2018.09.024.

References

Ambrosino, L., Colantuono, C., Monticolo, F., Chiusano, M.L., 2017. Bioinformatics resources for plant genomics: opportunities and bottlenecks in the-omics era. Curr. Issues Mol. Biol. 27, 71–88.

Anand, A., Rojas, C.M., Tang, Y., Mysore, K.S., 2012. Several components of SKP1/Cullin/F-box E3 ubiquitin ligase complex and associated factors play a role in Agrobacterium-mediated plant transformation. New Phytol. 195, 203–216.

Aronson, J., 1985. Plants for arid lands: proceedings of the kew international conference

on economic plants for arid lands held in the Jodrell laboratory, Royal Botanic Gardens, Kew, England. In: Wickens, G.E., Goodin, J.R., Field, D.V. (Eds.), Economic Halophytes-a Global Review. Springer Press, Dordrecht, pp. 177–188.
Attia, H., Arnaud, N., Karray, N., Lachaal, M., 2008. Long-term effects of mild salt stress on growth, ion accumulation and superoxide dismutase expression of *Arabidopsis* rosette leaves. Physiol. Plantarum 132, 293–305.
Brennan, T., Frenkel, C., 1977. Involvement of hydrogen peroxide in the regulation of senescence in pear. Plant Physiol. 59, 411–416.
Bressan, R.A., Reddy, M.P., SukHo, C., Yun, D.J., Hardin, L.S., Bohnert, H.J., 2011. Stress-adapted extremophiles provide energy without interference with food production. Food Secur. 3, 93–105.
Brown, B.A., Jenkins, G.I., 2008. UV-B signaling pathways with different fluence-rate response profiles are distinguished in mature *Arabidopsis* leaf tissue by requirement for UVR8, HY5, and HYH. Plant Physiol. 146, 576–588.
Byng, et al., 2016. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 181, 1–20.
Cao, M., Du, P., Wang, X., Yu, Y.Q., Qiu, Y.H., Li, W., Gal-On, A., Zhou, C., Li, Y., Ding, S.W., 2014. Virus infection triggers widespread silencing of host genes by a distinct class of endogenous siRNAs in *Arabidopsis*. Proc. Natl. Acad. Sci. U.S.A. 111, 14613–14618.
Caruso, A., Chefdor, F., Carpin, S., Depierreux, C., Delmotte, F.M., Kahlem, G., Morabito, D., 2008. Physiological characterization and identification of genes differentially expressed in response to drought induced by PEG 6000 in Populus canadensis leaves. J. Plant Physiol. 165, 932–941.
Cazalé, A.C., Clément, M., Chiarenza, S., Roncato, M.A., Pochon, N., Creff, A., Marin, E., Leonhardt, N., Noël, L.D., 2009. Altered expression of cytosolic/nuclear HSC70-1 molecular chaperone affects development and abiotic stress tolerance in *Arabidopsis thaliana*. J. Exp. Bot. 60, 2653–2664.
Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103, 551–560.
Chazen, O., Hartung, W., Neumann, P.M., 1995. The different effects of PEG 6000 and NaCl on leaf development are associated with differential inhibition of root water transport. Plant Cell Environ. 18, 727–735.
Chellamuthu, V.R., Ermilova, E., Lapina, T., Lüddecke, J., Minaeva, E., Herrmann, C., Hartmann, M.D., Forchhammer, K., 2016. A widespread glutamine-sensing mechanism in the plant kingdom. Cell 159, 1188–1199.
Chen, J.H., Jiang, H.W., Hsieh, E.J., Chen, H.Y., Chien, C.T., Hsieh, H.L., Lin, T.P., 2012. Drought and salt stress tolerance of an *arabidopsis* glutathione S-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. Plant Physiol. 158, 340–351.
Chen, K.M., Holmström, M., Raksajit, W., Suorsa, M., Piippo, M., Aro, E.M., 2010. Small chloroplast-targeted DnaJ proteins are involved in optimization of photosynthetic reactions in *Arabidopsis thaliana*. BMC Plant Biol. 10, 43.
Cheng, C., Krishnakumar, V., Chan, A.P., Thibaud-Nissen, F., Schobel, S., Town, C.D., 2017. Araport11: a complete reannotation of the arabidopsis thaliana reference genome. Plant J. 89, 789–804.
Chin, K., DeFalco, T.A., Moeder, W., Yoshioka, K., 2013. The *Arabidopsis* cyclic nucleotide-gated ion channels AtCNGC2 and AtCNGC4 work in the same signaling pathway to regulate pathogen defense and floral transition. Plant Physiol. 163, 611–624.
Clauw, P., Coppens, F., De Beuf, K., Dhondt, S., Van Daele, T., Maleux, K., Storme, V., Clement, L., Gonzalez, N., Inzé, D., 2015. Leaf responses to mild drought stress in natural variants of *arabidopsis*. Plant Physiol. 167, 800–816.
Dassanayake, M., Oh, D.H., Haas, J.S., Hernandez, A., Hong, H., Ali, S., Yun, D.J., Bressan, R.A., Zhu, J.K., Bohnert, H.J., Cheeseman, J.M., 2011. The genome of the extremophile crucifer *Thellungiella parvula*. Nat. Genet. 43, 913–918.
Dassanayake, M., Oh, D.H., Yun, D.J., Bressan, R.A., Cheeseman, J.M., Hans, Bohnert, J., 2012. The scope of things to come: new paradigms in biotechnology. Plant Biotechnol. Agric. 19–34.
Denancé, N., Ranocha, P., Oria, N., Barlet, X., Rivière, M.P., Yadeta, K.A., Hoffmann, L., Perreau, F., Clément, G., Maia-Grondard, A., van, den, Berg, G.C., Savelli, B., Fournier, S., Aubert, Y., Pelletier, S., Thomma, B.P., Molina, A., Jouanin, L., Marco, Y., Goffner, D., 2013. *Arabidopsis* wat1 (walls are thin1)-mediated resistance to the bacterial vascular pathogen, Ralstonia solanacearum, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. Plant J. 73, 225–239.
Dixon, D.P., Lapthorn, A., Edwards, R., 2002. Plant glutathione transferases. Genome Biol. 3 REVIEWS3004.
Ederli, L., Madeo, L., Calderini, O., Gehring, C., Moretti, C., Buonaurio, R., Paolocci, F., Pasqualini, S., 2011. The *Arabidopsis thaliana* cysteine-rich receptor-like kinase CRK20 modulates host responses to Pseudomonas syringae pv. tomato DC3000 infection. J. Plant Physiol. 168, 1784–1794.
Eshel, G., Shaked, R., Kazachkova, Y., Khan, A., Eppel, A., Cisneros, A., Acuna, T., Gutterman, Y., Tel-Zur, N., Rachmilevitch, S., Fait, A., Barak, S., 2017. Anastatica hierochuntica, an arabidopsis desert relative, is tolerant to multiple abiotic stresses and exhibits species-specific and common stress tolerance strategies with its halophytic relative, Eutrema (Thellungiella) salsugineum. Front. Plant Sci. 7, 1992.
Feller, U., Anders, I., Wei, S., 2015. Effects of PEG-induced water deficit in Solanum nigrum on Zn and Ni uptake and translocation in split root systems. Plants 4, 284–297.
Feng, J., Fan, P., Jiang, P., Lv, S., Chen, X., Li, Y., 2014. Chloroplast-targeted Hsp90 plays essential roles in plastid development and embryogenesis in *Arabidopsis* possibly linking with VIPP1. Physiol. Plantarum 150, 292–307.
Filichkin, S.A., Priest, H.D., Givan, S.A., Shen, R., Bryant, D.W., Fox, S.E., Wong, W.K., Mockler, T.C., 2010. Genome-wide mapping of alternative splicing in *Arabidopsis thaliana*. Genome Res. 20, 45–58.
Ford-Lloyd, B.V., Schmidt, M., Armstrong, S.J., Barazani, O., Engels, J., Hadas, R., Hammer, K., Kell, S.P., Kang, D., Khoshbakht, K., Li, Y., Long, C., Lu, B.R., Ma, K., Viet, T.N., Qiu, L., Ge, S., Wei, W., Zhang, Z., Maxted, N., 2011. Crop wild relatives—undervalued, underutilized and under threat? Bioscience 61, 559–565.
Frenkel, M., Külheim, C., Jänkänpää, H.J., Skogström, O., Dall'Osto, L., Agren, J., Bassi, R., Moritz, T., Moen, J., Jansson, S., 2009. Improper excess light energy dissipation in *Arabidopsis* results in a metabolic reprogramming. BMC Plant Biol. 9, 12.
FSIN. Food Security Information Network, 2017. Global Report on Food Crises 2017.
Gao, J., Sun, L., Yang, X., Liu, J.X., 2013. Transcriptomic Analysis of Cadmium Stress Response in the Heavy Metal Hyperaccumulator Sedum alfredii Hance. PloS One 8, e64643.
Giraud, E., Ho, L.H., Clifton, R., Carroll, A., Estavillo, G., Tan, Y.F., Howell, K.A., Ivanova, A., Pogson, B.J., Millar, A.H., Whelan, J., 2008. The absence of ALTERNATIVE OXIDASE1a in *Arabidopsis* results in acute sensitivity to combined light and drought stress. Plant Physiol. 147, 595–610.
Gong, B., Zhu, J., Zhao, H., 2009. Preliminary study on the mechanism for adaptation of stipagrosits pennata to desert. Int. J. Biol. 1, 94–100.
Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29, 644–652.
Guo, L., Ma, F., Wei, F., Fanella, B., Allen, D.K., Wang, X., 2014. Cytosolic phosphorylating glyceraldehyde-3-phosphate dehydrogenases affect *arabidopsis* cellular metabolism and promote seed oil accumulation. Plant Cell 26, 3023–3035.
Guo, L., Yang, H., Zhang, X., Yang, S., 2013. Lipid transfer protein 3 as a target of MYB96 mediates freezing and drought stress in Arabidopsis. J. Exp. Bot. 64, 1755–1767.
Hajjar, R., Hodgkin, T., 2007. The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156, 1–13.
Han, B., Duan, X., Wang, Y., Zhu, K., Zhang, J., Wang, R., Hu, H., Qi, F., Pan, J., Yan, Y., Shen, W., 2017. Methane protects against polyethylene glycol-induced osmotic stress in maize by improving sugar and ascorbic acid metabolism. Sci. Rep. 7, 46185.
Han, W., Xu, X.W., Li, L., Lei, J.Q., Li, S.Y., 2010. Chlorophyll a fluorescence responses of Haloxylon ammodendron seedlings subjected to progressive saline stress in the Tarim desert highway ecological shelterbelt. Photosynthetica 48, 635–640.
Harshavardhan, V.T., Van Son, L., Seiler, C., Junker, A., Weigelt-Fischer, K., Klukas, C., Altmann, T., Sreenivasulu, N., Bäumlein, H., Kuhlmann, M., 2014. AtRD22 and AtUSPL1, members of the plant-specific BURP domain family involved in *Arabidopsis thaliana* drought tolerance. PloS One 9, e110065.
He, L., Gao, Z., Li, R., 2009. Pretreatment of seed with H_2O_2 enhances drought tolerance of wheat (Triticum aestivum L.) seedlings. Afr. J. Biotechnol. 8, 6151–6157.
Heddad, M., Adamska, I., 2000. Light stress-regulated two-helix proteins in *Arabidopsis thaliana* related to the chlorophyll a/b-binding gene family. Proc. Natl. Acad. Sci. U.S.A. 97, 3741–3746.
Heilmann, I., Mekhedov, S., King, B., Browse, J., Shanklin, J., 2004. Identification of the *Arabidopsis* palmitoyl-monogalactosyldiacylglycerol delta7-desaturase gene FAD5, and effects of plastidial retargeting of *Arabidopsis* desaturases on the fad5 mutant phenotype. Plant Physiol. 136, 4237–4245.
Heusser, L.E., Kirby, M.E., Nichols, J.E., 2015. Pollen-based evidence of extreme drought during the last Glacial (32.6–9.0 ka) in coastal southern California. Quat. Sci. Rev. 126, 242–253.
Huang, Z.Y., Zhang, X.S., Zheng, G.H., Gutterman, Y., 2003. Influence of light, temperature, salinity and storage on seed germination of Haloxylon ammodendron. J. Arid Environ. 55, 453–464.
IPCC, 2014. Climate change 2014: synthesis report. In: Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Geneva, Switzerland.
Kebin, Z., 1989. The growth of man-made forests of Haloxylon ammodendron and their soil water contents in the Minqin desert region, Gansu Province, China. J. Arid Environ. 17, 109–115.
Kerchev, P.I., Pellny, T.K., Vivancos, P.D., Kiddle, G., Hedden, P., Driscoll, S., Vanacker, H., Verrier, P., Hancock, R.D., Foyer, C.H., 2011. The transcription factor ABI4 Is required for the ascorbic acid-dependent regulation of growth and regulation of jasmonate-dependent defense signaling pathways in *Arabidopsis*. Plant Cell 23, 3319–3334.
Kereïche, S., Kiss, A.Z., Kouril, R., Boekema, E.J., Horton, P., 2010. The PsbS protein controls the macro-organisation of photosystem II complexes in the grana membranes of higher plant chloroplasts. FEBS Lett. 584, 759–764.
Kissoudis, C., van de, Wiel, C., Visser, R.G., van, der, Linden, G., 2016. Future-proof crops: challenges and strategies for climate resilience improvement. Curr. Opin. Plant Biol. 30, 47–56.
Krämer, U., 2018. Conceptualizing plant systems evolution. Curr. Opin. Plant Biol. 42, 66–75.
Krishnakumar, V., Hanlon, M.R., Contrino, S., Ferlanti, E.S., Karamycheva, S., Kim, M., Rosen, B.D., Cheng, C.Y., Moreira, W., Mock, S.A., Stubbs, J., Sullivan, J.M., Krampis, K., Miller, J.R., Micklem, G., Vaughn, M., Town, C.D., 2015. Araport: the Arabidopsis information portal. Nucleic Acids Res. 43, D1003–D1009.
Lai, H.G., 1985. To stop the yellow dragon with a Green ocean. Commonw. For. Rev. 64, 251–257.
Lata, C., Prasad, M., 2011. Role of drebs in regulation of abiotic stress responses in plants. J. Exp. Bot. 62, 4731–4748.
Langmead, B., Trapnell, C., Pop, M., Salzberg, S.L., 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10, R25.
Li, W., Godzik, A., 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 22, 1658–1659.
Li, C., Li, Y., Ma, J., 2011. Spatial heterogeneity of soil chemical properties at fine scales induced by *Haloxylon ammodendron* (Chenopodiaceae) plants in a sandy desert. Ecol. Res. 26, 385–394.

Li, H., Li, X., Zhang, D., Liu, H., Guan, K., 2013. Effects of drought stress on the seed germination and early seedling growth of the endemic desert plant Eremosparton songoricum (Fabaceae). EXCLI J. 12, 89–101.
Li, L., Stoeckert, C.J., Roos, D.S., 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. 13, 2178–2189.
Li, X., Lu, J., Liu, S., Liu, X., Lin, Y., Li, L., 2014. Identification of rapidly induced genes in the response of peanut (Arachis hypogaea) to water deficit and abscisic acid. BMC Biotechnol. 14, 58.
Li, Y., Ma, X., Zhao, J., Xu, J., Shi, J., Zhu, X.G., Zhao, Y., Zhang, H., 2015. Developmental genetic mechanisms of C4 syndrome based on transcriptome analysis of C3 cotyledons and C4 assimilating shoots in Haloxylon ammodendron. PloS One 10, e0117175.
Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K., 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10, 1391–1406.
Liao, W., Ji, L., Wang, J., Chen, Z., Ye, M., Ma, H., An, X., 2014. Identification of glutathione S-transferase genes responding to pathogen infestation in Populus tomentosa. Funct. Integr. Genom. 14, 517–529.
Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S., Schulze-Lefert, P., 2005. Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. Science 310, 1180–1183.
Liu, M., Zhu, R., Zhang, Z., Liu, L., Hui, R., Bao, J., Zhang, H., 2016. Water use traits and survival mechanisms of psammophytes in arid ecosystems. Arid Land Res. Manag. 30, 166–180.
Long, Y., Zhang, J., Tian, X., Wu, S., Zhang, Q., Zhang, J., Dang, Z., Pei, X.W., 2014. De novo assembly of the desert tree Haloxylon ammodendron (C. A. Mey.) based on RNA-Seq data provides insight into drought response, gene discovery and marker identification. BMC Genomics 15, 1111.
Maere, S., Heymans, K., Kuiper, M., 2005. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics 21, 3448–3449.
Membre, N., Bernier, F., Staiger, D., Berna, A., 2000. *Arabidopsis thaliana* germin-like proteins: common and specific features point to a variety of functions. Planta 211, 345–354.
Meunier, J.D., Barboni, D., Anwar-Ul-Haq, M., Levard, C., Chaurand, P., Vidal, V., Grauby, O., Huc, R., Laffont-Schwob, I., Rabier, J., Keller, C., 2017. Effect of phytoliths for mitigating water stress in durum wheat. New Phytol. 215, 229–239.
Millar, A.H., Whelan, J., Soole, K.L., Day, D.A., 2011. Organization and regulation of mitochondrial respiration in plants. Annu. Rev. Plant Biol. 62, 79–104.
Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7, 405–410.
Molnar, G., Bancos, S., Nagy, F., Szekeres, M., 2002. Characterisation of BRH1, a brassinosteroid-responsive RING-H2 gene from *Arabidopsis thaliana*. Planta 215, 127–133.
Morgan, M.J., Lehmann, M., Schwarzländer, M., Baxter, C.J., Sienkiewicz-Porzucek, A., Williams, T.C., Schauer, N., Fernie, A.R., Fricker, M.D., Ratcliffe, R.G., Sweetlove, L.J., Finkemeier, I., 2008. Decrease in manganese superoxide dismutase leads to reduced root growth and affects tricarboxylic acid cycle flux and mitochondrial redox homeostasis. Plant Physiol. 147, 101–114.
Neill, S., Desikan, R., Hancock, J., 2002. Hydrogen peroxide signalling. Curr. Opin. Plant Biol. 5, 388–395.
NRC, 1980. Firewood Crops: Shrub and Tree Species for Energy Production: Report of an Ad Hoc Panel of the Advisory Committee on Technology Innovation, Board on Science and Technology for International Development. Commission on International Relations. National Academies.
Oh, D.H., Hong, H., Lee, S.Y., Yun, D.J., Bohnert, H.J., Dassanayake, M., 2014a. Genome structures and transcriptomes signify niche adaptation for the multiple-ion-tolerant extremophyte Schrenkiella parvula. Plant Physiol. 164, 2123–2138.
Oh, D.H., Barkla, B.J., Vera-Estrella, R., Pantoja, O., Lee, S.Y., Bohnert, H.J., Dassanayake, M., 2015. Cell type-specific responses to salinity – the epidermal bladder cell transcriptome of *Mesembryanthemum crystallinum*. New Phytol. 207, 627–644.
Oh, S.E., Yeung, C., Babaei-Rad, R., Zhao, R., 2014b. Cosuppression of the chloroplast localized molecular chaperone HSP90.5 impairs plant development and chloroplast biogenesis in *Arabidopsis*. BMC Res. Notes 7, 643.
Park, S.W., Li, W., Viehhauser, A., He, B., Kim, S., Nilsson, A.K., Andersson, M.X., Kittle, J.D., Ambavaram, M.M., Luan, S., Esker, A.R., Tholl, D., Cimini, D., Ellerström, M., Coaker, G., Mitchell, T.K., Pereira, A., Dietz, K.J., Lawrence, C.B., 2013. Cyclophilin 20-3 relays a 12-oxo-phytodienoic acid signal during stress responsive regulation of cellular redox homeostasis. Proc. Natl. Acad. Sci. U.S.A. 110, 9559–9564.
Parra, G., Bradnam, K., Korf, I., 2007. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 23, 1061.
Passaia, G., Queval, G., Bai, J., Margis-Pinheiro, M., Foyer, C.H., 2014. The effects of redox controls mediated by glutathione peroxidases on root architecture in *Arabidopsis thaliana*. J. Exp. Bot. 65, 1403–1413.
Perez, D.E., Hoyer, J.S., Johnson, A.I., Moody, Z.R., Lopez, J., Kaplinsky, N.J., 2009. BOBBER1 is a noncanonical *Arabidopsis* small heat shock protein required for both development and thermotolerance. Plant Physiol. 151, 241–252.
Porra, R.J., 2002. The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynth. Res. 73, 149–156.
Rabino, I., Mancinelli, A.L., 1986. Light, temperature, and anthocyanin production. Plant Physiol. 81, 922–924.
Ravet, K., Reyt, G., Arnaud, N., Krouk, G., Djouani, el-B., Boucherez, J., Briat, J.F., Gaymard, F., 2012. Iron and ROS control of the DownSTream mRNA decay pathway is essential for plant fitness. EMBO J. 31, 175–186.
Reyt, G., Boudouf, S., Boucherez, J., Gaymard, F., Briat, J.F., 2015. Iron- and ferritin-dependent reactive oxygen species distribution: impact on *Arabidopsis* root system architecture. Mol. Plant 8, 439–453.
Russell, B.L., Rathinasabapathi, B., Hanson, A.D., 1998. Osmotic stress induces expression of choline monooxygenase in sugar beet and amaranth. Plant Physiol. 116, 859–865.
Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2006. Functional analysis of an arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. Plant Cell 18, 1292–1309.
Samakovli, D., Margaritopoulou, T., Prassinos, C., Milioni, D., Hatzopoulos, P., 2014. Brassinosteroid nuclear signaling recruits HSP90 activity. New Phytol. 203, 743–757.
Santos, J., Al-Azzawi, M., Aronson, J., Flowers, T.J., 2016. eHALOPH a database of salt-tolerant plants: helping put halophytes to work. Plant Cell Physiol. 57, e10.
Savouré, A., Thorin, D., Davey, M., Hua, X.J., Mauro, S., Montagu, M.V., Inzé, D., Verbruggen, N., 2002. NaCl and $CuSO_4$ treatments trigger distinct oxidative defence mechanisms in Nicotiana plumbaginifolia L. Plant Cell Environ. 22, 387–396.
Savvides, A., Ali, S., Tester, M., Fotopoulos, V., 2016. Chemical priming of plants against multiple abiotic stresses: mission possible? Trends Plant Sci. 21, 329–340.
Schnaubelt, D., Queval, G., Dong, Y., Diaz-Vivancos, P., Makgopa, M.E., Howell, G., De Simone, A., Bai, J., Hannah, M.A., Foyer, C.H., 2015. Low glutathione regulates gene expression and the redox potentials of the nucleus and cytosol in *Arabidopsis thaliana*. Plant Cell Environ. 38, 266–279.
Schrammeijer, B., Risseeuw, E., Pansegrau, W., Regensburg-Tuïnk, T.J., Crosby, W.L., Hooykaas, P.J., 2001. Interaction of the virulence protein VirF of Agrobacterium tumefaciens with plant homologs of the yeast Skp1 protein. Curr. Biol. 11, 258–262.
Shamsutdinov, Z.Sh, Kosolapov, V.M., Shamsutdinova, E.Z., Savchenko, I.V., Zvolinskiy, V.P., Gunin, P.D., Shamsutdinov, N.Z., Goldvarg, B.A., Tsagan-Mandzhiev, N.L., 2016. Innovative technology for ecological restoration of degraded rangeland ecosystems based on new varieties of fodder halophytes in arid regions of Russia. Russ. Agric. Sci. 42, 348–352.
Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504.
Sheng, Y., Zheng, W., Pei, K., Ma, K., 2005. Genetic variation within and among populations of a dominant desert tree Haloxylon ammodendron (Amaranthaceae) in China. Ann. Bot. 96, 245–252.
Shin, L.J., Lo, J.C., Yeh, K.C., 2012. Copper chaperone antioxidant protein1 is essential for copper homeostasis. Plant Physiol. 159, 1099–1110.
Shindo, T., Misas-Villamil, J.C., Hörger, A.C., Song, J., van, der, Hoorn, R.A., 2012. A role in immunity for *arabidopsis* cysteine protease RD21, the ortholog of the tomato immune protease C14. PloS One 7, e29317.
Siefritz, F., Tyree, M.T., Lovisolo, C., Schubert, A., Kaldenhoff, R., 2002. PIP1 plasma membrane aquaporins in tobacco: from cellular effects to function in plants. Plant Cell 14, 869–876.
Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V., Zdobnov, E.M., 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31, 3210.
Song, J., Ding, X., Feng, G., Zhang, F., 2006. Nutritional and osmotic roles of nitrate in a euhalophyte and a xerophyte in saline conditions. New Phytol. 171, 357–366.
Song, J., Feng, G., Tian, C.Y., Zhang, F., 2005. Strategies for adaptation of Suaeda physophora, Haloxylon ammodendron and Haloxylon persicum to a saline environment during seed-germination stage. Ann. Bot. 96, 399–405.
Soto, I.C., Fontanesi, F., Liu, J., Barrientos, A., 2012. Biogenesis and assembly of eukaryotic cytochrome c oxidase catalytic core. Biochim. Biophys. Acta 1817, 883–897.
Sung, D.Y., Vierling, E., Guy, C.L., 2001. Comprehensive expression profile analysis of the *arabidopsis* Hsp70 gene family. Plant Physiol. 126, 789–800.
Taghvaei, M., Ghaedi, M., 2014. The effect of osmotic conditions on germination of (*Haloxylon aphyllum* L.) seeds and recovery under water stress. Agric. J. 9, 167–172.
Talke, I.N., Hanikenne, M., Krämer, U., 2006. Zinc-dependent global transcriptional control, transcriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis* halleri. Plant Physiol. 142 148 LP-167.
Tang, R.F., Gavin, M.C., 2010. Traditional ecological knowledge informing resource management: saxoul conservation in Inner Mongolia, China. 23. Society & Natural Resources, pp. 193–206.
Tarazona, S., Furió-Tarí, P., Turrà, D., Pietro, A.D., Nueda, M.J., Ferrer, A., Conesa, A., 2015. Data quality aware analysis of differential expression in RNA-seq with NOISeq R/Bioc package. Nucleic Acids Res. 43, e140.
Thatcher, L.F., Carrie, C., Andersson, C.R., Sivasithamparam, K., Whelan, J., Singh, K.B., 2007. Differential gene expression and subcellular targeting of *Arabidopsis* glutathione S-transferase F8 is achieved through alternative transcription start sites. J. Biol. Chem. 282, 28915–28928.
Tian, Y., Tashpolat, T., Li, Y., 2014. Causes of seedling mortality in desert for the small xeric tree Haloxylon ammodendron. Scand. J. For. Res. 29, 555–564.
Tobe, K., Li, X.M., Omasa, K., 2000a. Effects of sodium chloride on seed germination and growth of two Chinese desert shrubs, *Haloxylon ammodendron* and *H. persicum* (Chenopodiaceae). Aust. J. Bot. 48, 455–460.
Tobe, K., Li, X.M., Omasa, K., 2004. Effects of five different salts on seed germination and seedling growth of Haloxylon ammodendron (Chenopodiaceae). Seed Sci. Res. 14, 345–353.
Tobe, K., Li, X.M., Omasa, K., 2005. Effects of irrigation on seedling emergence and seedling survival of a desert shrub Haloxylon ammodendron (Chenopodiaceae). Aust. J. Bot. 53, 529–534.
Tobe, K., Li, X., Omasa, K., 2000b. Effects of sodium chloride on seed germination and growth of two Chinese desert shrubs, Haloxylon ammodendron and H. persicum (Chenopodiaceae). Aust. J. Bot. 48, 455–460.

Urzica, E.I., Casero, D., Yamasaki, H., Hsieh, S.I., Adler, L.N., Karpowicz, S.J., Blaby-Haas, C.E., Clarke, S.G., Loo, J.A., Pellegrini, M., Merchant, S.S., 2012. Systems and trans-system level analysis identifies conserved iron deficiency responses in the plant lineage. Plant Cell 24, 3921–3948.

Velasco, V.M., Mansbridge, J., Bremner, S., Carruthers, K., Summers, P.S., Sung, W.W., Champigny, M.J., Weretilnyk, E.A., 2016. Acclimation of the crucifer Eutrema salsugineum to phosphate limitation is associated with constitutively high expression of phosphate-starvation genes. Plant Cell Environ. 39, 1818–1834.

Wahid, A., Perveen, M., Gelani, S., Basra, S.M., 2007. Pretreatment of seed with H2O2 improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. J. Plant Physiol. 164, 283–294.

Wang, M., Zhang, S., Chu, G., 2014. Point pattern analysis of different life stages of haloxylon ammodendron in desert-oasis ecotone. Pol. J. Environ. Stud. 23, 2271–2277.

Winkel-Shirley, B., 2002. Biosynthesis of flavonoids and effects of stress. Curr. Opin. Plant Biol. 5, 218–223.

Xu, J., Tian, Y.S., Xing, X.J., Peng, R.H., Zhu, B., Gao, J.J., Yao, Q.H., 2015. Over-expression of AtGSTU19 provides tolerance to salt, drought and methyl viologen stresses in *Arabidopsis*. Physiol. Plantarum. https://doi.org/10.1111/ppl.12347.

Xue, J.G., Wang, X.G., Du, X.G., Mao, P.S., Zhang, T.J., Zhao, L., Han, J.G., 2012. Influence of salinity and temperature on the germination of haloxylon ammodendron and ceratoides arborescens. J. Anim. Vet. Adv. 11, 1306–1311.

Yamaguchi-Shinozaki, K., Urao, T., Iwasaki, T., Kiyosue, T., Shinozaki, K., 1994. Function and regulation of genes that are induced by dehydration stress in arabidopsis thaliana. Jircas J. Sci. Pap. 1, 69–79.

Yang, Y., Qin, Y., Xie, C., Zhao, F., Zhao, J., Liu, D., Chen, S., Fuglsang, A.T., Palmgren, M.G., Schumaker, K.S., Deng, X.W., Guo, Y., 2010. The *Arabidopsis* chaperone J3 regulates the plasma membrane H^+-ATPase through interaction with the PKS5 kinase. Plant Cell 22, 1313–1332.

Yang, Z.B., Eticha, D., Rotter, B., Rao, I.M., Horst, W.J., 2011. Physiological and molecular analysis of polyethylene glycol-induced reduction of aluminium accumulation in the root tips of common bean (Phaseolus vulgaris). New Phytol. 192, 99–113.

Youssef, A., Laizet, Y., Block, M.A., Maréchal, E., Alcaraz, J.P., Larson, T.R., Pontier, D., Gaffé, J., Kuntz, M., 2010. Plant lipid-associated fibrillin proteins condition jasmonate production under photosynthetic stress. Plant J. 61, 436–445.

Yu, T., Ren, C., Zhang, J., He, X., Ma, L., Chen, Q., Qu, Y., Shi, S., Zhang, H., Ma, H., 2012. Effect of high desert surface layer temperature stress on Haloxylon ammodendron (C.A. Mey.) Bunge. Flora 207, 572–580.

Zhang, X., Liu, S., Takano, T., 2008. Two cysteine proteinase inhibitors from *Arabidopsis thaliana*, AtCYSa and AtCYSb, increasing the salt, drought, oxidation and cold tolerance. Plant Mol. Biol. 68, 131–143.

Zhao, Y., Chow, T.F., Puckrin, R.S., Alfred, S.E., Korir, A.K., Larive, C.K., Cutler, S.R., 2007. Chemical genetic interrogation of natural variation uncovers a molecule that is glycoactivated. Nat. Chem. Biol. 3, 716–721.

Zheng, C., Wang, Q., 2015. Seasonal and annual variation in transpiration of a dominant desert species, haloxylon ammodendron, in central asia up-scaled from sap flow measurement. Ecohydrology 8, 948–960.

Zhong, L., Zhou, W., Wang, H., Ding, S., Lu, Q., Wen, X., Peng, L., Zhang, L., Lu, C., 2013. Chloroplast small heat shock protein HSP21 interacts with plastid nucleoid protein pTAC5 and is essential for chloroplast development in *arabidopsis* under heat stress. Plant Cell 25, 2925–2943.

Zhu, J., Kang, H., Tan, H., Xu, M., 2006. Effects of drought stresses induced by polyethylene glycol on germination of Pinus sylvestris var. mongolica seeds from natural and plantation forests on sandy land. J. For. Res. 11, 319–328.

Zhu, J.K., 2001. Plant salt stress. In: eLS. Wiley Online Library. https://doi.org/10.1038/npg.els.0001300.

Zong, W., Zhong, X., You, J., Xiong, L., 2013. Genome-wide profiling of histone H3K4-tri-methylation and gene expression in rice under drought stress. Plant Mol. Biol. 81, 175–188.

Zou, C., Chen, A., Xiao, L., Muller, H.M., Ache, P., Haberer, G., Zhang, M., Jia, W., Deng, P., Huang, R., Lang, D., Li, F., Zhan, D., Wu, X., Zhang, H., Bohm, J., Liu, R., Shabala, S., Hedrich, R., Zhu, J.K., Zhang, H., 2017. A high-quality genome assembly of quinoa provides insights into the molecular basis of salt bladder-based salinity tolerance and the exceptional nutritional value. Cell Res. 27, 1327–1340.

Zou, T., Li, Y., Xu, H., Xu, G.Q., 2010. Responses to precipitation treatment for *Haloxylon ammodendron* growing on contrasting textured soils. Ecol. Res. 25, 185–194.