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Research article

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

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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

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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