A comparative genomics examination of desiccation tolerance and sensitivity in two sister grass species

Ricardo A. Chavez Montes1,2, Anna Haber3,4, Jeremy Pardo3,4, Robyn F. Powell2, Upendra K. Divissetty5, Anderson T. Silva5, Tania Hernández-Hernández4,5, Vanildo Silveira6, Haibao Tang7, Eric Lyons8, Luis Rafael Herrera Estrella2,5,8, Robert VanBuren9,10,11, and Melvin J. Oliver1,12

1National Laboratory of Genomics for Biodiversity, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional 36824 Irapuato, Guanajuato, Mexico; 2Institute of Genomics for Crop Abiotic Stress Tolerance, Department of Plant and Soil Science, Texas Tech University, Lubbock, TX 79409; 3Department of Horticulture, Michigan State University, East Lansing, MI 48824; 4Plant Resilience Institute, Michigan State University, East Lansing, MI 48824; 5Department of Plant Biology, Michigan State University, East Lansing, MI 48824; 6Trait Diversity and Function, Royal Botanic Gardens, Richmond Surrey TW9 3AB, United Kingdom; 7CyVerse Collaborative, School of Plant Sciences, University of Arizona, Tucson, AZ 85721; 8Division of Plant Sciences, University of Missouri, Columbia, MO 65211; 9Department of Research, Conservation and Collections, Desert Botanical Garden, Phoenix, AZ; 10Laboratorio de Biotecnologia, Centro de Biocìencias e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ 28013-602, Brazil; 11School of Plant Sciences, BIOS Institute, University of Arizona, Tucson, AZ 85721; and 12Interdisciplinary Plant Group, University of Missouri, Columbia, MO 65211

Edited by Elizabeth Kellogg, Donald Danforth Plant Science Center, St. Louis, MO; received October 19, 2021; accepted December 14, 2021

Desiccation tolerance is an ancient and complex trait that spans all major lineages of life on earth. Although important in the evolution of land plants, the mechanisms that underlay this complex trait are poorly understood, especially for vegetative desiccation tolerance (VDT). The lack of suitable closely related plant models that offer a direct contrast between desiccation tolerance and sensitivity has hampered progress. We have assembled high-quality genomes for two closely related grasses, the desiccation-tolerant Sporobolus stapfianus and the desiccation-sensitive Sporobolus pyramidalis. Both species are complex polyploids; S. stapfianus is primarily tetraploid, and S. pyramidalis is primarily hexaploid. S. pyramidalis undergoes a major transcriptome remodeling event during initial exposure to dehydration, while S. stapfianus has a muted early response, with peak remodeling during the transition between 1.5 and 1.0 grams of water (gH2O) g−1 dry weight (dw). Functionally, the dehydration transcriptome of S. stapfianus is unrelated to that for S. pyramidalis. A comparative analysis of the transcriptomes of the hydrated controls for each species indicated that S. stapfianus is transcriptionally primed for desiccation. Cross-species comparative analyses indicated that VDT likely evolved from reprogramming of desiccation tolerance mechanisms that evolved in seeds and that the tolerance mechanism of S. stapfianus represents a recent evolution for VDT within the Chloridoideae. Orthogonal analyses of the significantly differentially abundant transcripts reconfirmed our present understanding of the response to dehydration, including the lack of an induction of senescence in resurrection angiosperms. The data also suggest that failure to maintain protein structure during dehydration is likely critical in rendering a plant desiccation sensitive.

desiccation tolerance | sister group contrast | comparative genomics | transcriptome remodeling | C4 grasses

Desiccation tolerance (DT) is a fundamental trait that is widespread and developed early in the evolution of the land plants (1, 2), and it is believed to have been critical in the colonization of the land by green algae (3). In tracheophytes, DT is generally limited to reproductive propagules, such as seeds and spores, while vegetative desiccation tolerance (VDT) occurs in only 0.086% of known vascular plant species (4). Our results revealed that the DT grass Sporobolus stapfianus is transcriptionally primed to tolerate a dehydration/desiccation event and that the desiccation response in the DT S. stapfianus is distinct from the water stress response of the desiccation-sensitive Sporobolus pyramidalis. Our results also show that the desiccation response is largely unique, indicating a recent evolution of this trait within the angiosperms, and that inhibition of senescence during dehydration is likely critical in rendering a plant desiccation tolerant.

Significance

This is a significant sister group contrast comparative study of the underpinning genomics and evolution of desiccation tolerance (DT), a critical trait in the evolution of land plants. Our results revealed that the DT grass Sporobolus stapfianus is transcriptionally primed to tolerate a dehydration/desiccation event and that the desiccation response in the DT S. stapfianus is distinct from the water stress response of the desiccation-sensitive Sporobolus pyramidalis. Our results also show that the desiccation response is largely unique, indicating a recent evolution of this trait within the angiosperms, and that inhibition of senescence during dehydration is likely critical in rendering a plant desiccation tolerant.

Author contributions: E.L., L.R.H.E., R.V., and M.J.O. designed research; J.P., R.F.P., T.H.H., H.T., and M.J.O. performed research; R.A.C.M., A.H., J.P., R.F.P., U.K.D., A.T.S., T.H.H., V.S., H.T., E.L., L.R.H.E., R.V., and M.J.O. analyzed data; and R.A.C.M., A.H., L.R.H.E., R.V., and M.J.O. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

1To whom correspondence may be addressed. Email: luis.herrera-estrella@ttu.edu, or olivermj@missouri.edu.

This article contains supporting information online at http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2118886119/-/DCSupplemental.

Published January 26, 2022.
A corollary to the ancestral reconstruction approach to understanding the evolution of VDT and the genomic aspect of its phenotypic expression is the comparison of the genomes of closely related species that contrast the two extremes: sensitivity and tolerance. Such closely related contrasting species pairings are rare in resurrection plants, but this approach has been applied, albeit with species pairs that are not as close as would be ideal. The genomes and dehybridization–rehydration transcriptomes of two resurrection eudicots within the Linderiaceae family (16), the desiccation-tolerant *L. brevifrons* and the desiccation-sensitive (DS) *Linaria subracemosa*, were sequenced and compared (9). The comparison revealed that at least in the *Linaria* lineage, VDT evolved via a combination of gene duplications in gene families that are functionally associated with the desiccation response and a network-level rewiring of gene expression in vegetative tissue commonly associated with seed desiccation. More recently, a comparative analysis of two contrasting grass genomes along with their respective desiccation-related transcriptomes, the desiccation-tolerant *E. nitidensis* and the related DS cereal *Eragrostis tef*, reinforced the potential role of gene duplications in the evolution of DT (10). Although there is still a significant phylogenetic distance between these two *Eragrostis* species (17), the comparative analysis and its extension to include other C4 grasses, including the desiccation-tolerant *O. thomaeum*, revealed chromatin restructuring and methylation patterns associated with down-regulated genes and specific seed-related orthologs whose expression is associated with VDT. The comparative transcriptome analyses indicated that genes having important roles in seed development and DT are broadly expressed under dehydration in both sensitive and tolerant species, with just a few genes uniquely expressed in the tolerant plants.

In this study, we have chosen two phylogenetically closely related C4 grasses, the homiochlorophyllous desiccation-tolerant *Sporobolus stapfianus* and the DS *Sporobolus pyramidalis*, to develop detailed comparative genomic and transcriptomic analyses to further explore genomic inferences into the evolution of VDT. *S. stapfianus* and *S. pyramidalis* are members of the same clade, clade A, in the *Sporobolus* family of the Sporobolinae subtribe of the Chloridoideae (18). *S. stapfianus* has been the subject of many mechanistic studies of its DT phenotype (19, 20) and along with *S. pyramidalis*, the subject of a detailed comparative leaf metabolomics study that highlighted differences in the metabolic responses of the two species to dehydration (21). We constructed Hi-C-derived assemblies of the sequenced genomes for both species and conducted transcript profiling analyses for parallel reductions in water contents for both species as well as a full desiccation drying series for *S. stapfianus*. We performed a detailed comparative genomic analysis for the two species and extended the analysis to include other grass species, both desiccation tolerant and DS. Our results offer insights into the mechanism and evolution of VDT in the Chloridoid grasses.

### Results

#### Generating Genomic Resources for *Sporobolus Grasses*

One-step flow cytometric assays generated size estimates for each of the *Sporobolus* genomes. The haploid genome of *S. stapfianus* had an average of 1,385 pg of DNA per nucleus, which is approximately equal to a complete genome sequence of 1.354 Gbp, and the haploid genome of *S. pyramidalis* had an average of 1.867 pg of DNA per nucleus, which is ∼1.826 Gbp (Table 1).

Draft genome assemblies were generated for each grass using Illumina whole-genome shotgun sequencing combined with Chicago and Hi-C proximity ligation (Materials and Methods). The final assemblies consisted of 11,574 scaffolds with an N50 of 19.4 Mb for *S. stapfianus* and 2,518 scaffolds with an N50 of 21.6 Mb for *S. pyramidalis*, with the longest scaffolds for both species greater than 60 Mb. Despite their high contiguity, the assembled genomes are smaller than the estimated genome size, at 1.080 and 1.055 Gbp for *S. stapfianus* and *S. pyramidalis*, respectively. These differences between the estimated and assembled genome sizes are likely caused by collapsed homologous regions in these complex polyploid species as described in detail below. Both genomes have similar levels of repetitive elements, 39.7 and 41.3% for *S. stapfianus* and *S. pyramidalis*, respectively (Table 2), with almost identical distributions of known repeat families (SI Appendix, Table S1). Gypsy and Copia retrotransposons are the most predominant families of the known repeats at 36 and 10 to 12%, respectively, for the two genomes.

The *Sporobolus* genomes were annotated using MAKER with a combination of RNASeq and PacBio Iso-Seq full-length transcripts as expressed sequence tag (EST) evidence and protein homology from other high-quality plant genomes. After filtering, the final annotations contained 52,208 and 51,207 gene models for *S. stapfianus* and *S. pyramidalis*, respectively (Table 2), with almost identical distributions of known repeat families (SI Appendix, Table S1). Gypsy and Copia retrotransposons are the most predominant families of the known repeats at 36 and 92.4% of complete BUSCOs for *S. stapfianus* and *S. pyramidalis*, respectively, indicating that both genomes were well annotated and contained the vast majority of the coding portion of these two genomes (Table 3). Gene models were functionally annotated using a simplified maize–GAMER pipeline; 96% of genes were annotated with InterProScan domain/family information, and 66% were annotated with Gene Ontology (GO) descriptions for both genomes.

#### Comparative Genomics of Chloridoid Grasses

*Sporobolus* belongs to the Chloridoideae subfamily of grasses, a large and diverse group of predominantly C4 species with remarkable drought, heat, and salinity tolerance. The orphan grain crops finger millet and teff are found within Chloridoideae, as are several model desiccation-tolerant plants in the genera *Oropetium*, *Eragrostis*, *Tripogon*, and *Sporobolus* among others. Most of the surveyed Chloridoideae species (∼90%) are polyploid, including species from many of the aforementioned taxa. The availability of several high-quality chloridoid genomes facilitates detailed comparative genomic comparisons within these grasses. Macro-synteny between *S. stapfianus* and *S. pyramidalis* shows a clear 2:3 pattern, consistent with the tetraploid and hexaploid nature of these grasses, respectively (Fig. 1 and SI Appendix, Fig. S1).

Comparisons with the closely related diploid chloridoid grass *O. thomaeum* also revealed 1:2 and 1:3 patterns of synteny for *S. stapfianus* and *S. pyramidalis*, respectively, supporting their polyploidy (Fig. 1 and SI Appendix, Fig. S2). Although neither *Sporobolus* genome is scaffolded into complete chromosomes, large 20-Mb+ sized scaffolds are highly collinear with the *Oropetium* genome with few structural large-scale rearrangements (SI Appendix, Fig. S2), which is consistent with the unusually high conservation of karyotype and collinearity observed among other chloridoid grass genomes (22).

#### Table 1. Estimation of the genome size (1C value) using flow cytometry

| Species        | Standard | Average: sample | Average: standard | GS (2C), pg | GS (1C), pg | SD sample  |
|----------------|----------|-----------------|-------------------|-------------|-------------|-----------|
| *S. stapfianus*| P. crispum | 305.2           | 367.85            | 3.734       | 1.867       | 0.011533  |
| *S. pyramidalis*| P. crispum | 206.57         | 335.67            | 2.769       | 1.385       | 0.02623   |

Estimated genome size (GS), in bold, for the diploid (2C) and haploid (1C) states.
Macrocentric analysis between the Sporobolus species and O. thomaeum exposed an overall more complex polyploid structure than the more straightforward tetraploid and hexaploid compositions (SI Appendix, Fig. S2). Roughly half the hexaploid S. pyramidalis genome has the expected 3:1 pattern of syntenic blocks compared with O. thomaeum, while 57% is only 2:1. The pattern is similar for tetraploid S. stapfianus, where ~44% of syntenic blocks are 2:1 to O. thomaeum as expected and ~42% of blocks are 1:1 (SI Appendix, Fig. S2). Similar assembly issues were observed in the tetraploid chloridoid grass E. nindensis, where one to four regions were assembled for each syntenic region in O. thomaeum (10). These discrepancies, combined with differences between the estimated and assembled genome sizes, suggest the Sporobolus genomes were partially collapsed during assembly in homologous regions. S. pyramidalis and S. stapfianus may be segmental allopolyploids with varying degrees of homology between chromosomes from separate subgenomes. Partial collapse during assembly would result in divergent homologous regions assembling separately and highly similar regions collapsing, which is supported by the observation that the ratio of assembled syntenic blocks is maintained across large syntenic blocks and whole chromosomes in O. thomaeum. For instance, two homologous regions are assembled in S. pyramidalis for chromosomes 3 and 4 from O. thomaeum, while three regions in S. pyramidalis were identified for most of chromosome 2 in O. thomaeum. Similar patterns were observed between S. stapfianus and O. thomaeum. To account for these issues related to polyploidy, syntenic gene pairs and orthogroups were used for downstream comparative genomics and transcriptomics analyses between the Sporobolus genomes and other chloridoid grasses.

### Table 2. S. pyramidalis and S. stapfianus genome assemblies

| Assembly size, bp | S. stapfianus | S. pyramidalis |
|------------------|--------------|---------------|
| No. of contigs   | 1,079,709,429| 1,055,099,634 |
| Longest contig, bp| 74,381,802  | 60,313,943    |
| N50              | 19,421,551  | 21,593,735    |
| N90              | 6,548,862   | 7,502,351     |
| Repetitive elements, bp (%) | 428,320,271 (39.7) | 435,795,325 (41.3) |
| Genome GC content, % | 44.0 | 43.1 |
| No. of genes     | 52,808      | 51,207        |
| No. of transcripts| 61,021      | 62,960        |
| No. of CDS       | 61,021      | 62,960        |
| Median mRNA length, bases | 1,239 | 1,694 |
| Median CDS length, bases | 1,071 | 1,221 |
| CDS GC content, % | 52.6 | 51.9 |
| Median exons per transcript | 4 | 5 |
| Median exon length, bases | 148 | 150 |
| Median intron length, bases | 157 | 154 |
| No. of transcription factors | 2,722 | 2,897 |
| No. of genes with InterProScan entry (%) | 52,601 (99.6) | 51,023 (99.6) |
| No. of genes with GO biological process entry (%) | 34,874 (66.0) | 34,039 (66.5) |

Genome assembly statistics for both S. stapfianus and S. pyramidalis. N50 and N90 represent the length of the shortest contig for which longer and equal length contigs cover at least 50 % and 90% of the assembly respectively. GC represents the guanine-cytosine contents and CDS represents the number of protein coding sequences for each genome.

Transcriptional Landscape of Desiccation in S. pyramidalis and S. stapfianus. We generated RNAseq data from RNA isolated from leaf tissues at different stages of dehydration for both species (SI Appendix, Fig. S3). Differentially expressed genes were identified using edgeR (25), and the resulting gene lists were assigned to GO biological process categories enrichment using the Cytoscape (23) plugin Bingo (24). These analyses indicate that S. pyramidalis and S. stapfianus transcriptomes respond differently to dehydration and share few biological process adaptations during the drying process. When water content decreases from 3 to 2 grams of water (gH2O) g−1 dry weight (dw), S. pyramidalis exhibits a strong response with 11,978 statistically differentially abundant transcripts (SDATs), in contrast to the more moderate response of 1,776 SDATs in S. stapfianus (Fig. 2 A and B). A GO enrichment analysis of SDAT lists further demonstrates that during the 3 to 2 gH2O g−1 dw water content transition, few biological processes are shared between the two species (Fig. 2 C and D and SI Appendix, Fig. S4). Some biological process categories, including response to heat and response to reactive oxygen species, are common to both species (SI Appendix, Fig. S4). Moreover, while S. pyramidalis responds to the change in water content from 3 to 2 gH2O g−1 dw by modulating processes involving the ribosome and the cell wall, S. stapfianus initiates alterations in the abundance of transcripts that relate to the response to oxidative stress, response to water deficit, and protein refolding (SI Appendix, Fig. S4).

As dehydration advances from 2 to 1.5 gH2O g−1 dw in S. pyramidalis, the functional categories of SDATs remain relatively unchanged from that activated at the initial loss of water, and as it is undergoing senescence during the 1.5 to 1 gH2O g−1 dw transition, further acclimation appears unlikely. By contrast, S. stapfianus exhibits an increase to 3,730 SDATs during the 2 to 1.5 gH2O g−1 dw transition, but starting at the 1.5 to 1 gH2O g−1 dw transition, it initiates a major remodeling of its transcriptome (SI Appendix, Fig. S3), as indicated by a significant increase to 14,557 and 16,047 SDATs during these two transitions in water content, respectively (Fig. 2D). Global transcriptional remodeling continues during the 0.75 to 0.5 gH2O g−1 dw transition.

### Table 3. Genome assemblies BUSCO v4 statistics vs. the grass (poales_odb10) dataset

| S. pyramidalis | S. stapfianus |
|----------------|--------------|
| Complete BUSCOs | 4,575 (93.5) | 4,521 (92.4) |
| Complete and single-copy BUSCOs | 2,162 (44.2) | 3,421 (69.9) |
| Complete and duplicated BUSCOs | 2,413 (49.3) | 1,100 (22.5) |
| Fragmented BUSCOs | 65 (1.3) | 76 (1.6) |
| Missing BUSCOs | 256 (5.2) | 299 (6.0) |

Data are presented as the no. of BUSCOs (%).
Sporobolus pyramidalis

Sporobolus stapfianus

Oropetium thomaeum

Fig. 1. Microsnytety within Chloridoideae grasses. A collinear region between O. thomaeum, S. stapfianus, and S. pyramidalis is highlighted, reflecting the ploidy of each species (diploid, tetraploid, and hexaploidy, respectively). Genes are shown in blue and green, and syntenic gene pairs are connected by gray lines.

dw transition, albeit at a lower degree, with 8,146 SDATs (Fig. 2D). When desiccated S. stapfianus plants are rehydrated, another strong transcriptome reprogramming, with 27,280 SDATs 12 h after rehydration, is evident and shifts to a transcriptome functional expression profile more similar to that of the fully hydrated control (SI Appendix, Fig. S3). Although S. stapfianus appeared morphologically fully recovered after 24 h of rehydration, the transcriptional profile is not equivalent to that observed in leaves of plants with a water content of 3 gH2O g⁻¹ dw (SI Appendix, Fig. S3), with 24,659 SDATs between the two conditions (Fig. 2F). Leaves from plants 24 h after rehydration have up-regulated SDATs classified in ribosome biogenesis GO categories and down-regulated SDATs in photosynthesis categories, as well as remnants of stress-responsive adaptations, including the response to water categories, and altered metabolism, suggested by the presence of glucose 6-phosphate, fructose 1,6-bisphosphate, and several other metabolism-related categories (SI Appendix, Fig. S4B).

Syntenic Ortholog Transcripts Comparison of the Hydrated State in S. pyramidalis and S. stapfianus. To directly compare the transcriptomes for S. stapfianus and S. pyramidalis and identify differentially regulated transcripts that relate to the differences between the two species in the hydrated state prior to dehydration, we created a custom list of syntenic ortholog “genes” (Materials and Methods). Differential expression was accomplished using a contrast S. stapfianus vs. S. pyramidalis in edgeR (23), and the resultant syntenic ortholog gene lists were probed with GO enrichment as described previously for the intraspecies dehydration transcriptomic analyses. The analyses demonstrate that S. stapfianus and S. pyramidalis have very different transcriptional landscapes under hydrated conditions that reflect functionally different priorities for each species. The S. stapfianus transcriptome significantly favors nitrogen, starch, and photosynthetic metabolic processes, whereas the S. pyramidalis transcriptome significantly favors processes involved in growth, primarily the biogenesis of cell wall components (SI Appendix, Fig. S5A). These differences are also reflected at the cellular component and molecular levels (SI Appendix, Fig. S5 B and C), with the majority of cellular functions related to the chloroplast and photosystems in S. stapfianus and the symplast, cytoskeleton, cell wall, and cell wall modification activities in S. pyramidalis.

Syntenic Ortholog Protein Comparisons of Desiccation Response in S. pyramidalis and S. stapfianus. To further compare the response of S. pyramidalis and S. stapfianus to dehydration, we performed a proteomic analysis using young leaves at 3 and 1.5 gH2O g⁻¹ dw and focused on proteins encoded by syntenic genes in a comparison of enriched GO biological process categories of accumulating and decreasing proteins in both water content conditions (SI Appendix, Fig. S6). At 1.5 gH2O g⁻¹ dw, S. pyramidalis had increased accumulation of proteins that are almost exclusively involved in stress responses; S. stapfianus had increased accumulation of stress response proteins but also, accumulated proteins involved in the response to misfolded proteins and protein catabolism (SI Appendix, Fig. S6A), and it decreased the abundance of proteins involved in energy production (SI Appendix, Fig. S6B). The protein data demonstrate that, as observed for the transcriptomic profiles, S. pyramidalis and S. stapfianus follow predominantly different approaches of protein accumulation in their response to dehydration.

Expression Dynamics of DS and Desiccation-Tolerant Grasses. To explore the evolution of VDT in the Chloridoideae subfamily of grasses, we made use of several high-quality genomes with similar dehydration expression datasets that were available for this group of grasses: the desiccation tolerant (S. stapfianus, O. thomaeum, and E. nindensis) and the DS (E. tef and S. pyramidalis). To facilitate comparisons between species with different ploidy, we
clustered genes into syntenic orthologs using MCScan (25) and orthologous groups (orthogroups) using OrthoFinder (26) and compared expression patterns between genes in the same orthogroups. We identified 49,418 orthogroups from OrthoFinder containing 806,075 genes across 23 diverse land plant genomes and focused the subsequent analyses on orthogroups, orthologs, or syntenic gene pairs present in the genome of all chloridoid grasses.

We first surveyed the global expression profiles of the five Chloridoid grasses under well-watered, drought/desiccation, and rehydration conditions using transformed expression data of 19,267 shared syntenic orthologs across all species. We applied a dimensionality reduction on the resulting expression matrix through principal component analysis. The first two principal components collectively explain 62% of the variance and separate the expression datasets by species and stress (Fig. 3). Well-watered RNASeq samples are found in a single tight cluster of all five species, while desiccation and rehydration samples are found in dispersed but distinct clusters. Samples from dehydration and rehydration time courses in the DT species fall into two clusters, with *E. nindensis* and *O. thomaeum* samples intertwined in one cluster and *S. stapfianus* in the second. The dehydration samples from the two DS species (*E. tef* and *S. pyramidalis*) clustered together in a third distinct cluster. *E. nindensis* and *O. thomaeum* are separated by relative water content in principal component (PC)1 and by dehydration vs. rehydration in PC2, but interestingly, they are not delineated by species. Together, these results indicate that expression patterns are broadly conserved in leaf samples of all species but that dehydration and rehydration samples are distinct between the three lineages of DT species and their DS relatives.

Samples from dehydration and rehydration time courses in the DT species fall into two clusters, with *E. nindensis* and *O. thomaeum* samples intertwined in one cluster and *S. stapfianus* in the second. The dehydration samples from the two DS species (*E. tef* and *S. pyramidalis*) clustered together in a third distinct cluster. Samples of *E. nindensis* and *O. thomaeum* are separated by relative water content in principal component (PC)1 and by dehydration vs. rehydration in PC2, but interestingly, they are not delineated by species. Together, these results indicate that expression patterns are broadly conserved in leaf samples of all species but that dehydration and rehydration samples are distinct between the three lineages of DT species and their DS relatives.

The same leaf RNASeq data were analyzed in a pairwise fashion to identify genes with significantly increased transcript abundance under dehydrating conditions in all five species. These SDATs were clustered based on orthogroup using OrthoFinder (as described above) and compared between species. Orthogroups were used in this set of analyses as they contained more genes than the synteny-based analyses, and orthogroups
have better resolution of recently duplicated genes. Across the five sequenced chloridoid grasses, the largest number of up-regulated orthogroups under dehydrating conditions was observed between the two *Sporobolus* species (Fig. 4), as expected since they are sister taxa. The second largest number of up-regulated orthogroups was shared between the two *Sporobolus* species and *O. thomaeum* (Fig. 4), which is consistent with their phylogenetic placement within the Chloridoideae. Many other orthogroups are up-regulated similarly in all VDT species are enriched in 214 biological process GO terms (*SI Appendix*, Fig. S7). Highly enriched GO terms include ultraviolet UV light response, chlorophyll catabolism, reactive oxygen species (ROS) metabolism, seed dormancy maintenance by abscisic acid (ABA), and gene expression in response to heat stress, among others (*SI Appendix*, Fig. S7A). These GO terms are consistent with well-characterized processes related to DT. Other GO terms with a lower magnitude of enrichment include those related to lipids, osmoprotectant biosynthesis, high light response, energy metabolism, protein degradation, and ABA signaling (*SI Appendix*, Fig. S7B and C). Seventy-one biological process GO terms were uniquely up-regulated in only the DS species (*SI Appendix*, Fig. S8). These included several terms related to salicylic acid as well as ethylene and ABA signaling, arabinose biosynthesis, cell wall biogenesis, and notably, leaf senescence, among others (*SI Appendix*, Fig. S4). We then asked whether any of the GO terms uniquely up-regulated in DT species would overlap with those uniquely down-regulated in DS species and vice versa (*SI Appendix*, Table S3). The GO term “protein folding” was uniquely up-regulated in DT and down-regulated in DS species. Across these five species, most seed-related orthogroups are up-regulated similarly (*SI Appendix*, Fig. S9). There are no seed orthogroups that are up-regulated in all three DT species without also being up-regulated in one or more DS species.

**Expansion of ELIPs.** ELIPs have a conserved role in photoprotection during desiccation, and they have undergone massive tandem gene duplication in all sequenced resurrection plant genomes surveyed to date (14). We observed a similar duplication of ELIPs in the *Sporobolus* genomes (Fig. 5A). The *S. stapfianus* genome has 65 ELIPs in three tandem arrays, and the *S. pyramidalis* genome has 30 ELIPs in two tandem arrays (Fig. 5B). The largest array in *S. stapfianus* has 49 ELIPs compared with 17 in its corresponding homologous region, suggesting the duplications occurred after the divergence of the two *S. stapfianus* subgenomes. Both *O. thomaeum* and *S. stapfianus* have large tandem arrays of ELIPs, but the duplication events originated from different syntenic orthologs. The total number of ELIPs in *S. pyramidalis* is higher than some other desiccation-tolerant species, but when gene counts are normalized for ploidy, the ELIPs are within the range of other sensitive grasses.

ELIPs have little to no detectable expression in well-watered tissue, but they are highly induced in desiccating *S. stapfianus* leaf tissue after they reach 1.0 g H2O g⁻¹ dw, and their expression continues 12 and 24 h postrehydration (Fig. 5). ELIPs are also up-regulated under drought in *S. pyramidalis*, and this occurs quickly in the dehydration process at 2.0 and 1.5 g H2O g⁻¹ dw. However, their combined expression is less than *S. stapfianus* (Fig. 5C), similar to what has been observed in other DS grasses (14).

**Discussion**

The genomic resources we developed for the sister species *S. stapfianus* and *S. pyramidalis* offer a robust contrast that facilitates a strong comparison between a VDT and a DS grass species. The addition of the genomic resources from other resurrection grasses, *O. thomaeum* (8) and *E. nindensis* (10), broadens the comparison further into the Chloridoideae subfamily of grasses. The two genome assemblies revealed the complex mixed ploidy of these two grasses, with *S. stapfianus* primarily tetraploid and *S. pyramidalis* primarily hexaploid. The structural complexity of the two genomes likely contributed to
the inability to assemble the genomes into chromosome-level contigs or to record sequenced genome sizes equivalent to those determined cytologically. The increase in ploidy between the two species probably occurred immediately after the divergence of the *S. pyramidalis* clade from the common ancestor of the two species (18). The assemblies did not reveal any genomic structural characteristics, with the exception perhaps of tandem arrays of ELIP genes (14), that could be attributed to the difference in VDT between the two species, which is consistent with the general observation that there is not a genomic “blueprint” for VDT in resurrection species (4). However, the assemblies did allow for a thorough comparative analysis, both structural and functional, of the gene space for each genome, and coupled with the in-depth transcriptome data, we were able to explore a detailed genomic assessment of the dehydration/desiccation responses within the *Sporobolus* sister species contrast.

The generation of transcriptomic and proteomic data for dehydrating young leaf tissue at specific water contents during a dry-down experiment such that the dehydration levels are survivable for both grasses provides a broad assessment of the stress response for each species. DS *S. pyramidalis* mounted a messenger RNA (mRNA)-level response to an initial drop in hydration as has been observed for the majority of dehydration-sensitive plants (27, 28). However, as dehydration to 1.5 gH₂O g⁻¹ dw was reached, the transcript abundance response declined dramatically (Fig. 2A), perhaps as the leaf water content reached a critical level for *S. pyramidalis*. The leaves of *S. pyramidalis* are wilted at 1.5 gH₂O g⁻¹ dw (21) but otherwise, appear undamaged, so it is tempting to speculate that the decline in the transcript abundance response may be related to wilting and perhaps, loss of turgor during wilting in *S. pyramidalis*. Although *S. pyramidalis* responds quickly to a loss of water, the early increased transcript abundance response appears to be focused on protein translational processes and transcripts common to heat and cold stress (SI Appendix, Fig. S4), and only later, as dehydration deepens, do transcripts associated with proline metabolism (osmoregulation) and redox proteins, common to water-deficit responses (27), accumulate. The early decline in transcripts involved in photosynthesis and...
cell wall homeostasis is also common to the dehydration response in most angiosperms (4). The later decline in transcripts that are associated with general biosynthetic processes is consistent with the general lack of a metabolic response to dehydration seen in metabolite profiling studies of *S. pyramidalis* at similar levels of water loss (21). Desiccation-tolerant *S. stapfianus*, in contrast, exhibited a significantly different qualitative transcriptional response to dehydration with a low-magnitude response in the early phase of dehydration. With the comparatively muted response and although there are some common transcript abundance responses between the two species, *S. stapfianus* clearly targets remodeling a completely different functional aspect of the transcriptome than does *S. pyramidalis* at similar water contents. Indeed, it appears that *S. stapfianus* targets the accumulation of transcripts that function more in stress-related activities unlike *S. pyramidalis*, which does not. The differences between the two transcriptional responses for the two species were unexpected as other studies have indicated that there was extensive overlap in functionality of the transcriptomes of both sensitive and tolerant grasses exposed to dehydration (10). Although there are a few common transcript abundance functional categories in the early response to dehydration in both species, it is clear that the overall transcriptome remodeling during dehydration is very different between the two species, exemplified by the different dehydration thresholds for the accumulation ELIP transcripts.

For *S. stapfianus*, the primary remodeling of the transcriptome during dehydration appears to occur as the plants reach the 1.0- to 0.75 gH₂O g⁻¹ dw part of the drying curve, which appears to be a critical period in the desiccation response of all resurrection angiosperms studied so far (29) and concurs with early microarray data (30). In *S. stapfianus*, the transition from 1.0 to 0.75 gH₂O g⁻¹ dw occurs during leaf curling (19) and is likely at water contents just prior to and during a change in membrane fluidity that occurs as leaf water potentials approach −12 MPa (4). The functional aspects of the transcriptome remodeling during desiccation of *S. stapfianus* leaves have been documented previously and are in accord with the observation that transcript abundance is concordant with changes in metabolism associated with cellular protection aspects of DT (30). There was a dramatic alteration of the transcriptome upon rehydration of *S. stapfianus* leaves, which likely reflects the complex nature of the dehydration event. The magnitude of the change in the transcriptome, reflecting a change in abundance of at least half of the known transcripts, and the functional processes they represent indicate not only the stress incurred from the inrush of water and mechanical aspects of cellular expansion but also, the need to repair damage (from both desiccation and rehydration), reactivate energy metabolism, and reactivate the physiological integrity of the cells and tissues (4). The observation that transcripts encoding proteins involved in ribosome biogenesis are accumulated and those encoding proteins involved in photosynthesis have not recovered control levels at 24 h following rehydration highlights the extent of the impact that desiccation and rehydration have on plant cells and tissues even in DT plants. *S. stapfianus* requires between 48 and 72 h to regain the structural and physiological integrity seen in well-watered plants (19, 31).

The remodeling of the transcriptome in response to dehydration starts from two very different resting-state (fully hydrated) transcriptomes. Our functional analysis of the gene-level expression of the syntenic orthologs of the sister grasses, although somewhat confounded by the structural complexity of the two genomes, revealed that for *S. stapfianus*, the biosynthesis of starch and nitrogen compounds was perhaps a priority for young leaves under normal conditions, while for young leaves of *S. pyramidalis*, the priority appeared more focused on the construction of cell walls. Although somewhat speculative, the increase in nitrogen compounds, primarily amino acids from a combination of new synthesis and redistribution, was the focus of a recent study that demonstrated that these compounds are apparently used to fuel central metabolism or for other metabolic adjustments related to the acquisition of DT, such as osmoregulation (32). The differences in priorities are consistent with the changes in protein abundance from 3 to 1.5 gH₂O 1 gDW. Although *pyramidalis* protein abundance changes did not reflect cell wall processes, perhaps due to the difficulty in extracting the majority of wall-related proteins (33), they show that *pyramidalis* was almost exclusively focused on the accumulation of stress response proteins. At the same desiccation stage, *S. stapfianus* had similarly accumulated stress response proteins but also, proteins involved in protein catabolism, and it had down-accumulated energy-related proteins, suggesting a scaling down, at the protein level, of the energy metabolism transcriptomic activity of the hydrated state and the continuation of N metabolism prioritization through protein salvage, possibly from misfolded proteins. Syntenic orthologs transcriptomic data are also consistent with information from the metabolomes of young leaves of these two grasses in that fully hydrated leaves of *S. stapfianus* were focused on the accumulation of a variety of amino acids and photosynthetic derivatives, while for *S. pyramidalis*, the metabolome was focused on energy metabolism and growth (21). The conclusion from these transcriptomics analyses was that leaves of *S. stapfianus* were prepared (primed) for a dehydration/desiccation event by accumulating osmolytes in times of water abundance and that *pyramidalis* needed to generate energy and components to support a faster growth rate, perhaps to deal with competition in its more mesic habitats. The hydrated transcriptome functional analysis fully supports this conclusion, and our transcriptomic and proteomic data, although somewhat speculative in nature, extend the hypothesis to include a focus on the maintenance of chloroplast function in *S. stapfianus* in the priming mechanism and cell wall biogenesis in *S. pyramidalis* as a target for the focus on energy metabolism and growth.

Although transcriptomic analyses were useful in comparing the functional aspects of the response to dehydration of the contrasting sister Sporobolus species and the desiccation and dehydration response of *S. stapfianus*, the ability of a high-quality genome for each of these two species allowed for a direct comparison of the genetic components (and their functions) of the response and allowed us to extend the comparison with other desiccation-tolerant and DS grass species. The broad comparison of the expression patterns of orthogroups and syntenic gene sets common in all five of the chloridoid grasses included in the analysis confirmed the disparate nature of the dehydration response between *S. stapfianus* and *S. pyramidalis*. It also revealed that the overall dehydration expression pattern for *S. stapfianus* was distinctly different from those observed for the other two desiccation-tolerant grasses, *E. nindensis* and *O. thomaeum*. The most recent phylogenetic analyses of the Chloridoideae indicate that the common ancestor of the Eragrostidaceae, which contains *E. nindensis* and *E. tef*, gave rise to the Zoysiae and the Cynodontaeae, within which *O. thomaeum* resides; the Zoysiae then diversified into the Zoysiinae and the Sporobolinae, within which the Sporobolus clade containing both *S. stapfianus* and *S. pyramidalis* is located (18, 34). The phylogeny indicates that *O. thomaeum* and *S. stapfianus* are closer to one another than either are to *E. nindensis*, which is consistent with results of our analysis of orthogroups representing SDAIs that increase in abundance. However, the overall expression response to dehydration for *O. thomaeum* appears to be more similar to the distantly (ancestrally) evolved response of *E. nindensis*. This might also explain why there is less overlap between the dehydration transcriptome of *S. stapfianus* and the transcriptomes of both sensitive and tolerant
grasses exposed to dehydration (10). Thus, although we have used only a three-way comparison, it does allow for the hypothesis that the desiccation response of *S. stapfianus* represents a more recent evolution of a mechanism for VDT within the Chloridoideae.

The orthogroup analysis of the SDATs that increase in abundance in all of the DS species underscored the importance of most of the well-characterized processes that deliver cellular DT (4). The orthogroup analysis of the SDATs that increase in abundance in all of the DS species also reconfirmed what we understand of the response of most plants to a water deficit stress and highlighted the induction of senescence, which is thought to be blocked in resurrection angiosperms during desiccation (reviewed in ref. 4). However, the observation that transcripts classified as involved in protein folding accumulate in the VDT species and decline in abundance in the DS species indicates not only that maintaining protein structure is important in VDT; as has been well documented, but that the lack of the necessary components to do so might be critical in rendering a plant DS. The observation that all seed-related orthogroups are up-regulated in all VDT species and in one or more of the DS species reinforces the hypothesis that VDT likely evolved from a reprogramming of DT mechanisms that evolved in seeds (10).

**Materials and Methods**

**Plant Material.** *S. stapfianus* Gandoger (original provenance: Verena, Transvaal, South Africa) and *S. pyramidalis* Beauv. (also known as *Sporobolus indicus var. pyramidalis*) were grown and maintained as described in ref. 21. For genome sequencing, a single, healthy 3-mo-old fully hydrated plant from each species was selected, and young leaf tissue was collected, flash frozen in liquid N₂, and stored at −80 °C.

**Sampling and Drying Treatments.** Plants were grown and maintained and seed stocks were included (as described in ref. 35) in 1-gallon pots under greenhouse conditions. Three-month-old plants were subjected to a drying event by withholding water. *S. stapfianus* plants were dried until desiccated (after 3 wk), whereas *S. pyramidalis* plants were dried to a water content of −1.5 g H₂O g⁻¹ dw before rewatering. Drying rates were as described by Oliver et al. (21) to simulate field drying rates that occur over a period of ~7 d to reach the 1.5 g H₂O g⁻¹ dw stage for both grasses and −14 d for full desiccation of *S. stapfianus* plants (were left dry for a further 7 d). Young leaf tissue was collected at daily intervals, between 9 and 10 AM, from individual plants, flash frozen in liquid N₂, and stored at −80 °C. Dried plants were maintained dry for a week before rehydration. Duplicate samples were harvested for leaf cotyledon measurements at the time of sampling. The water content was calculated as fresh weight minus the dry weight (dried to equilibrium at 70 °C for 4 h). Triplicate samples were chosen for RNA extraction. Rehydration was achieved by placing the desiccated *S. stapfianus* plants under a continuous misting system in the greenhouse, and young leaves were sampled in triplicate at 12 and 24 h following the addition of water.

**Estimation of the Genome Size (1C Value) Using Flow Cytometry.** The genome size was estimated using the one-step flow cytometry procedure described in ref. 36. Approximately 1 cm² of leaf material from the *Sporobolus* species and leaf material of the calibration standard *Petraselium crispum* (Mill.) Fuss (37) (haploid genome [1C] value = 2.201 Mbp) were diced in 1 mL of "general purpose buffer" (GBP) (38) supplemented with 3% polyvinylpyrrolidone of average molecular weight of 40,000. A further 1 mL of GBP was added, and the homogenate was filtered through a 30-μm nylon mesh (Celtrix 30-μM mesh; Sysmex); 100 μL propidium iodide (1 mg/mL) was added and incubated on ice for 10 min. The relative fluorescence of 5,000 particles was recorded using a Partec Cyflow SL3 flow cytometer (Partec GmbH) fitted with a 100-mW green solid-state laser (532 nm; Cobalt Samba). Three replicates of species were processed, and output histograms were analyzed using FlowMax software v2.4 (Partec GmbH).

**DNA Isolation, Library Preparations, and Sequencing.** High–molecular weight DNA was isolated from 5 g of flash-frozen young leaf tissue using the PacBio SampleHei-Shared Protocol (https://www.pacb.com/support/documentation) as described. Random shotgun genomic libraries with various insert sizes, both paired end and mate pair libraries, were constructed for the Illumina HiSeq 2000 sequencing system (Illumina) according to the manufacturer’s protocols. Sequencing of was conducted using an Illumina HiSeq 2500 ultrahigh-throughput DNA sequencing platform (Illumina) at the DNACore facility at the University of Missouri, Columbia, MO (https://dnacore.missouri.edu/ngs.html).

For Chicago sequencing, genomic DNA isolation, library preparation, sequencing, and assembly were conducted by Dowtell Genomics and are detailed in SI Appendix, Methods. Chicago genomic DNA libraries were prepared as described in ref. 39. Dowtell Hi-C library reads were prepared as described in ref. 40 after fixation of chromatin in place by incubation of leaf tissue for each species in 1% formaldehyde for 15 min under vacuum.

**Assembly of the *S. stapfianus* and *S. pyramidalis* Genomes.** A de novo assembly was conducted using a combination of paired end (mean insert size ~350 bp) libraries and mated pair libraries with insert ranging from 7 to 12 kbp. De novo assembly was performed using Meraculous v2.2.5 (diploid mode 1) (41) with a k-mer size of 109. Reads were trimmed for quality, sequencing adapters, and mate pair adapters using Trimmomatic (42). The de novo assembly, shotgun reads, Chicago library reads, and Dowtell Hi-C library reads were used as input data for HiRise, a software pipeline designed specifically for using proximity ligation data to scaffold genome assemblies (39) and detailed in SI Appendix, Methods.

**RNA Extraction and RNA Sequencing.** RNA was extracted from young leaf samples using the RNeasy (Qiagen) kit with RLC buffer following the manufacturer’s protocol. The RNA isolates were treated with deoxyribonuclease 1 and cleaned using the DNA-free RNA Kit (Zymo Technologies). RNA quality was assessed by use of a fragment analyzer (Advanced Analytical Technologies), and concentration was determined with a Nanodrop Spectrophotometer (ThermoFisher). RNA libraries were individually bar-coded from 2.7 μg of template total RNA utilizing the TruSeq RNA Sample Prep Kit (Illumina) as described in the manufacturer’s protocol. Libraries were pooled in groups of 12 and sequenced (12 samples per lane) on an Illumina HiSeq 2500 ultrahigh-throughput DNA sequencing platform (Illumina) at the DNACore facility at the University of Missouri.

**Full-Length Sequencing (Iso-Seq) and De Novo Transcriptome Assembly.** High-quality RNA was extracted from whole-roots tissues obtained from seedlings at the four-stage leaf when the first pair of leaves had matured, whole seedlings at the two-stage leaf, mature leaves, young leaves, floral inflorescences, and tissue samples identical to those used for the dehydration/desiccation/rehydration transcriptomes. The RNASeqs were pooled for each individual species for subsequent amplification. Bar-coded SMART MRRT libraries were prepared and sequenced on the PacBio platform with X SMRT cells by Novogene Corporation Inc. Sequence reads were processed using Iso-Seq (https://github.com/PacificBiosciences/IsoSeq).

**Genome Annotation.** Genome assemblies were annotated using three rounds of MAKER-P. Briefly, round 1 used full-length nonchimeric sequences from Pacbio transcriptome sequencing as EST evidence; a collection of Arabidopsis thaliana [Araport11 (43)], Zea mays [downloaded from Gr.amene’s ftp server at https://www.granome.org/ftp-download; ACPv4 release 59 (44, 45), Sorghum bicolor [downloaded from Phytozome; https://phytozome-next.jgi doe.gov/pz/portal.html; version 3.1.1 (46)], and O. thomaeum [downloaded from Phytozome, version 1.0 (7)] sequences as protein evidence; and a de novo repeats library obtained using LTR Finder (47), LTRharvest (48), LTR retriever (49), and RepeatModeler (50) as inputs. Round 2 used the round 1 maker gff file and an SNP (http://korfilab.ucsd.edu/software/html) hmm file obtained from the round 1 gff3 file. Round 3 used the round 2 maker gff3 file, the GeneMark-ES (51) HMM output file from a BLASTER (52) run from hisat (53) aligned RNASeq reads, and the corresponding Augustus (54) gene prediction models.

As a further filter, we decided to only keep genes that had expression evidence in our RNASeq illumina or Pacbio data and/or whose corresponding protein is homologous to a known plant protein. Evidence of expression was at least one of the following two criteria: 1) an expression value of at least one transcripts per million (TPM) in all replicates of at least one sample in the RNASeq data after bowtie2 (55) alignment and Salmon (56) quantification or 2) at least one TPM in the gff file obtained after a minimap2 (57) alignment and Stringtie (58) quantification of isoSeq3 polished long reads. *Sporobolus* proteins were considered as homologous if they satisfied at least one of three criteria: 1) a blastp match with an e value of 1e-6 or lower vs. either
Cross-Species Expression Analyses. Differential expression (DE) analyses were conducted using DESeq2 (63) (E. nidiness, E. tef, and O. thamaeum) or edger (23) (S. stapfianus and S. pyramidalis), and resulting outputs were processed using Pandas 0.25.0 in Python 3.6.8. Up-regulated and down-regulated genes were extracted, and GO enrichment was conducted using Fisher’s exact test via the weightGO algorithm. Following enrichment, unique biological process GO terms were extracted using the Python library Pandas. Unique GO terms for DS as compared with DT were also extracted for further study.

**GO Categories Enrichment Analysis for the spstansus vs. S. pyramidalis Comparison.** A comparison of gene expression of the S. stapfianus vs. S. pyramidalis leaves at 3 g H2O g−1 dw was achieved using tximport (66) and edgeR (23). We created a custom syntentic orthologs tx2gene file ([https://bioconductor.org/packages/release/bioc/vignettes/tximport/inst/doc/tximport.html](https://bioconductor.org/packages/release/bioc/vignettes/tximport/inst/doc/tximport.html)). GO annotation files for both species were merged, replacing each gene identifier with the custom gene identifier from our tx2gene file. In this way, each gene inherits the GO annotation of all its corresponding S. stapfianus and S. pyramidalis genes (SI Appendix, Methods). GO categories enrichment analysis was carried out for the list of up-regulated both_n genes and the list of down-regulated both_n genes using Bingo (24) in Cytoscape (67), with a false discovery rate (FDR)-adjusted P value cutoff of 0.05 and the list of genes in our tx2gene file as the universe.

**Protein Extraction and Proteomics Analysis.** Proteins were extracted from triplicate samples of 1 g of frozen leaf tissue, separated on 16-cm sodium dodecyl sulfate polyacrylamide gel electrophoresis gels, and cut into 10 equal slices; each slice was digested with trypsin, and liquid chromatograph mass spectrometer (LCMS) data were acquired on the LTQ Orbitrap at the Charles W. Gehrke Proteomics Center, University of Missouri using standard protocols ([http://proteomics.missouri.edu/protocols.php](http://proteomics.missouri.edu/protocols.php)). Raw data were analyzed with MaxQuant software v. 2.0.1.0 (68). Tandem mass spectrometer spectra were searched against the S. pyramidalis and S. stapfianus proteins, and potential contaminants by the built-in Andromeda search engine (69). Label-free quantification (LFQ) of the identified proteins was performed using normalized LFQ (LFQ intensity) using the MaxLFQ algorithms (70). The resulting identified proteins were filtered, keeping only proteins with an LFQ intensity greater than zero in all biological replicates or absent in all biological replicates. Proteins with significant Student’s t test (two tailed; P < 0.05) results were considered up- and down-accumulated (log, fold change > 0.5) or down accumulated (log, fold change < 0.5). The lists of up- and down-accumulated protein identifiers were translated to their corresponding syntentic ortholog identifiers, and GO biological process categories enrichment was done using Bingo previously.

**Data Availability.** The two genomes, Illumina DNA-seq, Chicago-seq, and Hi-c data are available from the CyVerse CoGe genome evolution site ([https://genomeweb.com/CoGe/index.pl](https://genomeweb.com/CoGe/index.pl), [https://genomeweb.com/ogoe/GenomeInfo.pl?gid=54807](https://genomeweb.com/ogoe/GenomeInfo.pl?gid=54807)). The S. stapfianus RNASEq data and the S. pyramidalis RNASeq data can be found at the National Center for Biotechnology Information Sequence Read Archive (BioProject accession nos. PRJNA19555 and PRJNA19804, respectively) (71, 72).

**ACKNOWLEDGMENTS.** We acknowledge the expert technical assistance of Jim Eldjer in the preparation and growth of the plant material. We also thank Dr. Brian Mooney and the Charles W Gehrke Proteomics Center for their expertise in the proteomics analysis. This work was partially supported by Gov- ernor University Research Initiative Program of the State of Texas Grant 05-2018 (to L.R.H.E.), NSF Grant MCB-1817347 (to R.V.), and Agricultural Research Services Project 5070-21000-038-00D (to M.J.O.).

1. M. J. Oliver, Z. Tuba, B. D. Mishler, The evolution of vegetative desiccation tolerance in land plants. Plant Biol. 151, 85–100 (2000).
2. M. J. Oliver, J. Velten, B. D. Mishler, Desiccation tolerance in bryophytes: A reevaluation of the primitive strategy for plant survival in dehydrating habitats? Integ. Comp. Biol. 45, 788–799 (2005).
3. C. F. Delwiche, E. D. Cooper, The evolutionary origin of a terrestrial flowering plant: A Drosophyllum morphiophyllum. Curr. Biol. 25, 8895–8910 (2015).
4. M. J. Oliver et al., Desiccation tolerance: Avoiding cellular damage during drying and rehydration. Annu. Rev. Plant Biol. 71, 435–460 (2020).
5. L. Xiao et al., The resurrection genome of Boea hygrometrica: A blueprint for survival of dehydration. Proc. Natl. Acad. Sci. U.S.A. 112, 5833–5837 (2015).
6. M. D. Costa et al., A framework of tolerance for desertification of the genome of Xerophyta viscosa. Nat. Plants 3, 17038 (2017).
7. R. VanBuren et al., Single-molecule sequencing of the desiccation-tolerant grass Oro- petum thomaeum. Nature 527, 508–511 (2015).
8. R. VanBuren, C. M. Wai, J. Kellwagen, J. Pardo, A chromosome-scale assembly of the model desiccation-tolerant grass Oryzopsis thomaeum. Plant Direct 2, e00096 (2018).
9. R. VanBuren et al., Desiccation tolerance evolved through gene duplication and network rewiring in Lindernia. Plant Cell 30, 2943–2958 (2018).
10. J. Pardo et al., Intertwined signatures of desiccation and drought tolerance in grasses. Proc. Natl. Acad. Sci. U.S.A. 117, 10879–10888 (2020).
11. Z. Xu et al., Genome analysis of the ancient tracheophyte Selaginella tamariscina reveals evolutionary features relevant to the acquisition of desiccation tolerance. Mol. Plant 11, 983–994 (2018).
12. R. VanBuren et al., Extreme haplotype variation in the desiccation-tolerant clubmoss Selaginella lepidophylla. Nat. Commun. 9, 13 (2018).
13. A. T. Silva et al., To dry perchance to live: Insights from the genome of the desiccation-tolerant biocrust moss Syntrichia caninervis. Plant J. 105, 1339–1356 (2021).
14. R. VanBuren et al., D. Man Wai, S. Evans, D. Bartels, Massive tandem proliferation of EF-Tu paralogs converges on evolution of desiccation tolerance across land plants. Plant Physiol. 179, 1040–1049 (2019).
15. M. C. D. Costa et al., Key genes involved in desiccation tolerance and dormancy across all life forms. Plant Sci. 251, 162–168 (2016).
16. E. Fischer, B. Schaferhoff, K. Muller, The phylogeny of Linderniaceae—The new genus Lindernia, and new combinations within Botanaya, Craterostigma, Lin- dernia, Micranthemum, Torenia and Vandellia. Willdenowia 43, 209–238 (2013).
