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Authors

Ma, Chao
Wang, Hong
Macnish, Andrew J
et al.

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ARTICLE

Transcriptomic analysis reveals numerous diverse protein kinases and transcription factors involved in desiccation tolerance in the resurrection plant *Myrothamnus flabellifolia*

Chao Ma[1]*, Hong Wang[2]*, Andrew J Macnish[1], Alejandro C Estrada-Melo[1], Jing Lin[2], Youhong Chang[2], Michael S Reid[1] and Cai-Zhong Jiang[3]

The woody resurrection plant *Myrothamnus flabellifolia* has remarkable tolerance to desiccation. Pyro-sequencing technology permitted us to analyze the transcriptome of *M. flabellifolia* during both dehydration and rehydration. We identified a total of 8287 and 8542 differentially transcribed genes during dehydration and rehydration treatments respectively. Approximately 295 transcription factors (TFs) and 484 protein kinases (PKs) were up- or down-regulated in response to desiccation stress. Among these, the transcript levels of 53 TFs and 91 PKs increased rapidly and peaked early during dehydration. These regulators transduce signal cascades of molecular pathways, including the up-regulation of ABA-dependent and independent drought stress pathways and the activation of protective mechanisms for coping with oxidative damage. Antioxidant systems are up-regulated, and the photosynthetic system is modified to reduce ROS generation. Secondary metabolism may participate in the desiccation tolerance of *M. flabellifolia* as indicated by increases in transcript abundance of genes involved in isopentenyl diphosphate biosynthesis. Up-regulation of genes encoding late embryogenesis abundant proteins and sucrose phosphate synthase is also associated with increased tolerance to desiccation. During rehydration, the transcriptome is also enriched in transcripts of genes encoding TFs and PKs, as well as genes involved in photosynthesis, and protein synthesis. The data reported here contribute comprehensive insights into the molecular mechanisms of desiccation tolerance in *M. flabellifolia*.

INTRODUCTION

Tolerance to desiccation can be defined as the ability of an organism to survive extreme dehydration and then to resume normal growth upon rehydration.[1] The phenomenon of desiccation tolerance is common in seeds and non-tracheophytes.[2] Vascular plants with the ability to revive from extreme desiccation are commonly called as resurrection plants.[3] To date, more than 300 angiosperms, including a few dicotyledonous plants, have been identified as resurrection plants.[1,4]

Tolerance to desiccation in resurrection plants is a complex process that involves many physiological and metabolic mechanisms.[1–3] The strategies adopted by resurrection plants to survive desiccation may resemble those seen in seeds.[5,6] Water deficit is initially accompanied by osmotic adjustment to protect against cellular damage, which is accomplished by the accumulation of large amounts of sugars, amino acids, and small polypeptides such as late embryogenesis abundant (LEA) proteins and dehydrins.[1] Increasing water deficit results in the activation of mechanisms to cope with desiccation-induced structural and functional alterations of macromolecules and membranes, the accumulation of toxic substances and free radicals, and mechanical damage associated with the loss of turgor.[1] Under desiccation stress, the oxidative damage caused by the production of reactive oxygen species (ROS) is enhanced, especially in chloroplasts. Two different strategies are adopted by resurrection plants to minimize ROS damage. Homoiochlorophyllous resurrection plants conserve the structure of their photosynthetic apparatus, synthesize anthocyanins, and increase the activity of antioxidants during desiccation, whereas poikilochlorophyllous resurrection plants demolish chloroplast pigments and membranes, thus reducing cellular sources of ROS.[6]

Resurrection plants are excellent models for studying molecular mechanisms that could potentially serve as biotechnological tools for enhancing drought tolerance.[7] There have been many studies of the molecular effects of drought on model plants such as *Arabidopsis* and rice,[8,9] but information is scarce on the desiccation tolerance mechanisms of resurrection plants.[2,10] Transcriptomics has been used to generate global gene expression atlases, such as for powdery mildew resistance,[11] horticultural traits in apple,[12] fruit ripening,[13] and cherry fruit development.[14] To understand desiccation tolerance mechanisms, transcriptomic and proteomic studies have been performed in resurrection plants such as *Sporobolus stapfianus*,[15] *Tortula ruralis*,[16] *Xerophyta viscosa*,[17] and *Boea hygrometrica*.[18] However, large-scale expressed sequence tag sequencing analysis using high-throughput sequencing and *de novo* assembly strategies have only been conducted in two herbaceous species, *Craterostigma plantagineum*[6,19] and *Haberlea rhodopensis*.[20]

Myrothamnus flabellifolia, a woody homoiochlorophyllous resurrection plant growing in the mountainous regions of central and southern Africa,[21] is probably the most primitive angiosperm to show extreme tolerance to desiccation.[22] It displays novel anatomical, ultra-structural, and biochemical adaptations to desiccation stress[23]

[1]Department of Plant Sciences, University of California, Davis, Davis, CA 95616, USA; [2]Institute of Horticulture, Jiangsu Academy of Agricultural Sciences/Jiangsu Key Laboratory for Horticultural Crop Genetic Improvement, Nanjing 210014, China and [3]Crops Pathology and Genetic Research Unit, United States Department of Agriculture, Agricultural Research Service, Davis, CA 95616, USA. *These authors contributed equally to this work.
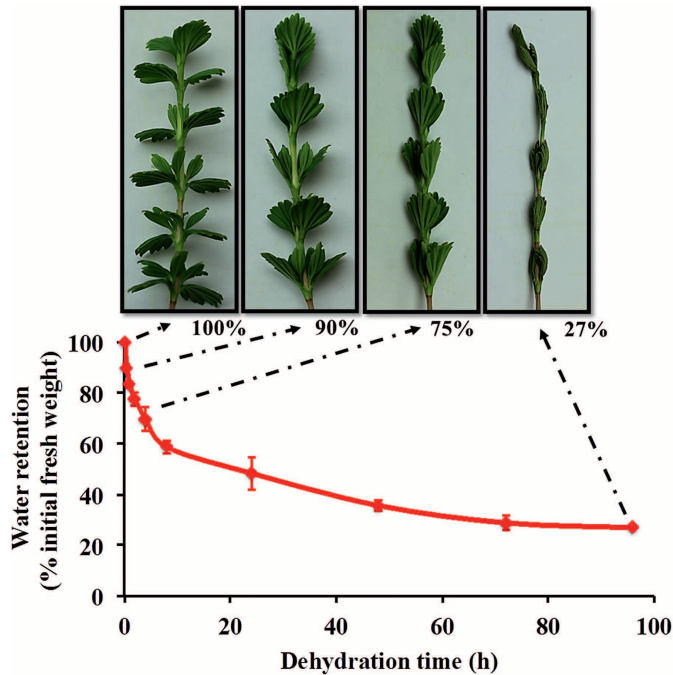Correspondence: Cai-Zhong Jiang (cjiang@ucdavis.edu)

Figure 1. Water loss curve of *M. flabellifolia*. Water loss is expressed as the percentage of IFW at each time point. Results are the means of three biological replicates ± SD.

and has been the subject of a number of physiological and biochemical studies. Under severe drought conditions, *M. flabellifolia* leaves can become air-dry, folding in a unique fan-like manner.[24] Upon re-watering, the leaves return to their original color and shape within 24 hours.[25] Biochemical studies of the cell walls have demonstrated an

abundance of arabinose polymer side chains, consisting of arabinans associated with the pectin matrix, which may explain the flexibility of the mesophyll cells, allowing leaf morphology to be rapidly recovered after rehydration.[22,26] During desiccation, presumably to protect from ROS, the leaves synthesize large amounts of anthocyanins and change their color from green to dull-brown.[21,23] High levels of phenols, including tannins, arbutin, and 3,4,5 tri-*O*-galloylquinic acid, have been reported in the leaves of *M. flabellifolia*.[27–29] Differences in phenolic content and composition may be associated with differences in tolerance to desiccation stress among populations of *M. flabellifolia*.[30] Moreover, desiccated leaves of *M. flabellifolia* contain high levels of saccharides, although the precise constituents differ.[29,31,32] The molecular mechanisms underlying the tolerance of *M. flabellifolia* and its ability to rapidly rehydrate are still largely unknown. In the present work, we attempt to investigate the transcriptome dynamics in response to dehydration and rehydration in *M. flabellifolia*. Most woody fruit and ornamental plants can encounter extreme drought stress. Therefore, understanding this plant's extreme tolerance to desiccation and drought can aid the development of strategies for improving drought stress resistance in horticultural crops.[33,34]

MATERIALS AND METHODS

Plant materials and dehydration treatment

M. flabellifolia plants were kindly provided by Dr. Matthew Opel (University of Connecticut) and grown in containers in the greenhouse at the University of California, Davis under a 16 h/8 h day/night cycle. Current season branches from two-year-old plants were used for the treatments. For the dehydration treatment, fully hydrated branches were harvested and placed in a high humidity chamber for 30 min to reduce any potential initial wounding effects. Dehydration was performed by placing the cut branches in a temperature-controlled (20 ℃) room at approximately 40% relative humidity. Mature leaves were collected when the branches weighed 90% (onset of leaf wilting), 75% (leaf wilted), or 27% (desiccated) of their initial fresh weight (IFW) (Figure 1). For rehydration, desiccated branches were submerged in water for 6 h (onset of leaf unfolding) and 12 h (leaves recovered). Mature leaves from all these stages were collected and immediately frozen in liquid
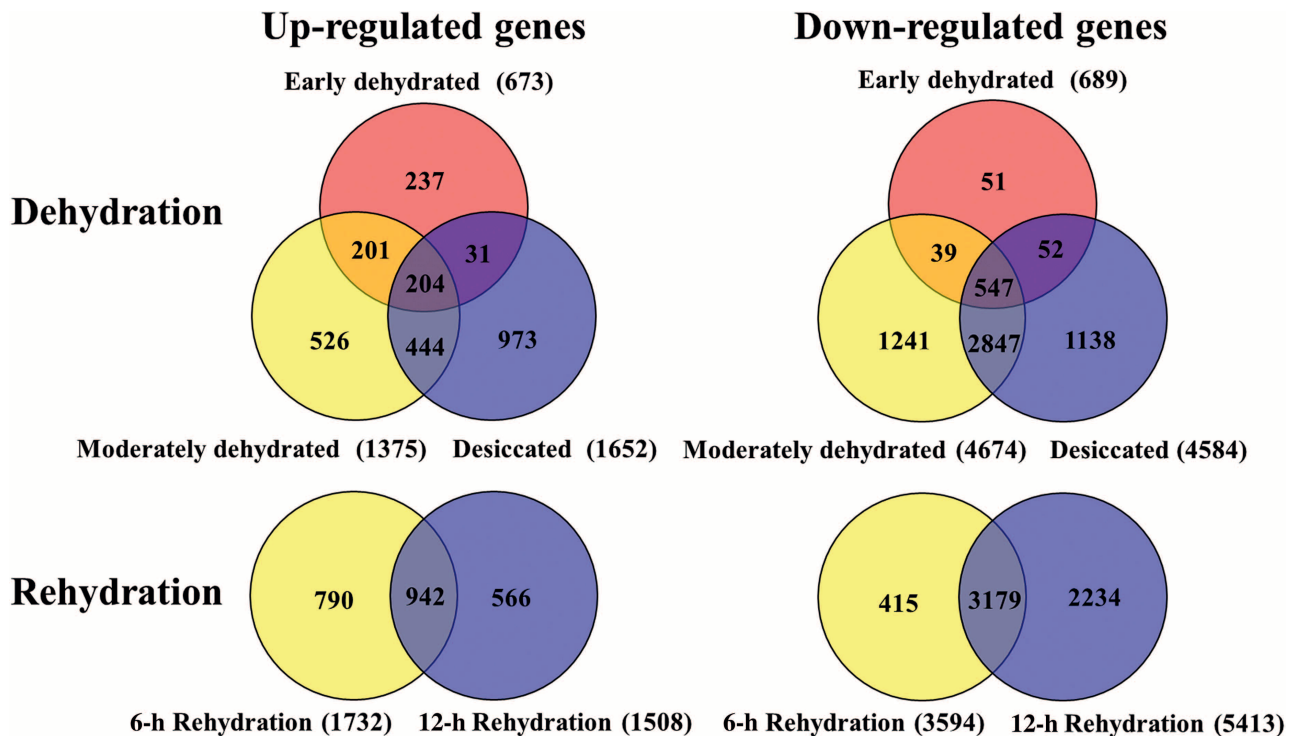

Figure 2. Venn diagrams showing common genes significantly up- or down-regulated in dehydration and rehydration processes.

Table 1. Summary of *M. flabellifolia* sequencing data

	Control	Early dehydration	Moderate dehydration	Desiccation	6-h rehydration	12-h rehydration	Total
No. of reads	20 329 227	23 606 535	22 923 710	23 274 062	23 792 776	24 482 833	13 840 9143
No. of contigs	23 686	23 625	22 895	22 619	22 751	22 837	25 235
Average contig length (bp)	2024	2034	2062	2070	2069	2050	1988

nitrogen and stored at −80℃ until use. Three biological replicates were mixed for the preparation of an RNA-Seq library.

Total RNA extraction and RNA-Seq library preparation
Total RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA) and treated with RNase-free DNase I (Promega, Madison, WI, USA) to remove any contaminating genomic DNA. RNA libraries

were constructed according to the manufacturer's instructions and sequenced on a HiSeq2000 Illumina platform.

Cleaning and assembly of RNA-Seq sequenced reads
The quality of the raw reads was examined before and after trimming using FastQC software (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Low-quality reads with a *Phred* quality score less than 20, sequences
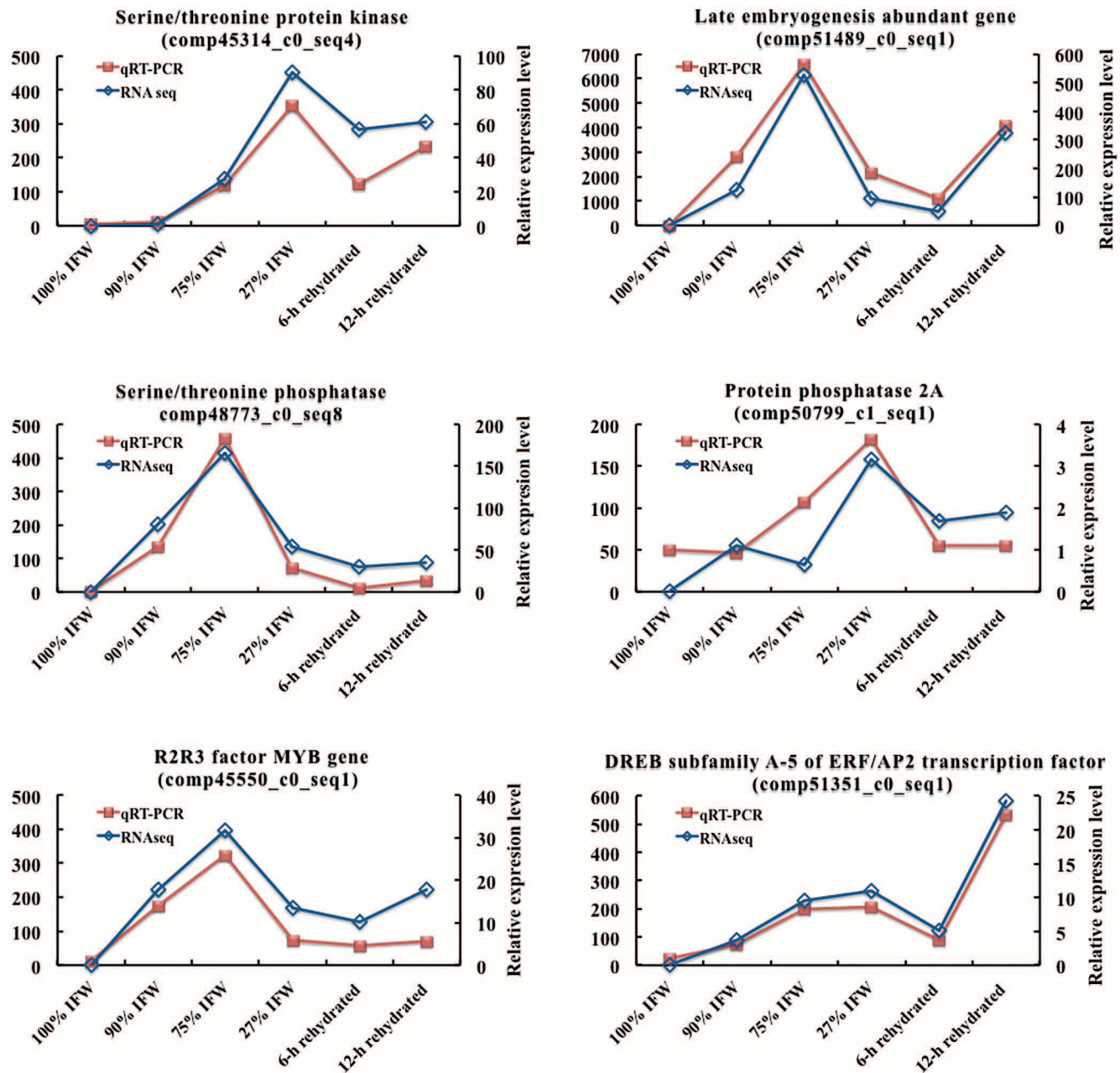

Figure 3. Validation of RNA-Seq data by qRT-PCR. RNA was extracted from leaf tissue taken at the indicated percentage of IFW under dehydration and at the indicated times of rehydration. Actin (comp22823_c0_seq1) was used as an internal control. The results are the means of three biological replicates ± SD.
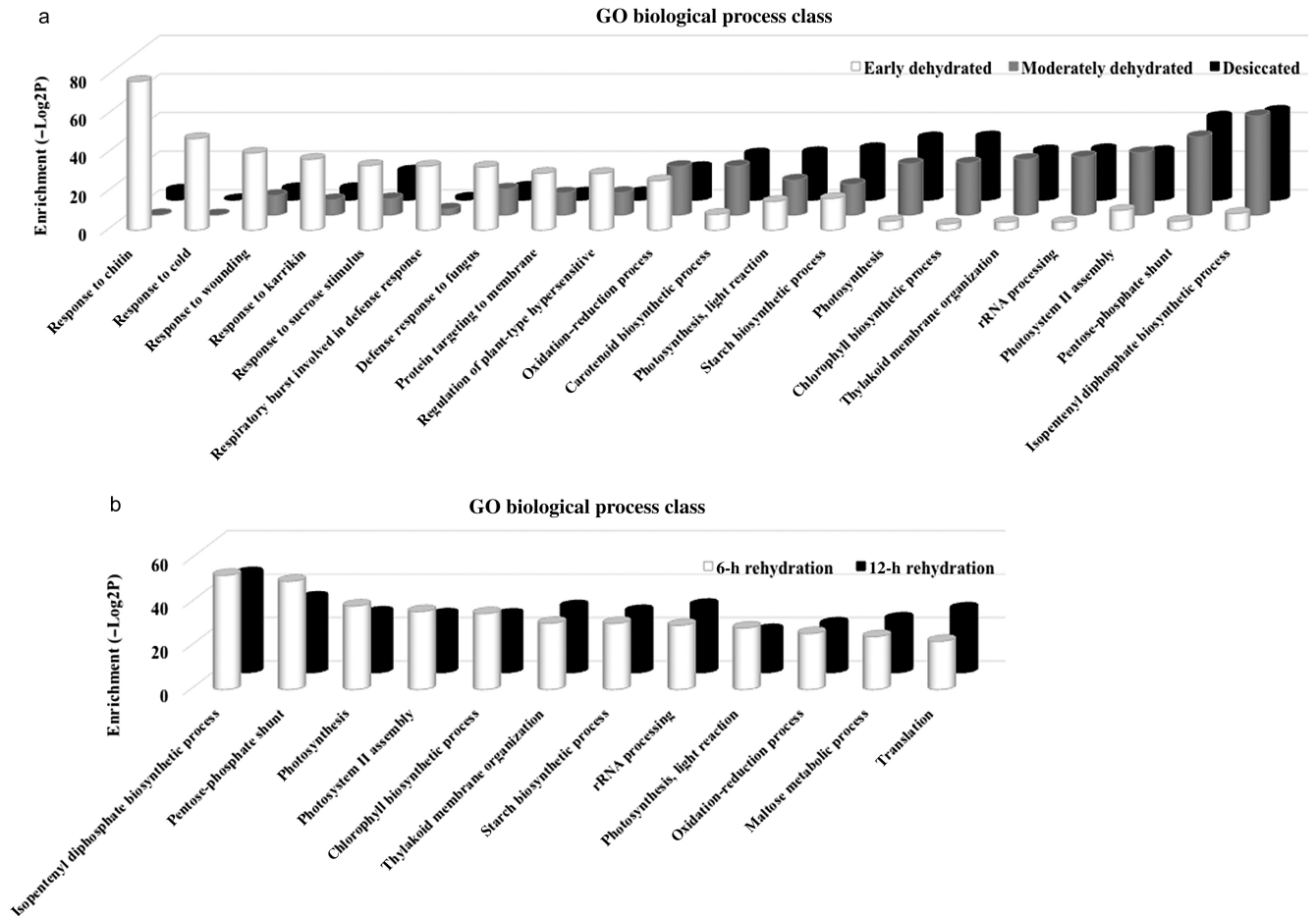
a

GO biological process class

b

GO biological process class

Figure 4. GO functional classification analysis of DTGs during dehydration (**a**) and rehydration (**b**). Histograms representing enriched functional distributions in each stage.

shorter than 40 bp, barcodes, polyA, polyT ends and adapter sequences were removed. High-quality clean reads were assembled *de novo* into contigs using the Trinity program.[35] The resulting contigs were blasted against the PlantGDB and *Arabidopsis* protein databases with a cutoff E-value of $1e^{-6}$.

Quantitative real-time PCR analysis
To examine the transcript abundance of selected genes, total RNA was extracted from leaves of three replicate branches. First strand cDNA was synthesized using 2 μg total RNA, oligo (dT) primers, random hexamers, and SuperscriptIII reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR (qRT-PCR) was performed with the 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Green Real-time PCR Master Mix (Applied Biosystems).[36] To normalize sample variance, *actin* (comp22823_c0_seq1) was used as an internal control.[37] Relative quantification of the expression of each gene was performed using the $2^{-\Delta\Delta CT}$ method.[38] The primers used for determining transcript abundance are listed in Supplementary File 3.

Annotation and pathway enrichment
Gene ontology (GO) terms were assigned to the assembled contigs based on their homologues in the *Arabidopsis* protein database. GO term enrichment was performed by a hypergeometric distribution test with Bonferroni multiple testing correction. To investigate metabolic pathways, we compared the contigs with their orthologs in the *Arabidopsis* KEGG (http://www.genome.jp/dbget/). Transcription factors (TFs) were identified and classified as such if their *Arabidopsis* homologs were identified in the *Arabidopsis* TF database (http://arabidopsis.med.ohio-state.edu/AtTFDB/). Protein kinases (PKs) were identified and classified as such if their *Arabidopsis* homologs

were identified in the list of PKs in the PlantsP database (http://plantsp.genomics.purdue.edu/index.html).

Hierarchical clustering
GenePattern (http://genepattern.broadinstitute.org/gp/pages/index.jsf) was used for hierarchical clustering. Default options were used and the Pearson correlation was performed on gene expression data.

RESULTS AND DISCUSSION

Sequencing and transcriptome assembly of *M. flabellifolia*
Six RNA-Seq libraries of *M. flabellifolia* were constructed from leaves of branches that were fully hydrated (control), slightly dehydrated (90% IFW), moderately dehydrated (75% IFW), desiccated (27% IFW), and rehydrated for 6 h or 12 h (Figure 1). A total of 141 262 112 paired-end raw reads of 100 nucleotides (nt) were generated using the Illumina platform. These raw sequences were further filtered and cleaned up, resulting in 138 409 143 paired-end clean reads (Table 1). The proportion of clean reads to total raw reads was over 98% in each library. These high-quality reads generated 25 235 contigs by *de novo* assembly, with an average contig length of 1988 nt (Table 1). We annotated the assembled contigs through homologous searching (BlastX) against the PlantGDB database. A total of 14 273 (56.6%) contigs had at least one homolog in the PlantGDB database. BlastX analysis against PlantGDB revealed that translated proteins from many of *M. flabellifolia*'s transcripts were similar to the protein
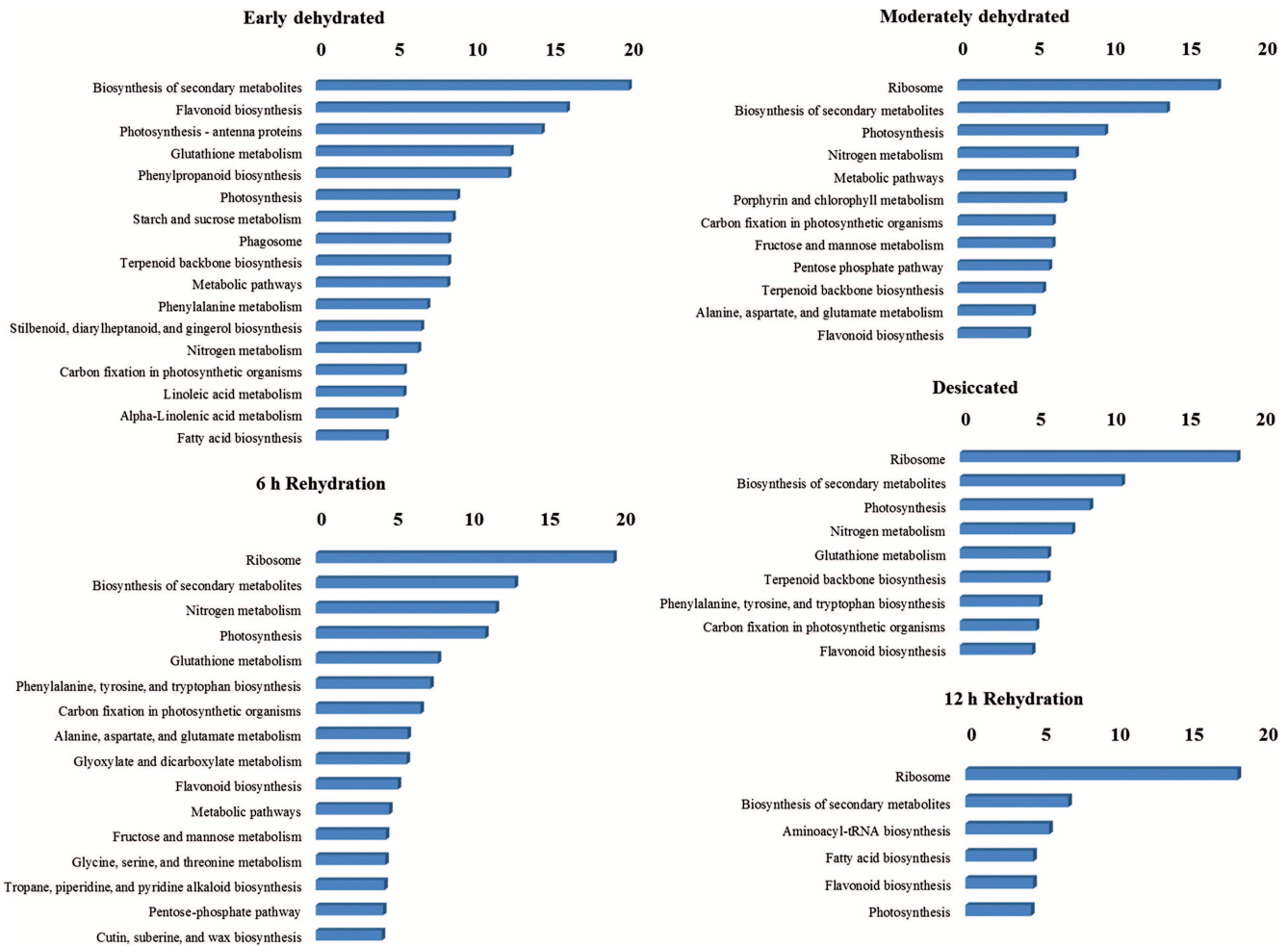
Figure 5. Enriched pathways of DTGs during dehydration and rehydration.

sequences in *Prunus persica* (30.4%), *Manihot esculenta* (28.5%), and *Populus trichocarpa* (24.9%) (Supplementary File 1).

Changes in transcriptome profiles during dehydration and rehydration

A total of 8287 differentially transcribed genes (DTGs) were detected in the dehydration treatments (Supplementary File 4). The overlapping regions of the Venn diagram in Figure 2 show that 204 DTGs were up-regulated and 547 DTGs were down-regulated during all the dehydration stages. The number of up- and down-regulated DTGs increased as desiccation progressed (Figure 2), indicating increasing molecular responses as dehydration stress increased. During rehydration, a total of 8542 DTGs were identified (Supplementary File 5). Among these DTGs, 942 up-regulated DTGs and 3179 down-regulated DTGs were present in both the 6 h and 12 h rehydration stages (Figure 2).

To validate the DTGs identified by RNA-Seq, we analyzed the transcript abundance of six randomly selected DTGs using qRT-PCR analysis. The results obtained from qRT-PCR analysis were generally consistent with the expression analysis generated from the RNA-Seq data (Figure 3).

Functional assignment of differentially transcribed genes during dehydration and rehydration

To evaluate the potential functions of genes that showed significant transcriptional changes during dehydration and rehydration in *M. flabellifolia*, we examined the GO enrichment of DTGs at each dehydration and rehydration stage. The biological processes that were significantly enriched early in dehydration included many defense responses such as response to chitin, cold, wounding, and fungi (Figure 4a). This is consistent with a previous transcriptomic study in the resurrection plant *H. rhodopensis* in which many genes involved in acquiring tolerance to a variety of abiotic and biotic stresses were induced by desiccation stress.[20] In striking contrast, the DTGs represented in moderately dehydrated and desiccated leaves participate in photosystem II assembly, thylakoid membrane organization, chlorophyll biosynthesis, and photosynthesis (Figure 4a). These data suggest that alteration of photosynthesis is an important strategy in response to desiccation for *M. flabellifolia*. Moreover, genes involved in isopentenyl diphosphate biosynthesis were enriched in moderately dehydrated, desiccated, and rehydrated leaves (Figure 4a and b). Isopentenyl disphosphate is a precursor of all plant isoprenoids,[39] which are a diverse group of metabolites including primary metabolites such as sterols, chlorophylls, carotenoids, quinones, and hormones (brassinosteroids, cytokinins, gibberellins, abscisic acid (ABA)) and secondary metabolites participating in plant defense and communication such as pigments, volatiles, and defense compounds.[40,41]

To further delineate the metabolic pathways participating in the dehydration and rehydration responses, we mapped the DTGs into the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. A total of 23 and 18 biochemical pathways were significantly enriched during dehydration and rehydration, respectively (Figure 5). During dehydration, consistent with the GO enrichment
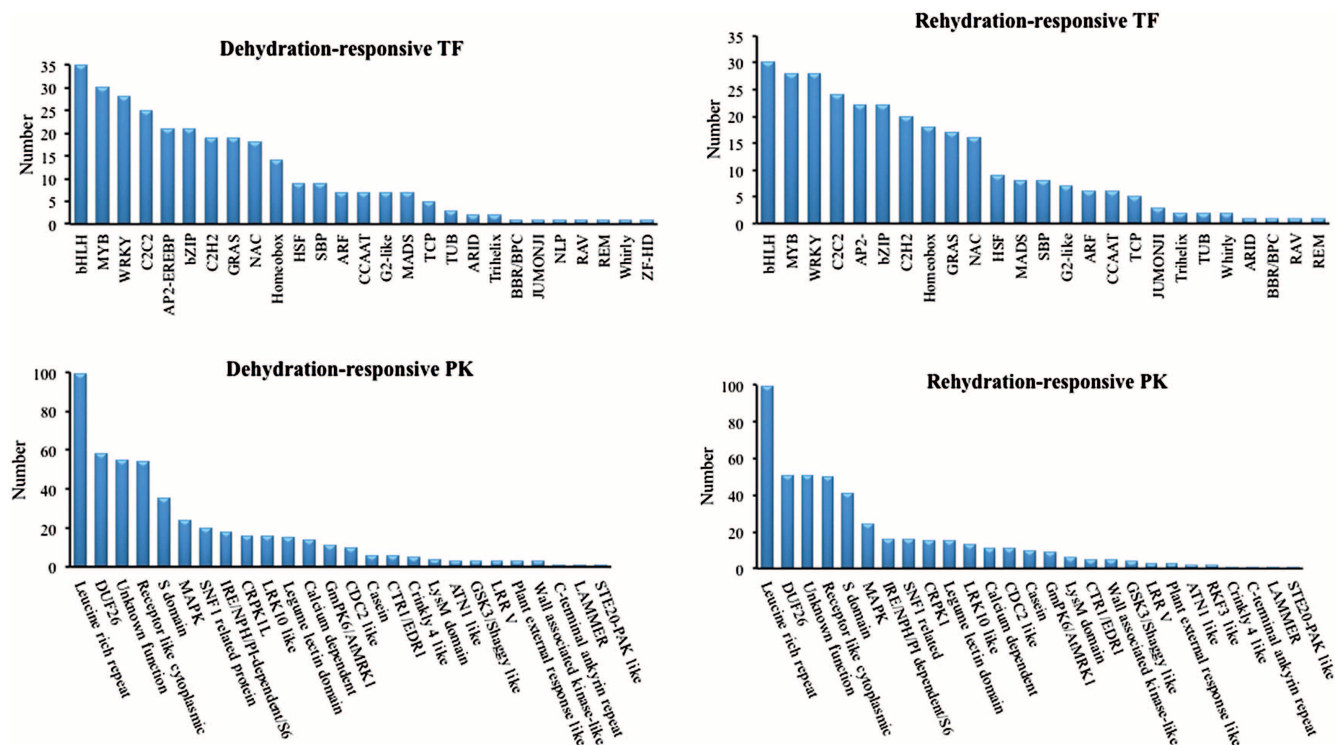
Figure 6. Distribution of dehydration- and rehydration-responsive transcription factors and protein kinases in *M. flabellifolia*.

analysis, the enriched biochemical pathways involved diverse metabolites, including secondary metabolites, starch, sucrose, fatty acids, fructose and mannose, and photosynthesis, suggesting a global response to desiccation in *M. flabellifolia*. As one example, the flavonoid biosynthesis pathway was significantly enriched during dehydration, consistent with the role of anthocyanins, a flavonoid class, in the 'green-to-brown'/'brown-to-green' leaf color alternation during dehydration and rehydration.[23]

During rehydration, photosynthetic genes were significantly enriched (Figure 5). This is consistent with a previous study in *M. flabellifolia* showing that photosynthesis resumes rapidly, reaching full capacity within three days of rehydration.[42] The recovery of vital functions and metabolic activities during rehydration is indicated by alterations of genes encoding a range of structural and physiological processes.[1] For example, genes encoding enzymes involved in ribosome and aminoacyl-rRNA biosynthesis, essential for the re-establishment of normal cellular metabolism, are enriched during rehydration (Figure 5).

Dehydration- and rehydration-responsive transcription factors and protein kinases in *M. flabellifolia*

Transcription factors (TFs) act as master switches of transcriptional reprogramming, inducing diverse protective mechanisms in response to abiotic stresses.[9,43,44] We identified a large number of TFs (295) responsive to dehydration, and 287 TFs were responsive to rehydration from among the DTGs (Supplementary Files 2 and 6). Among these TFs, the *bHLH*, *MYB*, and *WRKY* families were the largest groups during both dehydration and rehydration (Figure 6). Members of these three TF families are implicated in stress responses in model plants. In *Arabidopsis*, bHLH-type genes *AtMYC2* and *AtAIB* are involved in ABA signaling, and overexpression of *AtMYC2* or *AtAIB* enhances the drought tolerance of transgenic plants.[45,46] Multiple *MYB* genes have also been implicated in stress responses. For example, *AtMYB2* is involved in the ABA-dependent drought tolerance pathway,[45] and *AtMYB108* is associated with both biotic and abiotic stress responses.[47] *WRKY*

TFs are well known for their involvement in the regulation of plant development and in response to abiotic stresses.[48,49] Functional analyses of rice *OsWRKY11* and soybean *GmWRKY54* demonstrated that *WRKYs* are involved in drought signaling pathways.[50] SBP (SQUAMOSA promoter binding protein) and TCP TFs are known to be plant-specific developmental regulators.[51] Our results revealed that 9 SBP and 5 TCP type TFs also showed a response to dehydration in *M. flabellifolia* (Figure 6 and Supplementary File 6). Further functional characterization of these TFs may shed light on their roles in desiccation tolerance.

PKs are essential signaling regulators in the acquisition of desiccation tolerance. Among the DTGs, we identified strikingly large numbers that respond to dehydration (484 PKs) and rehydration (468 PKs) (Supplementary Files 2 and 7). PKs may play a key regulatory role in drought stress adaptation in *M. flabellifolia*, as corroborated by comparison of our data with *Arabidopsis*[52] and chrysanthemum drought stress expression profile data.[53] In *Arabidopsis*, only 121 PKs were found to be responsive to osmotic stress.[52] Xu *et al.* (2013) identified only 229 differentially expressed PKs during drought stress in chrysanthemum, a species with a large and complex polyploid genome and high heterozygosity.[54]

Protein family distribution analyses showed that three receptor-like kinase (RLK) type families – leucine-rich repeat (LRR) kinases, domain of unknown function DUF26, and receptor like cytoplasmic kinases – were the largest groups of DTGs in both the dehydration and rehydration treatments (Figure 6). RLKs constitute the largest PK family in plants[55] and have been implicated in the regulation of meristem proliferation, organ specification, reproduction, and hormone signal transduction.[55–57] Drought transcript profiling in *Arabidopsis* has revealed that the transcript abundance of many RLK genes peaks 1 h after the start of drought treatment, indicating that RLKs may also be part of a rapid drought response.[58] Several functional studies have also confirmed roles of RLKs in drought tolerance.[56] In *Arabidopsis*, an LRR kinase, receptor protein kinase 1 (RPK1), is induced by ABA. Repression of RPK1 decreases sensitiv-
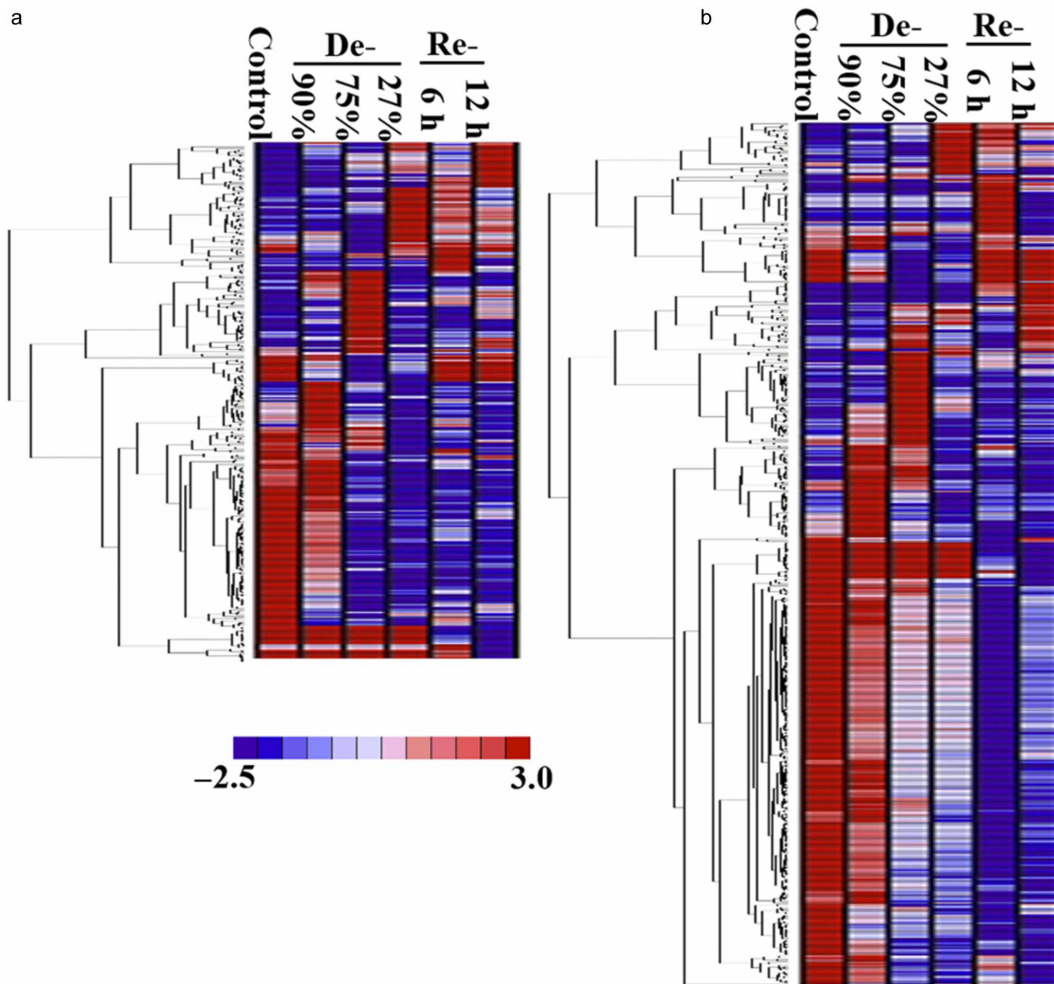
Figure 7. Hierarchical cluster analysis of differentially expressed transcription factors (**a**) and protein kinases (**b**) in *M. flabellifolia*. Log2 ratios are used to express the relative transcript abundance at the indicated percentage of IFW during dehydration (De-) or at the indicated time of rehydration (Re-) of leaves versus control hydrated leaves. Red squares indicate increased expression and blue squares indicate decreased.

ity to ABA, suggesting that RPK1 is involved in ABA perception.[59] A DUF26 type kinase, CRK36, and a receptor-like cytoplasmic type kinase, ARCK1, form a complex that negatively controls ABA and osmotic stress signal transduction.[60]

In addition, 24 DTGs encoding mitogen-activated protein kinases (MAPKs) were identified during dehydration stress in *M. flabellifolia* (Figure 6 and Supplementary File 7). MAPK cascades function in transducing environmental and developmental cues to intracellular responses.[61] In *Arabidopsis*, several MAPKs are involved in abiotic stress signaling pathways including MEKK1,[62] MPK1,[63] MPK3,[62,64,65] MPK4,[63] MPK6,[63] MAP9,[66] and MAP12.[66] The transcript abundance of homologs of all these MAPKs, apart from MPK6 and MAP12, was altered in response to dehydration in *M. flabellifolia* (Supplementary File 7).

To identify early responsive regulatory genes, the expression pattern of differentially transcribed TFs and PKs was analyzed by hierarchical clustering (Figure 7). The transcript abundance of 53 TFs and 91 PKs peaked early in dehydration (Figure 8, Supplementary Files 8 and 9), indicating that broad regulatory networks are quickly employed to coordinate global transcriptional reprogramming during desiccation of *M. flabellifolia*. Among the up-regulated TFs during early dehydration, the strongest induction was observed for genes encoding putative DREB family members (comp45691_c0_seq1, comp42806_c0_seq1, Supplementary File 8).

The DREB TFs are well-known regulators of ABA-independent drought stress signaling pathways in other plant species.[9,46,67] In addition, a total of nine genes encoding WRKY family members were included among the up-regulated TFs in early dehydration, making the WRKY family the biggest group of early dehydration up-regulated TFs (Supplementary File 8). Furthermore, among the up-regulated PKs, the LRK10 L kinase family is one of the largest groups in during early dehydration (13 out of total 91 PKs) (Supplementary File 9), suggesting that LRK10 L kinases may be important upstream regulators of rapid responses to water deficit. For example, the transcript abundance of a DTG (comp49162_c0_seq1) encoding an LRK10 L kinase increased nearly 120-fold in early dehydration (Supplementary File 9). LRK10 L genes have previously been reported to be associated with resistance to pathogen infection.[68–70] Functional characterization of these LRK10 L kinases will be an important step toward elucidating their roles in desiccation tolerance.

Induction of diverse mechanisms in response to desiccation stress in *M. flabellifolia*

ABA signaling. The plant hormone ABA plays a critical role during dehydration stress.[9,43] Evidence from earlier studies showed that ABA is also important for desiccation tolerance in resurrection plants such as *C. plantagineum*[71] and *Chamaegigas intrepidus*.[72] ABA con-
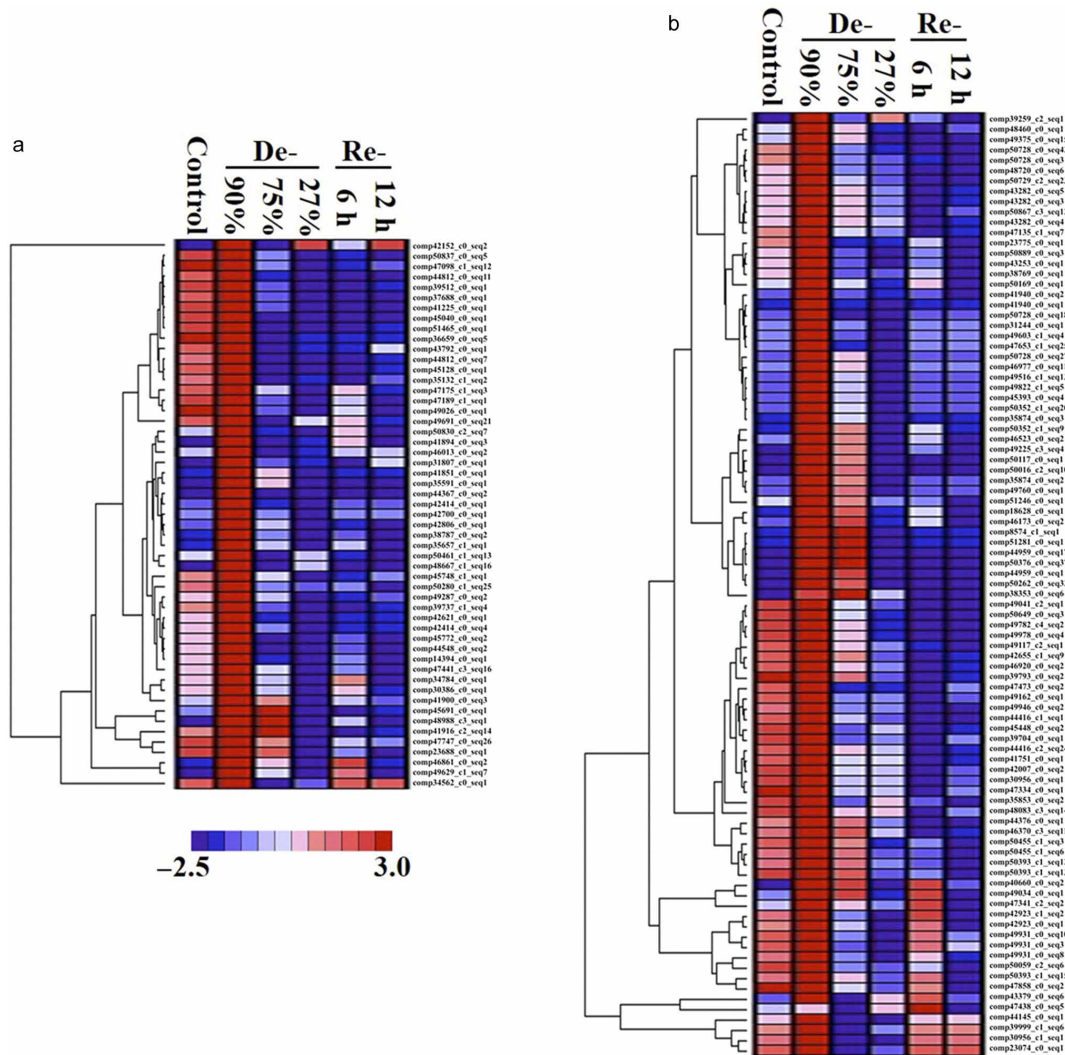
Figure 8. Hierarchical clustering analysis of early dehydration up-regulated transcription factors (**a**) and protein kinases (**b**) in *M. flabellifolia*. Log2 ratios are used to express relative transcript abundance at the indicated percentage of IFW under dehydration (De-) or at the indicated time of rehydration (Re-) of leaves versus hydrated leaves. Red squares indicate increased abundance and blue squares indicate decreased abundance.

tent increases in desiccated leaves of *M. flabellifolia*, and desiccation stress tolerance is enhanced by exogenous ABA treatment in *M. flabellifolia*.[73] In agreement with the physiological studies, our desiccation transcriptomic results showed that many ABA biosynthesis and signaling genes were up-regulated during desiccation (Figure 9 and Supplementary File 10). For example, transcripts of four homologs of the *nine-cis-epoxycarotenoid dioxygenase* (*NCED*) gene (comp41624_c0_seq1, comp47700_c0_seq4, comp49970_c0_seq2, comp40276_c0_seq1, Figure 9, Supplementary File 10), encoding the rate limiting enzyme in ABA biosynthesis, were increased during dehydration. Similarly, transcripts of several ABA signaling-related genes were also up-regulated, including *ABF2* (comp42333_c0_seq1), *ABI1* (comp47878_c0_seq10), and *HAI2* (comp48773_c0_seq8) (Figure 9 and Supplementary File 10).

Late embryogenesis abundant proteins. Many LEA proteins are synthesized when plants respond to desiccation, suggesting that these proteins may play an important role in desiccation tolerance.[43,74] In the resurrection plants *C. plantagineum* and *H. rhodopensis*, large-scale analysis of desiccation-tolerant transcrip-

tome data showed that up to nine LEA genes are highly accumulated in desiccated leaves.[6,20] In *M. flabellifolia*, transcripts of 18 DTGs encoding LEA proteins were highly accumulated and remained at relatively high levels throughout dehydration and rehydration (Table 2). Intriguingly, compared with those LEAs in *C. plantagineum*[6] and *H. rhodopensis*,[20] we found that highly related LEA orthologs were all up-regulated under desiccation stress among these three resurrection plants (Table 2), suggesting that specific LEA genes are employed to enhance desiccation stress tolerance in resurrection plants.

Photosynthesis. M. flabellifolia, as a homoiochlorophyllous resurrection plant, maintains its photosynthetic apparatus and chlorophyll under desiccation stress.[21] The advantage of this is the ability to rapidly resume photosynthesis during rehydration. However, retaining the photosynthetic apparatus during desiccation exposes plants to the risk of oxidative damage caused by the extra production of ROS by photosynthetic pigments, photosystems and cofactors. We found that the transcript abundance of several genes encoding antioxidants was increased by dehydration treatment
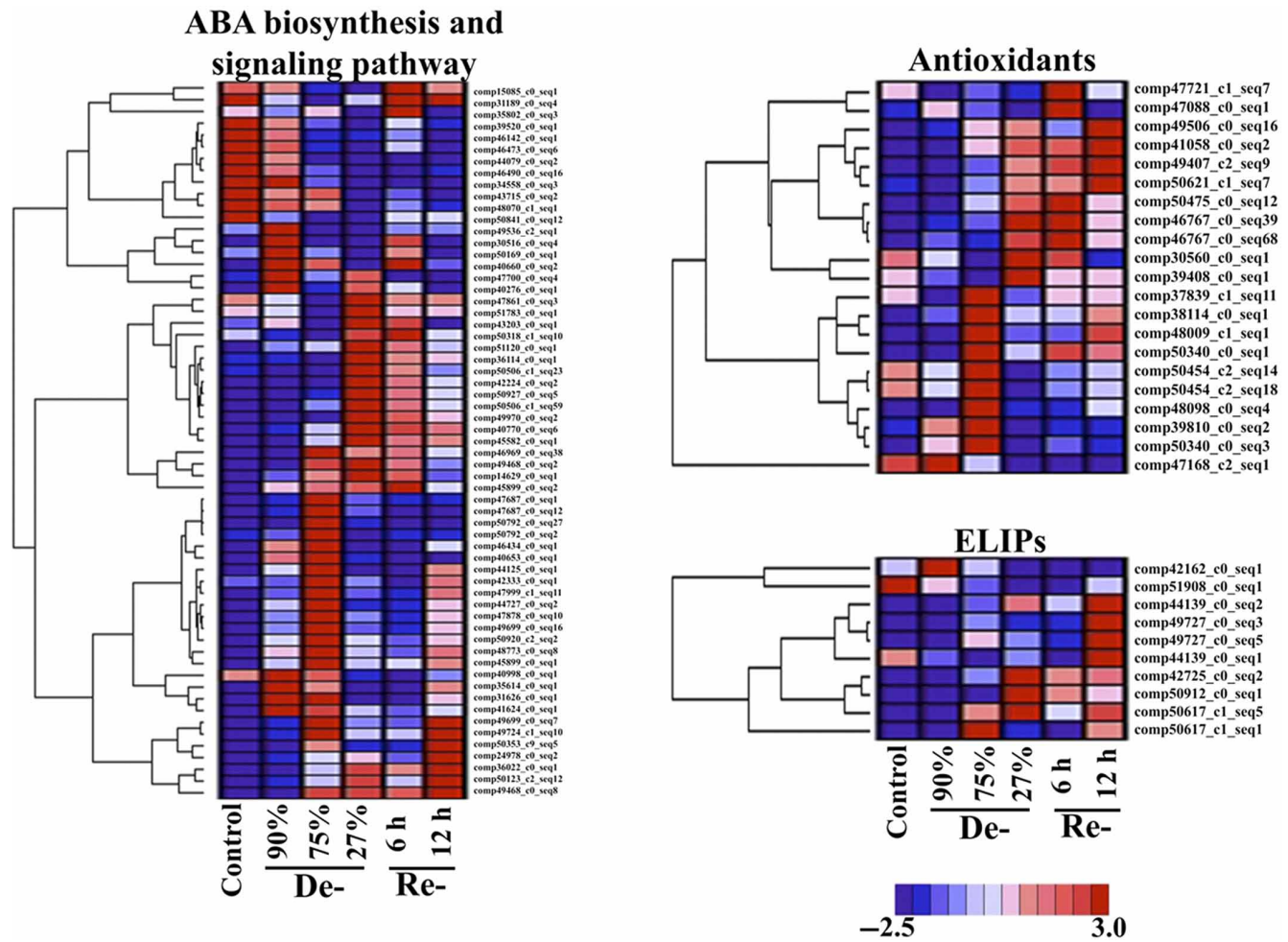
Figure 9. Representative pathways in de- and re-hydration treatments in *M. flabellifolia* transcript profiles. Log2 ratios are used to express relative transcript abundance at the indicated percentage of IFW during dehydration (De-) or at the indicated time of rehydration (Re-) of leaves versus hydrated leaves. Red squares indicate increased abundance and blue squares indicate decreased abundance.

Table 2. Expression profiles of LEA genes in *M. flabellifolia*

ContigID	Control	Early dehydration	Moderate dehydration	Desiccation	6-h rehydration	12-h rehydration	Putative ortholog	Other plants[a]
Comp40530_c0_seq1	2.25	268.53	556.06	67.76	28.93	301.87	At1 g72100	C
Comp50751_c0_seq1	7.69	787.21	3792.9	735.86	300.26	1945.18	At2 g21490	
Comp42145_c0_seq1	16.39	2159.51	9526.27	1810.41	841.27	6780.48	At2 g36640	H, C
Comp42145_c0_seq4	4.99	1117.04	4653.76	869.67	460.52	3248.48	At2 g36640	H, C
Comp46243_c0_seq2	1.11	17.86	159.84	122.67	70.21	626.39	At2 g36640	H, C
Comp46692_c0_seq4	1.03	14.69	169.17	135.22	83.26	635.56	At2 g42560	
Comp43396_c2_seq3	17.49	1741.06	11861.78	3305.72	1783.88	13218.9	At3 g15670	H, C
Comp43396_c2_seq4	12.52	1475.64	5200.8	261.22	154.19	1116.84	At3 g15670	H, C
Comp51407_c0_seq1	70.14	432.55	794.2	4.53	0.5	234.9	At3 g15670	H, C
Comp50084_c1_seq1	11.88	550.75	3750.64	760.76	547.69	1917.12	At3 g17520	
Comp39347_c0_seq2	29.03	36.63	85.01	10.08	7.06	1.55	At3 g53040	C
Comp51118_c0_seq1	45.46	794.72	1886.8	76.77	29.26	381	At3 g53040	C
Comp51489_c0_seq1	9.58	1480.88	6149.5	1117.44	577.98	3797.75	At3 g53040	C
Comp52111_c0_seq1	7.92	797.48	3334.65	625.14	309.71	2052.38	At3 g53040	C
Comp46226_c0_seq1	3.44	125.3	881.84	88.37	71.15	208.33	At4 g36600	
Comp49017_c4_seq10	17.51	146.51	474.57	43.51	21.4	128.54	At5 g06760	C
Comp45763_c0_seq1	25.1	1142.38	3559.46	1834.16	1579.35	4230.23	At5 g44310	C

The expression level values are the normalized read counts in the dehydration and rehydration processes.

[a]These *LEA* genes have been previously reported to be up-regulated during desiccation stress in the listed resurrection plant species.

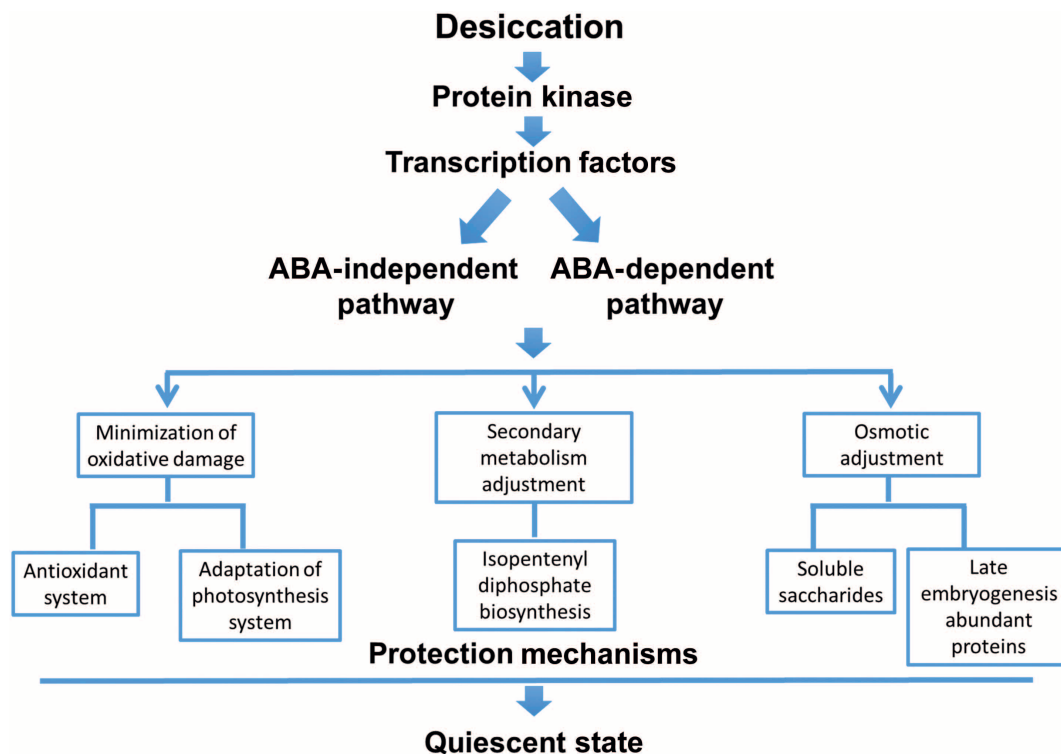H, *Haberlea rhodopensis*;[20] C, *Craterostigma plantagineum*.[6]

Figure 10. Strategies in the response to desiccation in *M. flabellifolia.*

and reduced following rehydration, including peroxiredoxins, peroxidases, UDP-glucose 6-dehydrogenases, and 6-phosphogluconate dehydrogenase (Figure 9). In addition, down-regulation of photosynthesis-regulated genes has been observed in other resurrection plants like *C. plantagineum*[6] and *H. rhodopensis*.[20] In *M. flabellifolia*, we observed down-regulation of genes encoding subunits of the photosystem reaction center (PsaA/PsaB, PsbP), electron transport chain proteins (ferredoxins, Cytochrome c), and ATP synthase (Supplementary File 11). This response presumably helps the desiccated plant to minimize excitation energy and to reduce ROS production. In striking contrast, several genes encoding light-harvesting antenna system proteins were up-regulated and remained at relatively high expression levels during dehydration and rehydration, including light-harvesting complex proteins (LHCs) of photosystems I and II and early light-inducible proteins (ELIPs) (Figure 9 and Supplementary File 11). For example, the strongest induction in the slightly dehydrated stage was observed for genes encoding chlorophyll A/B binding protein (comp51438_c0_seq1, induced 7438 times) and rubisco activase (comp40977_c0_seq5, induced 5443 times), and in the desiccated stage, it was for a gene encoding ELIP (comp50912_c0_seq1, induced 17809 times). In *Arabidopsis*, members of the LHC family play roles in the response to ABA. Reducing expression of one LHC member, *LHCB6*, decreases drought stress tolerance,[75] suggesting that up-regulation of LHCs serves as a protective strategy by transducing ABA signaling and modulating ROS homeostasis. Induction of ELIPs was reported to be involved in desiccation stress in resurrection plants like *T. ruralis*,[76] *C. plantagineum*,[77] and *H. rhodopensis*.[20] Functional analysis demonstrated that ELIPs play an important role in protection against chlorophyll-induced photo-oxidative damage in *Arabidopsis*.[78] In *H. rhodopensis*, a temporary desiccation-induced shift from C3-to-CAM metabolism is enabled by induction of genes encoding phosphoenolpyruvate carboxylase (PEPC). This C3-to-CAM shift may increase water use efficiency and decrease photo-respiratory H_2O_2 production.[20] However, in our analysis, the genes encoding PEPC (comp41025_c0_seq1, comp49907_c0_seq13, comp50447_c1_seq2) in *M. flabellifolia* decreased in response to desiccation stress (Supplementary File 4), suggesting that different strategies for photosynthetic protection may be employed by *M. flabellifolia*.

Osmotic responses. During dehydration stress, the initial avoidance of water loss in cells is accomplished by the production of compatible solutes for osmotic adjustment. Several soluble saccharides have been observed to accumulate in desiccated leaves of *M. flabellifolia*, including arbutin,[29] sucrose,[29,31,32] trehalose,[32] and glucopyranosyl-β-glycerol.[31] Our data showed that three DTGs encoding sucrose phosphate synthase (SPS, comp50138_c2, comp48008_c0, comp49692_c1) were up-regulated during dehydration (Supplementary File 4). In the resurrection plant *S. stapfianus*, increases in hexoses and sucrose were associated with increased activity of SPS at the initiation of dehydration, whereas starch content declined to undetectable levels.[79] The accumulation of SPS in *M. flabellifolia* may redirect the carbon flow from starch to soluble saccharides. Moreover, a DTG encoding UDP-dependent glycosyltransferase (comp46140_c0_seq1, Supplementary File 4) was highly induced under desiccation stress. In *Rauvolfia serpentina*, UDP-dependent glycosyltransferase is a key enzyme of arbutin biosynthesis.[80]

CONCLUSIONS

The resurrection plant *M. flabellifolia* has remarkable tolerance to desiccation. Next generation high-throughput sequencing and *de novo* assembly strategies permitted us to dissect the transcriptome of *M. flabellifolia* during both dehydration and rehydration. Adaptation to desiccation in *M. flabellifolia* is initiated by the induction of a large number of diverse PKs and TFs. These regulators transduce signal cascades of molecular pathways, including the

up-regulation of ABA-dependent and independent drought stress pathways and the activation of protective mechanisms for coping with oxidative damage. Antioxidant systems are up-regulated, and the photosynthetic system is modified to reduce ROS generation. Secondary metabolism may participate in desiccation tolerance by the adjustment of isopentenyl diphosphate biosynthesis. The up-regulation of genes encoding LEA proteins and sucrose phosphate synthases is also associated with increased tolerance to desiccation (Figure 10).

COMPETING INTERESTS

The authors declare no conflict of interest.

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