Tree peony (Paeonia suffruticosa Andrews; Paeoniaceae) has been cultivated for more than 1600 years in China, and there are approximately 2000 tree peony cultivars worldwide (Wang, 1997). The first written description of this genus was in 200 BC as a medicinal plant. In the fifth century, with the selection of multiple flower shapes and colors, it became known as an ornamental plant (Li et al., 2011). Studies have shown that the seed oil of tree peony contains abundant unsaturated fatty acids that are beneficial to human health (Sarker et al., 1999; Su et al., 2016). Owing to its multiple uses, the peony genus has spread through Asia, to the Mediterranean, Caucasus Mountains, the mountainous regions of Europe, the United States, and Australia (Rogers, 1995).

The greatest number of cultivated varieties and the largest distribution area of tree peony are found in central China, which has long been characterized by an arid climate. Nevertheless, severe water deficiency stress can limit the cultivation area, lead to smaller leaves and flowers, inhibit the synthesis of organic substances and flower pigments, and reduce the ornamental value and seed yield of tree peony (Li et al., 2011). Recent studies, however, have mainly focused on oil extraction (Chen et al., 2016a, b; Cui et al., 2016; Han et al., 2016). In addition, efficient protocols for the micropropagation of tree peony and the effects of different medium compositions and exogenous hormones on the browning of tree peony callus in tissue culture were recently investigated (Wen et al., 2016; Zhou et al., 2016a). Surprisingly, however, the desiccation tolerance strategies in tree peony cultivars have not yet been investigated.

Transcriptome analysis that uses deep sequencing technology now permits large-scale gene expression detection in the absence of a reference genome. Although there have been several investigations of transcriptome sequencing of tree peony (Gai et al., 2012; Zhou et al., 2013; Zhang et al., 2014, 2015; Zhao et al., 2014; Barghini et al., 2015; Li et al., 2015; Shi et al., 2015; Gao et al., 2016; Wang...
et al., 2016b), studies of drought-responsive differential expression genes in tree peonies have not yet been reported in the literature. Two separate studies of reference gene selection in tree peony—one in plants with different flower colors and another during flower development—were recently reported (Li et al., 2016; Zhou et al., 2016b).

Screening of drought-tolerant tree peony cultivars revealed that ‘Luo Yang Hong’ (LYH) is tolerant to drought, whereas ‘Wu Long Peng Sheng’ (WLPS) is tolerant to flooding (Kong et al., 2011), making the two cultivars ideal study material for investigating mechanisms of drought response in plants. With a view toward improving plant structure, perfecting bloom quality, and mitigating damage from desiccation, this study used LYH and WLPS with their contrasting water sensitivity to characterize unigenes during dehydration and rehydration to explore the complex mechanisms of drought response networks.

MATERIALS AND METHODS

Plant material treatment and sample collection

Four-year-old LYH and WLPS seedlings were cultured in pots using soil collected from the Luoyang National Peony Garden (Luoyang, China). The pots were buried deep in the ground from October until May to avoid freezing injury. The pots were then dug out and irrigated once every two days and cultured under natural conditions before they were used for the water deficiency treatments. Five individuals were used per treatment. The drought treatment (DR) was initiated by tap water irrigation until the soil moisture content reached 80%, after which plants were dehydrated naturally for seven days until the leaves had severely wilted. For the rehydration treatment (RE), the tree peonies were re-watered until the soil moisture content again reached 80%, after which they were cultured for one more day to let the leaf blades completely unfold. Tree peonies cultured in pots with a constant soil moisture content of 80% served as the control treatment (CK). The soil moisture content was measured by gravimetric methods (Bao, 2000). For all three treatments, the third and fourth leaves were sampled and immediately frozen in liquid nitrogen and stored at −80°C.

RNA extraction, cDNA library construction, and sequencing

The leaves sampled from the three different treatments (DR, RE, and CK) were assigned to six independent pools. Total RNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method (Gambino et al., 2008). The integrity of RNA was examined by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California, USA). An in-house library preparation method was used for mRNA sequencing, using fragment sizes of 200 bp. Libraries were quantified by an Agilent Bioanalyzer and qualified by the ABI StepOnePlus Real-Time PCR System (Thermo-Fisher Scientific, Waltham, Massachusetts, USA) during the quality control steps. Libraries were sequenced using the Illumina HiSeq 2000 (Illumina, Shenzhen, Guangzhou, China) at the Beijing Genomics Institute (BGI). Each library was run on a separate lane of the HiSeq. The cDNA library was deposited in the National Center for Biotechnology Information (NCBI) Transcriptome Shotgun Assembly database (BioSample accession no. SRS1180651).

De novo assembly and protein-coding region prediction

Reads with adapters, unknown nucleotides larger than 5%, and low-quality reads (bases quality ≤10) were discarded. Only reads longer than 90 bp were used for assembly. Reads from all treatments and/or cultivars were assembled together by Trinity 3.4 (Grabherr et al., 2011; open source code publicly available at http://TrinityRNASeq.sourceforge.net). Because a reference genome is not available for tree peony, reads were mapped to the assembled unigene set.

Unigenes were first aligned by BLASTX (E-value <0.00001) to protein databases in the following order of NCBI’s nonredundant protein database (nr), Swiss-Prot, Kyoto Encyclopedia of Genes and Genomes (KEGG), and NCBI’s Clusters of Orthologous Groups (COG) database. Unigenes aligned to a higher-priority database were not aligned to lower-priority databases. The best alignment results were used to decide sequence direction of unigenes. When a unigene was not aligned with any of the above databases, ESTScan was used to determine its sequence direction (Iseli et al., 1999).

Proteins with the highest ranks in the BLAST results were used to decide the coding region sequences of unigenes, then the coding region sequences were translated into amino sequences with the standard codon table. Unigenes that could not be aligned to any database were scanned by ESTScan (Iseli et al., 1999), producing nucleotide sequence (5′–3′) direction and amino sequence of the predicted coding region.

Gene ontology classification and metabolic pathway analysis

Unigene functional classification and annotation was performed by WEGO (http://wego.genomics.org.cn/) (Ye et al., 2006). Gene function in cellular processes and gene products during metabolism process were analyzed by KEGG (http://www.genome.jp/kegg) (Kanehisa et al., 2008).

Real-time quantitative PCR (qPCR) verification analysis

Twenty dehydrin-related unigenes were selected for the assessment of expression profiles. Total RNA was converted into single-stranded cDNA using an M-MLV reverse transcriptase (Promega Corporation, Madison, Wisconsin, USA). The ABI StepOnePlus Real-Time PCR System (Thermo-Fisher Scientific) was utilized to perform the expression profile verification. The reaction was carried out as described in our previous publication (Pang et al., 2015), using three biological replicates per sample. The relative gene expression levels of the selected unigenes were normalized to 18S rRNA and calculated using the 2-ΔΔCt method (Livak and Schmittgen, 2001). The primer sequences are shown in Table 1.

RESULTS

Sequencing output statistics, assembly metrics, and protein-coding region classification

The total number of clean nucleotides generated from the six libraries of the two tree peony cultivars (LYH and WLPS) by three treatments (CK, DR, and RE) exceeded 4.6 Gbp. The number of clean reads of LYH–CK, LYH–DR, LYH–RE, WLPS–CK, WLPS–DR, and WLPS–RE were 54, 52, 52, 51, and 52 million, respectively. The Q20 values exceeded 97%, and the GC contents were
approximately 46% of all samples, which meant that the sequencing data were robust for further analysis. After assembling all sequences from all samples, 81,725 unigenes were obtained. Their aggregate length was 62,310,011 nucleotides, with a mean length of 762 nucleotides.

A total of 41,808 protein-coding regions from the unigenes were translated into amino sequences. Detailed information of their length distributions is given in Appendix 1. The COG analysis showed that 14,768 unigenes were assigned to 25 classifications. The largest category was ‘General function prediction only’; ‘Transcription,’ ‘Replication, recombination, and repair,’ ‘Post transcriptional modification, protein turnover, chaperones,’ and ‘Signal transduction mechanisms’ were comparatively high; and ‘RNA processing and modification’ had the smallest number of responding unigenes (Fig. 1).

### Functional annotation of the unigenes

A total of 43,977 unigenes were successfully allocated to the three main gene ontology categories: biological process, molecular function, and cellular component (Fig. 2). The biological process category contained 22 classes subsumed under five larger groups: cellular process (18,310), metabolic process (17,809), single-organism process (12,415), stimulus (8,451), and biological regulation (6,850). The cellular components category consisted of 17 classes, dominated by cell (21,823), cell part (21,822), and organelle.
Identification of dehydration- and rehydration-responsive unigenes

A total of 971 drought-responsive unigenes in LYH were identified by comparison of LYH-CK vs. LYH-DR (8979), LYH-CK vs. LYH-RE (5650), and LYH-DR vs. LYH-RE (9397) (Fig. 3A), whereas 1064 drought-responsive unigenes in WLPS were identified by comparison of WLPS-CK vs. WLPS-DR (14,446), WLPS-CK vs. WLPS-RE (5593), and WLPS-DR vs. WLPS-RE (13,327) (Fig. 3B). Further comparison identified 373 unigenes in both LYH and WLPS. Excluding the 83 unigenes accessed by the comparison of LYH-CK vs. LYH-DR and WLPS-CK vs. WLPS-DR (Fig. 3C), 290 unigenes lacking any relationship with

Within a cultivar but also between the cultivars (LYH and WLPS) for a given treatment. The unigenes responded to stimuli, including abiotic, endogenous, biotic, and chemical stimuli that were identified extensively in LYH and WLPS. Unigenes’ response to stress was detected only in the CK vs. RE treatments of LYH (Appendix 2). For the molecular function category, unigenes were identified in DR vs. RE of LYH, while oxidoreductase and catalytic activity were largely detected both between cultivars and among the treatments. None of the other candidate molecular functions were identified, except the above two functions between LYH and WLPS (Appendix 3). For the cellular component category, the membrane, cell periphery, plasma membrane, and extracellular region were extensively detected in the LYH and WLPS treatments separately. Within the same treatment, however, none of the unigenes responded to the cellular component between the cultivars (LYH and WLPS) apart from two exceptions: the membrane detected in DR and the extracellular region identified in RE (Appendix 4).
genotype yet responding to dehydration and rehydration were detected. Hierarchical clustering of LYH-CK, LYH-DR, LYH-RE, WLPS-CK, WLPS-DR, and WLPS-RE indicated that CK, DR, and RE were clustered together, revealing dehydration- and rehydration-responsive unigenes (Fig. 4). We note that our differential expression results are preliminary, as our experiments lack replication. Follow-up experiments will be needed to fully validate our observations.

**Pathway analysis using the KEGG database**

BLAST analysis of 81,725 unigenes against the KEGG database was performed to analyze gene products during metabolism processes and related gene functions in cellular processes. A total of 23,518 unigenes were involved in 128 KEGG pathways. More than one-fifth (21.77%) were related to metabolic pathways, whereas 11.82% were related to the biosynthesis of secondary metabolites, 5.51% were related to plant–pathogen interaction, and 4.69% were related to plant hormone signal transduction (Table 2).

The KEGG pathways of unigenes with annotation for the two cultivars (LYH vs. WLPS) within the same treatment and among treatments (CK, DR, and RE) within the same cultivar were also analyzed. The metabolic pathways, biosynthesis of secondary metabolites, plant–pathogen interactions, and plant hormone signal transduction had similar percentages in all pathways identified. Abscisic acid, jasmonic acid, ethylene, brassinosteroids, salicylic acid, gibberellins, cytokinin, and auxin signaling pathways were all detected in this study. It is interesting to note that when analyzing the pathway between LYH and WLPS, the plant hormone signal transduction pathway was not detected in CK and RE (Table 3). LYH and WLPS exhibited higher plant–pathogen interaction after dehydration than the control, which might be caused by plant interaction with a pathogen such as arbuscular mycorrhizal fungi. However, it was unclear why a plant–pathogen interaction apparently went undetected after rehydration.

**Unigene validation by qPCR**

To validate the expression profiling of the dehydrin-responsive unigenes, 20 genes predicted to participate in the dehydrin response pathway were selected (1) to determine their relative expression in the dehydration (DR), rehydration (RE), and non-treatment of tree peony (CK) and (2) to validate the transcriptome sequencing results. Abundance of the target genes was normalized relative to the abundance of 18S RNA; the Ct values (i.e., the number of cycles corresponding to the inflection point from baseline to exponential growth) of 18S rRNA for all samples ranged from 24.0 to 26.0. The results of the qPCR verification showed a differential expression pattern under both the DR and RE treatments. Dehydrin Xero 2-like was significantly upregulated after dehydration but then downregulated during rehydration of tree peony seedlings (Table 4).

**DISCUSSION**

Drought stress is one of the main abiotic stresses, and it may alter plant growth, metabolism, and yield (Ajithkumar and Panneerselvam, 2014). In tree peonies cultivated in central and northwestern China, water deficiency is a common problem. This drought stress limits the growth of leaves and flowers, inhibits the synthesis of organic compounds and anthocyanin, and reduces seed yield (Li et al., 2011). Plants that receive drought signals initiate a range of physiological, morphological, and biochemical defense responses at both the cellular and molecular level (Verslues et al., 2006). An overexpression of genes in response to drought stress could alleviate drought-induced damage while promoting plant growth. Drought tolerance strategies, as revealed by transcriptome sequencing in poplar (Barghini et al., 2015) and sorghum (Fracasso et al., 2016), have uncovered a number of drought-responsive genes. To diminish the damage caused by water deficiency in tree peony, two cultivars with contrasting water sensitivity were selected for unigene characterization to investigate the molecular mechanisms driving their drought response.

Plants can adapt to desiccation stresses and stay alive by alternating the accumulation of osmolytes (Parida et al., 2007). Proline, one of the most important osmolytes, is quickly accumulated and involved in the plant response to dehydration to maintain a cellular balance of water content and turgor potential (Vendruscolo et al., 2007). A previous study showed that proline accumulates after
TABLE 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway statistics.

| Pathway ID | Pathway | No. of genes with pathway annotation (%)a |
|------------|---------|------------------------------------------|
| ko01100    | Metabolic pathways                      | 5121 (21.77%) |
| ko01110    | Biosynthesis of secondary metabolites   | 2781 (11.82%) |
| ko04626    | Plant–pathogen interaction              | 1296 (5.51%)  |
| ko04075    | Plant hormone signal transduction       | 1103 (4.69%)  |
| ko03040    | Spliceosome                             | 972 (4.13%)   |
| ko03013    | mRNA transport                          | 953 (4.05%)   |
| ko03018    | RNA degradation                         | 746 (3.17%)   |
| ko03008    | Ribosome biogenesis in eukaryotes       | 745 (3.17%)   |
| ko04141    | Protein processing in endoplasmic reticulum | 627 (2.67%) |
| ko00056    | Glycosphingolipid metabolism            | 538 (2.29%)   |
| ko00240    | Pyrimidine metabolism                   | 510 (2.17%)   |
| ko03015    | mRNA surveillance                      | 505 (2.15%)   |
| ko00230    | Purine metabolism                       | 503 (2.14%)   |
| ko04120    | Ubiquitin-mediated proteolysis          | 433 (1.84%)   |
| ko00564    | Phenylpropanoid biosynthesis            | 380 (1.62%)   |
| ko00565    | Ether lipid metabolism                  | 366 (1.56%)   |
| ko00908    | Zeatin biosynthesis                     | 310 (1.32%)   |
| ko00100    | Glycolysis/glucogenesis                 | 310 (1.32%)   |
| ko00190    | Oxidative phosphorylation               | 300 (1.28%)   |
| ko00900    | Terpenoid backbone biosynthesis         | 285 (1.21%)   |
| ko02010    | ABC transporters                        | 279 (1.19%)   |
| ko03020    | RNA polymerase                         | 259 (1.1%)    |
| ko00040    | Pentose and glucuronate interconversions | 245 (1.04%) |
| ko00520    | Amino sugar and nucleotide sugar metabolism | 244 (1.04%) |
| ko03440    | Homologous recombination                | 239 (1.02%)   |

*Pathway analysis of unigenes with pathway annotation (P ≤ 0.05).*

TABLE 3. Pathway analysis of unigenes with pathway annotation (P ≤ 0.05).*

| Unigene comparison | Metabolic pathways | Biosynthesis of secondary metabolites | Plant–pathogen interaction | Plant hormone signal transduction |
|--------------------|--------------------|---------------------------------------|----------------------------|----------------------------------|
| LYH-CK vs. LYH-DR  | 985/3972 = 24.8    | 622/3972 = 15.66                      | 317/3972 = 798             | 260/3972 = 65.5                 |
| LYH-CK vs. LYH-RE  | 574/2366 = 24.26   | 378/2366 = 15.98                      | 179/2366 = 7.57            | 170/2366 = 7.19                 |
| LYH-DR vs. LYH-RE  | 1131/4142 = 27.31  | 697/4142 = 16.83                      | 355/4142 = 8.57            | 331/4142 = 7.99                 |
| WLPS-CK vs. WLPS-DR| 1430/5905 = 24.22  | 838/5905 = 14.19                      | 411/5905 = 6.96            | 368/5905 = 6.23                 |
| WLPS-CK vs. WLPS-RE| 567/2234 = 25.38   | 334/2234 = 14.95                      | 172/2234 = 7.7             | 159/2234 = 7.12                 |
| WLPS-DR vs. WLPS-RE| 1455/5503 = 26.44  | 841/5503 = 15.28                      | 416/5503 = 7.56            | 383/5503 = 6.96                 |
| LYH-CK vs. WLPS-CK | 652/2332 = 27.96   | 409/2332 = 17.54                      | 170/2332 = 7.29            | —                               |
| LYH-CK vs. WLPS-DR | 835/2843 = 29.37   | 507/2843 = 17.83                      | 229/2843 = 8.05            | 160/2843 = 5.63                 |
| LYH-RE vs. WLPS-RE | 596/2011 = 29.64   | 348/2011 = 17.3                       | —                          | —                               |

*Values are number/total = percentage.
maximum efficiencies of PSII photochemistry (Ryan et al., 2014). Reduced photosynthetic pigment contents resulting from drought stress might decrease ROS formation by regulating chlorophyll synthesis and other components of the photosynthetic machinery.

ROS generation is considered to be closely related to lipid peroxidation under drought stress (Ryan et al., 2014; Uzilday et al., 2014). For example, lipid peroxidation analysis showed that transgenic Agrostis stolonifera L. root exhibited less cellular damage when compared with the wild type under drought stress conditions (Xu et al., 2016). The alleviation of adverse effects of drought stress is partially attributable to an increased antioxidant ability and decreased lipid peroxidation induced by early ROS accumulation (Xing et al., 2016). Tobacco plants treated with low and moderate levels of riboflavin accumulated higher levels of ROS and lipid peroxide with enhanced drought tolerance (Deng et al., 2014). In the present study, 666 unigenes involved in lipid transport and metabolism were identified according to the COG classification. This clearly illustrates the close relationship between lipid peroxidation and the drought stress response in plants.

Calcium mobilization is one of the downstream events modulated by H₂O₂ (Neill et al., 2002). The calcium ion (Ca²⁺) functions as a secondary messenger in modulating diverse physiological processes that are important for stress adaptation in plants. Both Ca²⁺ and Ca²⁺/calmodulin (CaM)-binding protein and transcription factors have been identified, and their functional analysis suggests that they play key roles in plant stress signaling pathways (Reddy et al., 2011). Previous studies have indicated that drought stress activates ABA-dependent and ABA-independent gene expression (Yoshida et al., 2014). The cis-regulatory element ABA-responsive element (ABRE) (CAGCTG [T/C/G]) and their coupling elements ([C/A]ACGCG[T/C/G]) in the upstream region were observed in the upregulated genes (Kaplan et al., 2006). Hence, it was concluded that for some specific Ca²⁺ transients, ABREs function as Ca²⁺-responsive cis-regulatory elements (Reddy et al., 2011).

ABRE and calcium-dependent protein kinase (CDPK) have been found to be related to drought stress in other plant species (Yoshida et al., 2010; Zou et al., 2010). In the present study, ABREs (CL3759.Contig1_All and CL3759.Contig2_All) and CDPKs (unigene21495_All, unigene21823_All, CL5185.Contig3_All, and CL3906.Contig3_All) were identified. In addition, transcript accumulation of the myeloblastosis (MYB) transcription factor, the APETALA2/Ethylene Responsive Factor (AP2/ERF), the NAM, ATAF, AND CUC (NAC) transcription factor, the basic helix-loop-helix (bHLH) protein, and the zinc RING finger protein (RING-H) were all identified after desiccation stress, which agrees

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**TABLE 4.** Real-time quantitative PCR (qPCR) validation of 20 unigenes in three tree peony treatment groups: dehydration, rehydration, and a control group.

| Gene ID         | Nr annotation                                      | 2-ΔΔCT | Log2 (DR_FPKM or RE_FPKM/CK_FPKM) |
|-----------------|----------------------------------------------------|--------|-----------------------------------|
| Unigene8873_All_LYH-DR | Dehydrin (Paeonia suffruticosa)                     | 5.3658 | 4.4933                            |
| CL8710.Contig2_All_LYH-DR | Ethylene response factor 11 (Actinidia delicosa) | −0.1155 | −1.4333                            |
| Unigene5006_All_LYH-DR | Ethylene responsive transcription factor 1A (Prunus salicina) | 5.0722 | 3.5543                            |
| Unigene16234_All_LYH-DR | Ethylene-responsive transcription factor 1B, putative ( Ricinus communis) | 9.1154 | 4.4226                            |
| CL2427.Contig2_All_LYH-DR | GDSL esterase/lipase EXL3 (Vitis vinifera)         | −2.7565 | −3.0232                            |
| Unigene383_All_LYH-DR | GDSL esterase/lipase 1 (Vitis vinifera)            | 4.5329 | 2.5685                            |
| Unigene18390_All_LYH-DR | RING-H2 finger protein ATL60-like (Vitis vinifera) | 5.7046 | 3.3146                            |
| CL9864.Contig2_All_LYH-DR | RING-H2 finger protein ATL78 (Vitis vinifera)      | −1.6611 | −2.444                            |
| Unigene1202_All_LYH-DR | RING-H2 zinc finger protein RH4A (Vitis vinifera)   | −3.5098 | −4.4392                            |
| Unigene1395_All_LYH-DR | Transcription factor bHLH135 (Vitis vinifera)     | −0.0729 | −2.4395                            |
| CL4531.Contig1_All_LYH-RE | Transcription factor bHLH63-like (Vitis vinifera) | −1.4411 | −1.5683                            |
| CL154.Contig2_All_LYH-RE | NAC domain-containing protein 72 (Vitis vinifera)   | −0.3531 | −2.7657                            |
| Unigene4037_All_LYH-RE | Zinc finger CCH domain-containing protein S3-like (Glycine max) | −1.0864 | −2.9649                            |
| Unigene25204_All_LYH-RE | MYBF1 (Vitis vinifera)                             | −0.5343 | −1.4983                            |
| CL3906.Contig3_All_LYH-RE | Uncharacterized calcium-binding protein At1g02270 (Vitis vinifera) | 6.7048 | 4.9768                            |
| CL10838.Contig2_All_LYH-RE | Universal stress protein A-like protein (Vitis vinifera) | 9.8757 | 3.6137                            |
| Unigene15264_All_LYH-RE | TIR-NBS type disease resistance protein (Populus trichocarpa) | 7.0632 | 5.5444                            |
| Unigene32639_All_LYH-RE | Heavy metal-associated isoprenylated plant protein 26-like ( Fragaria vesca subspp. vesca) | 13.1831 | 3.3610                            |
| CL7346.Contig2_ALL_LYH-RE | Glutamate dehydrogenase, putative (Ricinus communis) | 14.1086 | 7.3002                            |
| CL7474.Contig3_All_LYH-RE | 17.9 kDa class II heat shock protein isoform 1 (Vitis vinifera) | −1.4672 | −3.6081                            |

Note: CK = control treatment; DR = drought treatment; FPKM = fragments per kilobase of transcript per million mapped reads; Nr = National Center for Biotechnology Information nonredundant protein database; RE = rehydration treatment.
perfectly with ABA accumulation (Nakashima and Yamaguchi-Shinozaki, 2013; Furlan et al., 2014). Further studies are required to reveal their mechanisms of regulating drought resistance in tree peonies.

Heat stress can trigger the higher expression of heat-shock proteins (HSPs), which might coordinate with other stress-response mechanisms to mitigate cellular damage and re-establish cellular homeostasis (Wang et al., 2004). Copper applied to tree peony revealed an increase in dehydration-responsive element–binding (DREB) protein (Wang et al., 2016a). In the present study, we identified one class II HSP isoform 1 (CL7474.Contig3_All) and one heavy metal–associated isoprenylated plant protein (HIPP) (Unigene32639_All), both of which were unrelated to genotype but responsive to dehydration and rehydration. Regulation of HSP and HIPP by dehydration and rehydration in tree peony illustrates the synergistic interaction of drought with other stress-response mechanisms to alleviate cellular damage and re-establish cellular homeostasis.

CONCLUSIONS

Transcriptome profiling analysis demonstrated unigene response to dehydration and rehydration in tree peony, namely MYB, AP2/ERF, NAC, HILH, RING-H, HSP, and HIPP. These newly identified unigenes will increase our understanding of drought stress–responsive mechanisms, and they may be quite useful as novel genes for the molecular breeding of tree peony to improve its drought tolerance. Further research is necessary to reveal and understand how antioxidant enzymes interact with key hormones in the signaling responses of plants to drought stress.

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DATA ACCESSIBILITY

The cDNA library was deposited in the National Center for Biotechnology Information (NCBI) Transcriptome Shotgun Assembly database (BioSample accession no. SRS1180651).

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**APPENDIX 1.** Length distribution of the protein-coding region (CDS) prediction. (A) Length distribution of CDS using BLASTX. (B) Length distribution of CDS using ESTScan. (C) Length distribution of proteins using BLASTX. (D) Length distribution of proteins using ESTScan.

**APPENDIX 2.** Gene ontology enrichment analysis of biological processes (*P* ≤ 0.05).^a^

| Biological process               | LYH-CK vs. LYH-DR (3785) | LYH-CK vs. LYH-RE (2241) | LYH-DR vs. LYH-RE (4038) | WLPS-CK vs. WLPS-DR (2073) | WLPS-CK vs. WLPS-RE (5482) | WLPS-DR vs. WLPS-RE (2614) | LYH-CK vs. WLPS-CK (2758) | LYH-DR vs. WLPS-DR (1759) |
|----------------------------------|--------------------------|--------------------------|--------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Oxygen-containing compound       | 14.5%                    | 18.3%                    | 14.6%                    | 14.5%                      | 16.4%                      | 14.4%                      | 14.0%                      |                           |
| Oxidation-reduction process      | 15.7%                    | 15.7%                    | 15.1%                    | 16.5%                      | 15.1%                      | 16.6%                      | 16.3%                      |                           |
| Stimulus                         | 41.7%                    | 45.9%                    | 43.1%                    | 40.9%                      | 43.1%                      | 41.7%                      |                           |                           |
| Abiotic stimulus                 | 19.2%                    | 22.3%                    | 19.7%                    | 19.2%                      | 19.9%                      | 18.8%                      |                           |                           |
| Endogenous stimulus              | 10.5%                    | 13.4%                    | 11.6%                    | 10.9%                      | 11.9%                      | 10.8%                      |                           |                           |

(continues)
### APPENDIX 2. (continued)

| Biological process | LYH-CK vs. LYH-DR (3785) | LYH-CK vs. LYH-RE (2241) | LYH-DR vs. LYH-RE (4038) | WLPS-CK vs. WLPS-DR (5682) | WLPS-CK vs. WLPS-RE (2073) | WLPS-DR vs. WLPS-RE (5482) | LYH-CK vs. WLPS-CK (2087) | LYH-DR vs. WLPS-DR (2614) | LYH-RE vs. WLPS-RE (1759) |
|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Biotic stimulus    | 10.5%                    | 10.9%                    | 10.2%                    | 10.4%                    | 10.6%                    |                          |                           |                           |                          |
| Chemical stimulus  | 23.2%                    | 27.0%                    | 23.6%                    | 22.4%                    | 22.9%                    |                          |                           |                           |                          |
| Stress             |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Organic substance  |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Single-organism    |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| metabolic process  | 35.2%                    | 36.6%                    | 36.1%                    | 35.1%                    | 34.5%                    |                          |                           |                           |                          |
| Single-organism    |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| biosynthetic process |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Single-organism    |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| transport         |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Single-organism    |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| signaling         |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Signaling         |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Signal transduction |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Cell communication |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Other organism    |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| inorganic substance |                          |                          |                          |                          |                          |                          |                           |                           |                          |
| Organic acid metabolic process |          |                          |                          |                          |                          |                          |                           |                           |                          |
| Oxoacid metabolic process |            |                          |                          |                          |                          |                          |                           |                           |                          |
| Carboxylic acid metabolic process |      |                          |                          |                          |                          |                          |                           |                           |                          |

Note: CK = control treatment; DR = drought treatment; LYH = ‘Luo Yang Hong’ cultivar; RE = rehydration treatment; WLPS = ‘Wu Long Peng Sheng’ cultivar.

*Numbers in parentheses in the column headings represent the number of unigenes.

### APPENDIX 3. Gene ontology enrichment analysis of molecular functions (P ≤ 0.05).*

| Molecular function | LYH-CK vs. LYH-DR (3697) | LYH-CK vs. LYH-RE (2196) | LYH-DR vs. LYH-RE (3996) | WLPS-CK vs. WLPS-DR (5522) | WLPS-CK vs. WLPS-RE (2017) | WLPS-DR vs. WLPS-RE (5340) | LYH-CK vs. WLPS-CK (2106) | LYH-DR vs. WLPS-DR (2634) | LYH-RE vs. WLPS-RE (1792) |
|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Oxidoreductase activity | 17.6%                    | 19.3%                    | 19.1%                    | 16.4%                    | 15.9%                    | 18.2%                    | 17.0%                    | 17.6%                    | 18.2%                    |
| Catalytic activity  | 68.8%                    | 70.8%                    | 67.1%                    | 68.1%                    | 69.4%                    | 68.5%                    | 70.3%                    |                          |                          |
| Transporter activity | 11.5%                    | 2.1%                     | 11.5%                    | 11.5%                    |                          |                           |                           |                           |                          |
| Protein kinase activity | 11.0%                    |                          |                          |                          |                          |                           |                           |                           |                          |
| Phosphotransferase activity, alcohol group as acceptor | 11.9% | 11.5% |                          |                          |                          |                           |                           |                           |                          |
| Kinase activity    |                          |                          |                          |                          |                          |                           |                           |                           | 13.8%                    |
| Transmembrane transporter activity |            |                          |                          |                          |                          |                           |                           |                           | 10.0%                    |

Note: CK = control treatment; DR = drought treatment; LYH = ‘Luo Yang Hong’ cultivar; RE = rehydration treatment; WLPS = ‘Wu Long Peng Sheng’ cultivar.

*Numbers in parentheses in the column headings represent the number of unigenes.

### APPENDIX 4. Gene ontology enrichment analysis of cellular components (P ≤ 0.05).*

| Cellular component | LYH-CK vs. LYH-DR (3683) | LYH-CK vs. LYH-RE (2031) | LYH-DR vs. LYH-RE (3800) | WLPS-CK vs. WLPS-DR (5503) | WLPS-CK vs. WLPS-RE (1879) | WLPS-DR vs. WLPS-RE (5235) | LYH-CK vs. WLPS-CK (1969) | LYH-DR vs. WLPS-DR (2373) | LYH-RE vs. WLPS-RE (1609) |
|--------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Membrane           | 43.6%                    | 43.2%                    | 48.3%                    | 43.8%                    | 41.6%                    | 46.3%                    | 42.3%                    |                          |                          |
| Cell periphery     | 27.8%                    | 28.4%                    | 31.0%                    | 27.3%                    | 26.8%                    | 28.2%                    |                          |                          |                          |
| Plasma membrane    | 22.3%                    | 23.7%                    | 25.7%                    | 23.1%                    |                          |                          |                          |                          |                          |
| Extracellular region | 10.7%                    | 9.8%                     | 10.9%                    | 9.1%                     | 9.7%                     | 9.2%                     | 9.3%                     |                          |                          |
| Chloroplast        | 25.7%                    |                          |                          |                          |                          |                          |                          |                          |                          |
| Plastid            | 29.2%                    |                          |                          |                          |                          |                          |                          |                          |                          |

Note: CK = control treatment; DR = drought treatment; LYH = ‘Luo Yang Hong’ cultivar; RE = rehydration treatment; WLPS = ‘Wu Long Peng Sheng’ cultivar.

*Numbers in parentheses in the column headings represent the number of unigenes.