Transcriptome sequencing and drought resistance gene annotation in *Quercus liaotungensis* leaves

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Abstract

Q. liaotungensis is an important drought-resistant tree species in Northeast China where the climate is dry and rainless. In this study, we performed a deep transcriptomic sequencing in *Q. liaotungensis* leaves, including de novo assembly and functional annotation for screening the candidate genes involved in drought avoidance. A total of 25,593 unigenes were obtained from Illumina sequencing platform. According to Gene Ontology annotation and KEGG pathway enrichment analysis, we screened a series of candidate genes encoding SOD, POD, CAT, DREB, MYB, WRKY, bZIP, and NAC from the *Q. liaotungensis* leaf transcriptome, all of which are potentially involved in drought resistance. The results of this study expanded the genetic resources of *Q. liaotungensis* and provided a theoretical basis for further exploring the functional gene information of *Q. liaotungensis*.

Keywords *Quercus liaotungensis* · Leaf · Transcriptome · Drought-related factors

Introduction

Oaks belong to the genus *Quercus*, which comprises several hundred diploid and highly heterozygous species spreading throughout the northern hemisphere, from the tropical to the boreal regions (Abrams 1990). Among the oak species, *Q. liaotungensis*, which leaf is the main food source for *Anthe-raea pernyi*, is an important drought-resistant tree species in the northern warm temperate zone. The leaf morphology of *Q. liaotungensis* is relative small which could adapt to the dry and rainless climate in Northeast China. However, a detailed characterization of genomic information of *Q. liaotungensis* leaf coping with drought is lacking.

Recent transcriptomic and gene expression profiling studies in oaks have led to the construction of large cDNA

libraries (Ueno et al. 2010; Kremer et al. 2012; Tarkka et al. 2013; Torre et al. 2014), and RNA-Seq studies have made it possible to identify genes involved in drought-resistant (Torre et al. 2014). In this study, the leaves of *Q. liaotungensis* were used for transcriptome analysis for expanding the genetic resources of *Q. liaotungensis*. Besides, a series of drought-related factors of *Q. liaotungensis* leaf were also screened. The results of this study may provide a theoretical basis for further research on drought resistance mechanism of *Q. liaotungensis*.

Materials and methods

Sample preparation and RNA extraction

Q. liaotungensis used in this study were located at the research base of Shenyang Agricultural University under natural environment at 23 ± 2 °C with $70 \pm 5\%$ relative humidity. Juvenile and mature leaves (Fig. 1A) from 30 individuals of 4-years old plants were collected and immediately frozen in liquid nitrogen and stored at -80 °C until processing. Total RNA was extracted using Trizol reagent (Invitrogen) according to the manual. The quantity and purity of RNA were analyzed using NanoDrop ND-1000

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Fig. 1 **A** The juvenile leaf (up) and matured leaf (down) of *Q. liaotungensis*. **B** Number of unigenes annotated in NCBI_nr, GO, KEGG, Swiss-Prot Pfam, Swiss-Prot, and eggNOG databases. **C** The number

of different putative candidate factors involved in drought avoidance of *Q. liaotungensis* leaf

(Wilmington, DE, USA). The integrity of RNA was detected by Bioanalyzer 2100 (Agilent, CA, USA).

cDNA library construction and transcriptome sequencing

The high-quality RNA with concentration > 50 ng/μL, RIN number > 7.0, OD_{260/280} > 1.8, and total RNA

amount > 1 μg was sent for cDNA library construction and RNA-seq which were commissioned by Lianchuan Biotechnology Co., Ltd (Hangzhou, China). Briefly, approximately 10 μg of total RNA representing a specific adipose type was subjected to isolate Poly (A) mRNA with poly-T oligo-attached magnetic beads (Invitrogen). Following purification, the poly(A)- or poly(A)+ RNA fractions is fragmented into small pieces using divalent cations under

elevated temperature. Then the cleaved RNA fragments were reverse-transcribed to create the final cDNA library in accordance with the protocol for the mRNA-seq sample preparation kit (Illumina, San Diego, USA), the average insert size for the paired-end libraries was 300 bp (± 50 bp). And then the paired-end sequencing was performed on an Illumina Hiseq 4000 (Ic-bio, China).

De novo transcriptome assembly

The adaptor reads of the raw data were removed using Cutadapt software (<https://cutadapt.readthedocs.io/en/stable/>) (Martin 2011). The clean data were obtained by removing the low quality and repeat reads. The sequence quality was verified by FastQC software (<http://www.bioinformatics.babraham.ac.uk/projects/FastQC/>), including Q20, Q30, and GC content. The clean data were de novo assembled using Trinity software (Grabherr et al. 2011). Trinity grouped transcripts into clusters based on the shared sequence content. Each transcript cluster was referred to as a 'unigene', and the longest transcript sequence was chosen as the gene sequence of 'unigene'.

Unigene annotation

The annotations of the unigenes obtained from the assembled transcriptome were obtained by aligning against the databases including Gene ontology (<http://www.geneontology.org>), Kyoto Encyclopedia of Genes and Genomes (<http://www.genome.jp/kegg/>), eggNOG (<http://eggNOGdb.embl.de/>), NCBI_nr (<http://www.ncbi.nlm.nih.gov/>), and Pfam (<http://pfam.xfam.org/>) using DIAMOND software (Buchfink et al. 2015).

Validation of RNA-Seq by quantitative RT-PCR

Ten unigenes were selected for qRT-PCR to validate the transcriptome data. Total RNA from the *Q. liaotungensis* leaf samples used for qRT-PCR were the same as for RNA-Seq. The gene-specific primers were designed using the predicted CDSs as reference sequences. qRT-PCR was performed on a CFX Connect™ Real-Time System (Bio-Rad) using a 20-μL reaction system with a procedure as follows: 95 °C for 30 s, followed by 39 cycles of 95 °C for 5 s, 60 °C for 30 s. Melting

curves were generated after each run to confirm a single PCR product. Each reaction was run in triplicate. mRNA quantity of each gene was calculated with the $2^{-\Delta CT}$ method (Livak and Schmittgen 2001).

Results and discussion

The RNA-seq data generated 54,153,182 raw reads. After filtering out the low-quality reads, 53,021,436 clean reads were obtained, which assembled into 41,207 transcripts with a mean length of 704 bp and GC content of 42.17%, and 25,593 unigenes with a mean length of 687 bp and GC content of 42.31%, based on Trinity assembly platform (Table 1). The raw sequence reads of this study were deposited in the Gene Expression Omnibus (GEO) under the accession number GSE125798. According to the *Q. liaotungensis* leaf transcriptome, 16,021, 17,935, 9290, 14,476, 19,806, 20,529 unigenes were matched to the Pfam, GO, KEGG, Swiss-Prot, eggNOG, NCBI_nr database, respectively (Table 2, Fig. 1B). The species distribution of the unigenes via blasting into NCBI_nr is shown in Fig. 2. The species that most contributed to the annotation was *Juglans regia* (59.43%), following with *Ziziphus jujuba* (4.04%), *Vitis vinifera* (3.52%), *Theobroma cacao* (3.3%), *Prunus persica* (2.41%), *Prunus mume* (2.07%), which suggested that there may be a closely genetic relationship between *Q. liaotungensis* and *J. regia*. The qRT-PCR results showed that the expression patterns of the candidate genes were consistent with those from RNA-Seq (Fig. 3),

Table 2 Unigenes annotated in different databases

Database	Number of unigenes	Ratio (%)
All	25,593	100.00
GO	17,935	70.08
KEGG	9290	36.30
swissprot	14,476	56.56
eggNOG	19,806	77.39
NCBI_nr	20,529	80.21
Pfam	16,021	62.60

Table 1 Statistical analysis of the transcriptome sequence data

Index	All	GC%	Min length	Median length	Max length	Total assembled bases	N50
Transcript	41,207	42.17	201	704	12,190	41,133,221	1515
Gene	25,593	42.31	201	687	12,190	25,541,255	1550

Fig. 2 Species distribution of the unigenes via blasting into NCBI_nr database. Different colors represent different species. The number represents the proportion of unigene matched to different species

Fig. 3 Verification of the selected unigenes by qRT-PCR as compared with RNA-Seq data. X-axis represents the 10 genes selected for qRT-PCR validation. Y-axis represents the relative expression. DN6399_c0_g1, a superoxide dismutase 5 [*Betula platyphylla*]. DN9704_c1_g3, putative peroxidase 48 [*Juglans regia*]. DN9174_c2_g8, PREDICTED: basic leucine zipper 61 [*Juglans regia*]. DN10208_c2_g3, PREDICTED: putative peroxidase 48 [*Juglans regia*]. DN5663_c0_g1, PREDICTED: putative dehydration-responsive element-binding protein 2H [*Juglans regia*]. DN10418_c0_g5, PREDICTED: probable WRKY transcription factor 23 [*Juglans regia*]. DN7168_c0_g1, PREDICTED: probable WRKY transcription factor 75 [*Juglans regia*]. DN809_c0_g1, PREDICTED: bZIP transcription factor 53 [*Juglans regia*]. DN6101_c0_g1, PREDICTED: basic leucine zipper 23 [*Vitis vinifera*]. DN9760_c2_g6, PREDICTED: transcription factor MYB44 [*Juglans regia*]

confirming the expression of the unigenes identified in the deep sequencing analysis.

The unigenes were classified into three categories including cellular component, molecular function and biological process via GO analysis, with 675, 1905 and 2849 GO terms corresponding to each category, respectively. The top 25 significantly clustered GO terms of the unigenes for each category are shown in Fig. 4. Among the subcategories of cellular component, assignments were mostly given to nucleus, integral component of membrane, plasma membrane, cytoplasm, chloroplast, mitochondrion, and cytosol. The majority of the annotated unigenes were assigned to protein binding, ATP binding, protein serine/threonine kinase activity and DNA binding in molecular function. Dominant GO terms of the biological process subcategories were grouped into regulation of transcription, defense response, protein phosphorylation, oxidation–reduction process, and signal transduction.

KEGG pathway enrichment analysis was conducted for unigenes obtained from the transcriptome. The results showed that the unigenes in *Q. liaotungensis* could be assigned to 138 pathways. Among the 25 top KEGG pathways with the highest representation of unigenes, the abundant genes mapped onto endocytosis, plant hormone signal transduction, starch and sucrose metabolism, RNA transport, biosynthesis of amino acids, carbon metabolism, and amino sugar and nucleotide sugar metabolism (Fig. 5).

We screened a series of candidate genes potentially involved in drought adaptation via GO and KEGG analysis

Fig. 4 The top 25 enriched GO terms in cellular component, molecular function and biological process categories of the unigenes from *Q. liaotungensis* leaf transcriptome

from those unigenes, including the genes encoding superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and several drought resistance-related transcription factors (TFs) such as dehydration-responsive element binding (DREB), v-myb avian myeloblastosis viral oncogene

homolog (MYB), WRKY, basic leucine zipper (bZIP), and NAC (Fig. 1C, Table 3).

Drought has several effects on plant growth and development, one of which is oxidative damage. We identified 41 genes encoding superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Table 3) which compose the antioxidant defense system in plants that can cooperate to resist the damage of active oxygen to cells. Seven candidate

Fig. 5 The top 25 enriched pathways of the unigenes from *Q. liaotungensis* leaf transcriptome

genes encoding DREBs (Table 3) including DREB1 and DREB2 were obtained. Overexpression of DREB1 increased tolerance of *Malus baccata* to low temperature, drought, and salt stresses (Yang et al. 2010). When treated with dehydration, the expression of DREB2A increased in *Arabidopsis thaliana* (Sakuma et al. 2007). Several drought resistance-related TFs such as MYB and bZIP which were founded in *Quercus pubescens* leaves (Torre et al. 2014) were also identified in this study. We screened 5 genes encoding MYB TFs, which is one of the largest transcription factor families and play regulatory roles in developmental processes and defense responses in plants, including MYB108, 24, 44, 12 (Table 3). The bZIP and NAC TFs are known to play a

crucial role in response to various processes in plant as well as abiotic or biotic stress challenges such as drought (Xiang et al. 2008; Huang et al. 2015). WRKY proteins are newly identified TFs that are also involved in drought tolerance in plants (Ren et al. 2010). Overall, we screened 10, 42, and 6 candidate genes encoding bZIPs, WRKYs, and NACs, respectively (Table 3).

Through high-throughput sequencing, we can quickly obtain the desired target genes. As a consequence, the results of this study expanded the genetic resources of *Q.*

Table 3 The putative candidate genes involved in drought avoidance

Gene ID	Annotation
DN6399_c0_g1	Superoxide dismutase 5 [<i>Betula platyphylla</i>]
DN7263_c0_g1	PREDICTED: superoxide dismutase [Fe], chloroplastic [<i>Juglans regia</i>]
DN7744_c0_g1	Superoxide dismutase, partial [<i>Fagus sylvatica</i>]
DN11311_c2_g3	Cu/Zn-superoxide dismutase copper chaperone precursor [<i>Corylus heterophylla</i>]
DN4365_c0_g1	PREDICTED: superoxide dismutase [Cu–Zn] [<i>Juglans regia</i>]
DN7248_c0_g1	PREDICTED: superoxide dismutase [Cu–Zn], chloroplastic [<i>Fragaria vesca</i> subsp. <i>vesca</i>]
DN10225_c2_g4	[Fe] superoxide dismutase, partial [<i>Betula platyphylla</i>]
DN8775_c0_g1	PREDICTED: L-ascorbate peroxidase 2, cytosolic-like [<i>Juglans regia</i>]
DN3904_c0_g1	PREDICTED: L-ascorbate peroxidase 3, peroxisomal-like [<i>Juglans regia</i>]
DN9185_c0_g11	PREDICTED: probable L-ascorbate peroxidase 6, chloroplastic isoform X2 [<i>Ziziphus jujuba</i>]
DN10135_c1_g3	PREDICTED: L-ascorbate peroxidase, cytosolic [<i>Gossypium raimondii</i>]
DN7015_c0_g1	PREDICTED: cationic peroxidase 1-like [<i>Lupinus angustifolius</i>]
DN10800_c0_g1	PREDICTED: probable glutathione peroxidase 2 [<i>Juglans regia</i>]
DN7056_c0_g1	PREDICTED: phospholipid hydroperoxide glutathione peroxidase 1, chloroplastic-like [<i>Juglans regia</i>]
DN10459_c2_g1	PREDICTED: putative L-ascorbate peroxidase 6 [<i>Juglans regia</i>]
DN8556_c0_g1	PREDICTED: peroxidase A2-like [<i>Juglans regia</i>]
DN7254_c0_g1	PREDICTED: peroxidase 25-like [<i>Juglans regia</i>]
DN9704_c1_g3	putative peroxidase 48 [<i>Juglans regia</i>]
DN11127_c1_g2	PREDICTED: peroxidase 73-like [<i>Juglans regia</i>]
DN277_c0_g1	PREDICTED: peroxidase 47 [<i>Juglans regia</i>]
DN11207_c1_g2	PREDICTED: putative peroxidase 48 [<i>Juglans regia</i>]
DN9110_c0_g1	PREDICTED: peroxidase 17-like [<i>Juglans regia</i>]
DN10911_c0_g1	PREDICTED: peroxidase 3-like [<i>Juglans regia</i>]
DN11029_c1_g2	PREDICTED: putative peroxidase 48 [<i>Juglans regia</i>]
DN4001_c0_g1	peroxidase [<i>Betula platyphylla</i>]
DN11503_c2_g1	PREDICTED: putative peroxidase 48 [<i>Malus domestica</i>]
DN5675_c0_g1	PREDICTED: peroxidase P7-like [<i>Populus euphratica</i>]
DN2342_c0_g1	PREDICTED: peroxidase 55 [<i>Vitis vinifera</i>]
DN10208_c2_g5	PREDICTED: putative peroxidase 48 [<i>Ziziphus jujuba</i>]
DN7165_c0_g1	PREDICTED: peroxidase 31-like [<i>Juglans regia</i>]
DN7165_c0_g2	PREDICTED: peroxidase 31-like [<i>Juglans regia</i>]
DN9704_c1_g1	PREDICTED: putative peroxidase 48 [<i>Ziziphus jujuba</i>]
DN11057_c0_g2	PREDICTED: peroxidase 4-like [<i>Vitis vinifera</i>]
DN11457_c0_g2	PREDICTED: putative peroxidase 48 [<i>Glycine max</i>]
DN10208_c2_g3	PREDICTED: putative peroxidase 48 [<i>Juglans regia</i>]
DN11661_c0_g1	PREDICTED: peroxidase 4-like [<i>Juglans regia</i>]
DN8556_c0_g1	PREDICTED: peroxidase A2-like [<i>Juglans regia</i>]
DN11057_c0_g2	PREDICTED: peroxidase 4-like [<i>Vitis vinifera</i>]
DN9704_c1_g2	PREDICTED: putative peroxidase 48 [<i>Malus domestica</i>]
DN11029_c1_g3	PREDICTED: putative peroxidase 48 [<i>Juglans regia</i>]
DN10358_c0_g3	PREDICTED: catalase isozyme 1 [<i>Juglans regia</i>]
DN9997_c2_g1	transcription factor MYB5 [<i>Fragaria x ananassa</i>]
DN10502_c1_g1	PREDICTED: transcription factor MYB108-like [<i>Populus euphratica</i>]
DN330_c0_g1	PREDICTED: transcription factor MYB24-like [<i>Juglans regia</i>]
DN9760_c2_g6	PREDICTED: transcription factor MYB44 [<i>Juglans regia</i>]
DN1817_c0_g1	PREDICTED: transcription factor MYB108-like [<i>Juglans regia</i>]
DN10064_c2_g2	PREDICTED: transcription factor MYB12-like isoform X2 [<i>Juglans regia</i>]
DN9464_c0_g1	PREDICTED: dehydration-responsive element-binding protein 1A-like [<i>Juglans regia</i>]
DN5663_c0_g1	PREDICTED: putative dehydration-responsive element-binding protein 2H [<i>Juglans regia</i>]
DN5351_c0_g1	PREDICTED: dehydration-responsive element-binding protein 2A-like isoform X1 [<i>Juglans regia</i>]

Table 3 (continued)

Gene ID	Annotation
DN10470_c0_g2	PREDICTED: dehydration-responsive element-binding protein 1D-like [<i>Nelumbo nucifera</i>]
DN5663_c0_g3	Dehydration-responsive element-binding protein 2A [<i>Carica papaya</i>]
DN10595_c0_g6	PREDICTED: dehydration-responsive element-binding protein 1A-like [<i>Ziziphus jujuba</i>]
DN10470_c0_g4	PREDICTED: dehydration-responsive element-binding protein 1A-like [<i>Nicotiana attenuata</i>]
DN9174_c2_g8	PREDICTED: basic leucine zipper 61 [<i>Juglans regia</i>]
DN1381_c0_g1	PREDICTED: basic leucine zipper 25 [<i>Juglans regia</i>]
DN11089_c0_g2	PREDICTED: basic leucine zipper 23-like [<i>Juglans regia</i>]
DN6101_c0_g1	PREDICTED: basic leucine zipper 23 [<i>Vitis vinifera</i>]
DN6968_c0_g1	PREDICTED: bZIP transcription factor 53 [<i>Ricinus communis</i>]
DN9193_c0_g1	PREDICTED: bZIP transcription factor 17 [<i>Juglans regia</i>]
DN9677_c0_g1	PREDICTED: bZIP transcription factor 53-like [<i>Prunus mume</i>]
DN10955_c1_g4	PREDICTED: bZIP transcription factor 60-like [<i>Juglans regia</i>]
DN8966_c0_g2	PREDICTED: bZIP transcription factor 16-like [<i>Juglans regia</i>]
DN809_c0_g1	PREDICTED: bZIP transcription factor 53 [<i>Juglans regia</i>]
DN11516_c0_g1	PREDICTED: NAC transcription factor 29-like [<i>Juglans regia</i>]
DN11389_c0_g2	PREDICTED: NAC transcription factor 56 [<i>Juglans regia</i>]
DN10815_c0_g1	NAC transcription factor, partial [<i>Fagus sylvatica</i>]
DN11389_c0_g4	PREDICTED: NAC transcription factor 56 [<i>Juglans regia</i>]
DN1786_c0_g1	PREDICTED: NAC transcription factor 25 [<i>Juglans regia</i>]
DN1874_c0_g1	PREDICTED: NAC transcription factor 29 [<i>Theobroma cacao</i>]
DN3070_c0_g1	PREDICTED: probable WRKY transcription factor 29 isoform X2 [<i>Juglans regia</i>]
DN10418_c0_g5	PREDICTED: probable WRKY transcription factor 23 [<i>Juglans regia</i>]
DN9002_c6_g3	PREDICTED: probable WRKY transcription factor 33 [<i>Juglans regia</i>]
DN10303_c3_g4	PREDICTED: probable WRKY transcription factor 32 isoform X2 [<i>Vitis vinifera</i>]
DN7541_c0_g2	PREDICTED: probable WRKY transcription factor 2 [<i>Prunus mume</i>]
DN4882_c0_g1	PREDICTED: probable WRKY transcription factor 40 [<i>Juglans regia</i>]
DN9140_c1_g1	PREDICTED: probable WRKY transcription factor 53 [<i>Juglans regia</i>]
DN6992_c0_g1	PREDICTED: probable WRKY transcription factor 51 isoform X1 [<i>Juglans regia</i>]
DN9650_c1_g6	PREDICTED: probable WRKY transcription factor 21 [<i>Juglans regia</i>]
DN9208_c4_g1	PREDICTED: probable WRKY transcription factor 57 [<i>Juglans regia</i>]
DN10157_c0_g3	PREDICTED: probable WRKY transcription factor 3 isoform X1 [<i>Juglans regia</i>]
DN8858_c1_g5	PREDICTED: probable WRKY transcription factor 13 [<i>Theobroma cacao</i>]
DN11151_c2_g4	PREDICTED: probable WRKY transcription factor 65 [<i>Juglans regia</i>]
DN9530_c2_g5	PREDICTED: probable WRKY transcription factor 31 [<i>Juglans regia</i>]
DN9986_c3_g1	PREDICTED: probable WRKY transcription factor 31 [<i>Nelumbo nucifera</i>]
DN9087_c2_g1	PREDICTED: probable WRKY transcription factor 4 [<i>Juglans regia</i>]
DN9333_c1_g1	PREDICTED: probable WRKY transcription factor 7 [<i>Juglans regia</i>]
DN9569_c1_g2	WRKY transcription factor 43 [<i>Manihot esculenta</i>]
DN9140_c2_g2	PREDICTED: probable WRKY transcription factor 20 isoform X1 [<i>Juglans regia</i>]
DN9632_c0_g4	PREDICTED: probable WRKY transcription factor 4 [<i>Juglans regia</i>]
DN9002_c4_g1	PREDICTED: probable WRKY transcription factor 20 isoform X1 [<i>Juglans regia</i>]
DN10418_c0_g4	PREDICTED: probable WRKY transcription factor 48 [<i>Juglans regia</i>]
DN9553_c1_g6	PREDICTED: probable WRKY transcription factor 70 isoform X2 [<i>Juglans regia</i>]
DN9282_c1_g2	PREDICTED: WRKY transcription factor 44-like [<i>Juglans regia</i>]
DN7168_c0_g1	PREDICTED: probable WRKY transcription factor 75 [<i>Juglans regia</i>]
DN9652_c4_g3	PREDICTED: probable WRKY transcription factor 28 [<i>Juglans regia</i>]
DN2165_c0_g1	PREDICTED: probable WRKY transcription factor 69 [<i>Ziziphus jujuba</i>]
DN9423_c0_g1	PREDICTED: probable WRKY transcription factor 31 isoform X2 [<i>Juglans regia</i>]
DN11844_c0_g1	PREDICTED: probable WRKY transcription factor 61 [<i>Juglans regia</i>]
DN8225_c0_g1	PREDICTED: WRKY transcription factor 55-like [<i>Juglans regia</i>]

Table 3 (continued)

Gene ID	Annotation
DN9947_c0_g1	PREDICTED: probable WRKY transcription factor 50 isoform X2 [<i>Prunus mume</i>]
DN9148_c1_g5	PREDICTED: probable WRKY transcription factor 2 [<i>Juglans regia</i>]
DN9553_c1_g9	PREDICTED: probable WRKY transcription factor 70 [<i>Juglans regia</i>]
DN9279_c4_g6	WRKY transcription factor 47–2 [<i>Dimocarpus longan</i>]
DN9002_c7_g3	PREDICTED: WRKY transcription factor 1 isoform X1 [<i>Juglans regia</i>]
DN9767_c3_g7	PREDICTED: probable WRKY transcription factor 53 [<i>Juglans regia</i>]
DN4756_c0_g1	PREDICTED: probable WRKY transcription factor 69 isoform X1 [<i>Juglans regia</i>]
DN7477_c0_g1	PREDICTED: WRKY transcription factor 44-like [<i>Nelumbo nucifera</i>]
DN10872_c0_g11	PREDICTED: probable WRKY transcription factor 53 [<i>Juglans regia</i>]
DN9899_c0_g5	PREDICTED: probable WRKY transcription factor 40 [<i>Juglans regia</i>]
DN9140_c2_g8	PREDICTED: WRKY transcription factor WRKY24-like isoform X2 [<i>Juglans regia</i>]
DN10364_c0_g1	PREDICTED: WRKY transcription factor 18-like [<i>Juglans regia</i>]

liaotungensis and provided useful information for further research on drought resistance mechanisms of *Q. liaotungensis*.

Author contribution statement GW and LQ conceived the project; GW performed the experiments; GW analyzed the data and wrote the paper.

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