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Research article

Characterization of the transcriptional profiles in common buckwheat (*Fagopyrum esculentum*) under PEG-mediated drought stress

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ABSTRACT

Background: Common buckwheat (*Fagopyrum esculentum*) is an important staple food crop in southwest China, where drought stress is one of the largest limiting factors that lead to decreased crop production. To reveal the molecular mechanism of common buckwheat in response to drought stress, we performed a comprehensive transcriptomics study to evaluate gene expression profiles of common buckwheat during PEG-mediated drought treatment.

Results: In total, 45 million clean reads were assembled into 53,404 unigenes with an average length of 749 bp and N50 length of 1296 bp. A total of 1329 differentially expressed genes (DEGs) were identified by comparing well-watered and drought-treated plants, out of which 666 were upregulated and 663 were downregulated. Furthermore, we defined the functional characteristics of DEGs using GO and KEGG classifications. GO enrichment analysis showed that the DEGs were significantly overrepresented in four categories, namely, "oxidoreductase activity," "oxidation–reduction process," "xyloglucan:xyloglucosyl transferase activity," and "apoplast." Using KEGG pathway analysis, a large number of annotated genes were overrepresented in terms such as "plant hormone signal transduction," "phenylpropanoid biosynthesis," "photosynthesis," and "carbon metabolism."

Conclusions: These results can be further exploited to investigate the molecular mechanism of common buckwheat in response to drought treatment and could supply with valuable molecular sources for abiotic-tolerant elite breeding programs in the future.

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1. Introduction

Drought stress is one of the most severe environmental constraints that adversely affects growth and yields of crops [1]. Water shortage could inevitably lead to oxidative damage and protein denaturation as well as mechanical damage to plant cells [2]. Previous studies indicated that plant morphological and physiological indices were changed when suffered from drought stress, reflected by repressed growth rate, enhanced antioxidant enzymes activities, and injured cells [3,4]. Drought stress usually triggers a considerable variety of molecular responses, ranging from altered gene expression to cellular metabolism, which are thought to be related to resistance or drought adaptation in plants [5]. Reduced activities of chlorophyll-binding proteins such as PsbB and PsbQ under drought stress conditions indicated that the photosynthesis process could be severely disrupted [6]. In addition, drought could also influence the mobilization of sugar reserves by decreasing the contents of phosphoribulose kinase (PRK) and fructose 1,6-bisphosphatase (FBP) in carbon metabolism [7]. Transcription factors (TFs) have been proven to play vital roles in plant growth and development as well as in abiotic stress responses [8]. For example, DREB1 and DREB2 functioned as trans-acting factors in signal transduction pathways under drought conditions [9]. The WRKY transcription factor superfamily was found to be involved in drought resistance in wheat [10]. The transcription factor CBF4 was a crucial regulator during drought adaptation in *Arabidopsis thaliana* [11].

Recent studies have demonstrated that investigation of the genome-wide gene expression levels is an effective way of studying

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the molecular activities under abiotic stress conditions [12]. Recently, the high-throughput and accurate deep sequencing method (RNA-seq) has been used to discover functional genes and transcript changes in cell morphology as well as physiological and biochemical metabolism in plants under abiotic stress conditions [13]. RNA-seq data could provide insight into response to drought stress, gene discovery, and molecular marker identification [14]. Using RNA-seq methods, a large number of differentially expressed genes related to abiotic stress progresses have been identified in various plant species [15,16,17,18,19].

Common buckwheat (*Fagopyrum esculentum*) is an important short season crop widely distributed in China, Japan, and Europe. Drought stress is one of the largest adverse environmental factors that are a threat to crop production [20]. Previously, RNA-seq technology has been used to examine the expression profile of aluminum-responsive genes in aluminum-accumulating buckwheat species [19]. Using RNA-seq analysis, some flavonoid synthesis-related genes were identified in *F. esculentum* [21]. In addition, RNA-seq results showed that a number of transporter genes were highly expressed in the roots and leaves of common buckwheat subjected to A1 stress [18]. However, only very few functional genes associated with drought response were discovered, such as *FeDREB1* and *FtMYB10* [22]. As expected, lack of transcriptomic data has seriously limited the understanding of the drought-responsive molecular mechanisms in *F. esculentum*. In this study, we provide a comprehensive drought-responsive expression profile of common buckwheat. By analyzing genome-wide transcript expression patterns of common buckwheat grown under control and drought conditions, we found that many key differentially expressed genes (DEGs) participate in several important metabolic pathways. Our results could provide a global view of drought-tolerant regulatory mechanisms in common buckwheat.

2. Materials and methods

2.1. Plant materials and drought treatment

Hong Hua Tian Qiao variety was used for *F. esculentum* transcriptome analysis. Seeds were germinated in nutritional soil under growth chamber conditions. The growth conditions were set as previously reported [23]: temperature 25°C during day and 23°C at night, photon flux density of 100 μmol·m^{-2}·s^{-1}, a photoperiod of 12/12 h day/night cycle, and a relative humidity of 65%. The 14-day-old seedlings were treated with a 20% polyethylene glycol (PEG)-6000 (Sigma-Aldrich, USA) solution for 24 h as previously described [24]. Each sample with three independent biological replicates was collected under both control (well water) and drought-treated conditions for RNA-seq. For each biological replicate, aerial parts from 7 to 10 seedlings were collected and immediately frozen in liquid nitrogen for RNA isolation.

2.2. Measurement of the buckwheat physiological index

After drought treatment, control and drought-treated samples in quadruplicate were used for measurement of physiological indices. Relative water content (RWC) and electrolyte leakage (EL) were measured as previously described [25]. Malondialdehyde (MDA) content, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities were determined by the protocols used in a previous study [26].

2.3. RNA extraction, cDNA library construction, and Illumina sequencing

Total RNA was extracted using a Plant RNA Extraction kit (TIANDZ, China) according to the manufacturer's instructions. Total RNA pooled with equal quantity from all three biological replicates was used for cDNA library construction. Column DNA Erasol (TIANDZ, China) was used to avoid DNA contamination. The quality and quantity of total RNA were determined using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) and Bioanalyzer RNA nano chip (Agilent Technologies, Singapore). Only samples with an RIN between 6 and 7 and a 260/280 ratio within the range of 1.8–2.0 were qualified for cDNA library preparation. mRNAs were enriched from total RNA using oligo(dT)-attached magnetic beads. The derived mRNAs were fragmented into approximately 300 bps using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5X). The fragmented mRNAs were reverse-transcribed into first-strand cDNAs with a random hexamer, and then the second-strand cDNAs were synthesized using a NEB Next Ultra™ RNA Library Prep Kit for Illumina (NEB). The double-stranded cDNAs were purified and ligated to adaptors. Suitable cDNA fragments were selected for PCR amplification. An Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA) and an ABI 7500 real-time PCR machine (Applied Biosystems) were used to determine average molecular lengths. Then, qualified libraries were amplified on cBot to generate a cluster on flow cell (TruSeq PE Cluster Kit V3-cBot-HS, Illumina), and paired-end clusters amplified using flow cell were sequenced on a HiSeq2500 platform.

2.4. De novo assembly and annotation of buckwheat transcriptome

Adaptor, poly-N, and low-quality bases containing sequences were removed to obtain clean data. Using the Trinity program [27], the obtained clean reads were assembled *de novo* to generate unigenes. To annotate the assembled transcripts, BLASTX searches [28] were performed against the NR [29], Swiss-Prot [30], KOG (euKaryotic Orthologous Groups) [31], and Kyoto Encyclopedia of Genes and Genomes (KEGG) [32] with the threshold of e-value = 1e−5 for NR and Swiss-Prot, e-value = 1e−3 for KOG, and e-value = 1e−10 for KEGG. Blast2GO [33] (e-value = 1e−6) was used to obtain GO annotation provided by the UniProt Consortium [34]. To evaluate differences in gene expression, the FPKM method (Fragments Per Kilobase of transcript per Million fragments mapped) was employed to calculate read density. Read count data were normalized using trimmed mean of M-values (TMM) [35]. A stringent cutoff with a q-value <0.005 and an absolute value of $|\log_2$ Fold Change$|$ >1 was used to identify differentially expressed genes (DEG). Subsequently, the DEGs were further subjected to GO and KEGG enrichment analyses.

2.5. Real-time PCR analysis

After DNA digestion and reverse transcription by applying EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit (Transgen Biotech, Beijing, China), the synthesized cDNAs of 3 μg total RNA were used for real-time PCR. PCR amplifications were conducted in a reaction volume of 20 μl containing 15 ng templates, 6 μl ddH_2O, 10 μl PCR mix, and 6 pmol of each primer. The reaction conditions were 95°C for 5 min and 40 cycles of 95°C for 5 s and 59°C for 15 s. Real-time RT-PCR was carried out using the Line-Gene 9600 system (BIOER, Hangzhou, China). All the primers used in this study are listed in Table S1. The melting curve was obtained by applying increasing temperature from 60 to 90°C. With the *Actin* gene set as internal control [23], the relative fold change for each gene was calculated using the $2^{-\Delta\Delta Ct}$ method [36]. Then we compared the fold change value between the results obtained from real-time PCR and those from RNA-seq through the Pearson correlation analysis.

3. Results

3.1. Physiological evaluation of buckwheat seedlings in response to drought treatment

To determine whether the drought stress conditions in this study could influence the physiological activities in *F. esculentum* seedlings, we measured a series of key physiological indices, namely, relative water content (RWC), electrolyte leakage (EL), malondialdehyde

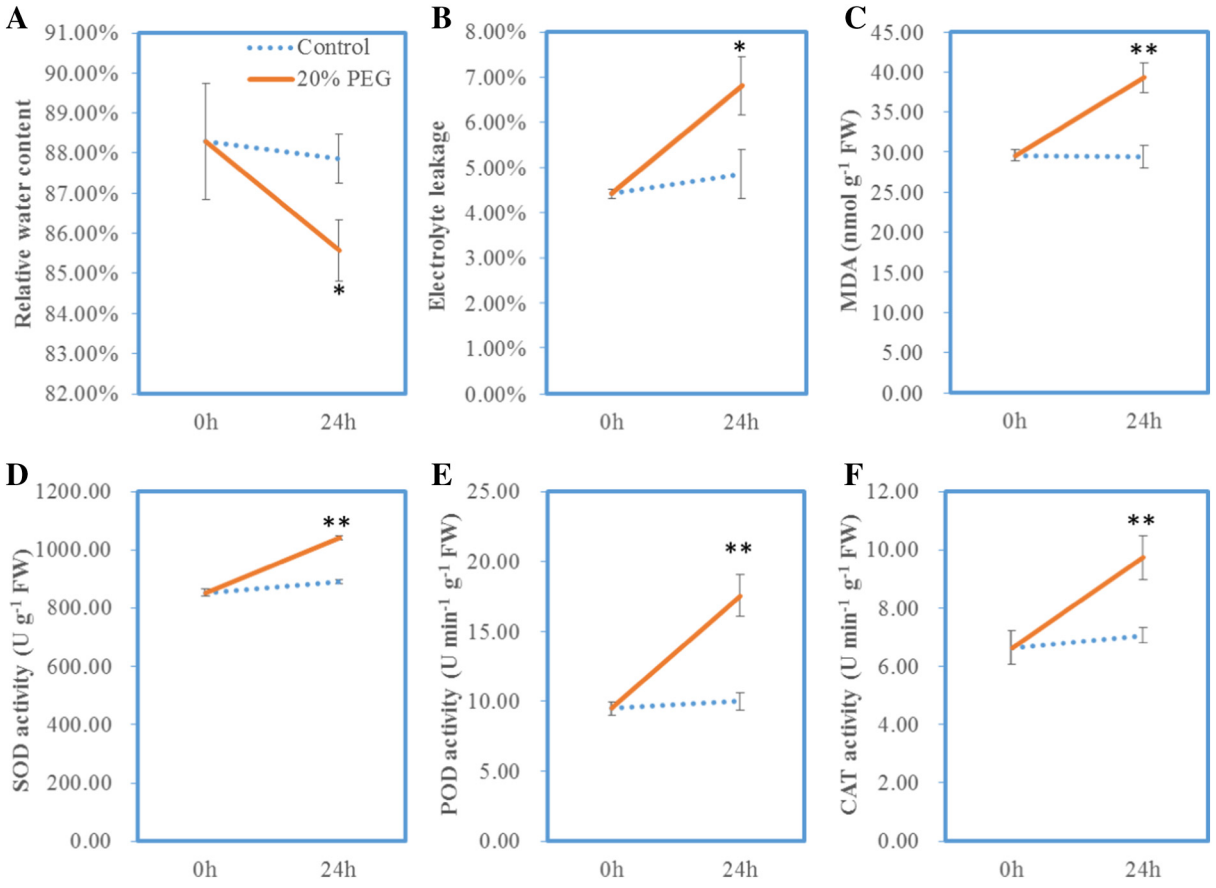

Fig. 1. Measurement of relative water content (RWC) (A), electrolyte leakage (EL) (B), malondialdehyde (MDA) (C), superoxide dismutase (SOD) (D), peroxidase (POD) (E), and catalase (CAT) activities in control and drought-treated plants. Control and drought treatment conditions are represented as 0 h and 24 h. Values are presented as mean ± SD (n = 3). Single asterisk (*) and double asterisks (**) indicate $P < 0.05$ and $P < 0.01$, respectively, determined by Student's *t*-test.

Table 1
Summary of Illumina paired-end sequencing data.

Sample name	Raw reads	Clean reads	Q20 (%)	Q30 (%)	GC content (%)	Transcripts	Unigenes
HH_0	25,126,888	25,114,173	99.02	97.10	49.91		
HH_PEG24	19,870,816	19,861,667	96.65	91.79	51.35		
Mean length						862 bp	749 bp
N50 length						1412 bp	1296 bp
Total number						78,053	53,404

HH_0h and HH_PEG24h represent control and drought-treated samples, respectively.

(MDA) content, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities. The aerial parts of two-week-old seedlings under control and drought stress conditions were collected for physiological evaluation.

Our results demonstrated that RWC in buckwheat seedlings was relatively decreased as they suffered from drought treatment (Fig. 1A). EL in drought-treated buckwheat was increased by 40% compared with control (Fig. 1B). Further, we found that the MDA content, SOD, POD, and CAT activities in drought stress plants were severely greater than that in control plants (Fig. 1C, Fig. 1D, Fig. 1E, Fig. 1F).

3.2. RNA sequencing, assembly, and functional annotation of drought transcriptome

To explore in-depth molecular changes in response to drought stress, we applied high-throughput paired-end sequencing approaches by using the Illumina HiSeq2500 platform to discover which categories of genes were mostly involved in drought adaption. Approximately 19 and 25 million raw reads of length 300 bp were produced from control and drought-treated samples, respectively. After data processing, in total, approximately 45 million clean reads were assembled using the Trinity program [27] to generate 78,053 transcripts with a mean length of 862 bp and N50 length of 1412 bp. Subsequently, the transcript sequences were subjected to similarity test, generating 53,404 unigenes. The mean length and N50 length of unigenes reached 749 bp and 1296 bp, respectively (Table 1).

Subsequently, we mapped the transcriptome sequences onto public genome databases for functional annotation. As shown in Table 2, a total

Table 2
Annotated unigenes mapped onto different databases.

	Number of unigenes	Percentage (%)
Annotated in NR	30,819	57.7
Annotated in Swiss Prot	23,903	44.75
Annotated in GO	21,006	39.33
Annotated in KOG	11,986	22.44
Total unigenes	53,404	100

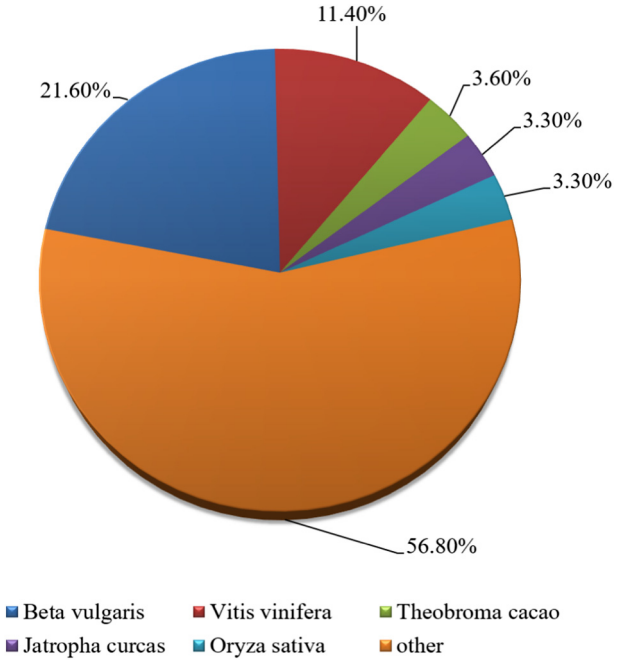

Fig. 2. The percentage of unigenes annotated in genomes of different species.

of 30,819 and 23,903 unigenes were well annotated against the NR and SwissProt databases, accounting for 57.7% and 44.75% of the total number, respectively. Further, only 21,006 (39.33%) and 11,986 (22.44%) unigene sequences could find their matches in GO and KOG databases (Table 2). Through NR annotation, we found that the unigenes were significantly mapped onto *Beta vulgaris*, *Vitis vinifera*, *Theobroma cacao*, *Jatropha curcas,* and *Oryza sativa* genomes (Fig. 2).

Gene Ontology (GO) enrichment analysis was conducted to identify drought-responsive transcriptome sequences, and these genes were categorized into 56 GO terms (Fig. 3). In biological process category, "cellular process" and "metabolic process" groups were mostly enriched compared with other categories. Among the cellular component category, unigenes were mostly enriched in "cell" (312; 1.5%) and "cell part" terms. In the molecular function category, "binding" and "catalytic activity" were most significantly enriched (Fig. 3).

To better understand the biological functions of these unigenes, we performed euKaryotic Orthologous Groups (KOG) enrichment analysis. As illustrated in Fig. 4, 26 KOG terms were highly enriched, among which the key metabolic processes including "General function prediction only," "Posttranslational modification, protein turnover, chaperones," "Signal transduction mechanisms," "Translation, ribosomal structure and biogenesis," "Intracellular trafficking, secretion, and vesicular transport," "Transcription," and "Carbohydrate transport and metabolism" were the most significantly represented (Fig. 4).

Moreover, through the Kyoto Encyclopedia of Genes and Genomes (KEGG) classification, we found KEGG items such as "Transport and catabolism" and "Cell growth and death" in "Cellular Processes"; "Signal transduction" and "Membrane transport" in "Environmental Information Processing;" "Translation," "Folding, sorting and degradation," and "Transcription" in "Genetic Information Processing"; "Carbohydrate metabolism," "Overview," and "Energy metabolism" in "Metabolism"; and "Endocrine system," "Nervous system," and "Environmental adaptation" in "Organismal Systems" were the most highly represented (Fig. 5). Consequently, these functional classification results will be very useful for downstream evaluation of the drought-responsive mechanisms in common buckwheat.

3.3. Differentially expressed genes under drought conditions

Gene expression levels were calculated and normalized by the FPKM method. The genes harboring FPKM values larger or less than 0.3 were defined as expressed genes or not expressed genes, respectively. Using such criteria, we found 10,238 and 6680 genes, respectively, showed a specific expression pattern under control and drought conditions, and 29,694 genes were co-expressed under both conditions (Fig. 6A). Further, we identified 1329 differentially expressed genes (DEGs) by utilizing the threshold of absolute fold change values larger than 1 (Fig. 6B). Out of 1329 DEGs, 666 were upregulated, while 663 were downregulated (Fig. 6B).

GO enrichment analysis showed that the DEGs were significantly overrepresented in four categories, namely, "oxidoreductase activity," "oxidation–reduction process," "xyloglucan:xyloglucosyl transferase activity," and "apoplast" (Table 3).

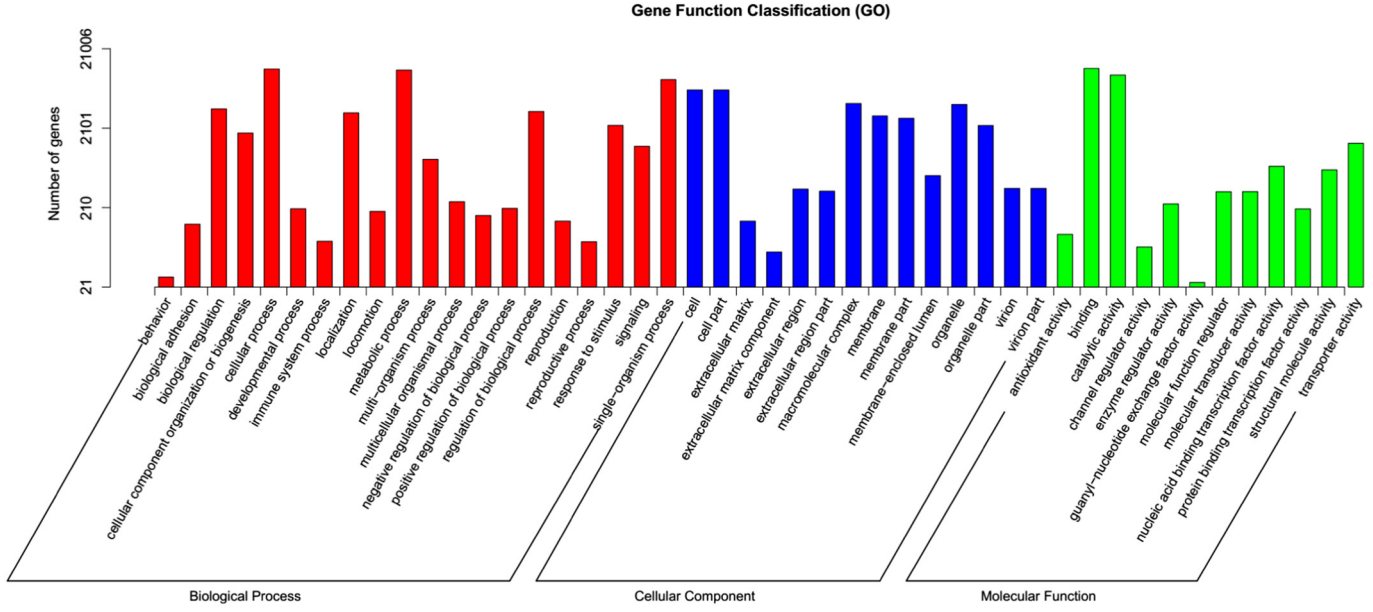

Fig. 3. Gene ontology (GO) analysis of the *F. esculentum* unigenes. The *F. esculentum* unigenes were categorized into 56 GO terms, mainly distributed in Biological Process, Cellular Component, and Molecular Function categories.

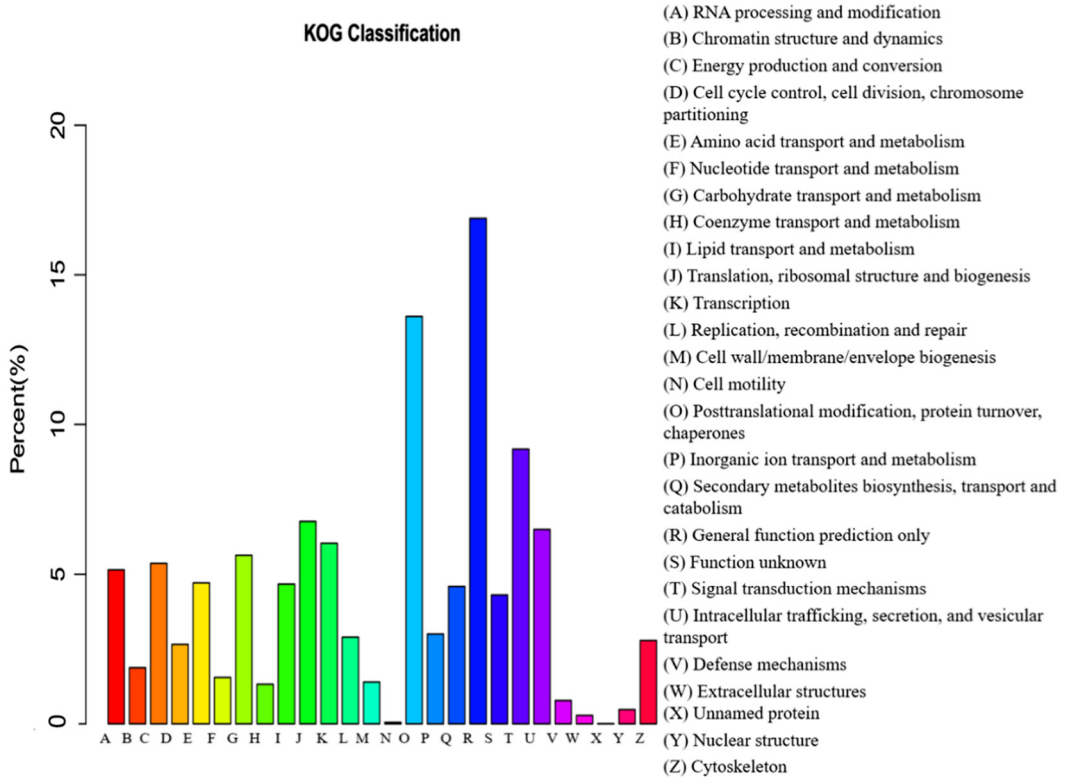

Fig. 4. euKaryotic Orthologous Groups (KOG) functional classification of the *F. esculentum* unigenes. The *F. esculentum* unigenes in this study were categorized into 26 KOG terms, out of which "General function prediction only," "Posttranslational modification, protein turnover, chaperones," and "Signal transduction mechanisms" harbor the most numbers.

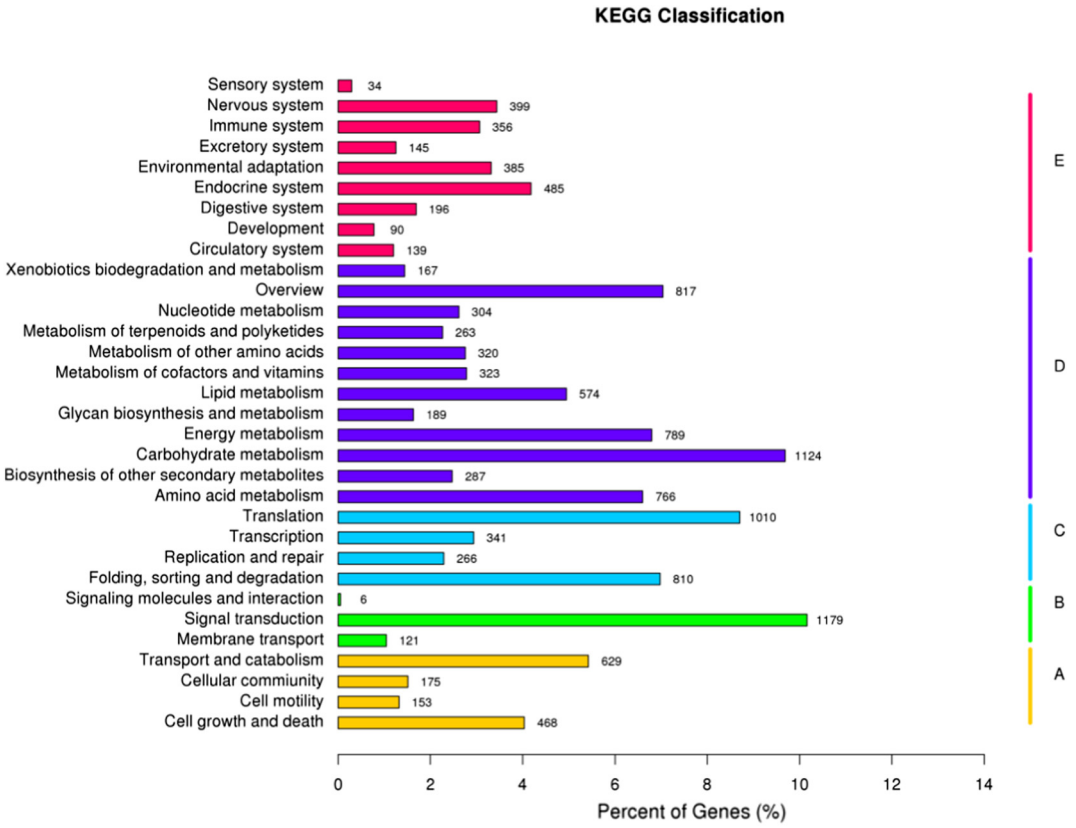

Fig. 5. Kyoto Encyclopedia of Genes and Genomes (KEGG) functional classification of the *F. esculentum* unigenes. A–E on the right side indicates five different KEGG functional groups. (A) Cellular Processes; (B) Environmental Information Processing; (C) Genetic Information Processing; (D) Metabolism; (E) Organismal Systems.

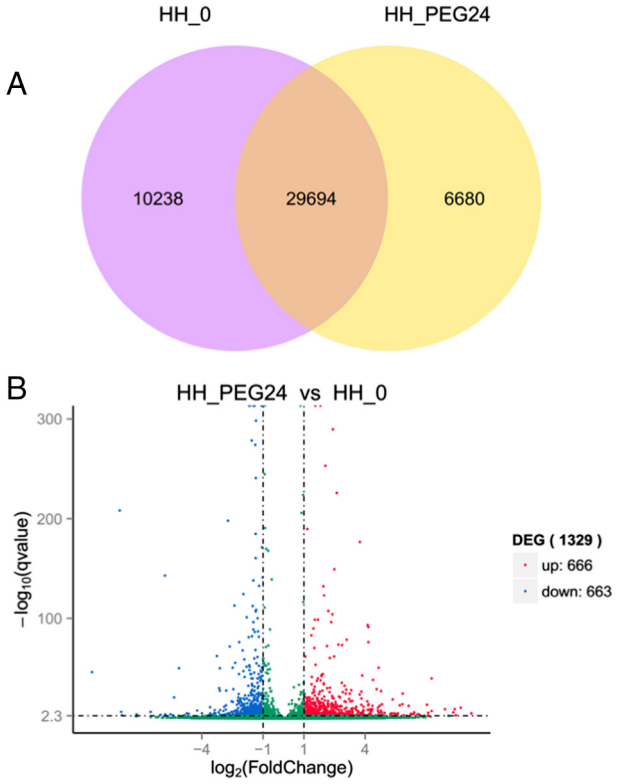

Fig. 6. (A) Venn diagram of co-expressed and specifically expressed genes. (B) Volcano plot of differentially expressed genes (DEGs). The red dots on the right region indicate upregulated genes, whereas the green dots on the left region indicate downregulated genes.

The DEGs were also subjected to KEGG pathway enrichment analysis (Fig. 7A, Fig. 7B). Interestingly, compared with the control, a large number of upregulated genes were involved in the pathway terms "plant hormone signal transduction," "phenylpropanoid biosynthesis," and "oxidative phosphorylation" (Fig. 7A). By contrast, the downregulated genes were observed to be enriched in "photosynthesis," "carbon metabolism," and "biosynthesis of amino acids" (Fig. 7B). These results indicate the involvement of these metabolic processes in drought tolerance in buckwheat.

3.4. Real-time PCR validation of differentially expressed genes from RNA-seq

To test the reliability of RNA-seq data, several drought-responsive genes that showed up- and downregulation were chosen for real-time PCR. The relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. Clearly, the expression trends of these genes obtained by real-time PCR were in strong agreement with those of RNA-Seq data (Fig. 8), and a significant correlation of fold changes was identified ($R^2 = 0.935$ according to Pearson correlation analysis).

3.5. Characterization of key drought-responsive genes in F. esculentum

The changes at the transcription levels of regulatory genes are biologically meaningful, which may be linked to core metabolic processes under stress conditions [37]. As mentioned above, a large number of upregulated genes encoding transcription factors were significantly involved in phytohormone metabolic pathways (Table 4), such as *Brassinosteroid Insensitive 1* (*BRI1*), *Auxin Response Factors* (*ARF*), *EIN3-Binding F-box protein* (*EBF1/2*), *Brassinazole Resistant 1/2* (*BZR1/2*), *DELLA* protein, and *Gibberellin-insensitive Dwarf 2* (*GID2*). In addition, increased expression of genes encoding antioxidant enzymes was observed, including *phenylalanine ammonia-lyase* (*PAL*), *cinnamic acid 4-hydroxylase* (*CYP73A*) and *Cinnamyl Alcohol Dehydrogenase* (*CAD*) (Table 4).

On the other hand, an almost equal amount of downregulated genes was also identified (Table 4). Most of them were related to photosynthesis metabolism, including *PSBB, PSBP*, *PSBM*, *PSBQ, PSBY*, and *PSBW* involved in photosystem II; *PSAF*, *PSAG*, *PSAK*, *PSAL*, *PSAN*, and *PSAO* involved in photosystem I; and *PETF*, *PSAH* involved in photosynthetic electron transport. Many key enzyme-encoding genes related to carbon metabolism dramatically decreased when common buckwheat seedlings were treated with drought stress. Specifically, these downregulated genes were *Phosphofructokinase* (*PFKA*), *Glyceric acid* (*GLYA*), *Serine Hydroxymethyl Transferase* (*SHMT*), *Glutamate/Glyoxylate Aminotransferase* (*GGAT*), *Guanidinopolymyxin* (*GPMB*), *Glyceraldehyde-3-phosphate Dehydrogenase* (*GAPDH*), *Glyceraldehyde 3-Phosphate Dehydrogenase A* (*GAPA*), *Glycine Decarboxylase* (*GCVT*), *Malate Dehydrogenase* 2 (*MDH2*), *Glycine Decarboxylase* (*GLDC*), *Phosphoribulose Kinase* (*PRK*), *Phosphoribulokinase* (*PRKB*), *Ribose 5-Phosphate Isomerase A* (*RPIA*) and *Fructose 1 6-Bisphosphatase* (*FBP*).

4. Discussion

F. esculentum is an important pseudo cereal in Asia. Recently, many researchers have focused on the effect of unfavorable environmental factors on buckwheat, especially the effect of aluminum stress [38,39]. For another buckwheat species of the genus *Fagopyrum* (*Fagopyrum tataricum)*, many vital salt-resistant genes were identified by RNA-seq analysis [23]. In agriculture production, drought severely affects buckwheat growth. Unfortunately, to our knowledge, only one investigation on drought-induced morphological changes in *F. esculentum* was carried out thus far [24]. By contrast, little is known about drought-related genes that contribute to stress response in buckwheat at a molecular level, which is the aim of our current study. We identified a large number of drought-responsive genes based on our transcriptome data that are distinct from those reported in Al-tolerant buckwheat varieties. To understand the function of drought-responsive genes, we used GO and KEGG analysis tools to classify the DEGs into items related to different pathways. Interestingly, some human- or disease-related KEGG items such as "nervous system," "digestive system," "Parkinson's disease," and "systemic lupus" were presented in the functional classification results of transcriptome or DEGs. We speculated that this may arise from the homology of the genes between different species. Development of plant-specific functional classification tools will solve this problem.

Phytohormone plays a crucial role in controlling plant physiological and developmental processes [40]. Previous research has confirmed the importance of brassinosteroids (BRs) on conferring tolerance to various abiotic stressors in *Arabidopsis* [41]. *Brassinosteroid-insensitive 1* (*BRI1*) has been reported to initiate BR signaling and regulate plant growth

Table 3
GO enrichment analysis of the DEGs.

GO accession	Description	Term type	Over represented *P*-value	Corrected *P*-value	DEG number
GO: 0016491	Oxidoreductase activity	Molecular function	6.4717e−09	2.8197e−05	1031
GO: 0055114	Oxidation–reduction process	Biological process	1.8557e−07	0.00040426	1031
GO: 0016762	Xyloglucan:xyloglucosyl transferase activity	Molecular function	1.2733e−06	0.0011096	1031
GO: 0048046	Apoplast	Cellular component	1.2733e−06	0.0011096	1031

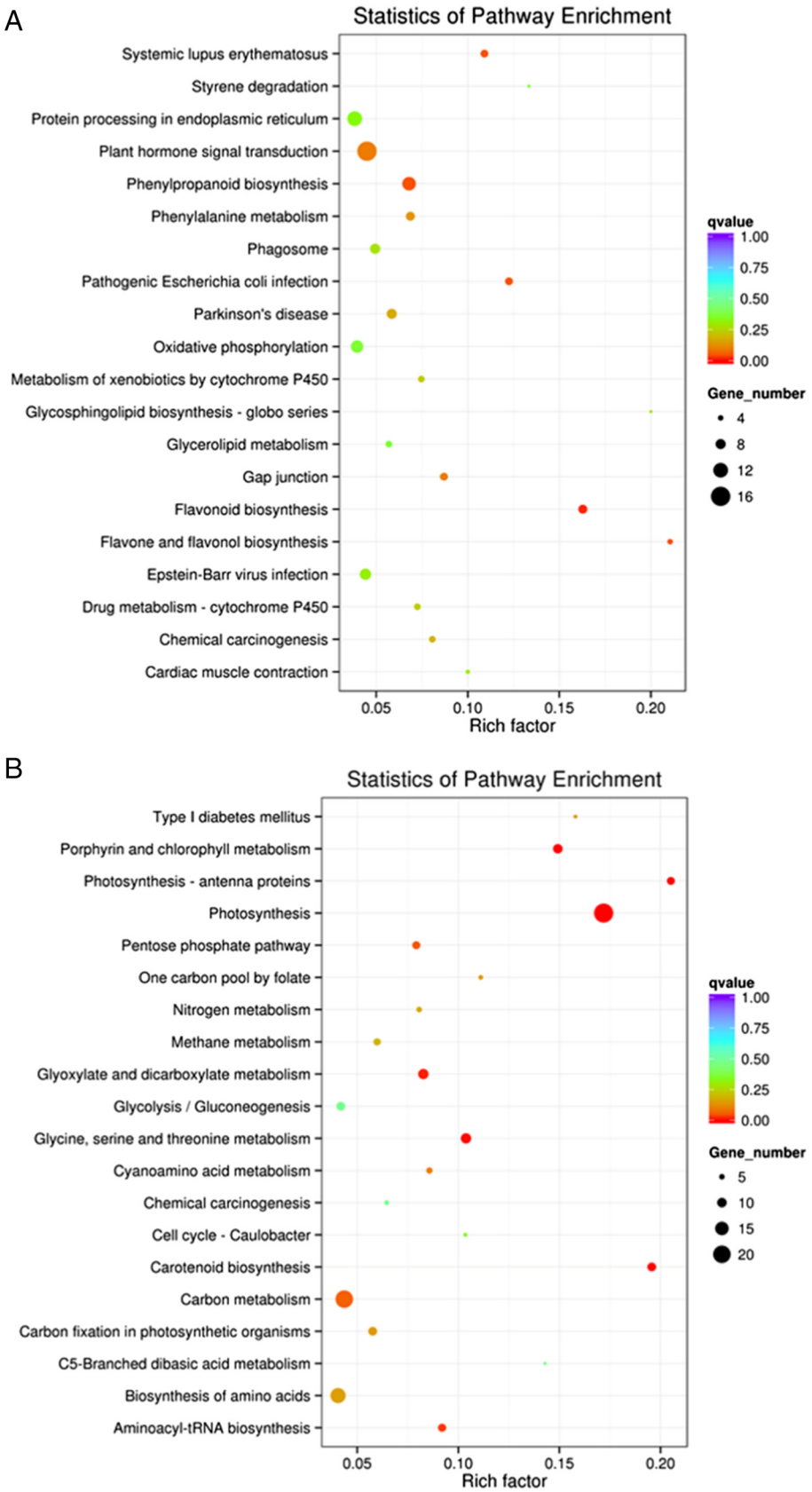

Fig. 7. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of up- (A) and downregulated (B) genes during drought treatment.

and development [42,43]. *Brassinazole resistant 1/2* (*BZR1/2*) acts as a master regulator in BR-induced transcriptional change [44]. Our results indicate that transcription-level changes of these genes (*FeBRI1*, gene ID: c41182_g1) (*FeBZR1/2*, gene ID: c34225_g1) might be highly linked to drought stress. Recent reports indicated that the expression of genes encoding ARFs in rice (*O. sativa* L.) and poplar

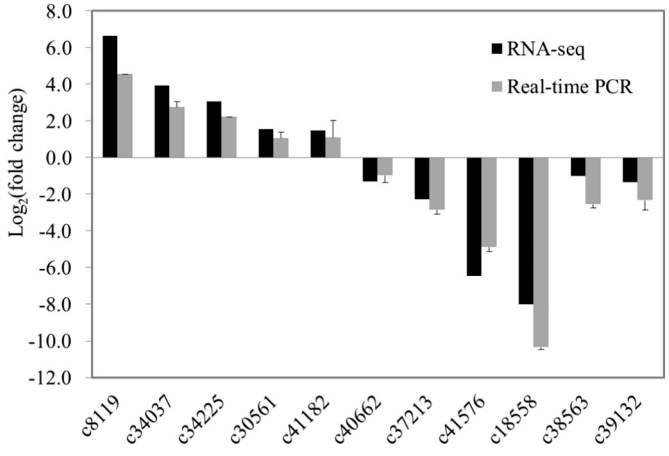

Fig. 8. Validation of RNA sequencing (RNA-seq) expression profiles by real-time PCR. The black columns represent fold changes of relative expression levels that were obtained by real-time PCR based on the $2^{-\Delta\Delta Ct}$ algorithm. The gray columns represent fold changes based on read count number from normalized RNA-seq data.

(*Populus trichocarpa*) was changed under drought stress [45,46]. Coincidently, in this study, significant upregulation of genes encoding ARFs (gene ID: c35693_g1, c28011_g1, c38058_g1, c38575_g1) were observed, suggesting that these transcription factors might immensely contribute to common buckwheat's ability to adapt to drought stress.

In addition, drought-induced DELLA proteins play a prime role in jasmonic acid (JA) and gibberellin (GA) signaling pathways in promoting plant survival rates [47,48]. Gibberellin signaling was realized through conformational changes of DELLA proteins that lead to recognition by the F-box protein Sleery1 (SLY1) [49] and Gibberellin-insensitive dwarf 2 (GID2) [50], and increased DELLA content likely enhances its binding to SLY1 and GID2 [51]. Our results showed that drought promoted the expression of genes encoding the DELLA proteins (gene ID: c36622_g1) and GID2 (gene ID: c30561_g1) in common buckwheat, suggesting gibberellin signaling is tightly involved in drought adaption in buckwheat. Phenylalanine ammonia-lyase (PAL) is considered to be the key enzyme in the biosynthetic pathway of salicylic acid (SA), which was upregulated in response to drought stress [52]. Cinnamyl alcohol dehydrogenase (CAD) is known to be an important enzyme in lignin biosynthesis [53]. Our results indicated that CAD (gene ID: c36561_g1, c31631_g1, c33174_g1) and *PAL* (gene ID: c34247_g2) were significantly upregulated in common buckwheat seedlings grown under drought conditions. Previous studies indicated that photosynthesis activity was repressed by abiotic stress [54], and the abundance of *PSBB* and *PSBQ* related to photosystem II was downregulated in *Arabidopsis* under high-temperature stress [55,56]. Our results also indicated that not only PSII but also PSI and photosynthetic electron transport-related genes are downregulated under drought stress in common buckwheat. Furthermore, downregulation of genes involved in carbon metabolism suggested that drought might lead to lower energy availability, as resources are shifted to defense needs in common buckwheat.

5. Conclusions

This study provides comprehensive transcriptome data for the characterization of drought-related gene expression profiles in common buckwheat. Well-watered common buckwheat seedlings exhibited gene expression profiles that showed significant difference with seeds grown under drought stress. By analyzing genome-wide expression patterns related to drought response in common buckwheat, the functional characteristics of the differentially expressed genes were identified, suggesting various metabolic pathways including plant hormone signal transduction, phenylpropanoid biosynthesis, and photosynthesis and carbon metabolism were highly related to drought adaptation. In detail, after drought treatment, phytohormone signaling-related genes such as *FeBRI1* and *FeBZR1/2* in BR signaling, *FeDELLA* and *FeGID2* in GA signaling, and *FeARF* in auxin signaling, as well as phenylpropanoid biosynthesis-related enzymes such as *FePAL*, *FeCYP73A*, and *FeCAD,* were largely enhanced. By contrast, photosynthesis-related genes including *FePSBB*, *FePSBP*, *FePSBM*, *FePSBQ, FePSBY*, and *FePSBW*, as well as carbon metabolism-related genes including *FePFKA*, *FeGLYA*, *FeSHMT*, *FeGGAT,* and *FeGPMB,* were dramatically depressed. We speculated that the balance between phytohormone signaling, photosynthesis, and carbon metabolism is very important for drought adaption in common buckwheat. Functional characterization of these genes will shed light on their synergistic action against drought stress. Thus, the results of this study will be very useful sources for further uncovering the drought-responsive mechanisms in common buckwheat.

Conflict of interest statement

The authors declare that they have no competing interests.

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Table 4
KEGG classification of up- and downregulated DEGs.

	Item	Gene name
Upregulated	Plant hormone signal transduction	Brassinosteroid insensitive 1 (BRI1) (gene ID: c41182_g1); IAA (gene ID: c35693_g1; c40564_g1; c38575_g1); Auxin response factors (ARF) (gene ID: c35693_g1, c28011_g1, c38058_g1, c38575_g1); EIN3-binding F-box protein (EBF1/2) (gene ID: c40564_g1, c36651_g1, c16779_g1); Brassinazole resistant 1/2 (BZR1/2) (gene ID: c34225_g1); DELLA (gene ID: c36622_g1); Gibberellin-insensitive dwarf2 (GID2) (gene ID: c30561_g1)
Upregulated	Phenylpropanoid biosynthesis	Phenylalanine ammonia-lyase (PAL) (gene ID: c34247_g2); Trans-cinnamate 4-monooxygenase (CYP73A) (gene ID: c39277_g1); Cinnamyl alcohol dehydrogenase (CAD) (gene ID: c36561_g1, c31631_g1, c33174_g1)
Downregulated	Photosynthesis	PSBB (gene ID: c32385_g1); PSBP (gene ID: c38341_g2, c11708_g1); PSBM (gene ID: c629_g2); PSBQ (gene ID: c26636_g1, c34189_g1, c37640_g1); PSBY (gene ID: c36545_g1); PSBW (gene ID: c36016_g1); PSAF (gene ID: c55305_g1); PSAG (gene ID: c39039_g1); PSAK (gene ID: c25484_g1); PSAL (gene ID: c25975_g1); PSAN (gene ID: c29544_g1), PSAO (gene ID: c30798_g1); PETF (gene ID: c31706_g1); PSAH (gene ID: c 33110_g1)
Downregulated	Carbon metabolism	Phosphofructokinase (PFKA) (gene ID: c37364_g1); Glyceric Acid (GLYA) (gene ID: c27035_g1); Serine Hydroxymethyl Transferase (SHMT) (gene ID: c36399_g1); Glutamate/Glyoxylate Aminotransferase (GGAT) (gene ID: c44310_g1); Guanidinopolymyxin (GPMB) (gene ID: c32408_g1); Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) (gene ID: c40342_g2); Glyceraldehyde 3-Phosphate Dehydrogenase A (GapA) (gene ID: c30626_g1); Glycine Decarboxylase (GCVT) (gene ID: c40971_g1); Ammonium Transporters (AMT) (gene ID: c40342_g1); Malate Dehydrogenase 2 (MDH2) (gene ID: c37868_g1); Serine Hydroxymethyl Transferase (SHMT) (gene ID: c40342_g2); Glycine Decarboxylase (GLDC) (gene ID: c30626_g1); Phosphoribulose Kinase (PRK) (gene ID: c37728_g1); Phosphoribulokinase (PRKB) (gene ID: c32625_g1); Ribose 5-Phosphate Isomerase A (RPIA) (gene ID: c40342_g1); Fructose 1 6-Bisphosphatase (FBP) (gene ID: c36404_g1)

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Supplementary material

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References

[1] Ihsan MZ, El-Nakhlawy FS, Ismail SM, et al. Wheat phenological development and growth studies as affected by drought and late season high temperature stress under arid environment. Front Plant Sci 2016;7(14). https://doi.org/10.3389/fpls.2016.00795. PMID: 27375650.

[2] Al Abdallat AM, Ayad JY, Abu Elenein JM, et al. Overexpression of the transcription factor HvSNAC1 improves drought tolerance in barley (*Hordeum vulgare* L.). Mol Breed 2014;33(2):401–14. https://doi.org/10.1007/s11032-013-9958-1.

[3] Wu N, Li Z, Wu F, et al. Comparative photochemistry activity and antioxidant responses in male and female *Populus cathayana* cuttings inoculated with arbuscular mycorrhizal fungi under salt. Sci Rep 2016;6(15). https://doi.org/10.1038/srep37663. PMid: 27898056.

[4] Guo YM, Samans B, Chen S, et al. Drought-tolerant *Brassica rapa* shows rapid expression of gene networks for general stress responses and programmed cell death under simulated drought stress. Plant Mol Biol Report 2017;35(4):416–30. https://doi.org/10.1007/s11105-017-1032-4. PMID: 28751801.

[5] Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K. The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. Front Plant Sci 2014;5(7). https://doi.org/10.3389/fpls.2014.00170. PMid: 24904597.

[6] Foyer CH, Noctor G. Managing the cellular redox hub in photosynthetic organisms. Plant Cell Environ 2012;35(2):199–201. https://doi.org/10.1111/j.1365-3040.2011.02453.x. PMid: 22070467.

[7] Kovács G, Sorvari S, Scott P, et al. Pyrophosphate:fructose 6-phosphate 1-phosphotransferase is involved in the mobilization of sugar reserves in the taproots of cold- and drought-stressed carrot plants. Acta Agronomica Hung 2007;55(1):71–82. https://doi.org/10.1556/AAgr.55.2007.1.8.

[8] Kasuga M, Liu Q, Miura S, et al. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol 1999;17(3):287–91. https://doi.org/10.1038/7036 PMid: 10096298.

[9] Liu Q, Kasuga M, Sakuma Y, et al. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. Plant Cell 1998;10(8):1391–406. https://doi.org/10.2307/3870648. PMid: 9707537.

[10] Okay S, Derelli E, Unver T. Transcriptome-wide identification of bread wheat WRKY transcription factors in response to drought stress. Mol Genet Genomics 2014;289(5):765–81. https://doi.org/10.1007/s00438-014-0849-x. PMid: 24748053.

[11] Haake V, Cook D, Riechmann JL, et al. Transcription factor CBF4 is a regulator of drought adaptation in *Arabidopsis*. Plant Physiol 2002;130(2):639–48. https://doi.org/10.1104/pp.006478. PMID: 12376631.

[12] Jo K, Kwon HB, Kim S. Time-series RNA-seq analysis package (TRAP) and its application to the analysis of rice, *Oryza sativa* L. ssp *Japonica*, upon drought stress. Methods 2014;67(3):364–72. https://doi.org/10.1016/j.ymeth.2014.02.001. PMID: 24518221.

[13] Pan L, Zhang XQ, Wang JP, et al. Transcriptional profiles of drought-related genes in modulating metabolic processes and antioxidant defenses in *Lolium multiflorum*. Front Plant Sci 2016;7(15). https://doi.org/10.3389/fpls.2016.00519. PMid: 27200005.

[14] Yates SA, Swain MT, Hegarty MJ, et al. *De novo* assembly of red clover transcriptome based on RNA-Seq data provides insight into drought response, gene discovery and marker identification. BMC Genomics 2014;15:453. https://doi.org/10.1186/1471-2164-15-453. PMid: 24912738.

[15] Wang Z, Gerstein M, Snyder M. RNA-Seq: A revolutionary tool for transcriptomics. Nat Rev Genet 2009;10(1):57–63. https://doi.org/10.1038/nrg2484. PMid: 19015660.

[16] Jin H, Dong D, Yang Q, et al. Salt-responsive transcriptome profiling of *Suaeda glauca* via RNA sequencing. PLoS One 2016;11(3):e0150504. https://doi.org/10.1371/journal.pone.0150504. PMID: 26930632.

[17] Duan Y, Gong Z, Wu R, et al. Transcriptome analysis of molecular mechanisms responsible for light-stress response in *Mythimna separata* (Walker). Sci Rep 2017;7:45188. https://doi.org/10.1038/srep45188. PMID: 28345615.

[18] Yokosho K, Yamaji N, Ma JF. Global transcriptome analysis of Al-induced genes in an Al-accumulating species, common buckwheat (*Fagopyrum esculentum* Moench). Plant Cell Physiol 2014;55(12):2077–91. https://doi.org/10.1093/pcp/pcu135. PMid: 25273892.

[19] Zhu H, Wang H, Zhu Y, et al. Genome-wide transcriptomic and phylogenetic analyses reveal distinct aluminum-tolerance mechanisms in the aluminum-accumulating species buckwheat (*Fagopyrum tataricum*). BMC Plant Biol 2015;15:16. https://doi.org/10.1186/s12870-014-0395-z. PMID: 25603892.

[20] Zhou ML, Wieslander G, Tang Y, et al. In: Zhou M, Kreft I, Woo SH, et al, editors. Characterization of functional genes in buckwheat. London: Academic Press Ltd-Elsevier Science Ltd.; 2016. p. 327–34. https://doi.org/10.1016/B978-0-12-803692-1.00025-0.

[21] Yao HP, Li CL, Zhao HX, et al. Deep sequencing of the transcriptome reveals distinct flavonoid metabolism features of black tartary buckwheat (*Fagopyrum tataricum* Garetn.). Prog Biophys Mol Biol 2017;124:49–60. https://doi.org/10.1016/j.pbiomolbio.2016.11.003. PMid: 27836511.

[22] Fang ZW, Xu XY, Gao JF, et al. Characterization of FeDREB1 promoter involved in cold- and drought-inducible expression from common buckwheat (*Fagopyrum esculentum*). Genet Mol Res 2015;14(3):7990–8000. https://doi.org/10.4238/2015.July.17.7. PMid: 26214481.

[23] Wu Q, Bai X, Zhao W, et al. De novo assembly and analysis of tartary buckwheat (*Fagopyrum tataricum* Garetn.) transcriptome discloses key regulators involved in salt-stress response. Genes 2017;8(10). https://doi.org/10.3390/genes8100255. PMid: 28972562.

[24] Jamwal A, Puri S, Sharma S, et al. Polyethylene glycol induced morphological changes in *Fagopyrum esculentum* Moench of Indian Himalayan Region. Asian J Adv Basic Sci 2015;3:142–6.

[25] Li Z, Baldwin CM, Hu Q, et al. Heterologous expression of *Arabidopsis* H+-pyrophosphatase enhances salt tolerance in transgenic creeping bentgrass (*Agrostis stolonifera* L.). Plant Cell Environ 2010;33(2):272–89. https://doi.org/10.1111/j.1365-3040.2009.02080.x. PMid: 19930128.

[26] Merewitz EB, Gianfagna T, Huang B. Protein accumulation in leaves and roots associated with improved drought tolerance in creeping bentgrass expressing an *ipt* gene for cytokinin synthesis. J Exp Bot 2011;62(15):5311–33. https://doi.org/10.1093/jxb/err166. PMID: 21831843.

[27] Grabherr MG, Haas BJ, Yassour M, et al. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol 2011;29(7):644–52. https://doi.org/10.1038/nbt.1883. PMID: 21572440.

[28] Mount DW. Using the Basic Local Alignment Search Tool (BLAST). CSH protocols 2007; 2007. p. pdb.top17. https://doi.org/10.1101/pdb.top17. PMID: 21357135.

[29] Pruitt KD, Tatusova T, Maglott DR. NCBI reference sequences (RefSeq): A curated non-redundant sequence database of genomes, transcripts and proteins. Nucleic Acids Res 2007;35(Database issue):D61–5. https://doi.org/10.1093/nar/gkl842. PMID: 17130148.

[30] Boutet E, Lieberherr D, Tognolli M, et al. UniProtKB/Swiss-Prot, the manually annotated section of the UniProt KnowledgeBase: How to use the entry view. Methods Mol Biol 2016;1374:23–54. https://doi.org/10.1007/978-1-4939-3167-5_2. PMid: 26519399.

[31] Mao X, Cai T, Olyarchuk JG, et al. Automated genome annotation and pathway identification using the KEGG Orthology (KO) as a controlled vocabulary. Bioinformatics 2005;21(19):3787–93. https://doi.org/10.1093/bioinformatics/bti430. PMid: 15817693.

[32] Kanehisa M, Araki M, Goto S, et al. KEGG for linking genomes to life and the environment. Nucleic Acids Res 2008;36(Database issue):D480–4. https://doi.org/10.1093/nar/gkm882. PMID: 18077471.

[33] Conesa A, Gotz S, Garcia-Gomez JM, et al. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics 2005;21(18):3674–6. https://doi.org/10.1093/bioinformatics/bti610. PMid: 16081474.

[34] Dimmer EC, Huntley RP, Alam-Faruque Y, et al. The UniProt-GO Annotation database in 2011. Nucleic Acids Res 2012;40(D1):D565–70. https://doi.org/10.1093/nar/gkr1048. PMid: 22123736.

[35] Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol 2010;11(3):R25. https://doi.org/10.1186/gb-2010-11-3-r25. PMID: 20196867.

[36] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$ method. Methods 2001;25(4):402–8. https://doi.org/10.1006/meth.2001.1262. PMID: 11846609.

[37] Gehan MA, Greenham K, Mockler TC, et al. Transcriptional networks-crops, clocks, and abiotic stress. Curr Opin Plant Biol 2015;24:39–46. https://doi.org/10.1016/j.pbi.2015.01.004. PMid: 25646668.

[38] Reyna-Llorens I, Corrales I, Poschenrieder C, et al. Both aluminum and ABA induce the expression of an ABC-like transporter gene (*FeALS3*) in the Al-tolerant species *Fagopyrum esculentum*. Environ Exp Bot 2015;111:74–82. https://doi.org/10.1016/j.envexpbot.2014.11.005.

[39] Lei GJ, Yokosho K, Yamaji N, et al. Functional characterization of two half-size ABC transporter genes in aluminium-accumulating buckwheat. New Phytol 2017;215(3):1080–9. https://doi.org/10.1111/nph.14648. PMid: 28620956.

[40] Misra PC. Transplasma membrane electron-transport in plants. J Bioenerg Biomembr 1991;23(3):425–41. https://doi.org/10.1007/bf00771013. PMid: 1864850.

[41] Li JM, Nam KH, Vafeados D, et al. BIN2, a new brassinosteroid-insensitive locus in *Arabidopsis*. Plant Physiol 2001;127(1):14–22. https://doi.org/10.1104/pp.127.1.14. PMid: 11553730.

[42] Khew CY, Teo CJ, Chan WS, et al. Brassinosteroid insensitive 1-associated kinase 1 (OsI-BAK1) is associated with grain filling and leaf development in rice. J Plant Physiol 2015;182:23–32. https://doi.org/10.1016/j.jplph.2015.05.003. PMid: 26037695.

[43] Wang QN, Wang SF, Gan SF, et al. Role of specific phosphorylation sites of *Arabidopsis* brassinosteroid-insensitive 1 receptor kinase in plant growth and development. J Plant Growth Regul 2016;35(3):755–69. https://doi.org/10.1007/s00344-016-9580-7.

[44] Deng XG, Zhu T, Peng XJ, et al. Role of brassinosteroid signaling in modulating tobacco mosaic virus resistance in *Nicotiana benthamiana*. Sci Rep 2016;6(14). https://doi.org/10.1038/srep20579. PMID: 26838475.

[45] Jung H, Lee DK, Choi YD, et al. OsIAA6, a member of the rice Aux/IAA gene family, is involved in drought tolerance and tiller outgrowth. Plant Sci 2015;236:304–12. https://doi.org/10.1016/j.plantsci.2015.04.018. PMid: 26025543.

[46] Liu QG, Wang ZC, Xu XM, et al. Genome-wide analysis of C2H2 zinc-finger family transcription factors and their responses to abiotic stresses in poplar (*Populus trichocarpa*). PLoS One 2015;10(8):25. https://doi.org/10.1371/journal.pone.0134753. PMID: 26237514.

[47] An J, Hou L, Li C, et al. Cloning and expression analysis of four DELLA genes in peanut. Russ J Plant Physiol 2015;62(1):116–26. https://doi.org/10.1134/s1021443715010021.

[48] Golldack D, Li C, Mohan H, et al. Tolerance to drought and salt stress in plants: Unraveling the signaling networks. Front Plant Sci 2014;5(10). https://doi.org/10.3389/fpls.2014.00151. PMid: 24795738.

[49] Nelson SK, Steber CM. Transcriptional mechanisms associated with seed dormancy and dormancy loss in the gibberellin-insensitive sly1-2 mutant of *Arabidopsis thaliana*. PLoS One 2017;12(6):32. https://doi.org/10.1371/journal.pone.0179143. PMid: 28628628.

[50] Ueguchi-Tanaka M, Nakajima M, Katoh E, et al. Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. Plant Cell 2007;19(7):2140–55. https://doi.org/10.1105/tpc.106.043729. PMid: 17644730.

[51] Zentella R, Sui N, Barnhill B, et al. The *Arabidopsis* O-fucosyltransferase SPINDLY activates nuclear growth repressor DELLA. Nat Chem Biol 2017;13(5):479–85. https://doi.org/10.1038/nchembio.2320 PMID: 28244988.

[52] Zhang Y, Fu XQ, Hao XL, et al. Molecular cloning and promoter analysis of the specific salicylic acid biosynthetic pathway gene phenylalanine ammonia-lyase (*AαPAL1*) from *Artemisia annua*. Biotechnol Appl Biochem 2016;63(4):514–24. https://doi.org/10.1002/bab.1403. PMid: 26040426.

[53] Deng WW, Zhang M, Wu JQ, et al. Molecular cloning, functional analysis of three cinnamyl alcohol dehydrogenase (CAD) genes in the leaves of tea plant, *Camellia sinensis*. J Plant Physiol 2013;170(3):272–82. https://doi.org/10.1016/j.jplph.2012.10.010. PMid: 23228629.

[54] Ayyappan V, Saha MC, Thimmapuram J, et al. Comparative transcriptome profiling of upland (VS16) and lowland (AP13) ecotypes of switchgrass. Plant Cell Rep 2017;36(1):129–50. https://doi.org/10.1007/s00299-016-2065-0. PMid: 27812750.

[55] de Marcos A, Trivino M, Perez-Bueno ML, et al. Transcriptional profiles of *Arabidopsis stomataless* mutants reveal developmental and physiological features of life in the absence of stomata. Front Plant Sci 2015;6(14). https://doi.org/10.3389/fpls.2015.00456. PMid: 26157447.

[56] Shi JZ, Chen YT, Xu Y, et al. Differential proteomic analysis by iTRAQ reveals the mechanism of *Pyropia haitanensis* responding to high temperature stress. Sci Rep 2017;7(11). https://doi.org/10.1038/srep44734. PMID: 28303955.