

Screening and transcriptome analysis of water deficiency tolerant germplasms in peanut (*Arachis hypogaea*)

Yi Shen^{1,2} · E. Zhiguo³ · Xiaojun Zhang⁴ · Yonghui Liu¹ · Zhide Chen¹

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Abstract Drought is one of the main limiting factors for peanut yield worldwide. However, the underlying genetic and molecular mechanisms remain unclear. In this study, we identified several water deficiency tolerant peanut germplasms based on physiological properties in the seedling stage. One of the germplasms was further characterized by transcriptome sequencing of samples that had been untreated or treated with PEG 6000 at three different times. A total of 370,145 non-redundant transcripts and 141,289 unigenes were obtained, and differentially expressed transcripts were identified among samples. Based on functional annotation, transcripts involved in drought response pathways were selected for qPCR analysis. Similar patterns but different levels of gene expression were found between drought-tolerant and drought-sensitive germplasms. These findings provide useful insights into drought tolerance of peanut.

Keywords Transcriptome sequencing · Water deficiency stress · Peanut · *Arachis hypogaea*

Introduction

As an important oil and economic crop, peanut is cultured all over the world, especially in semiarid zones. In China, except for the main producing provinces, peanut is commonly planted in relatively dry land, which is difficult to irrigate, while more suitable land is reserved for other staple grains. Drought is one of the main limiting factors for peanut yield, especially in the (1) germination and young seedling stages, which determine plant survival and health, and (2) pod development and mature stages, which affect the final production. Furthermore, drought may easily aggravate *Aspergillus flavus* contamination, as well as infection with other diseases and insect pests (Collino et al. 2001). Therefore, it is vital to understand the mechanisms of drought tolerance in peanut and identifying tolerant germplasms.

Several studies assessing peanut drought tolerance have been reported. For instance, water deficit was shown to induce the expression of stress-responsive genes, including phospholipase D α (PLD α), cysteine protease (CP), serine protease (SP) and late embryogenesis abundant (LEA) protein (Dramé et al. 2007; Govind et al. 2009). In addition, *Arachis hypogaea* serine-rich protein (*AhSrp*) and *A. hypogaea* leucine-rich protein (*AhLrp*) were identified using rapid amplification of cDNA ends (RACE) (Devaiah et al. 2007). Furthermore, PLD1 and PLD2, which belong to the PLD α family, were shown to be involved in drought response and upregulated in *A. hypogaea* (Nakazawa et al. 2006). Two ethylene-responsive factor (ERF) genes with

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✉ Yi Shen
shenyi1202@gmail.com

✉ Zhide Chen
chen701865@aliyun.com

¹ Institute of Industrial Crops, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu, China

² Provincial Key Laboratory of Agrobiolgy, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu, China

³ China National Rice Research Institute, No. 359, Tiyuchang Road, Hangzhou 310006, China

⁴ College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao 266109, China

abiotic stress response have been cloned recently (Wan et al. 2014).

Several homologous/heterogeneous expression analyses have been carried out. *N*-Acetyl-Cysteine (NAC) from *A. hypogaea* was shown to enhance drought tolerance in *Arabidopsis* (Liu et al. 2011) and tobacco (Liu et al. 2013). An important transcriptional factor in peanut, abscisic acid-responsive element binding protein (AREB), was confirmed to modulate reactive oxygen species (ROS) scavenging and maintain endogenous abscisic acid (ABA) content in *Arabidopsis*, thereby positively affecting drought stress tolerance (Li et al. 2013). Heterogenous genes, including DNA helicase (PDH45) in pea *Pisum sativum* (Manjulatha et al. 2014), NAC in horsegram *Macrotyloma uniflorum* (Pandurangiah et al. 2014), and DREB in *Arabidopsis* (Sarkar et al. 2014) could all enhance the drought tolerance in peanut.

The peanut genome still lacks detailed information and little has been done in quantitative trait loci (QTL) location of drought tolerance (Ravi et al. 2010; Gautami et al. 2011). Considering the size (~4400 Mb) and the high repetition (~70 %) of the genome, high-throughput sequencing, especially transcriptome sequencing (RNA-Seq), is a convenient technique for the study of general gene expression changes occurring at a given growth stage or as a result of a biological response. Transcriptome data, combined with annotations based on bioinformatics, constitute a valuable tool for gene identification and pathway construction. To date, several transcriptome studies on peanut have been carried out, including on wild *Arachis* species under drought and fungi infection (Guimaraes et al. 2012), the Spanish botanical type *A. hypogaea* L. (Wu et al. 2013), the seed development and oil accumulation stage of *A. hypogaea* L. (Zhang et al. 2012; Xia et al. 2013; Yin et al. 2013), and the different patterns between aerial and subterranean young pods (Chen et al. 2013; Zhu et al. 2014).

One recent study found that many genes could be rapidly induced in response to water deficit with or without ABA pretreatment through RNA-Seq, indicating the importance of the ABA-dependent pathway in drought tolerance of peanut (Li et al. 2014).

In this study, we identified several water deficiency tolerant peanut germplasms based on physiological properties in the seedling stage. One of the germplasms was further characterized by transcriptome sequencing. As well as describing the RNA-Seq data, we focused on transcripts putatively involved in drought-related pathways. These findings provide important insights into drought tolerance and useful data for future gene investigations.

Materials and methods

Screening for water deficiency tolerant germplasms

Fresh peanut seeds were harvested, dried and kept at the Liuhe Science Base of Jiangsu Academy of Agricultural Sciences (JAAS) in 2012. Experiments were carried out in Jiangsu Provincial Platform for Conservation and Utilization of Agricultural Germplasm.

Fifty germplasms (Supplemental Table 1), regionally selected from a total of 617 peanut collections, were treated with 15 % PEG 6000, which acted as a drought simulant in the seedling stage. Control samples were grown in water. Seedling heights of the treated samples and the controls were measured and compared after 7 days.

Plant materials, sample treatment and RNA extraction

Peanut germplasm #11 (Fenghua 1, FH1) was identified as water deficiency tolerant in this study (see “Results” 1). After germination and growth to the trefoil stage, young seedlings were treated with 15 % PEG 6000 for 24 h (sample 1), 48 h (sample 2) or 7 days (sample 3). Control untreated seedlings (sample 0) were also analyzed. After treatment, total RNA was extracted from young leaves as previously described (Marioni et al. 2008) for RNA sequencing.

RNA sequencing, de novo assembly and transcript/unigene analysis

RNA quality and quantity were determined on a NanoDrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE). Then, mRNA in each sample was fragmented, and sequences between 300 and 1000 bp were selected by AMPure SPRI bead with Oligo (dT). First strand cDNA was synthesized using random hexamer primers, followed by synthesis of the second strand.

Illumina HiSeqTM 2000 (Solexa) was employed to sequence the libraries using PCR amplification (LC-Bio, Hangzhou, China). Since a reference genome is not yet available for peanut, the Trinity method (Grabherr et al. 2011) was used for de novo assembly. The Chrysalis clusters module in Trinity was used for unigene acquisition. Length distribution and GC content analysis of unigenes were conducted using histogram tools in Microsoft Excel. Reads per kilobase of gene per million reads (RPKM) were used to assess gene expression in various samples, and bowtie 0.12.8 (<http://bowtie-bio.sourceforge.net/index.shtml>) was employed for calculations (Mortazavi et al. 2008).

Functional annotation and differential transcript expression analysis

Unigenes were compared with the non-redundant GenBank database (<http://blast.ncbi.nlm.nih.gov/>) and *E* value $<1e-5$, followed by analysis using the COG (cluster of orthologous groups of proteins) program to classify gene functions (<http://www.ncbi.nlm.nih.gov/COG/>). Blast2GO and WEGO (Conesa et al. 2005; Ye et al. 2006) were employed to obtain the functional classification based on the annotations in SWISS-PROT/TrEMBL (<http://www.uniprot.org/>). The KEGG database (<http://www.genome.jp/kegg/>) was used to annotate the unigene pathways.

Fisher and Chi square tests were used to assess the differences in transcript expression. Transcripts with >twofold changes were considered differentially expressed transcripts (DETs) and further analyzed. Significantly altered transcripts of drought related pathways were identified and their expression patterns were confirmed by qRT-PCR.

Quantitative real time PCR (qRT-PCR)

qRT-PCR primers were designed according to transcript sequences and qRT-PCR was performed on an AB 7900 real-time PCR System (Applied Biosystems, USA) with SYBR Premix Ex TaqTM (DRR041A, TaKaRa). 18S was selected as an internal reference gene and three biological replicates were analyzed per sample. The relative gene transcript amounts were determined according to the real-time PCR application guide (Bio-Rad Laboratories; <http://www.bio-rad.com>).

Results

Germplasm screening for water deficiency tolerance

Fifty peanut germplasms were selected for water deficiency tolerance screening. Relative seedling heights (compared with untreated plants) were measured after treatment with CK and PEG, respectively. The PEG/CK ratio (Fig. 1)

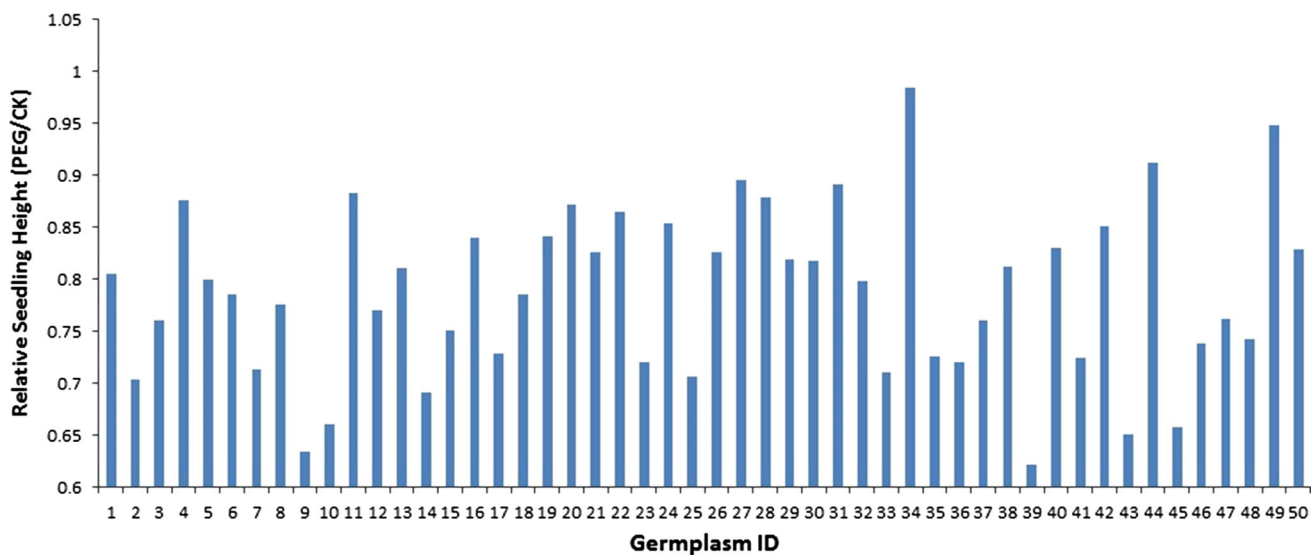
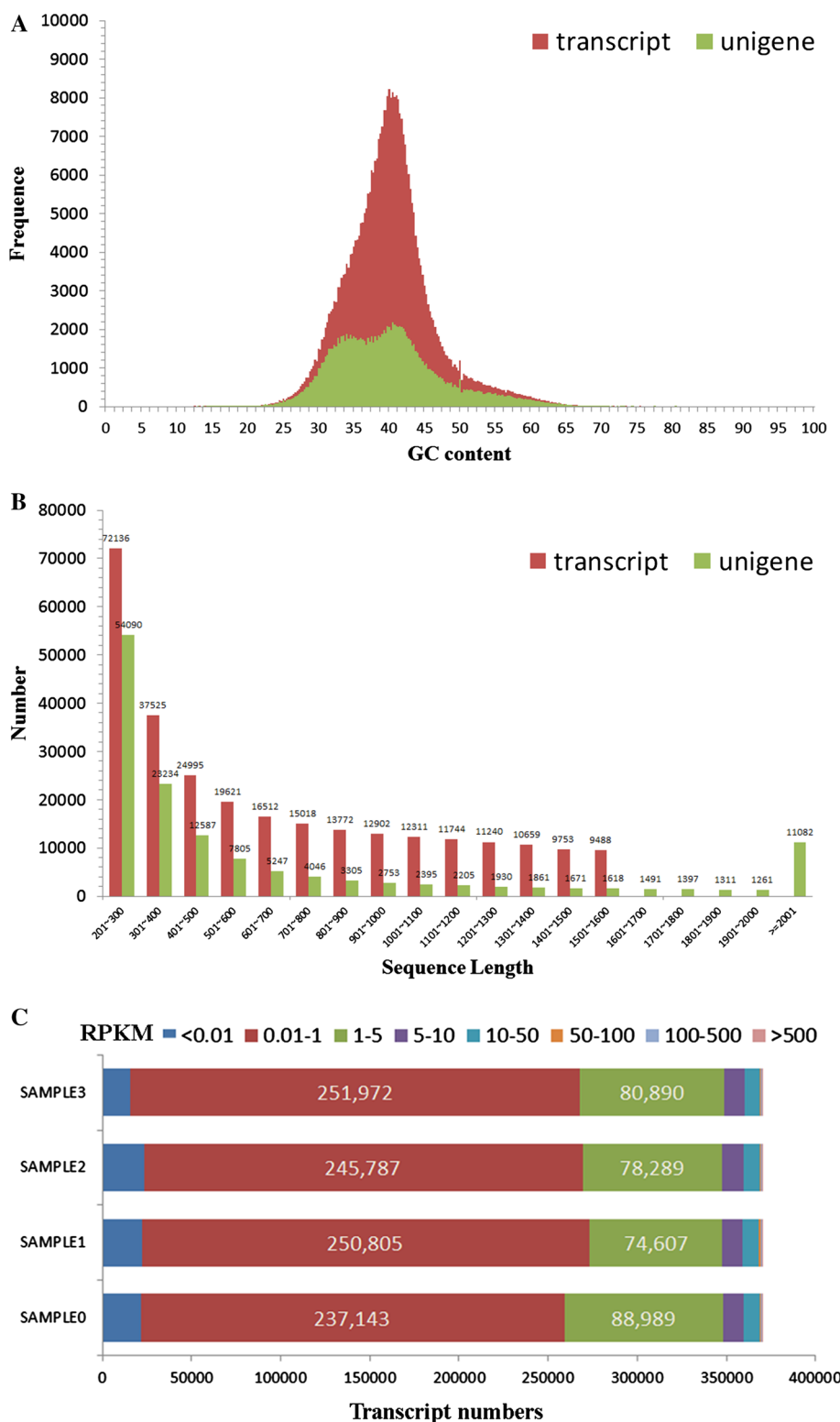


Fig. 1 Ratio (PEG/CK) of relative seedling heights after 7 days of treatment with water (CK) and PEG 6000 of 50 peanut germplasms

Table 1 Sample reads, and transcript and unigene numbers

Sample ID	Reads		Transcripts	Unigenes
	Raw data	Valid data		
0	43,717,856	36,742,486	348,353	94.11
1	42,361,974	36,120,046	347,866	93.98
2	43,886,486	38,026,326	346,717	93.67
3	47,923,852	40,500,012	354,324	95.73
Total		151,388,870		
Non-redundant			370,145	141,289

Fig. 2 GC content, length and RPKM analysis in transcriptome data. **a** GC content distributions of transcripts (*red*) and unigenes (*green*) in non-redundant mixed library. **b** Sequence length distribution of transcripts (*red*) and unigenes (*green*) in non-redundant mixed library. **c** RPKM (reads per kilobase of gene per million reads) distribution of transcripts in various samples (color figure online)



and actual performance in PEG (Supplemental Fig. 1) were used as indicators of tolerance. Germplasms #11, #34 and #49 were found to be tolerant, while #9, #39 and #43 were sensitive to water deficiency. #34 and #49 grew slowly in

both treatments, and #11 (Fenghua 1, FH1) was ultimately selected for transcriptome sequencing analysis.

Corresponding field experiments were carried out to verify our screening results. Without manual irrigation in

drought years, #11, #34 and #49 maintained 80–90 % production, while other germplasms only yielded about 60 % of their potential, indicating that performance in the seedling stage, as found in the lab, is valuable for evaluating drought tolerant of peanut.

Transcriptome sequencing and de novo assembly

A cDNA library for each of the samples 0, 1, 2 and 3 was constructed and sequenced on Illumina HiSeq™ 2000. Paired-end Illumina read numbers were 36,742,486, 36,120,046, 38,026,326, and 40,500,012 for samples 0, 1, 2 and 3, respectively.

After length filtering and redundancy cleaning, 370,145 non-redundant transcripts covering 417 Mb were obtained. 93.67–95.73 % of all transcripts were found in separate samples (Table 1). Considering the longest transcript read for each locus as a unigene, 141,289 unigenes (also known as transcript assembly contigs, TACs) with a total length of 100 Mb were obtained for functional annotation and expression comparison (Table 1, Supplemental Table 2).

The average GC contents of transcripts and unigenes were 39.96 and 39.84 % (Fig. 2a), with 1127.22 and 709.82 bp long reads, respectively (Fig. 2b), which accord with existing transcriptome analyses in peanut. The average transcript RPKMs were 2.18, 2.48, 2.25 and 2.1 in samples 0, 1, 2 and 3, respectively, indicating that most transcripts were expressed at a low level (mostly 0.01–5) (Fig. 2c), which may be associated with the relatively higher number of transcripts in our experiment compared with other RNA-Seq results in peanut (Table 1).

Unigene functional annotation

A total of 62,510 unigenes were annotated in the non-redundant GenBank database with E value $<1e-5$ (Supplemental Table 2), which is relatively less than other similar researches (Xia et al. 2013; Yin et al. 2013). Mostly, it is due to the different materials, considering the sequencing platform and the query parameter were the same.

Of these, 74.26 % were annotated to five other species besides *A. hypogaea* itself (1.79 %, Fig. 3a), including *Glycine max* (50.8 %), *Medicago truncatula* (13.43 %), *Lotus japonicas* (3.31 %), *Vitis vinifera* (3.18 %) and *Populus trichocarpa* (2.65 %), indicating the possible use of these species as references for gene function studies in *A. hypogaea*.

20,636 unigenes, about one third of the total, were categorized into 25 functional groups by COG analysis, and mostly assigned to “signal transduction mechanisms” (T, 4486), and “posttranslational modification, protein turnover, chaperones” (O, 4143) (Fig. 3b).

Furthermore, 43,427 (30.74 %) unigenes were functionally annotated in SWISS-PROT or TrEMBL, and

classified by gene ontology (GO). As shown in Fig. 3c and Supplemental Table 2, the genes analyzed belonged to biological process (42.03 %), cellular component (31.41 %) and molecular function (26.56 %) groups. The top seven processes (sequence number >100), including metabolic processes, cellular processes, organelle, cell part, molecular binding, catalytic activity and cell, accounted for 66.17 % of all gene annotations, and commonly thought as the basic structural and functional unit of all organisms.

To understand the gene interactions, the KEGG (Kyoto Encyclopaedia of Genes and Genomes) database was used for pathway analysis. A total of 21,495 unigenes, belonging to 1104 ECs, were assigned to 302 KEGG pathways, of which genetic information processing (ribosome translation and protein processing) displayed the most anchored unigenes (Supplemental Table 3).

Expression of drought related transcripts

Four samples were collected before and at different times as described in the “Materials and methods”, and 6 comparison pairs were obtained. Genes expressed with $>$ two-fold change were considered DETs (differentially expressed transcripts) and results are shown in Table 2. The expression levels of 130/107 DETs with KEGG annotations were gradually increased/decreased, respectively, after water deficiency treatment (Supplemental Table 4). This could help trace the genes involved in drought response. For example, homogentisate phytyltransferase (HPT), a key enzyme in Vitamin E (which is helpful in aldehyde elimination) biosynthesis, was induced dramatically after PEG treatment.

Transcripts with annotations related to drought response pathways, such as ABA, sugar, amino acid and protein kinase, are summarized in Supplemental Table 5. The expression of ABA biosynthesis genes, including ABA1, ABA3 and NCED, seemed to be induced continuously after PEG treatment, and similar results were obtained for ABF and mtID. Tryptophan and raffinose synthesis were increased at 24 h, but dropped to normal levels afterwards. Drought stress induced proline synthesis as shown by increased proline iminopeptidase (pip) levels, and reduced its degradation (decreased proline dehydrogenase amounts), probably to maintain in planta proline levels. Distinct changes were found in CPK transcripts, implying complex roles of CPK in drought response.

qPCR was carried out to verify these findings in germplasms #9 and #11 (Fig. 4). Similar expression patterns were observed for each transcript in the two germplasms, with the highest diversity observed in proline iminopeptidase (pip) and proline dehydrogenase, suggesting that proline plays an important role in drought tolerance.

Fig. 3 Annotation analysis of unigenes. **a** Species homogenously annotated to all unigenes. **b** COG analysis of unigenes: *A* RNA processing and modification; *B* chromatin structure and dynamics; *C* energy production and conversion; *D* cell cycle control, cell division, chromosome partitioning; *E* amino acid transport and metabolism; *F* nucleotide transport and metabolism; *G* carbohydrate transport and metabolism; *H* coenzyme transport and metabolism; *I* lipid transport and metabolism; *J* translation, ribosomal structure and biogenesis; *K* transcription; *L* replication, recombination and repair; *M* cell wall/membrane/envelope biogenesis; *N* cell motility; *O* posttranslational modification, protein turnover, chaperones; *P* inorganic ion transport and metabolism; *Q* secondary metabolites biosynthesis, transport and catabolism; *R* general function prediction only; *S* function unknown; *T* signal transduction mechanisms; *U* intracellular trafficking, secretion, and vesicular transport; *V* defense mechanisms; *W* extracellular structures; *Y* nuclear structure; *Z* cytoskeleton. **c** Gene ontology analysis of unigenes

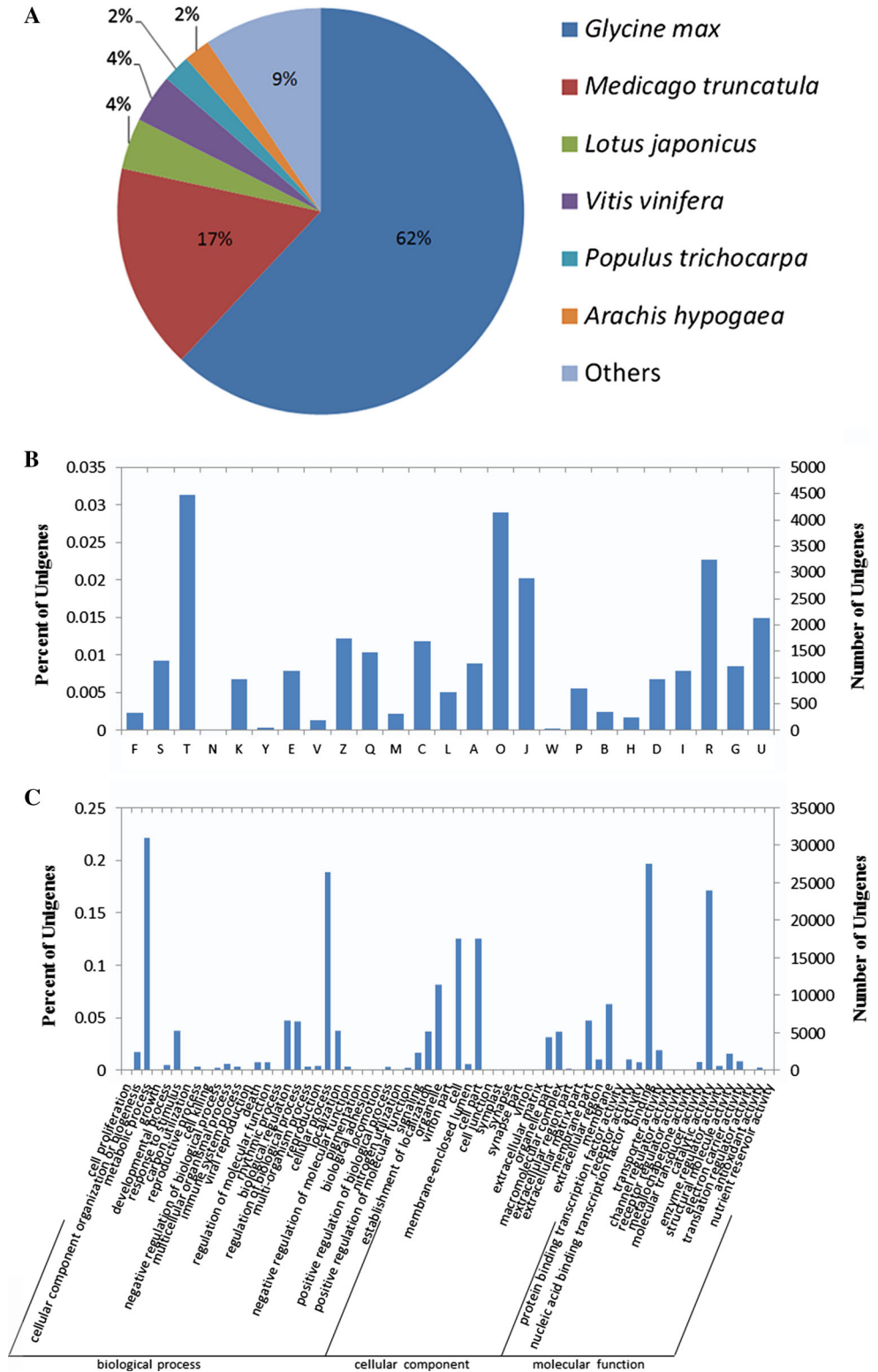


Table 2 DET (differentially expressed transcript) analysis between sample pairs

	FC. p	FC. n	FT. g	FT. l	CT
Sample 0 vs sample 1	62,884	114,346	5155	5432	19,972
Sample 0 vs sample 2	36,810	65,018	1757	1383	16,974
Sample 0 vs sample 3	47,844	67,068	2137	2662	16,704
Sample 1 vs sample 2	71,531	60,289	3600	3889	25,974
Sample 1 vs sample 3	93,261	65,032	3910	4549	23,645
Sample 2 vs sample 3	58,864	50,990	1640	2550	20,071

FC (fold change) $\log_2(B/A)$. FC. p (FC positive) $FC \geq 1$. FC. n (FC negative) $FC \leq 1$. FT. g (Fisher test greater) Fisher test: $B > A$, $p \leq 0.05$. FT. l (Fisher test less) Fisher test: $B < A$, $p \leq 0.05$. CT (Chi square test) Chi square test: $B = A$, $p \leq 0.05$

Discussion

Water deficiency tolerance screening in peanut

Water deficiency tolerance levels significantly vary by peanut genotype (Jongrunklang et al. 2013). Generally, creeping types have better tolerance capabilities compared with erect ones due to their stronger roots. Cultivated peanut germplasms are generally erect for practical reasons and it is necessary to select suitable germplasms for drought tolerance breeding. Here, 50 peanut germplasms

from distinct regions were chosen for tolerance testing. Some specimens, like #34 and #49, grew slowly both in normal and PEG conditions (Fig. 1a), and were not further studied. Variety #11 (FH 1) was finally selected for transcriptome sequencing for its drought tolerant ability. Germplasms #22 and #42 also performed well, although their ratios in screening were not outstanding. Large scale field experiments for drought screening as well as physiological studies assessing photosynthetic parameters of germplasms under different irrigative conditions are ongoing. Furthermore, these tolerant germplasms were also able to maintain good yield in salty soil, indicating their value in both drought zones and saline fields.

Drought tolerance breeding in peanut

The genetic improvement of peanut is still very challenging, mainly because of its complex genetic architecture and the lack of genomic information (Pandey et al. 2012). Therefore, identification and application of valuable germplasms are very important. In our experiment, samples #11 and #49 were both derived from the same peanut germplasm ‘baisha 171’, which also performed well in the water deficiency test. Germplasm #34 was obtained from distant hybridization between the cultivated species ‘baisha 1016’ and *A. chacoense*, successfully inheriting excellent

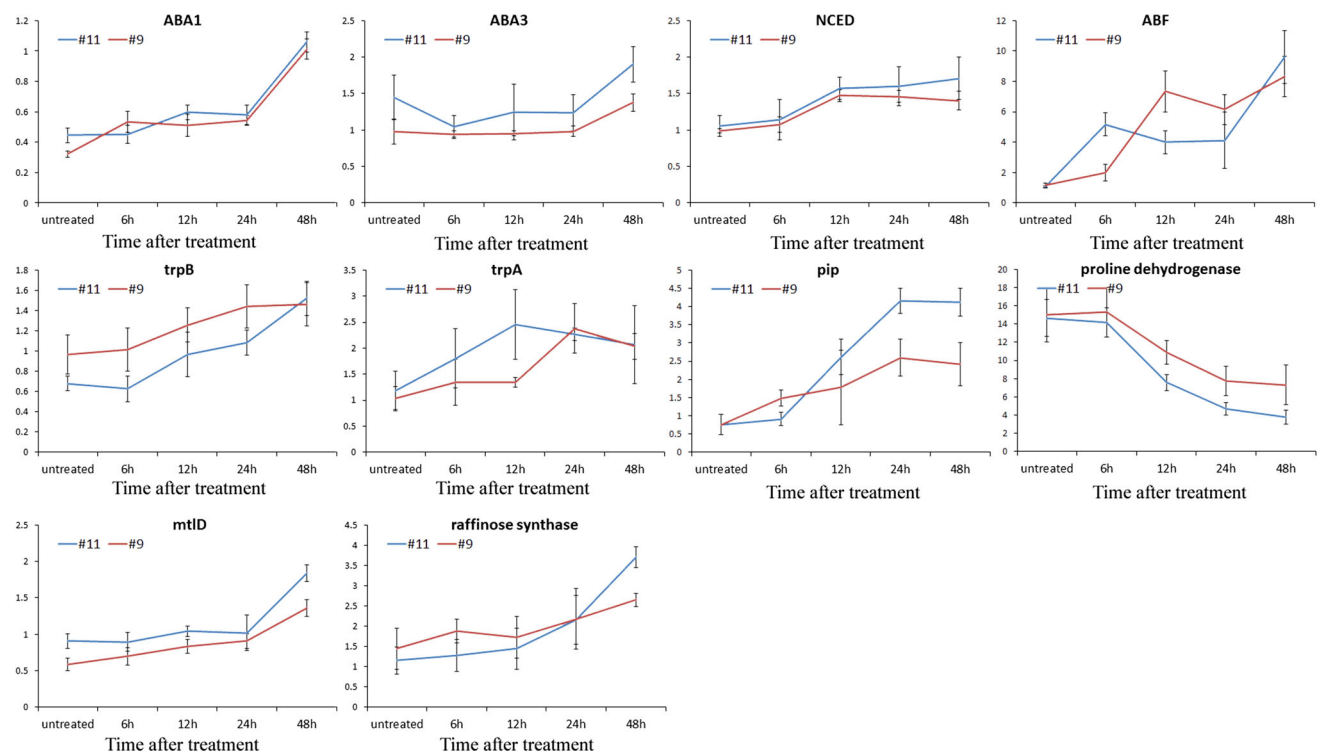


Fig. 4 qPCR analysis of drought unigenes (ABA, sugar, amino acid and protein kinase related unigenes) in #11 (tolerant germplasm) and #9 (sensitive germplasm)

characters of the wild species, probably including the tolerance ability.

Genomic resources, such as expressed sequenced tags (ESTs), genetic maps based on molecular markers, and genome sequencing have rapidly evolved in recent years. Next generation high-throughput sequencing technology, especially the transcriptome sequencing employed in this study, provides a novel tool for obtaining gene expression profiles in complex biological processes, discovering new genes and developing molecular markers including SSR and SNP (Zhang et al. 2012). For instance, transcriptome data revealed physiological and genetic information related to drought resistance in wheat (Reddy et al. 2014) and transgenic poplar (Zhang et al. 2014).

Drought-related genes in peanut and mechanism of resistance

We paid special attention to pathways of ABA and osmolytes, including sugar/sugar alcohol (such as mannitol), amino acids (such as proline) and amines, which are thought to be involved in plant drought response (Seki et al. 2007). Similar expression patterns were observed in both transcriptome sequencing and qPCR data, and differences in proline related gene expression were observed in various germplasm, implying a possibility of proline-driven drought tolerance ability in peanut, which was partly verified with the corresponding physiological analyses. Full cDNA sequences from certain transcripts in Supplemental Table 5 were acquired by online tools (Genemark, <http://opal.biology.gatech.edu/GeneMark/>) or RACE (Smart Race, Clotech), and can help in vector construction and transgenic research.

From the transcriptome sequencing data, we also obtained the complete cDNA sequences of several stress related genes, including 4 MYBs and 8 acetaldehyde dehydrogenase (ALDHs) (unpublished data), and analysis of their specific roles could help better understand the complex drought tolerance process in peanut.

Small RNAs are also believed to participate in stress response (Sunkar et al. 2012). Indeed, two studies on peanut microRNA have been performed (Zhao et al. 2010; Chi et al. 2011), with one using the same germplasm assessed in this work (Zhao et al. 2010). Future studies will further analyze drought related microRNA changes.

Author contribution statement Yi Shen and Zhide Chen are responsible for experiment design, data analysis and paper writing. Zhiguo E is responsible for qPCR. Xiaojun Zhang helped for data arrangement. Yonghui Liu is responsible for sample collection.

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Conflict of interest The authors declare that they have no conflict of interest.

References

- Chen X, Zhu W, Azam S, Li H, Zhu F, Hong Y, Liu H, Zhang E, Wu H, Yu S et al (2013) Deep sequencing analysis of the transcriptomes of peanut aerial and subterranean young pods identifies candidate genes related to early embryo abortion. *Plant Biotechnol J* 11:115–127
- Chi X, Yang Q, Chen X, Wang J, Pan L, Chen M, Yang Z, He Y, Liang X, Yu S (2011) Identification and characterization of microRNAs from peanut (*Arachis hypogaea* L.) by high-throughput sequencing. *PLoS One* 6:e27530
- Collino D, Dardanelli J, Sereno R, Racca R (2001) Physiological responses of argentine peanut varieties to water stress: light interception, radiation use efficiency and partitioning of assimilates. *Field Crop Res* 70:177–184
- Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21:3674–3676
- Devaiah KM, Bali G, Athmaram TN, Basha MS (2007) Identification of two new genes from drought tolerant peanut up-regulated in response to drought. *Plant Growth Regul* 52:249–258
- Dramé KN, Clavel D, Repellin A, Passaquet C, Zuily-Fodil Y (2007) Water deficit induces variation in expression of stress-responsive genes in two peanut (*Arachis hypogaea* L.) cultivars with different tolerance to drought. *Plant Physiol Bioch* 45:236–243
- Gautami B, Pandey MK, Vadez V, Nigam SN, Ratnakumar P, Krishnamurthy L, Radhakrishnan T, Gowda MVC, Narasu ML, Hoisington DA et al (2011) Quantitative trait locus analysis and construction of consensus genetic map for drought tolerance traits based on three recombinant inbred line populations in cultivated groundnut (*Arachis hypogaea* L.). *Mol Breed* 30:757–772
- Govind G, Vokkaliga Thamme Gowda H, Jayaker Kalaiarasi P, Iyer DR, Muthappa SK, Nese S, Makarla UK (2009) Identification and functional validation of a unique set of drought induced genes preferentially expressed in response to gradual water stress in peanut. *Mol Genet Genomics* 281:591–605
- Graherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q et al (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol* 29:644–652
- Guimaraes PM, Brasileiro AC, Morgante CV, Martins AC, Pappas G, Silva OB Jr, Togawa R, Leal-Bertioli SC, Araujo AC, Moretzsohn MC et al (2012) Global transcriptome analysis of two wild relatives of peanut under drought and fungi infection. *BMC Genom* 13:387
- Jongrunklang N, Toomsan B, Vorasoot N, Jogloy S, Boote KJ, Hoogenboom G, Patanothai A (2013) Drought tolerance mechanisms for yield responses to pre-flowering drought stress of peanut genotypes with different drought tolerant levels. *Field Crop Res* 144:34–42

- Li X-Y, Liu X, Yao Y, Li Y-H, Liu S, He C-Y, Li J-M, Lin Y-Y, Li L (2013) Overexpression of *Arachis hypogaea* AREB1 gene enhances drought tolerance by modulating ROS scavenging and maintaining endogenous ABA content. *Int J Mol Sci* 14:12827–12842
- Li X, Lu J, Liu S, Liu X, Lin Y, Li L (2014) Identification of rapidly induced genes in the response of peanut (*Arachis hypogaea*) to water deficit and abscisic acid. *BMC Biotechnol* 14:58
- Liu X, Hong L, Li X-Y, Yao Y, Hu B, Li L (2011) Improved drought and salt tolerance in transgenic *Arabidopsis* overexpressing a NAC transcriptional factor from *Arachis hypogaea*. *Biosci Biotech Biochem* 75:443–450
- Liu X, Liu S, Wu J, Zhang B, Li X, Yan Y, Li L (2013) Overexpression of *Arachis hypogaea* NAC3 in tobacco enhances dehydration and drought tolerance by increasing superoxide scavenging. *Plant Physiol Biochem* 70:354–359
- Manjulatha M, Sreevathsa R, Kumar AM, Sudhakar C, Prasad TG, Tuteja N, Udayakumar M (2014) Overexpression of a pea DNA helicase (PDH45) in peanut (*Arachis hypogaea* L.) confers improvement of cellular level tolerance and productivity under drought stress. *Mol Biotechnol* 56:111–125
- Marioni JC, Mason CE, Mane SM, Stephens M, Gilad Y (2008) RNA-seq: An assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res* 18:1509–1517
- Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B (2008) Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods* 5:621–628
- Nakazawa Y, Sato H, Uchino M, Takano K (2006) Purification, characterization and cloning of phospholipase D from peanut seeds. *Protein J* 25:212–223
- Pandey MK, Monyo E, Ozias-Akins P, Liang X, Guimaraes P, Nigam SN, Upadhyaya HD, Janila P, Zhang X, Guo B et al (2012) Advances in *Arachis* genomics for peanut improvement. *Biotechnol Adv* 30:639–651
- Pandurangaiah M, Lokanadha Rao G, Sudhakarbabu O, Nareshkumar A, Kiranmai K, Lokesh U, Thapa G, Sudhakar C (2014) Overexpression of horsegram (*Macrotyloma uniflorum* Lam. Verdc.) NAC transcriptional factor (MuNAC4) in groundnut confers enhanced drought tolerance. *Mol Biotechnol* 56:758–769
- Ravi K, Vadez V, Isobe S, Mir RR, Guo Y, Nigam SN, Gowda MVC, Radhakrishnan T, Bertoli DJ, Knapp SJ et al (2010) Identification of several small main-effect QTLs and a large number of epistatic QTLs for drought tolerance related traits in groundnut (*Arachis hypogaea* L.). *Theor Appl Genet* 122:1119–1132
- Reddy SK, Liu S, Rudd JC, Xue Q, Payton P, Finlayson SA, Mahan J, Akhunova A, Holalu SV, Lu N (2014) Physiology and transcriptomics of water-deficit stress responses in wheat cultivars TAM 111 and TAM 112. *J Plant Physiol* 171:1289–1298
- Sarkar T, Thankappan R, Kumar A, Mishra GP, Dobarra JR (2014) Heterologous expression of the AtDREB1A Gene in transgenic peanut-conferred tolerance to drought and salinity stresses. *PLoS One* 9:e110507
- Seki M, Umezawa T, Urano K, Shinozaki K (2007) Regulatory metabolic networks in drought stress responses. *Curr Opin Plant Biol* 10:296–302
- Sunkar R, Li Y-F, Jagadeeswaran G (2012) Functions of microRNAs in plant stress responses. *Trends Plant Sci* 17:196–203
- Wan L, Wu Y, Huang J, Dai X, Lei Y, Yan L, Jiang H, Zhang J, Varshney RK, Liao B (2014) Identification of ERF genes in peanuts and functional analysis of AhERF008 and AhERF019 in abiotic stress response. *Funct Integr Genomics* 14:467–477
- Wu N, Matand K, Wu H, Li B, Li Y, Zhang X, He Z, Qian J, Liu X, Conley S et al (2013) De novo next-generation sequencing, assembling and annotation of *Arachis hypogaea* L. Spanish botanical type whole plant transcriptome. *Theor Appl Genet* 126:1145–1149
- Xia H, Zhao C, Hou L, Li A, Zhao S, Bi Y, An J, Zhao Y, Wan S, Wang X (2013) Transcriptome profiling of peanut gynophores revealed global reprogramming of gene expression during early pod development in darkness. *BMC Genom* 14:517
- Ye J, Fang L, Zheng H, Zhang Y, Chen J, Zhang Z, Wang J, Li S, Li R, Bolund L (2006) WEGO: a web tool for plotting GO annotations. *Nucleic Acids Res* 34:W293–W297
- Yin D, Wang Y, Zhang X, Li H, Lu X, Zhang J, Zhang W, Chen S (2013) De novo assembly of the peanut (*Arachis hypogaea* L.) seed transcriptome revealed candidate unigenes for oil accumulation pathways. *PLoS One* 8:e73767
- Zhang J, Liang S, Duan J, Wang J, Chen S, Cheng Z, Zhang Q, Liang X, Li Y (2012) De novo assembly and characterisation of the transcriptome during seed development, and generation of genic-SSR markers in peanut (*Arachis hypogaea* L.). *BMC Genom* 13:90
- Zhang W, Chu Y, Ding C, Zhang B, Huang Q, Hu Z, Huang R, Tian Y, Su X (2014) Transcriptome sequencing of transgenic poplar (*Populus × euramericana* ‘Guariento’) expressing multiple resistance genes. *BMC Genet* 15(Suppl 1):S7
- Zhao CZ, Xia H, Frazier TP, Yao YY, Bi YP, Li AQ, Li MJ, Li CS, Zhang BH, Wang XJ (2010) Deep sequencing identifies novel and conserved microRNAs in peanuts (*Arachis hypogaea* L.). *BMC Plant Biol* 10:3
- Zhu W, Chen X, Li H, Zhu F, Hong Y, Varshney RK, Liang X (2014) Comparative transcriptome analysis of aerial and subterranean pods development provides insights into seed abortion in peanut. *Plant Mol Biol* 85:395–409