Comparative transcriptome analysis of genes involved in two peanut varieties under drought stress

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Research article

Keywords: RNA-seq, phytohormone, Peanut, Drought stress

Posted Date: May 31st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23240/v1

Version of Record: A version of this preprint was published on January 27th, 2021. See the published version at https://doi.org/10.1186/s12870-020-02761-1.

Abstract

Background

Peanut is one of the most important world oil crops. Peanut qualities and yields are restricted dramatically by abiotic stresses particularly by drought. Therefore, it would be beneficial to gain a comprehensive understanding on regulatory mechanisms of the peanut genomic transcriptional activities responding to drought, and hopefully extracting peanut molecular drought-resistance mechanisms.

Results

In this study, two peanut varieties NH5 (resistant) and FH18 (sensitive) which showed significantly differential drought-resistance were screened from twenty-three main commercial peanut cultivars and used for physiological characterization and transcriptomic analysis. NH5 leaves showed higher water and GSH contents, faster stomatal closure and lower relative conductivity (REC) than FH18. Under the time-course of 0 h (CK), 4 h (DT1), 8 h (DT2) and 24 h (DT3), drought-treatments tent to exert repressive impacts on peanut transcriptomes since the number of down-regulated differential expressed genes (DEGs) increased with the progression of treatments in both varieties.

Conclusions

Nevertheless, NH5 seemed to maintain stabler transcriptomic dynamics than FH18. Furthermore, annotations of identified DEGs implicated that signal transduction, elimination of reactive oxygen species, maintenance of cell osmotic potential were key drought-resistance-related pathways. Last, examination of ABA and SA components suggested that the fast stomata closure in NH5 was likely to be mediated through SA rather than ABA signaling. In all, these results have not only provided us comprehensive pictures of peanut drought transcriptomic changes, but also laid a foundation for further identification of the molecular drought tolerance mechanism in peanut and other oil crops.

Background

The peanut (*Arachis hypogaea* L.) is one of the main human oil and protein food sources. Its rich nutritional values are especially beneficial to the human cardiovascular system. The plantation of peanuts is distributed widely across developing countries from semi-arid tropical to subtropical regions [40, 46] Historically, peanuts have played important roles in the Chinese agricultural economy and are currently the top ranking exported-crops from China. The annual Chinese peanut output had reached 1.3 × 10 tons in 2008 [13]. Nevertheless, the quality and yield of peanuts are often seriously diminished by the drought. According to the public statistical data, the annual worldwide loss of peanut production caused by drought is about 6 million tons [41]. The frequency and severity of global drought are on the rise today and are expected to become severer in the next 30–90 years [13]. Future droughts have already exhibited

the tendency of high frequency, long duration and wide range. Once struck by drought, the normal growth of crops will be prohibited then yield reduction and even no-grain harvest will be caused. Up to date, studies have shown that drought stresses affect various biological processes, including water physiology, nutrient absorption, enzyme activity, photosynthesis and assimilate transport [15, 26, 53]. Plants under drought stresses can adjust their morphological, physiological and metabolic processes by regulating gene expression patterns [10]. Firstly, the expression of transcription factors can be regulated through the plant hormone signaling transduction. Then, multiple stress-responsive genes are induced [43, 49, 60]. In detail, drought stresses usually up-regulate abscisic acid (ABA), ethylene (ETH) and salicylic acid (SA) signaling pathways thus directing plants to produce osmo-regulatory substances to maintain cell osmotic potentials and antioxidant enzymes to re-establish the oxidation balances [30, 37, 50]. In addition, plants can also close stomata, thicken cuticle and harden cell walls to increase their drought resistance.

Until recently, transcriptomic studies have been conducted attempting to gain insights on molecular mechanisms underlying various perspectives of peanut biology. For example, Chen et al. have used young pods of Yueyo7 peanut variety for transcriptome sequencing trying to answer the question why peanut young pods will not enter developmental programs in the air [11]. Also, Wu et al. have used leaf, stem and root tissues from different developmental stages of the Spanish botanical type peanut *A. hypogaea* L to investigate peanut development by transcriptomic analysis [58]. In addition, Cui et al. have conducted transcriptome sequencing with salt-stressed LH14 shoot and root tissues to understand impacts of salt-stress on peanut [12]. However, there are only a few reported transcriptomic studies aiming at drought-related molecular mechanisms in peanuts. Shen et al. have studied transcriptomes in peanut leaves which were drought-stressed for seven days from FH1 a drought-resistant variety. In this study, information on peanut molecular responses to long-term drought stresses was revealed [44]. Another transcriptomic study was conducted by Zhao et al. [65]. They specifically studied peanut molecular responses to short-term drought (two-days) in root tissues from J1 another characterized drought-resistant peanut variety. Last, Brasileiro et al. have analyzed transcriptomes from wild-peanut tissues which were stressed for eleven-days [9]. Results from these three drought-transcriptome studies have demonstrated that drought stresses could induce the differential changes of expression levels in a suite of genes such as ABA-related, carbon metabolism-related, proline-related and photosynthesis-related genes. Despite above studies, molecular researches on drought-resistance mechanisms in peanut is still in a preliminary stage because of its huge-size allotetraploid genome.

Transcriptome sequencing technology has become an important tool for analyzing the molecular mechanism of drought-resistance in plants. At present, RNA-Sequencing (RNA-Seq) can provide increasing amount of information on differentially expressed genes, transcript structures, new transcripts and isomers, RNA alternative splicing and allele-specific expression etc [57]. RNA-Seq has been successfully applied to cuckoo, Yerba Mate and cotton [1, 10, 21], as well as many crop plants such as lentils, buckwheat and millet [23, 32, 64], to analyze their molecular mechanisms of drought resistance. These studies have supplied helpful information and improved our understanding of the molecular mechanism of plant resistance to drought stresses.

Transcriptome sequencing comparing varieties with significantly different resistance is proved to be an effective strategy for analyzing the stress-responsive molecular mechanism in a certain crop [55]. Since early drought-responses usually indicate the upstream regulatory events to the whole drought-responsive mechanism, it would be especially valuable to fill the blank of understanding on peanut drought-induced early molecular dynamic changes. Therefore, we chose two commercial peanut varieties which demonstrated differential drought-resistance in our screening as study materials (FH18 the sensitive type and NH5 the resistant type). PEG-6000 treatments were used to simulate drought stressful conditions during the seedling stage. Signature physiological indexes were further measured to monitor the physiological status of peanut seedlings under continuous drought stress. The RNA-Seq technology was used to sequence leaf transcriptomes of FH18 and NH5 at different stress time-points. The peanut transcriptomic spectrum under drought was studied, from which insights into the molecular mechanism of peanut drought resistance in the seedling stage were gained.

Result

Peanut drought-resistances

The plantation acreage of peanuts in the Northeastern provinces of China has been constantly increasing during recent years. To evaluate the performances of our current peanut germplasms under drought and to search for suitable research materials for peanut drought biology, we examined twenty-three representative commercial peanut varieties for their drought-resistances. After 24 h of simulated drought stress, all tested varieties had exhibited differential relative fresh-weight (FW), wilting-index (WI), leaf water-loss and conductivity (Table S1). And the level of drought-resistance was represented by a calculated “the membership function” (described as in the “materials and methods”). By this approach, the most drought-resistant varieties were NH5 and HY22 with ratings of 0.884 and 0.833 respectively. The least drought-resistant varieties were FH18 and NH16 with ratings of 0.304 and 0.288, ~ 36% of NH5 (Fig. 1). Showing synchronized paces of plant development, FH18 and NH5 were chosen as drought sensitive and drought-resistant peanut varieties for further analysis.

Analysis of Drought Stress Responses

Since FH18 (sensitive type) and NH5 (resistant type) seedlings showed vigorous growth during the 4th - leaf stage (Fig. 1), these seedlings were examined for phenotypic changes after being subjected to continuous simulated drought stresses. First, leaves of both varieties had exhibited an obvious wilting phenotype when the drought treatment prolonged but to a severer extent in FH18 than NH5 (Fig. 2). For example, FH18 leaves started drooping at DT1 (4 h), while no obvious change was observed in NH5 leaves at the same time-point. Furthermore, at DT2 (8 h), FH18 leaves significantly wilted but NH5 leaves only partially wilted (Fig. 2), indicating that NH5 could preserve a higher leaf water content than FH18 under drought conditions.

Stomata are important gateways for plants to control carbon and water exchange between leaves and the atmosphere. Based on the above observations, different stomatal-closure patterns should be

identified between FH18 and NH5 during the time-course of drought treatments. As expected, the stomata of both peanut varieties remained open at 0 h of drought stress (Fig. 3). NH5 showed stomatal closure at DT1 but not in FH18 (Fig. 3). For DT2 and DT3, the stomata in both peanut varieties were all closed (Fig. 3). These results suggested that drought- induced quick stomatal closure in NH5 leaves compared to FH18 leaves, which might contribute to a relatively slower water-loss and a higher leaf water content as displayed by NH5 in Fig. 2.

Relative conductivity (REC) is an index which can be used to reflect the ability of osmotic adjustment to stresses in the plasma membrane. Under drought conditions, the lower the REC value correlates with the better abilities of adjusting osmotic balance and thus stronger drought tolerance. As shown in the Fig. 4a, the REC values of NH5 were lower than those of FH18 at DT1 and DT2 time points (relative REC increase compared with CK: 1.81% for NH5 and 7.36% for FH18 at DT1; 5.85% for NH5 and 16.36% for FH18 at DT2) ($P < 0.01$). These data indicated better osmotic adjustment ability in NH5 than in FH18.

Reduced glutathione (GSH) is one of the most effective scavengers for reactive oxygen species (ROS). Next, the GSH contents in FH18 and NH5 were determined (Fig. 4B). Under the control conditions, there was no significant difference in the GSH content between these two peanut varieties. As the process of drought treatment progressed, the GSH content in both peanuts showed an increase trend but to different extent. Compared with the CK group, DT1, DT2 and DT3 of NH5 increased by 0.15 mol/g, 0.37 mol/g and 1.4 mol/g respectively, while DT1, DT2 and DT3 of FH18 increased by 0.15 mol/g, 0.26 mol/g and 0.52 mol/g respectively ($P < 0.01$). These results showed that stressed NH5 contained more GSH and therefore stronger ROS scavenging capabilities than FH18.

Transcriptome sequencing and assembly

Transcriptomes from the FH18 and NH5 seedlings which were stressed to different levels were sequenced using Illumina 2000, and totally twenty-four transcriptome libraries were constructed (three library repeats for each variety at every time-point). After removing the low-mass readings, 177.69 Gb of clean data were obtained. The clean data for each sample reached 5.90 Gb and the percentage of Q30 bases was 94.62% or more. The “Clean Reads” of each sample were sequenced with the designated reference genome, and the alignment efficiency ranged from 94.47% to 97.49%. Based on comparisons, alternative splicing prediction analysis, gene structure optimization analysis and discovery of new genes were carried out, and 6,940 new genes were discovered (Table S1).

Expression Analysis of Differential Genes

Gene expression patterns could be significantly affected by drought stresses. Therefore, differentially expressed genes (DEGs) were extracted according to their differential expression levels in different samples. Then functional annotation and enrichment analysis were carried out with these identified DEGs. DEGs at DT1, DT2 and DT3 of FH18 were identified as 7,989 (up-regulated 3,709/ down-regulated 4,280), 9,386 (up-regulated 4,052/down-regulated 5,334) and 11,218 (up-regulated 4,881/down-regulated 6,337), respectively. In contrast, 4,497 (up-regulated 2,448/down-regulated 2,049) DT1, 5,780 (up-

regulated 2,673/down-regulated 3,107) DT2 and 5,762 (up-regulated 2,585/down-regulated 3,177) DT3 DEGs for NH5 were identified. It was obvious that at each time point DEGs of FH18 significantly outnumbered those of NH5, for example almost twice of NH5 DEGs at DT3. This difference indicated that drought stresses would induce more volatile transcriptomic dynamics in FH18 than in NH5. From another aspect, NH5 seemed to be able to maintain a stabler transcriptome under drought conditions. Furthermore, when carefully examined, the number of down-regulated FH18 DEGs was ~ 30% more than up-regulated DEGs at both DT2 and DT3. For NH5 DEGs, the ratios were ~ 15% at DT2 and ~ 20% at DT3. These results suggested that drought stresses within 24 h tended to exert more of a down-regulation impact on peanut transcriptomes. Also the comparatively lesser extent of down-regulation in NH5 transcriptomes than FH18 might reflect and confirm previous physiological characterizations of NH5 as drought-resistant and FH18 as drought-sensitive. Next, cluster analysis was carried out with identified differential genes (Fig. 5b).

Functional Annotation of DEGs

Next, functional annotation was carried out for DEGs (refer to Table S2 for statistical numbers of genes annotated in each differential gene set). In order to determine subordinate categories of the responsive genes, we used GO classification for the DEGs in FH18 and NH5 respectively. And the matched DEGs were divided into three functional categories: biological processes, molecular functions and cell components (Fig. 6a and b). In the category of biological processes, the most abundant genes belonged to metabolic processes and cellular processes. In the category of cell components, the number of genes in cell parts and cells was the highest. In the category of molecular function, DEGs mainly belonged to binding and catalytic activity subgroups. In order to identify active biological pathways enriched with DEGs in both peanut varieties, KEGG pathway database was used (Figure S1). The results of KEGG enrichment analysis were shown in the following Figure with the first twenty top-ranking pathways by smallest significant Q values (Fig. 6C and D). Although FH18 and NH5 had shared similar pathway-enrichment results, the number of enriched genes and the expression levels of enriched genes were quite different (Table S3 and S4). The enriched-pathways included GSH-related glutathione metabolism, glycolysis, glyoxylic acid and dicarboxylic acid ester metabolism associated with pyruvic acid. Pathways of corneal and wax anabolism, fatty acid degradation related to stratum corneum, carbon fixation, photosynthesis-antenna protein, photosynthesis, degradation of amino acids valine, leucine and isoleucine, and porphyrin and chlorophyll metabolism were also enriched. In addition, several pathways were only enriched in the drought-resistant variety NH5, including alanine metabolism, sulfur metabolism, sphingolipid metabolism, phenylpropane biosynthesis, isoquinoline alkaloid biosynthesis and biosynthesis of tropane, piperidine and pyridine alkaloid.

Peanut Drought Resistant- Related Genes and Pathways

In order to explore the drought-resistance mechanism of peanut, we examined transcriptional changes of potential drought-resistance genes in FH18 and NH5 with drought treatments. It was found that genes related to ABA and SA signal-transduction were significantly up-regulated, specifically sixteen ABF genes

and twenty-two TGA (TGACG motif-binding factor) genes (Table S5). Compared with FH18 transcriptomes, some genes were differentially expressed only in NH5. These NH5-specific DEGs could be categorized into various biological pathways. Among them, fourteen genes were identified as ROS-scavenging genes (Table S5), which belonged to glutathione metabolism and proline metabolism respectively. Thirty-three osmotic-potential-regulating genes (Table S5) were subordinate to the metabolism of arginine, proline, sucrose and starch. In addition, fourteen cell wall sclerosis-related genes and fourteen cutin and wax metabolism genes were also enriched from NH5 transcriptomes, which were believed to be able to affect water loss (Table S5). Another set of genes involved in peanut defense-responses showed much higher expression levels in NH5 than in FH18. On the other hand, FH18-specific differential genes were also identified, however their expression patterns indicated that these genes were suppressed by drought treatments. Furthermore, another 126 DEGs genes were identified to enrich in main drought-responsive metabolic pathways (Table S5) such as sphingolipid metabolism, photosynthesis, pyruvate metabolism, fatty acid degradation and tricarboxylic acid cycle. In conclusion, a diagram of interactions of above-described enriched-pathways was drawn and shown as in Fig. 7.

Real-time qPCR Validation

In order to validate the accuracy of transcriptome data sets, the real-time qPCR technology was applied to analyze transcriptional levels ten genes which were randomly selected from drought-resistant-related pathways. The relative expression levels of genes were measured and calculated with ARAH1 as the internal reference gene. These ten genes were: pyruvate dehydrogenase; glutamate synthetase, agmatine deiminase isoenzyme X2, PXG, trehalose 6-phosphate synthase/phosphatase, inositol oxygenase 2, glutathione S-transferase, cinnamyl alcohol dehydrogenase, glycerol kinase and enoyl-CoA hydratase. RT-PCR results confirmed that the transcription changes of these ten genes were comparable with the fold-changes gained from our transcriptome analysis (Fig. 8).

Discussion

Adaptation of Peanuts to Drought

Drought stress is one of the main limiting factors for crop growth and productivity. In general, plant drought tolerance involves the combination of a variety of physiological and biochemical changes based on coordinated expression of hierarchy of genes. This complex mechanism is the result of interaction between the plant heredity and changes in the external environment [20, 61, 62]. In this study, we used PEG-6000 to simulate drought stresses in combination with the transcriptome sequencing technology to analyze the drought-resistance in two peanut varieties (FH18 and NH5). Compared to FH18, the drought-resistant variety NH5 showed stronger capabilities of adjusting osmotic-potential of the plasma membrane and scavenging reactive oxygen species (ROS). We also observed that the stomata of FH18 and NH5 closed to reduce water loss, and particularly the quicker stomatal closure in NH5 than in FH18.

Stratum Corneum Biosynthesis and Cell-Wall Sclerosis

The stratum corneum is a membrane structure composed of wax, cutin and polysaccharides. As a barrier against environmental stresses, its contents change under drought stresses and therefore play vital roles in reducing plant water loss [2, 29, 54]. Typical cutin is represented by epoxy C16/C18 fatty acids, which are crosslinked by ester bonds to form elastic polyester structures [5, 16]. Waxes consist of various aliphatic molecules, mainly long-chain fatty acids (VLCFAs), containing more than 20 carbon atoms and their derivatives including primary alcohols, secondary alcohols, aldehydes, alkanes, ketones and wax esters [36]. In this study, we found that the transcriptional abundances of C18/C22 synthetic genes were induced by drought stress in both FH18 and NH5. It has been speculated that the drought-stressed peanut stratum corneum may be mainly composed of C18 fatty-acid cutin and docosan-acid wax. Our results showed that drought induced cutin and wax-related genes in FH18 faster than in NH5, however the induction levels were mostly higher in NH5 than in FH18.

Cell-wall hardening of leaves is considered as another main response of crops to drought stresses. Glucosyluronic acid kinase (GLCAK) participates in the precursor-synthesis of pectin and hemicellulose [59]. Plants harden cell walls by covalently combining lignin and hemicellulose molecules to form interwoven networks. Under drought stresses, the biosynthesis of lignin can be affected by regulating the phenylpropane biosynthesis pathway, thus the modification of cell walls. The phenylpropane biosynthesis would also affect the biosynthesis of anthocyanins through the formation of anthocyanins, thus promoting the formation of plant keratins [4]. It is known that plants contain lower water potential and higher level of cell-wall hardening under drought. The hardening of plant cell- walls will effectively lead toreduction in leaf growth and water transpiration. According to Xiao et al. arabidopsis GLCAK mutant (deletion mutant) has exhibited lower drought-resistance and soluble-sugar content than WT [59]. The observed drought-induction of GLCAK gene in this study may contribute to the hardening of plant cell-walls and the accumulation of soluble sugars to balance osmotic potential so to resist drought stresses. Additionally, the phenylpropane biosynthesis pathway, which is enriched only in NH5, might be another significant contributing factor why NH5 is more drought-resistant than FH18.

ROS Scavenging and Steady Osmotic Potential

The regulation of plant osmotic potentials is considered as a defensive mechanism against drought stresses [28]. Under drought conditions, osmotic-adjusting substances will accumulate in plants, maintaining the balance of cell osmotic potential, turgor pressure and cell volume [3]. Proline is a protective agent for osmotic regulation. High levels of proline can reduce the cell water potential and enhance the ability of removing ROS by antioxidants [39, 51]. Sucrose, a soluble sugar, also plays an important role in plant osmotic regulation. The accumulation of soluble sugars can enhance the cell water absorption to protect cells [4, 34]. Glutamine can be another osmotic regulator to help plants with resisting drought stresses [48]. The results from the present study have suggested that peanuts could maintain the balance of osmotic potential under drought stresses by inducing the expression of synthetic genes of proline, sucrose and glutamic acid.

Drought stressed plants tend to accumulate reactive oxygen species and thus peroxidize plasma membrane, which will lead to cell death in severe cases [22]. Glutathione reductase (GR) and dehydroascorbate reductase (DHAR), as antioxidant enzymes, can effectively scavenge free radicals in cells and protect plant organisms [7, 8]. GR can reduce oxidized-glutathione (GSSH) to reduced-glutathione (GSH) which is the scavenger for free radicals and particular organic peroxides [19, 63]. In this study, GR and DHAR genes were up-regulated under drought stress conditions. Although the content of GSH in both FH18 and NH5 varieties showed an increasing trend with the progress of drought treatments, the transcription of GSH in the resistant variety NH5 was higher than that in the sensitive variety FH18. Since the metabolism of glutathione and ascorbic acid are important protective mechanisms against drought stresses, our findings also indicated their vital involvements as peanut drought-resistance mechanisms.

The Roles of ABA and SA Signal Transduction Pathways

Usually, plants respond to external stimuli by activation of signaling cascades in order to modify downstream gene expression patterns, finally to realize physiological and metabolic adaptations [33]. Abscisic acid (ABA) and salicylic acid (SA) signaling pathways were significantly induced by drought in this study. ABA and SA are two well-known plant hormones whose biosynthesis and signaling play key roles in drought-stress responses [17, 31, 35]. The core factors of ABA signal transduction pathway include ABA receptors (PYL/PYR), protein phosphatase 2C (PP2C), SNF1-related kinase (SNRK2) and ABA response-element-binding-factors (ABFs). Under drought stresses, ABA binds to PYLs/PYRs to inhibit PP2C which will lead to the promotion SnRK2. Then SnRK2 activates ABFs to regulate downstream transcription factors and to initiate ABA signal responses [18, 36]. Drought stresses often induce an elevated ABA level in plant which will cause the binding of ABI1 (Abel son interactor protein 1) to PYL/PYR receptors. Once ABI1 binds to PYLs/PYRs, the inhibition of SLAC1 kinase by ABI1 will be released, which in turn will result in the closure of anion channels and eventually stomatal closure [66, 67]. In the present study, the transcription of an ABA-biosynthesis-related gene NCED in both FH18 and NH5 was found to be significantly induced under all drought treatments. On the other hand, the ABA-receptor PYL/PYR-related genes were repressed by all drought treatments in NH5, while in FH18 they were partially repressed by 4 h and 8 h treatments. Taken together the observation that NH5 but not FH18 showed stomatal closure under the 4 h treatment, it was reasonable to postulate that this fast stomatal closure response might not be mediated through PYLs/PYRs. Furthermore, our results showed that the negative ABA signaling regulator PP2C was also induced and the positive component SNRK2 was repressed by drought treatments, suggesting decreased ABA-sensitivities. However, the SNRK2 targets ABFs transcription factors showed significant induction pattern in drought-stressed leaves. These seemingly different or even contradicting results were exactly the evidence for the complicate and intricate involvement of ABA signaling in peanut drought-resistance mechanisms.

The synthesis of SA in peanut is the phenylalanine pathway mediated by phenylalanine ammonia lyase (PAL). Previous research has shown that drought stress can promote the increase of SA content by increasing PAL activity, thus improving plant drought-resistance [6]. As Miura et al. have pointed out SA

can promote stomatal closure and induce defense-genes [31]. In this study, PAL and TGA genes were expressed at high levels indicating that SA signal transduction participated in peanut drought stress responses. Although SA might dominate the peanut stomatal closure, some TGA genes were only induced in NH5 which could explain the observation that NH5 stomatal closure was faster than FH18. All these findings on the drought- induction of ABA and SA related genes strongly implicated that the signal transduction under drought stress in peanuts was initiated by both ABA and SA hormones, thus comprised a highly complex drought-combating molecular mechanism in peanuts.

Conclusion

In conclusion, we first characterized the phenotype and physiology of drought-treated peanuts. Then we obtained peanut transcriptome data sets of different genetic materials by the RNA-Seq technology, in order to explore the drought-related key genes and metabolic pathways. Our results showed that the signal transduction pathways of ABA and SA hormones were activated in peanut under simulated-drought stress. The expression patterns of genes related to stratum corneum biosynthesis, cell wall hardening, ROS clearance and osmotic potential were also changed in favor of resisting drought stress. All these findings expanded our knowledge of mechanisms of peanut drought-resistance and could facilitate future breeding of elite peanut germplasms.

Methods

Materials and growth

A total of twenty-three major commercial peanut varieties in the Northeastern China were obtained from Shenyang Agricultural University. Sixteen of them were undertaken the formal identification by national and local approval committee, respectively, and the others are under review. The more detail information were listed in Table S8. Peanut seeds were pre-soaked in de-ionized water and germinated in the dark for 24 h in a 28 °C incubator. Germinated seeds were planted in sand and grew under 16 h/8h light cycle, 60% humidity and 28 °C supplemented with ½ Hoagland solution every other day. Seedlings at the 4th true-leaf stage with similar height were washed, dried and then root-cultured in Hoagland solution for another three days. Addition of 20% PEG6000 to Hoagland solution was adopted as the simulated-drought condition and the untreated Hoagland solution was the control condition.

Drought-resistance screen:

After 24-hours of treatment, stressed (S) and control (CK) seedlings were collected for the following measurements. All measurements were performed with three independent biological replicates if not specified.

Determination of Water loss rate (RWL): The second compound leaf (1.0 g) was detached from plants and weighed immediately for FW_1. Then detached leaves were placed in the yarn net and air-dried for 2 hours (kept from the wind and direct sunlight). Next, the air-dried leaves were weighed for FW_2. Then

leaves were dried in the oven at 80 °C to constant weight (DW). The oven-drying time-duration was represented as (t1-t2). RWL was calculated using the following equations: $RWL(mg \cdot g^{-1} \cdot min^{-1}) = (FW_1 - FW_2)/DW(t_1 - t_2)$.

Determination of relative plant fresh weight (RFW): first, the average fresh weights of seedling of drought-stressed and CK groups were respectively measured and calculated using three randomly-chosen seedlings as independent biological replicates for each group. Relative plant fresh weight RFW was calculated as the following: RFW = average fresh-weight of drought-treated plants/ average fresh-weight of CK plants.Conductivity: The conductivity was measured using a conductivity meter (model, maker) at room temperature (24 °C) and calculated as described by Xu et al. [62].

Determination of wilt index: the peanut wilt index grades were visually evaluated. Peanut seeds were germinated as described above. Germinated seeds were planted in 15 cm-diameter flowerpots with the same amount of sand and under 16 h/8h light cycle, 60% humidity and 28 °C. Seedlings were supplemented with ½ Hoagland solutionevery other day. Once reaching the 3rd true-leaf stage, watering was stopped and the soil was allowed to dry naturally. When the soil reached 75% relative water content, digital pictures of peanut plants were taken every day. Namely: grade 0: the peanut leaves were naturally expended and were bright and glossy; the culm was firm as well. Grade 1: the leaves began to lose water; the leaves were dull and the top one or two leaves were slightly drooping. Grade 2: the plants continued to lose more water; the drooping of leaves was aggravated. Grade 3: some leaves were dry, hard and curly. Grade 4: all leaves were drooping and shrinking, and turned yellow. Grade 5: leaves were completely dry and hard, and the plants died. If the wilting degree was between two levels, it would be treated as a grade and half

Calculation of comprehensive index:

The relative drought tolerance of peanuts was determined by the method of average “membership function” [68].

Formula for “membership function” was: $\mu_{xj} = (X_j - X_{min})/(X_{max} - X_{min})$

For a certain variety, μ_{xj} was the “membership function” for the “J” trait; x_j is the value of the “J” trait; X_{max} and X_{min} were respectively the maximum and minimum values for the trait among all considered varieties. In order to avoid errors caused by variety-differences, X_j, X_{max} and X_{min} were all calculated “relative values” instead of “measured values”. Relative value = the measured value under stress/ the measured value under the control.

Drought-treatment time-course

FH18 and NH5 seedlings were prepared and treated as described in “materials and growth”. The drought-treatment time-course was composed of a series of treatment time points: 0 h (CK), 4 h (DT1), 8 h (DT2)

and 24 h (DT3). The secondcompound leaves of seedlings were respectively collected at each time point which were frozen in liquid nitrogen then stored in a refrigerator at -80 °C for further analysis.

Physiological index measurements and stomatal observation

In order to compare the different effects by drought stress on FH18 and NH5, the second-compound leaf of seedlings were randomly selected from the treatment group and the control group, and then selected physiological indexes were measured. The conductivity was measured as described above. The reduced glutathione was measured by using a kit (Suzhou Keming Biotechnology Company) following manufacture's protocols. Observation of peanut Stomata after the simulated-drought treatments were carried out on a fluorescence positive microscope by Zeiss [47]. All measurements were performed with three independent biological replicates.

RNA extraction and RNA-seq

RNA samples were prepared from 24 harvests (4 treatments × 2 genotypes × 3 biological replicates) of peanut plants. Total RNA was extracted using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer instructions. The quality of RNA was assessed by Agilent 2100. The transcriptional group was sequenced using the method described by Wang et al [56]. Even if via magnetic beads with Oligo (dT), the mRNA of the samples were enriched. Briefly, a single stranded and double stranded cDNA was synthesized from the mRNA using random hexamers and AMPure XP beads (Beckman Coulter, Beverly, CA, USA), respectively, and finally, PCR enrichment was performed to obtain final cDNA libraries. In order to separate the cDNA fragments with a length of 240 bp, the library was purified by AMPure XP and the library quality was evaluated in Agilent Bioanalyzer 2100 system. Finally, Illumina 2000 was used to PCR amplify the library.

Data analysis

The built-in software "perl scripts" was used to clear the inferior quality readings from the original data DEseq was used for differential expression analysis and genes with $p < 0.01$ were assigned as differentially expressed genes (DEGs). In order to analyze the functional relations of DEGs, we performed the GO and KEGG enrichments based on GOseq R language pack and pathways in the KEGG database [52]. Hypergeometric test was used to test the enrichment- significance of enriched pathways against the whole genomic background.

QRT-PCR verification

To verify the accuracy of RNA-seq sequencing, ten putative drought- tolerance-related differential genes were randomly selected for qRT-PCR verification. The Arah 1 gene was used a reference gene and the genes-specific primers of the selected DEGs were designed using PRIMER5. QRT-PCRs were performed on an ABI Stepone plus platform with three reactions for each biological replicate and a total of three biological replicates for each gene.

Abbreviations

cDNA
Complementary DNA
CK
Control group
DEGs
Differentially expressed genes
FPKM
Fragments per kb per million fragments
GO
Gene ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
NCBI
National Center for Biotechnology Information
qRT-PCR
Quantitative real-time PCR
RNA-seq
RNA sequence
ABA
Abscisic acid
SA
salicylic acid
PAL
phenylalanine ammonia lyase
PP2C
protein phosphatase 2C
SNRK2
SNF1-related kinase
ABFs
ABA response-element-binding-factors
ABI1
Abel son interactor protein 1
GSSH
oxidized-glutathione
GSH
reduced-glutathione
GRG
lutathione reductase

DHAR
dehydroascorbate reductase
GLCAKG
lucosyluronic acid kinase
ROS
scavenging reactive oxygen species

Declarations

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available due [These data are being used for the next part of the research] but are available from the corresponding author on reasonable reques.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the National Key Research and Development Program of China (2018YFD1000906). The funding bodies have no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Authors' contributions

CJ J, XL L, JX Z, H J and HQ Y conceived the idea. XL L,JJ R and JX Z carried out the lab work. XL L, CY J, H Z performed data analysis. XL L wrote the manuscript, H J completes final revision. All authors read and approved the final manuscript.

Acknowledgments

We thank Biomarker Company (Beijing) for helping with transcriptome sequencing.

References

1. Acevedo R M, Avico EH, González S, *Salvador AR*, *Rivarola M*, *Paniego N*. *Transcript and metabolic adjustments triggered by drought in Ilex paraguariensis leaves*. *Planta*. 2019; *250*: *445–462*. https://doi. org/10.1007/s00425-019-03178-3
2. Aharoni A. Dixit S, Jetter R. Thoenes E, *van* Arkel G, Pereira A. The SHINE clade of AP2 domain transcription factors activates wax biosynthesis, alters cuticle properties, and confers drought tolerance when overexpressed in Arabidopsis. Plant Cell. 2004; 16:2463–80. https:// *doi: 10.1105/tpc.104.022897*.
3. Ashraf M. Foolad MR.. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007; 59: 206–16. https://doi. org/10.1016/j.envexpbot.2005.12.006.
4. Bai Z. Wang T, Wu Y. Wang K, Liang QY. Pan YZ, *et al.* Whole-transcriptome sequence analysis of differentially expressed genes in Phormium tenax under drought stress. Sci Rep. 2017; 7: 41700.https:///doi. org/10.1038/srep41700.
5. Bakan B. Marion D. Assembly of the cutin polyester: from cells to extracellular cell walls. Plants. 2017;. 6:, E57. https://doi. org/10.3390/plants6040057.
6. Bandurska H. Cieślak M. The interactive effect of water deficit and UV-B radiation on salicylic acid accumulation in barley roots and leaves. Environ Exp Bot. 2013; 94: 9–18. https://doi. org/10.1016/j.envexpbot.2012.03.001.
7. Begaramorales JC. Sánchezcalvo B, Chaki M. Matapérez C, Valderrama R. Padilla MN. Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and S-nitrosylation. J Exp Bot. 2015; 66 (19): 5983–96. https://doi. org/10.1093/jxb/erv306.
8. Bowler C. Montagu MV, Inze D. Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol. 1992; 43: 83–116. https://doi.org/10.1146/annurev.
9. Brasileiro ACM. Morgante CV, AraujoACG, Leal-Bertioli SCM, Silva AK. Silva A. Transcriptome profiling of wild Arachis from water-limited environments uncovers drought tolerance candidate genes. Plant Mol Biol Rep. 2015; 33: 1876–92. https://doi: 10.1007/s11105-015-0882-x.
10. Cai YF. Wang JH, Zhang L. Song J, Peng LC. Zhang SB.. Physiological and transcriptomic analysis highlight key metabolic pathways in relation to drought tolerance in Rhododendron delavayi. Physiol Mol Biol Plants. 2019; 25: 991–1008. https:// *doi: 10.1007/s12298-019-00685-1*.
11. Chen X. Zhu W, Azam S. Li H, Zhu F. Li H, *et al.* Deep sequencing analysis of the transcriptomes of peanut aerial and subterranean young pods identifies candidate genes related to early embryo abortion. Plant Biotech J. 2013; 11: 115–27.https://doi. org/10.1111/pbi.12018.
12. Cui F. Sui N, Duan G. Liu Y, Han Y. Liu S, Wan S. Li G. Identification of metabolites and transcripts involved in salt stress and recovery in peanut. Front Plant Sci. 2018; 9: 217. https:// *doi: 10.3389/fpls.2018.00217*.
13. Dai A. Increasing drought under global warming in observations and models. Clim Change. 2013;3:52–8. https://doi:10.1038/nclimate1633.
14. Dai CC. Chen Y, Wang XX. Li PD.. Effects of intercropping of peanut with the medicinal plant Atractylodes lancea on soil microecology and peanut yield in subtropical China. Agrofor Syst. 2013;

87: 417–26. https://doi.org/10.1007/s10457-012-9563-z.

15. Fahad S. Bajwa AA, Nazir U. Anjum S A, Farooq A. Zohaib A. Crop production under drought and heat stress: plant responses and management options. Front Plant Sci. 2017; 8: 1147. https:// *doi: 10.3389/fpls.2017.01147*.
16. Fich EA. Segerson NA, Rose JKC. The plant polyester cutin: biosynthesis, structure, and biological roles. Ann Rev Plant Biol. 2016; 67: 207–33. https:// *doi: 10.1146/annurev-arplant-043015-111929*.
17. Fragnière C. Serrano M, Abou-Mansour E. Métraux JPL, Haridon F.Salicylic acid and its location in response to biotic and abiotic stress. FEBS Lett. 2011; 585: 1847–52. https://doi. org/10.1007/s10457-012-9563-z.
18. Fujita Y. Yoshida T, Yamaguchi-Shinozaki K. Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. Physiol Plant. 2013; 147: 15–27. https://doi. org/10.1111/j.1399-3054.2012.01635.x.
19. Gill SS. Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 2010; 48: 909–30. https:// *doi: 10.1016/j.plaphy.2010.08.016*.
20. https://doi. 10.1155/2014/701596
    Gupta B. Huang B. *Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genomics.* 2014; *2014: 2314–4378.* https://doi. org/10.1155/2014/701596.
21. 10.3390/ijms20092076
    Hasan MU. Ma F, Islam F. Sajid M, Prodhan ZH. Li F. *Comparative transcriptomic analysis of biological process and key pathway in three cotton (Gossypium spp.) species under drought stress. Int J Mol Sci.* 2019; *20: 2076. doi*: 10.3390/ijms20092076.
22. Haupt H. Fock HP. Oxygen exchange in relation to carbon assismilation in water stress leaves during photosynthesis. Ann Bot. 2002; 89: 851–95. https:// *doi: 10.1093/aob/mcf023*.
23. Hou Z. Yin J, Lu Y. Song J, Wang S. Wei S. Transcriptomic analysis reveals the temporal and spatial changes in physiological process and gene expression in common buckwheat (Fagopyrum esculentum Moench) grown under drought stress. Agronomy. 2019; 9: 569. https://doi. org/10.3390/agronomy9100569.
24. Hughes NM. Reinhardt K, Feild TS. Gerardi AR, Smith WK. Association between winter anthocyanin production and drought stress in angiosperm evergreen species. J Exp Bot. 2010; 61(6): 1699–709. https:// *doi: 10.1093/jxb/erq042*.
25. Kanehisa M. Araki M, Goto S. Hattori M, Hirakawa M. Itoh M. KEGG for linking genomes to life and the environment. Nuc Acids Res. 2008; 36: D480–4. https:// *doi: 10.1093/nar/gkm882*.
26. Khan FP. Upreti R, Singh P. Shukla K, Shirke PA.Physiological performance of two contrasting rice varieties under water stress. Physiol Mol Biol Plants. 2017; 23(1): 85–97. https://doi: 10.1007/s12298-016-0399-2.
27. Li W. Xiang F, Zhong M. Zhou L, Liu H. Li S, *et al.* Transcriptome and metabolite analysis identifies nitrogen utilization genes in tea plant (Camellia sinensis). Sci Rep. 2017; 7: 1693. https://doi:

10.1038/s41598-017-01949-0.

28. Lorenz WW. Alba R, Yu YS. Bordeaux JM, Simões M. Dean JFD. Microarray analysis and scale-free gene networks identify candidate regulators in drought-stressed roots of loblolly pine (P. taeda L.). BMC Genom. 2011; 12: 264. https:// *doi: 10.1186/1471-2164-12-264*.
29. Menard R. Verdier G, Ors M. Erhardt M, Beisson F. Shen WH. Histone H2B monoubiquitination is involved in the regulation of cutin and wax composition in Arabidopsis thaliana. Plant Cell Physiol. 2014; 55: 455–66. https://doi. org/10.1093/pcp/pct182.
30. Miller G. Suzuki N, Ciftci-Yilmaz S. Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ. 2010; 33: 453–67. https:// *doi: 10.1111/j.1365-3040.2009.02041*.
31. Miura K. Tada Y. Regulation of water, salinity, and cold stress responses by salicylic acid. Front Plant Sci. 2014; 5: 4. https:// *doi: 10.3389/fpls.2014.00004*.
32. Morgil H. Tardu M, Cevahir G. Kavakli IH. Comparative RNA-seq analysis of the drought-sensitive lentil (Lens culinaris) root and leaf under short- and long-term water deficits. Fun Integ Genomics. 2019; 19: 715–27. https:// *doi: 10.1007/s10142-019-00675-2*.
33. Osakabe Y. Osakabe K, Shinozaki K. Tran LS. Response of plants to water stress. Front Plant Sci. 2014; 5: 86. https:// *doi: 10.3389/fpls.2014.00086*.
34. Pan HY. Zhou R, Louie GV. Muhlemann JK, Bomati EK. Bowman ME. Structural studies of cinnamoyl-CoA reductase and cinnamyl-alcohol dehydrogenase, key enzymes of monolignol biosynthesis. Plant Cell. 2014; 26: 3709–27. https:// *doi: 10.1105/tpc.114.127399*.
35. Pizzio GA. Rodriguez L, Antoni R. Gonzalez-Guzman M, Yunta C. The PYL4 A194T mutant uncovers a key role of PYR1-LIKE4/PROTEIN PHOSPHATASE 2CA interaction for abscisic acid signaling and plant drought resistance. Plant Physiol. 2013; 163: 441–55. https:// *doi: 10.1104/pp.113.224162*.
36. Raghavendra AS. Gonugunta VK, Christmann A. Grill E. ABA perception and signalling. Trends Plant Sci. 2010; 15: 395–401. *https:// doi*: 10.1016/j.tplants.2010.04.006.
37. Ranjan A. Pandey N, Lakhwani D. Dubey NK, Pathre UV. Sawant SV. Comparative transcriptomic analysis of roots of contrasting Gossypium herbaceum genotypes revealing adaptation to drought. BMC Genom. 2012; 13: 680. https:// *doi: 10.1186/1471-2164-13-680*.
38. Samuels L. Kuns L, Jetter R. Sealing plant surfaces: cuticular wax formation by epidermal cells. Ann Rev Plant Bio. 2008; 59: 683–707. https:// *doi: 10.1146/annurev.arplant.59.103006.093219.*.
39. Sánchez FJ. Manzanares M, *de* Andres EF, Tenorio JL. Ayerb, L. Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. Field Crop Res. 1998; 59: 225–35. https:// *doi. org/10.1016/S0378-4290(98)00125-7*.
40. Sarkar T. Thankappan R, Kumar A. Mishra GP, Dobaria JR. Heterologous expression of the AtDREB1A gene in transgenic peanut-conferred tolerance to drought and salinity stresses. PLoS ONE 2014; 9 (12): e110507. https:// *doi: 10.1371/journal.pone.0110507*.
41. Sarkar T. Thankappan R, Kumar A. Mishra GP, Dobaria JR. Stress inducible expression of AtDREB1A transcription factor in transgenic peanut (Arachis hypogaea L.) conferred tolerance to soil-moisture

deficit stress. Front Plant Sci. 2016; 7: 935. https:// *doi: 10.3389/fpls.2016.00935*.

42. Savoi S. Wong DCJ, Arapitsas P. Miculan M, Bucchetti B. Peterlunger E. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (Vitis vinifera L.). BMC Plant Biol. 2016; 16: 67. https:// *doi: 10.1186/s12870-016-0760-1*.
43. Shen H. Liu C, Zhang Y. Meng XP, Zhou X. Chu CC. OsWRKY30 is activated by MAP kinases to confer drought tolerance in rice. Plant Mol Biol. 2012; 80: 241–53. https:// *doi: 10.1007/s11103-012-9941-y*.
44. Shen Y. Zhiguo E, Zhang X. Liu YH, Chen ZD. Screening and transcriptome analysis of water deficiency tolerant germplasms in peanut (Arachis hypogaea). Acta Physiol Plant. 2015; 37: 103. https:// *doi:10.1007/s11738-015-1840-9*.
45. Shereen A. Khanzada MA, Baloch MAW. Asma, Shirazi MU, Khan MA. Effects of PEG induced water stress on growth and physiological responses of rice genotypes at seedling stage. Pak J Bot. 2019; 51(6): 2013–21. https:// *doi. org/10.30848/PJB2019-6(13)*.
46. Shoba D. Manivannan N, Vindhiyavarman P. Nigam SN. SSR markers associated for late leaf spot disease resistance by bulked segregant analysis in groundnut (Arachis hypogaea L.). Euphytica. 2012; 188: 265–72. https:// *doi. org/10.1007/s10681-012-0718-9*.
47. Sun MX. Peng FT, Xiao YS. Yu W, Zhang YF. Gao HF. Exogenous phosphatidylcholine treatment alleviates drought stress and maintains the integrity of root cellmembranes in peach. Sci Hortic. 2020;259. https:// *doi. org/10.1016/j.scienta.2019.108821*.
48. Tegeder M. Masclaux-Daubresse C. Source and sink mechanisms of nitrogen transport and use. New Phytol. 2018; 217: 35–53. https:// *doi. org/10.1111/nph.14876*.
49. Ullah A. Sun H, Yang X. Zhang XL. Drought coping strategies in cotton: increased crop per drop. Plant Biotechl J. 2017; 15: 271–84. https:// *doi: 10.1111/pbi.12688*.
50. Yamada M. Morishita H, Urano K. Shiozaki N, Yamaguchi-Shinozaki K. Shinozaki K. Effects of free proline accumulation in petunias under drought stress. J Exp Bot. 2005; 56: 1975–81. https:// *doi. org/10.1093/jxb/eri195*.
51. Yang YQ. Li X, Kong XX. Ma L, Hu XY. Yang YP. Transcriptome analysis reveals diversified adaptation of Stipa purpurea along a drought gradient on the Tibetan Plateau. Func Integ Genomics. 2015;15(3): 295–307. https:// *doi: 10.1007/s10142-014-0419-7*.
52. Young MD. Wakefield MJ, Smyth GK. Oshlack A. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Bio. 2010; 11(4): 14. https:// *doi: 10.1186/gb-2010-11-2-r14*.
53. Yousfi S. Marquez AJ, Betti M. Araus JL, Serret MD. Gene expression and physiological responses to salinity and water stress of contrasting durum wheat genotypes. J Integ Plant Biol. 2016; 58: 48–66. https:// *doi. org/10.1111/jipb.12359*.
54. Wang M. He XM, Jiang B. Liu WR, Lin YE. Xie DS, *et al.* Transcriptome analysis in different chieh-qua cultivars provides new insights into drought-stress response. Plant Biotech Rep. 2019; 13: 1–13. https:// *doi. org/10.1007/s11816-019-00564-x*.

55. Wang M. Jiang B, Liu WR. Lin YE, Liang ZJ. He XM. Transcriptome analyses provide novel insights into heat stress responses in chieh-qua (Benincasa hispida Cogn. var. Chieh-Qua How). Int J Mol Sci. 2019; 20: 883. https:// *doi: 10.3390/ijms20040883*.

56. Wang WS. Zhao XQ, Li M. Huang LY, Xu JL. Zhang F. Complex molecular mechanisms underlying seedling salt tolerance in rice revealed by comparative transcriptome and metabolomic profiling. J Exp Bot. 2016; 67: 405–19. https:// *doi: 10.1093/jxb/erv476*.

57. Wang Z, Gerstein M, Snyder M. *RNA-Seq*: *a revolutionary tool for transcriptomics*. *Nat Rev Genet.* 2009; *10* (*1*): *57*. https:// *doi: 10.1038/nrg2484*

58. Wu N. Matand K, Wu H. Li B, Li Y. Zhang X. De novo next-generation sequencing, assembling and annotation of Arachis hypogaea L. Spanish botanical type whole plant transcriptome. Theo App Genet. 2013; 126: 1145–9. https:// *doi. org/10.1007/s00122-013-2042-8*.

59. Xiao WJ. Hu S, Zhou XX. Yao RY, Luo JR. Yuan CY, *et al.* A glucuronokinase gene in Arabidopsis, AtGlcAK, is involved in drought tolerance by modulating sugar metabolism. Plant Mol Biol Rep. 2017; 35: 298–311. https://doi.org/10.1007/s11105-017-1023-5.

60. Xiong L. Zhu JK. Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell Environ. 2002; 25: 131–9. https:// *doi. org/10.1046/j.1365-3040.2002-00782-x*.

61. Xiong L. Zhu J. K. Regulation of abscisic acid biosynthesis. Plant Physiol. 2003; 133: 29–36. *https:// doi*: 10.1104/pp.103.025395.

62. Xu JQ. Jin JJ, Zhao H. Li KL. Drought stress tolerance analysis of Populus ussuriensis clones with different ploidies. J Forestry Res. 2019; 30: 1267–75. https:// *doi. org/10.1007/s11676-018-0729-z*.

63. Zhang F. Wan X, Zheng Y. Sun L, Chen Q. Zhu X, *et al.* Effects of nitrogen on the activity of antioxidant enzymes and gene expression in leaves of Populus plants subjected to cadmium stress. J Plant Interact. 2013; 9: 599–609. https:// *doi. org/10.1080/17429145.2013.879676*.

64. Zhang YY. Gao XL, Li J. Gong X W, Yang P. Gao JF, *et al.* Comparative analysis of proso millet (Panicum miliaceum L.) leaf transcriptomes for insight into drought tolerance mechanisms. BMC Plant Biol. 2019; 19: 397. https:// *doi: 10.1186/s12870-019-2001-x*.

65. Zhao XB. Li CJ, Wan SB. Zhang TT, Yan CX. Shan SH. Transcriptomic analysis and discovery of genes in the response of Arachis hypogaea to drought stress. Mol Bio Rep. 2018; 45: 119. https:// *doi: 10.1007/s11033-018-4145-4*.

66. *Ma Y*, *Szostkiewicz I*, *Korte A*, *Moes D*, *Yang Y*, *Christmann A*, *Grill E*, et al.*Regulators of PP2C phosphatase activity function as abscisic acid sensors.Science*. *2009*; *22*:*324*(*5930*):*1064-8*. https:// *doi: 10.1126/science.1172408*. *Epub 2009 Apr 30*.

67. *Scherzer S*, *Maierhofer T*, *Al-Rasheid KA*, *Geiger D*, *Hedrich R.Multiple calcium-dependent kinases modulate ABA-activated guard cell anion channels*. *Mol Plant*. *2012*; *5*(*6*):*1409-12*. https:// *doi: 10.1093/mp/sss084*

68. *Wassie M*, *Zhang WH*, *Zhang Q*, *Ji K*, *Chen L.Effect of Heat Stress on Growth and Physiological Traits of Alfalfa (Medicago sativa L.) and a Comprehensive Evaluation for Heat Tolerance*. *AGRONOMY-BASEL*. *2019*; *9*(*10*):*597*. https://doi.org/10.3390/agronomy9100597

Figures

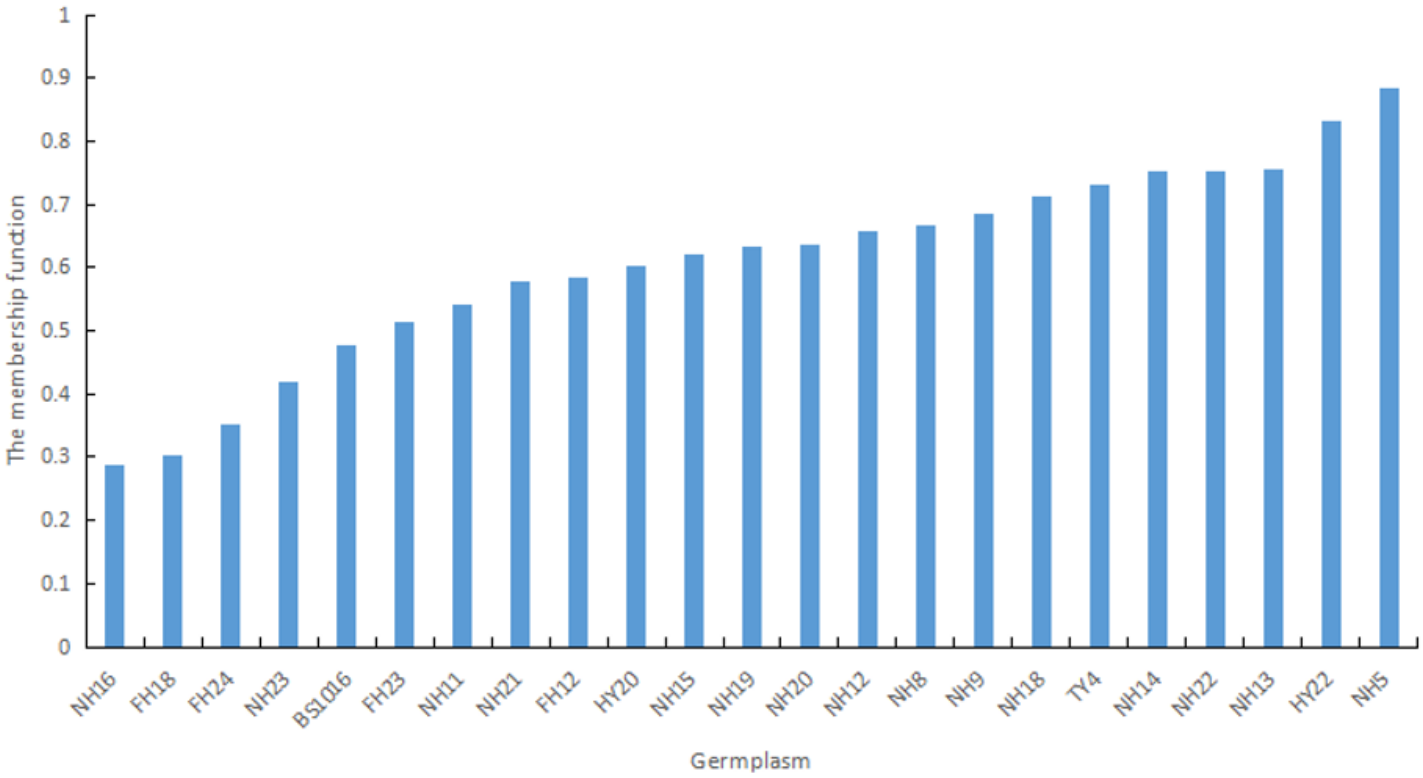

Figure 1

Comprehensive evaluation of drought resistance of peanut under drought stress

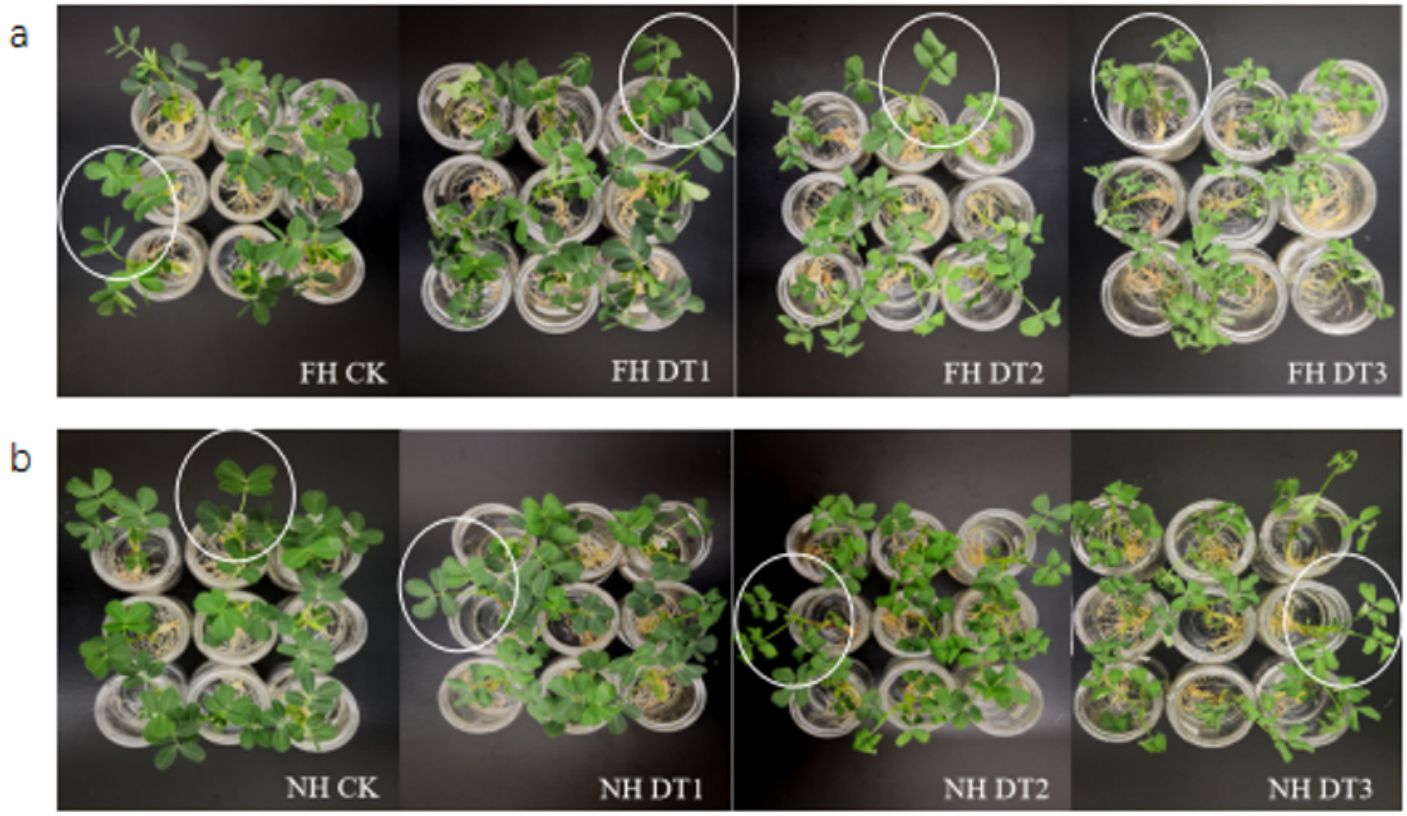

Figure 2

Phenotypic analysis of FH18 and NH5 under drought stress. (a) FH18 seedlings sustained drought stress 0 h, 4 h, 8 h, 24 h; (b) NH5 seedlings sustained drought stress 0 h, 4 h, 8 h, 24 h

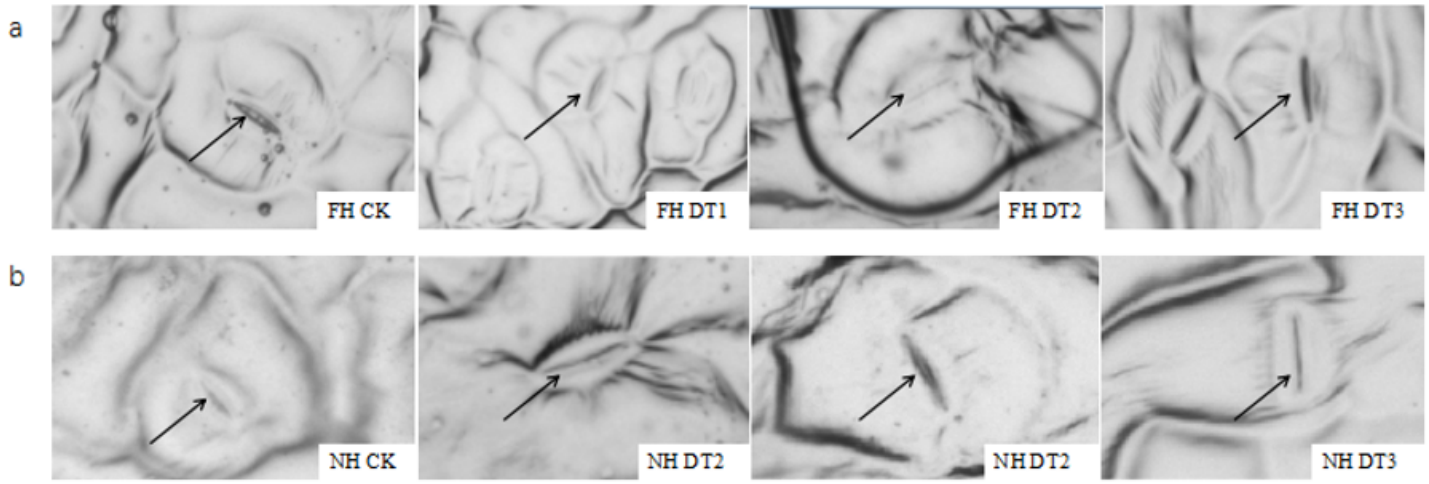

Figure 3

Stomatal analysis of FH18 and NH5 under drought stress. (a) stomatal state of FH5 seedlings under continuous drought stress for 0 h, 4 h, 8 h and 24 h; (b) stomatal state of NH5 seedlings under continuous drought stress for 0 h, 4 h, 8 h and 24 h

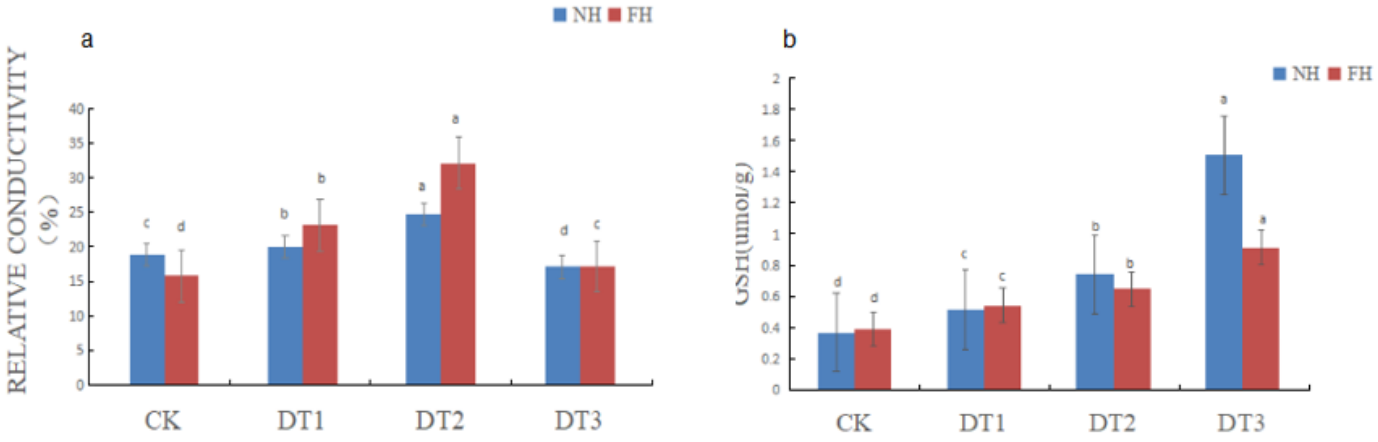

Figure 4

Determination of physiological indexes between FH18 and NH5. (a) Conductivity of FH18 and NH5; (b) Reduced glutathione content of FH18 and NH5

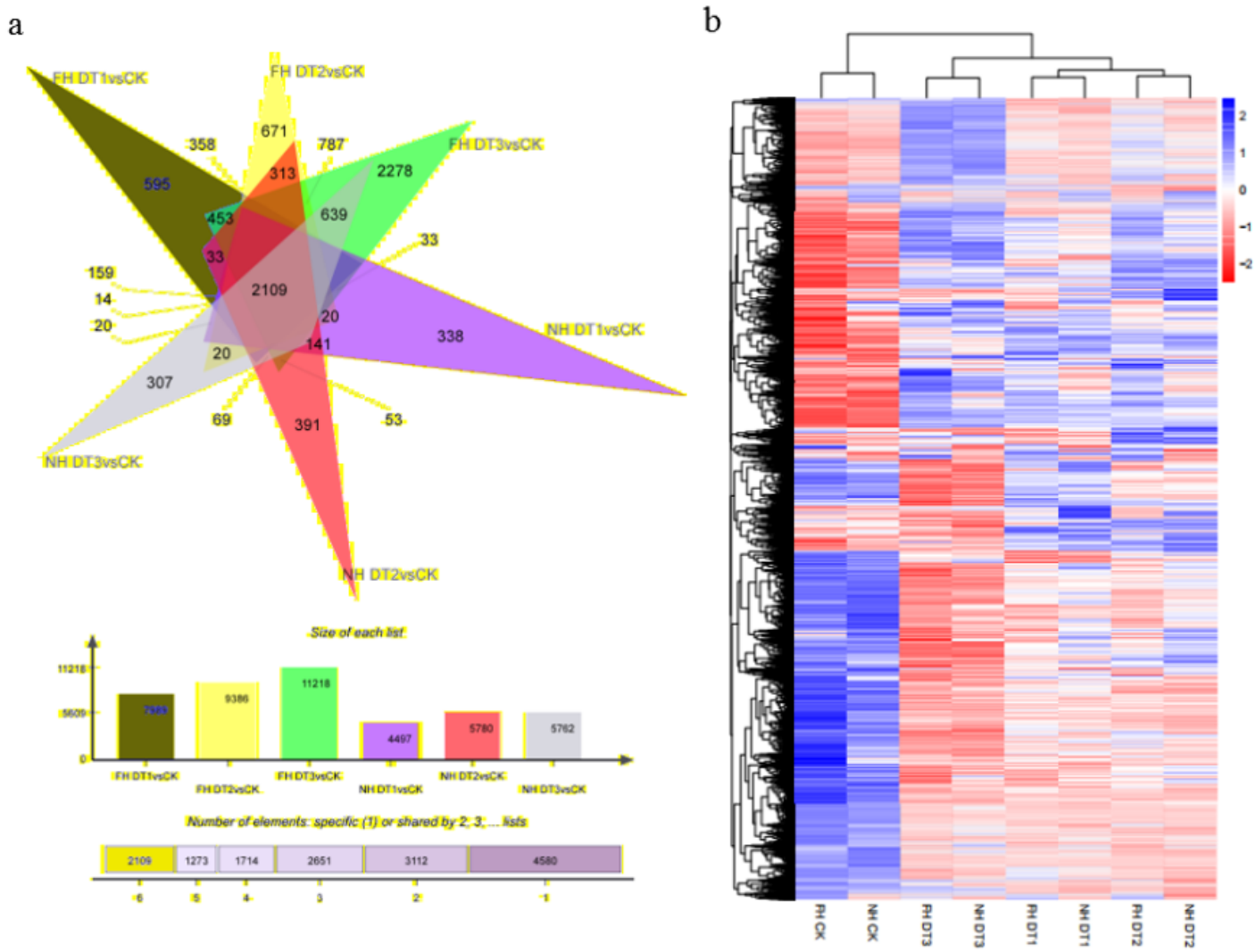

Figure 5

Differentially expressed genes between FH18 and NH5 under drought stress. (a) venn map of differentially expressed genes in two species of peanut under drought stress, and (b) thermographic analysis of transcriptional levels of differentially expressed genes in two species of peanut under drought stress.

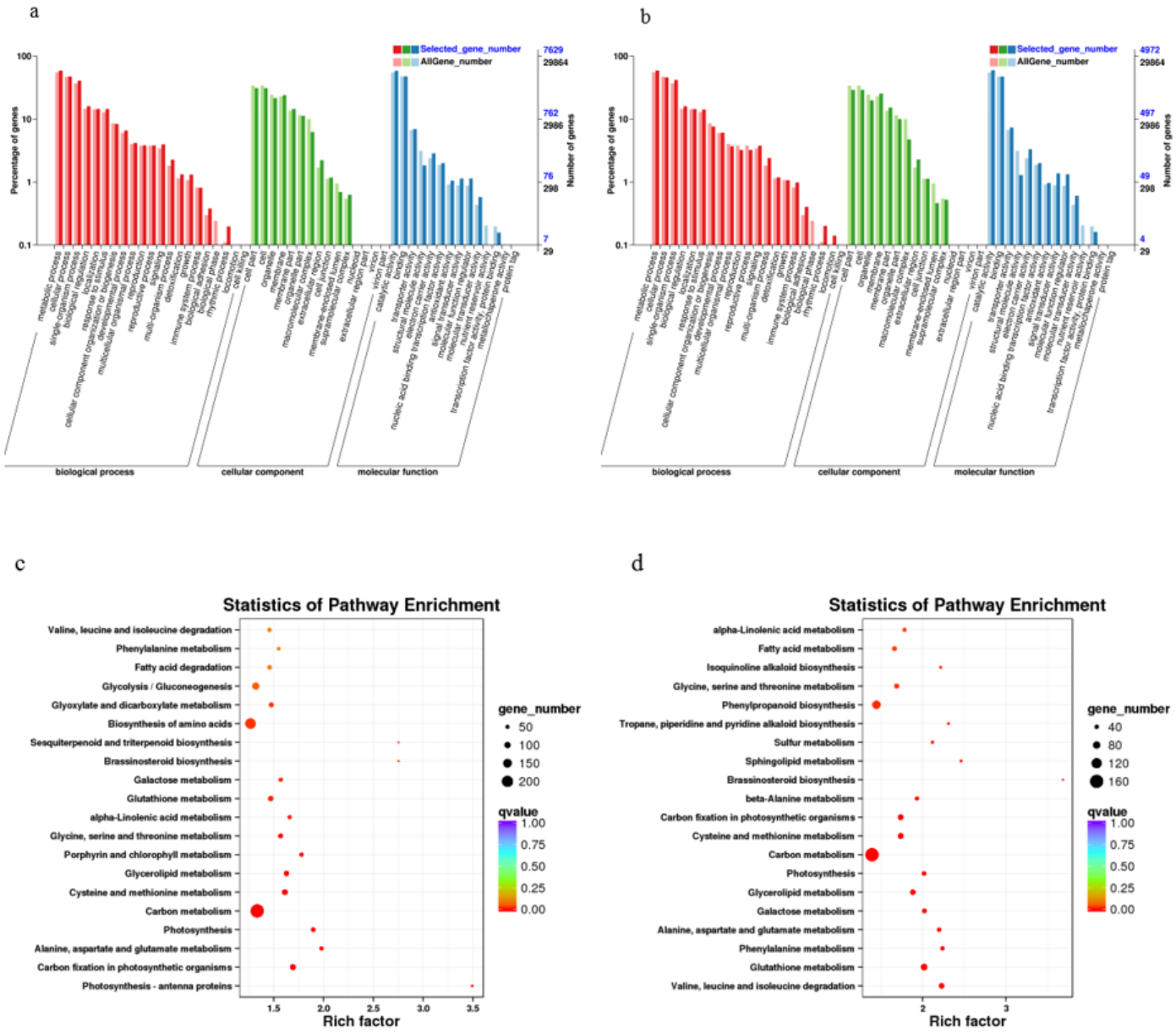

Figure 6

Functional notes of differential genes between FH18 and NH5 under drought stress. (a) GO classification of differentially expressed genes in FH18; (b) GO classification of differentially expressed genes in NH5, (c) KEGG pathway enrichment and dispersion map in FH18 compared with CK, (d) KEGG pathway enrichment and dispersion point map in NH5 compared with CK

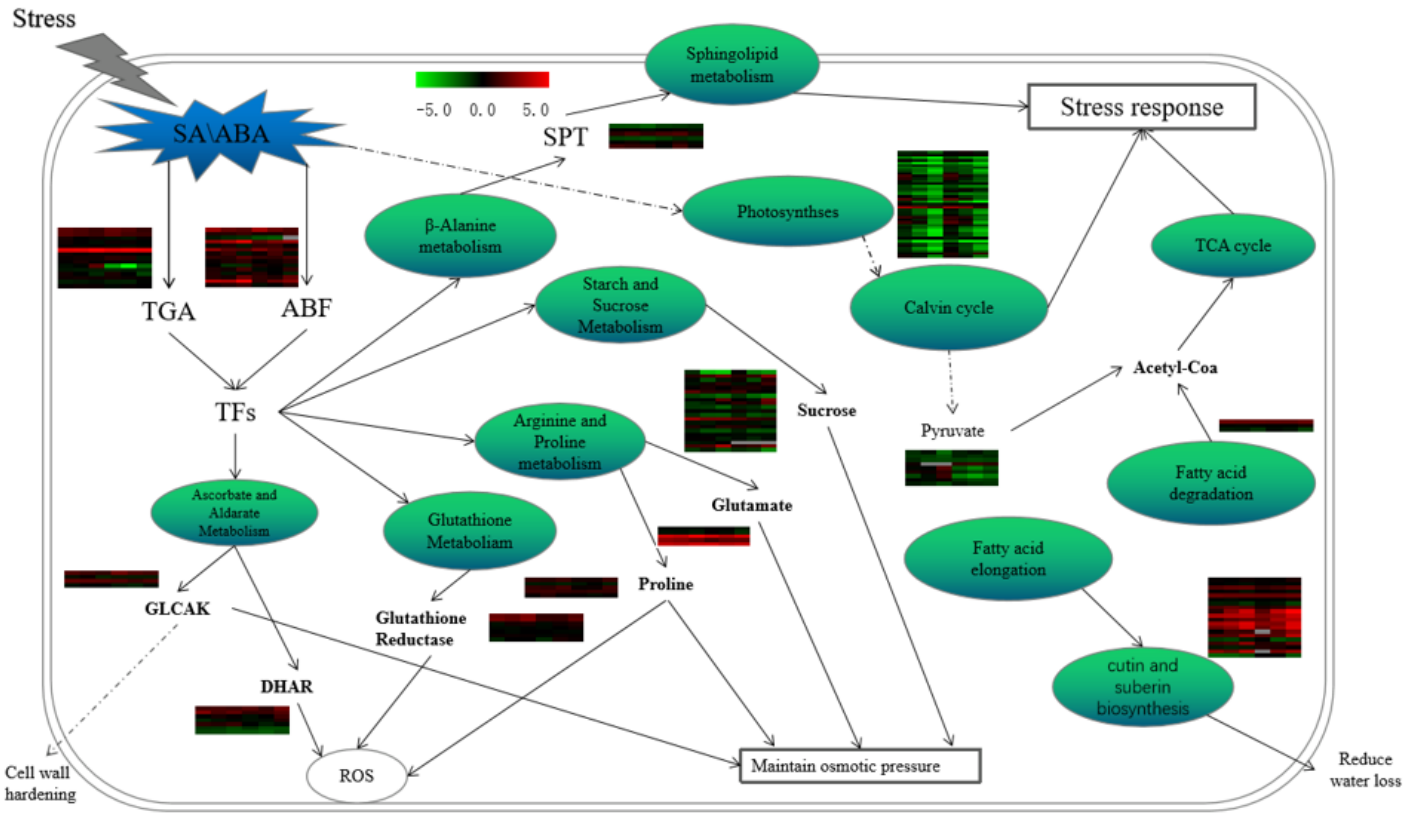

Figure 7

The schematic diagram of predicting the main process of peanut response to drought stress shows that peanut can resist drought stress by regulating the expression of stress genes by ABA and SA under drought stress. Thermography showed that FH18 and NH5 response genes were up-regulated (red) and down-regulated (green) under drought stress.

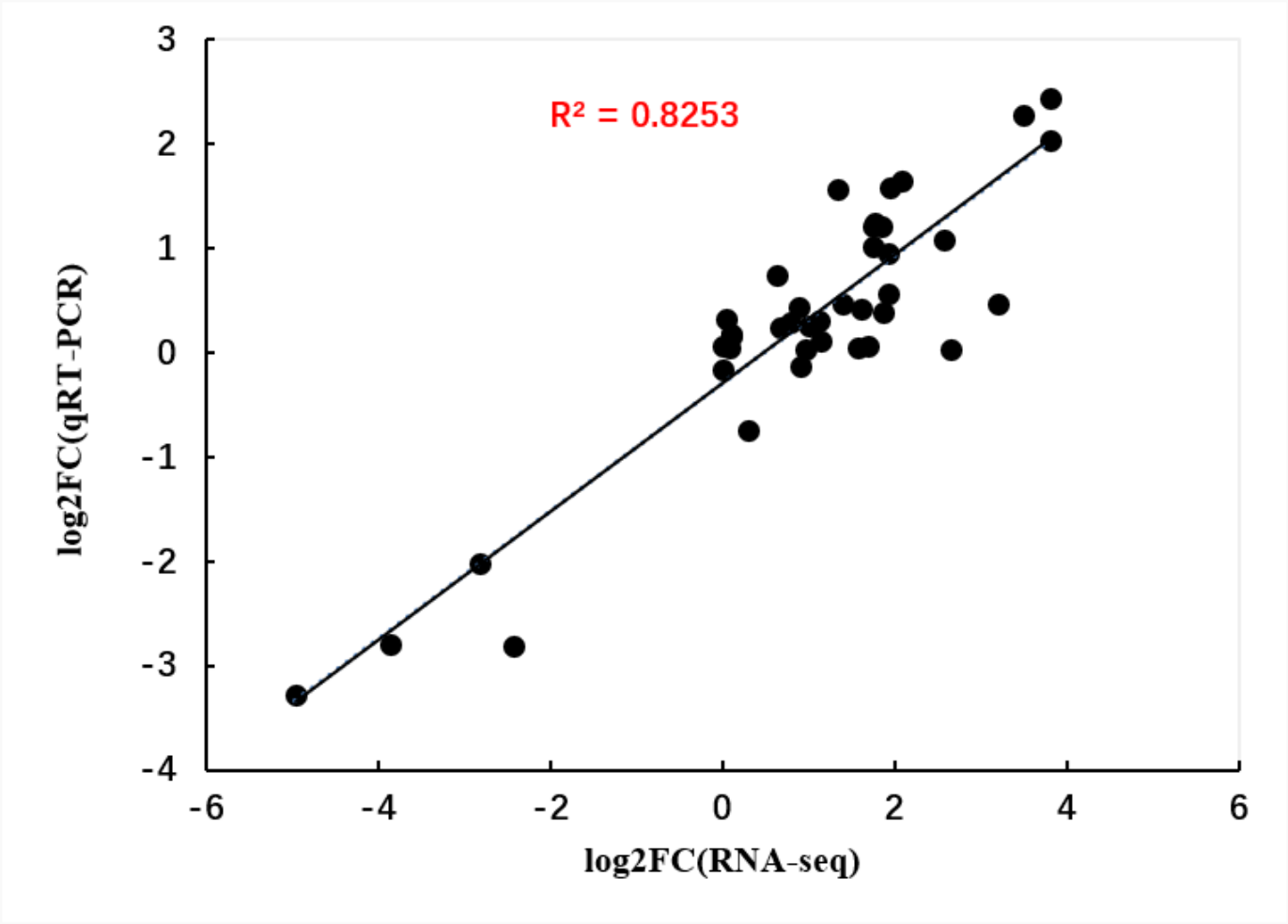

Figure 8

Correlation between RNA-Seq expression profile and qRT-PCR results

Supplementary Files

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- Allgenefpkm.list.xls