

Transcriptome changes induced by drought stress in Jerusalem artichoke (*Helianthus tuberosus* L.) and weighted gene co-expression network analysis (WGCNA)

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Abstract

Background: Jerusalem artichoke (*Helianthus tuberosus* L.) is strongly resistant to stress and an important plant used for ecological management in northern China in recent years. Currently, Jerusalem artichoke has been widely planted in the area around Qinghai Lake in Qinghai Province, China. Jerusalem artichoke can not only prevent land desertification but also has maintain most of its level of production. However, there is little research on the mechanism of drought resistance of Jerusalem artichoke.

Results: We conducted transcriptome sequencing under drought stress and normal watering treatment for two varieties, QY1 and QY3, with differing degrees of drought tolerance. In the three stress periods of QY1 and QY3, 5,613, 12,985 and 24,923 differentially expressed genes (DEGs) were identified, respectively. GO analysis showed that there were more DEGs in QY1 than in QY3, but there were more up-regulated genes in QY3 than in QY1. Based on an additional analysis of the metabolic pathways under drought stress using MapMan, the most different types of metabolism included secondary metabolism, light reaction metabolism and cell wall. The up-regulated genes in QY3 were significantly more prevalent than those in QY1 and were primarily concentrated in flavor IDS, phenylpropanoids, and the shikimate and terpenoids pathway. As a whole, QY1 and QY3 both had a large number of up-regulated genes in the flavor pathway. In addition, the gene analysis of the ABA key metabolic pathway showed that QY3 had more genes in NAC and WRKY than QY1. A weighted gene co-expression network was constructed and divided into modules. By specifically analyzing the expressed modules, four modules were found to have the highest correlation with drought. Further research on the genes revealed that all 16 genes related to histone, ABA and protein kinase were the most significant in these pathways.

Conclusions: In summary, these findings represent the first RNA-Seq analysis of drought stress in Jerusalem artichoke, which is of substantial significance to explore the function of drought resistance in Jerusalem artichoke and the unearthing of related genes.

Background

Drought is a major and direct environmental stress factor that threatens plant health, damages the structure and composition of ecological communities and leads to plant death. It usually leads to significant economic losses in agriculture and forestry. Current climate change, environmental degradation and soil salinization aggravate the frequency and severity of environmental drought [1]. The increasing severity of soil drought has led to changes in plant gene expression and cell metabolism, posing a growing threat to grassland and animal husbandry. One third of the land area of the world is arid and semiarid, which has a substantial impact on plant production and economic development. Therefore, improving the drought resistance of plants has become a hot topic of research [2]. Owing to the constant changes of a growing environment, plants are often under different environmental stresses. Under stress, plants will respond to and adapt to stress through changes in gene expression, metabolites and physiology. Therefore, there should be a variety of stress receptors in plant cells that produce and transduce signals [3]. However, it is possible that the sensing of stress signals is a redundant system, in

which the mutations of a single gene or protein will have its function fulfilled by other genes, resulting in a lack of obvious phenotype in stress signal sensing. Moreover, it could be that these genes are necessary for the growth of plants, and the functionally deficient mutants lead to the failure of normal growth and subsequent study. Therefore, only a few plant signal receptors have been identified. For example, the ABA signaling pathway is a widely studied drought response signaling pathway in plants that is considered to be the "core" signaling pathway under difficult levels of stress [4]. Under drought stress, ABA can regulate stomatal closure, inhibit growth and promote leaf senescence, thus improving the rate of survival of plant. When the plants were subjected to drought stress, their ABA levels increased under the control of SnRK2s (sucrose non-fermenting SNF1-related protein kinase 2s) [5]. ABA activates ABFs and RAV1 through SnRK2s and binds the ABRE (ABA-responsive element-binding proteins) or RAV1-binding cis-element in the promoter region of the NAC transcription factor gene, thus promoting the expression of ORE1, AtNAP and other genes, thereby controlling leaf senescence and enhancing the drought resistance of plants [6, 7]. Qinghai Province in China is located on the Qinghai-Tibet Plateau, the roof of the world, with a fragile ecological environment, high altitude and a severe climate. Once the local vegetation has been destroyed, it is difficult for the plants to recover. With the influence of grazing in the surrounding areas and other factors, sandstorm activities north and east of Qinghai Lake have gradually increased. Land desertification seriously restricts the production of agriculture and animal husbandry in the surrounding arid areas, and the implementation of sandy vegetation has become an important part of ecological construction in the Qinghai Plateau. However, owing to the low amount of precipitation and large evaporation in the plateau area, water has become the most important ecological constraint factor for the transformation of sandy land. In addition, the primitive grazing industry also seriously damages the grassland ecosystem. Therefore, during the process of the restoration of vegetation and construction of grassland ecological sandy land, it is necessary to select ecologically economical plants that can restore the environmental ecology and increase income of farmers and ranchers [8-10]. Jerusalem artichoke (*Helianthus tuberosus* L.) is a type of plant that is highly adaptable to infertile lands, drought, cold and salt and is suitable for growing in dry, cold, sunny climates and soil with a high concentration of sediment [11]. Jerusalem artichoke tubers are rich in inulin, which comprises 70-90% of their dry matter. Inulin is a polymer of fructose. It can be decomposed into a single fructose by exo-inulinase and then fermented to produce ethanol with a conversion rate of up to 83-99%. Jerusalem artichoke has the advantages of a low planting cost and high biological yield. Therefore, Jerusalem artichoke will be an important plant for fuel ethanol and the large-scale development of the inulin industry with substantial strategic significance and broad prospects for application [12, 13]. In recent years, the planting area of Jerusalem artichokes has been increasing yearly, particularly in the desertification and saline-alkali areas of north China. However, studies on the drought stress of Jerusalem artichoke are primarily only focused on the selection of varieties, physiological responses, sugar metabolism and water use efficiency [14-17] in the tropics. Because Jerusalem artichoke is a hexaploid species ($2n = 6x = 102$), and its genetic background is highly heterozygous and complex [18], the study of its mechanism of drought resistance has lagged behind that of other crops. To our knowledge, no comprehensive study on the drought adaptation mechanisms of Jerusalem artichoke has been reported. However, the study of the mechanism of the drought adaptation of Jerusalem artichoke is a prerequisite for its increased utilization in arid

areas and also an important basis for its cultivation and management. Currently, Jerusalem artichoke has been widely planted in the area surrounding Qinghai Lake in Qinghai Province, China. Jerusalem artichoke can not only prevent land desertification but also has loses little production. It is valuable in edible and industrial development, can create a long industrial development chain, and is of substantial significance to the improvement of regional ecological environment and economic development. This study explores the drought-resistant physiological mechanism of Jerusalem artichoke by simulating the drought stress of Jerusalem artichoke in the desert environment.

Results

2.1. Effects of drought stress on the growth of Jerusalem artichoke and the determination of physiological indices

This experiment simulated natural drought to cause stress to two genotypes of Jerusalem artichoke at the seedling stage, and the season in which Jerusalem artichoke was planted in Qinghai was selected for the experiment. The seeds were planted on April 28, and the Jerusalem artichoke plant was approximately 25 cm high in early June. Jerusalem artichoke with the same height and similar growth levels were selected for the experiment, and samples were collected from June 4 to June 10. Figure 1 indicates that, compared with the water treatment (WT), there was no significant change in both genotypes of Jerusalem artichoke plants under drought treatment (DT) at 2 d. QY1 wilted at 4 d, and most of the leaves of the plant withered at 10 d, while QY3 showed no significant wilting at 4 d, and most of the leaves remained normal at 10 d. Moreover, it can be seen from the color changes at the root that at 2 d of stress, QY1 became darker, while the color of QY3 did not become significantly darker until 10 d (Fig 1).

Fig 2a indicates that with the development of drought stress, the water content of DT group decreased to less than 10% in 10 days, while that of the WT group remained basically stable at 35%, and the water content of QY3 decreased faster than that of QY1. In contrast, Fig 2b shows that the content of dry matter in roots of two genotypes of Jerusalem artichoke increased significantly under drought stress. On the 2nd and 4th days after the plants were subjected to drought stress, there was no significant difference between the WT and DT groups. However, on the 10th day, the DT group reached its highest value. The difference between the WT and DT groups became highly significant, and the difference between the two genotypes of Jerusalem artichoke in DT group also became highly significant, with QY3 exhibiting higher levels of drought stress than QY1. The rate of accumulation of dry matter by the DT group was higher than that of the WT group. Next, we measured the contents of protein and chlorophyll. With an increase in treatment time, there was no significant difference in the contents of protein between DT and WT groups, but the DT group exhibited a trend of higher protein contents than those of the WT group. There were also significant differences between the two genotypes of Jerusalem artichoke in DT group on the 2nd and 10th days, with QY3 exhibiting higher trends than QY1. There was no significant difference between the WT and DT groups in their level of chlorophyll content on the 2nd and 4th days, but there was a very significant difference between these groups on the 10th day. QY1 and QY3 exhibited the same type of

trend under drought stress. However, the range of change in trend of QY3 was larger than that of QY1 (Fig 2c and d). The results showed that continuous drought stress has a substantial effect on the growth of Jerusalem artichoke.

2.2. Characterization of RNA-Seq

The leaves of two Jerusalem artichoke varieties were sequenced under normal water and drought conditions. A total of 89,550,751 raw reads were amplified. The sequenced clean reads and clean bases were 89,551,965 and 13,508,637,885, respectively. The average values of Q20 and Q30 were 97.66% and 93.265%, respectively, indicating that the sequencing quality was reliable. After data filtering and quality control, 89,551,965 clean reads were produced with an average of 3,198,284 clean reads per sequencing library and an average GC content of 45.585%. After assembly with Trinity, 309,323 unigenes and 455,817 transcripts were obtained. The average GC content of the unigenes and transcripts was 42.24% and 41.48%, respectively; the average N50 of the unigenes and transcripts was 727 bp and 788 bp, and the mean length of the unigenes and transcripts was 571 bp and 619 bp, respectively (Table 1). After splicing and assembling the clean reads, 455,817 transcripts were obtained with an average length of 619 bp and N50 of 788 bp. The number of transcripts between 200 and 400 bp was the most frequent at 200,512, accounting for 18.3% of the total (Fig 3).

The differentially expressed genes (DEGs) between the two Jerusalem artichoke varieties were screened using the DESeq method for subsequent gene function annotation and screening. The results of the difference data are shown in Figure 3. Under drought stress, genes in the QY1 and QY3 leaves diverged significantly. At the second day of drought stress, there were 5,613 genes differentially expressed in QY1/QY3, of which 4,091 genes were up-regulated, and 1,522 genes were down-regulated. At the fourth day of drought stress, there were 12,985 genes differentially expressed in QY1/QY3, of which 6,346 genes were up-regulated, and 6,639 genes were down-regulated. At the tenth day of drought stress, there were 24,923 genes differentially expressed in QY1/QY3, of which 12,081 genes were up-regulated, and 12,842 genes were down-regulated. With the increase in the trend of stress time, the number of up-regulated and down-regulated unigenes both increased, indicating that drought stress inhibited the expression of some unigenes and induced the expression of a number of other unigenes (Fig 4).

Fragments per kilobase of transcript per million (FPKM) > 0.3 was used as the standard of expression to calculate the gene expression of the two Jerusalem artichoke varieties under normal water and drought stress, as shown in Fig 5. Under WT treatment, 39,569 unigenes were screened out in QY1, including 16,972 nonshared unigenes. A total of 3,635 unigenes were shared between two groups, 1,865 unigenes among three groups and 2,433 unigenes among four groups. In QY1, 9,601, 9,374, 8,632 and 11,962 unigenes were screened at 0 d, 2 d, 4 d and 10 d, respectively. The overall trend of expression of QY1 under the DT treatment was similar to that of WT group, but the number of unigenes increased significantly. A total of 43,650 unigenes were screened out, including 18,866 nonshared unigenes, 3,927 shared unigenes between two groups, 2,066 shared unigenes among three groups and 2,683 shared unigenes among four groups. In QY1, 9,601, 9,462, 11,132 and 13,485 unigenes were screened at 0 d, 2 d,

4 d and 10 d, respectively. More genes were expressed in the DT group than the WT. In QY3, there were more unigenes screened out under the WT and DT treatments than those in QY1. Among them, there were 44,666 unigenes under WT, including 19,091 in nonshared unigenes, 4,129 shared unigenes between two groups, 2,191 shared unigenes among three groups and 2,686 shared unigenes among four groups. In QY3, 13,031, 9,723, 11,084 and 10,828 unigenes were screened out at 0 d, 2 d, 4 d and 10 d, respectively.

According to the unigene detection results under drought stress, GO function classification was conducted for the DEGs. Since the difference was the most obvious at 10 d, the DEG of the two varieties at 10 d of the WT and DT treatments were selected for further functional analysis. After 10 d of drought stress in QY1, 9,394 DEGs were annotated with 43 functions, among which 19 belonged to biological process, 13 to cellular component and 11 to molecular function. After 10 d of drought stress in QY3, 10,796 DEGs were annotated with 41 functions, among which 18 belonged to biological process, 13 to cellular component and 11 to molecular function. The three pathways with the highest differential expression in biological process, cellular component and molecular function were analyzed. In biological process, the differences primarily existed in the metabolic process, cellular process and single organism process, with the DEGs in QY1 comprised of 561, 493 and 451 more than those in QY3. A similar situation also occurred in cellular component and mobile function. In the cellular component, most of the different pathways were membrane, cell part and cell, with the DEGs in QY1 numbering 294, 451 and 268 more than those in QY3, respectively. In molecular function, QY1 had 553, 299 and 64 more DEGs in the quantitative activity, binding and transporter activity channels, respectively. QY1 had more DEGs than QY3 in most pathways, but in general, there were more up-regulated genes than down-regulated genes in QY3. According to the data of differential expression, drought stress has a significant impact on the biological functions described above (Fig 6).

To further understand the metabolic pathway and other processes of drought stress genes in Jerusalem artichoke, MapMan was used to compare the metabolic pathways of differential genes of QY1 and QY3 under the DT and WT treatments based on the results of RNA-Seq. A visual analysis found that there were significant differences in the expression of drought-sensitive genes in the metabolic pathways. As shown in Figure 7, the metabolic pathway was divided into minor CHO metabolism, cell wall metabolism, lipids metabolism, secondary metabolism, amino acids metabolism, light reaction metabolism, carbohydrate metabolism, nucleus metabolism and tetrapyrrole metabolism. Through the comparison of the QY1 and QY3 DT/WT, we can observe that there was a large difference in the overall metabolic pathway between QY1 and QY3. The most different metabolic pathways were secondary metabolism, light reaction metabolism and cell wall. Compared with QY1 (Fig 8a.), the up-regulated genes of QY3 were primarily cell wall, secondary metabolism and light reaction metabolism. The number of up-regulated genes was 130, 98 and 83, respectively, and the down-regulated genes were also primarily concentrated in these three pathways. Secondary metabolism helps plants to resist environmental stress, improve their self-preservation and ability to compete and maintain their survival and development. Therefore, the study of this area is highly significant for the study of the secondary metabolic accumulation of industrial cash crops and their production. Drought, as one of the primary environmental stresses, affects the accumulation of secondary metabolites. We used the secondary metabolic pathway with the largest

number of differential genes for further mining. In Fig 7c and d, we continue to use red for up-regulation and blue for down-regulation. The up-regulated genes in QY3 were significantly more abundant than those in QY1 and were primarily concentrated in flavonoids, phenylpropanoids, and the shikimate and terpenoids pathway. The results described above show that under the stress of continuous drought, two types of Jerusalem artichoke exhibited different drought resistance mechanisms, and the synthesis of cell wall, lipid, photosynthesis and secondary metabolism of QY3 were significantly higher than that of QY1. The primary component of the plant cell wall is cellulose, and the primary component of the intercellular layer of cell wall is pectin. Waxy synthesis, cellulose synthesis and the up-regulated expression of pectin methylesterase all indicated the enhancement of cell wall synthesis. QY1 has more up-regulated and down-regulated genes in carbohydrate metabolism than does QY3.

Endogenous hormones, as response factors to plant stress, play an important role in the adaptation to drought stress. ABA, as one of the primary endogenous hormones, plays an important role in plant stress-resistant growth, particularly under drought stress, and is even considered to be a potential indicator of crop drought resistance. In this study, 14 ABA signaling, 21 NAC, 7 DREB and 24 WRKY genes were selected for expression analysis. Some of them were both up-regulated in QY1 and QY3, including HAB2 (PP2C member of ABA signaling), ABA responsive element binding protein AREB1, NAC29 (NAC family), DREB2a (DREB family) and eight WRKY transcription factors: WRKY13, WRKY5, WRKY7, WRKY9, WRKY42, WRKY4, WRKY68, and WRKY8 (Fig 8.). NAC1 and some WRKY genes were down-regulated in both varieties of Jerusalem artichoke. On the whole, QY3 showed more variation in the NAC and WRKY genes than did QY1, particularly in the NAC family. In addition, QY3 showed an obvious trend of change in three SNRK genes, but the number of genes responding to drought stress was significantly higher in QY1.

To better understand the drought-tolerant gene expression regulatory network of Jerusalem artichoke, weighted gene co-expression network analysis (WGCNA) was used to analyze the drought-related genes. Based on the results of high mean connectivity and low scale independence, we finally chose $\beta=14$ as the threshold. The analytical results showed that 6,643 drought-related genes of QY1 could be clustered into 27 modules under drought stress, while 7,224 drought-related genes in QY3 could be clustered into 28 modules (Fig 9.). The WGCNA analysis showed that under drought stress, re-writing and de-writing existed in the co-expression network of a large number of genes of QY1 and QY3. We selected the dark slate blue ($r = 0.45$, $p = 0.02$), dark red ($r = 0.38$, $p = 0.04$), ivory ($r = 0.42$, $p = 0.03$) and brown ($r = -0.39$, $p = 0.04$) modules with the highest correlation with stress to perform a separate comparative analysis. The dark slate blue module had a significant correlation of expression in variety, stress and period traits (Fig 9d.). In the module of the trend of expression diagram that was drawn, QY1 and QY3 showed a consistent trend of expression in the dark slate blue module under the WT, but they showed an opposite trend of expression under the DT treatment. In addition, QY1 and QY3 had the same trend of expression in the ivory, dark red and brown modules in the DT group.

The functions of the genes in four stress resistance-related co-expression modules were analyzed. The GO enrichment results showed that 319 genes in the brown module were enriched in the items of

molecular function (GO:0003674), catalytic activity (GO:0003824) and metabolic process (GO:0008152). A total of 130 genes in the dark red module were enriched in the items of cell component (go: 0005575), cell (go: 0005623) and cell part (go: 0044464). Four genes in the dark slate blue module were enriched in transferase activity (go: 0016740), and 24 genes in the ivory module were enriched in response to hormone stimulus (go: 0009725), response to endogenous stimulus (go: 0009719), response to organic substance (go: 0010033) and response to chemical stimulus (go: 0042221). In general, the genes enriched from the brown and dark red modules had the greatest number of molecular functions, while the primary GO items enriched from brown and ivory modules did not contain a molecular function (Fig 10.). To further explore the candidate genes involved in the drought stress of Jerusalem artichoke, the candidate genes with the highest correlation with drought stress were screened out from the GO classification of four modules. Through the function of gene annotation, it was found that the 16 genes selected by annotation belong to histone, ABA regulation and protein kinase among others. From the annotated species, there were eight candidate genes annotated to *Cynara cardunculus*, indicating that the mechanism of drought stress of Jerusalem artichoke is similar to that of *C. cardunculus* (Table 2).

Discussion

Plant growth and development are affected by various external environmental factors. Drought stress is one such factor. The comprehensive analysis of the transcriptome helps to understand the expression and regulatory mechanism of genes in plant systems. Currently, the application of transcriptome technology to the research of multiple species has advanced substantially [19-22]. In this study, RNA-Seq was used to conduct a transcriptome analysis on two Jerusalem artichoke varieties after drought stress treatment and compare the changes of gene expression in plants after drought stress treatment and a control of water treatment. We obtained important drought-stress response genes by comparing 0 d, 2 d, 4 d and 10 d drought-stress treatment with the watered control. These results will help to understand the regulatory mechanism of Jerusalem artichoke seedlings under drought stress.

The Jerusalem artichoke is a highly heterozygous hexaploid crop and basically reproduces asexually using tubers. In addition, due to the long term of introduction, many planting resources with similar genetic background lead to the single planting resources of Jerusalem artichoke [23]. Although Jerusalem artichoke did not originate in an arid area, and the species has not undergone long-term selective evolution, the tuber of Jerusalem artichoke is rich in fructan, which can greatly improve the resistance of crops. It is reported that drought and high temperature stress will cause a loss of 29% of the tubers and a loss of 53% of biomass in tropics [16]. Studies that have been conducted on the stress resistance of Jerusalem artichoke are mostly related to salt stress. Under salt stress, the increase in the metabolism of proline in Jerusalem artichoke promotes osmotic regulation and protection [24]. However, the accumulation of underground dry matter and efficiency of transport of non-reducing sugar to tubers continued to decrease, and the synthesis of IAA and GA3 in the leaves and tubers was inhibited, while the synthesis of ABA significantly improved [25]. A comparison between the northern variety of QY2 and the southern variety of NY1 indicated that the ABA content in the tuber of QY2 was higher [26]. The microbial community in the root of the crops could also play an important role under salt stress [27, 28]. With the

development of research on the abiotic stress of Jerusalem artichoke, the stress resistance of Jerusalem artichoke has been widely recognized. The goal of this research on the drought resistance of Jerusalem artichoke is to clarify the mechanism of the response of Jerusalem artichoke to drought. The most economical and effective way to solve the problem of drought is to select and cultivate new drought-resistant varieties. Current studies have found that drought stress can affect the physiological and biochemical characteristics of Jerusalem artichoke. Varieties differ in their susceptibility to drought. Drought can reduce the dry weight of the tuber and biomass of Jerusalem artichoke, but the water use efficiency of the yield of tubers and the water use efficiency of biomass are enhanced [14, 16].

Effects of drought stress on the metabolic pathway of flavonoids in Jerusalem artichoke

In this experiment, "flavor biosynthesis" was only significantly enriched in QY3 under severe drought stress, indicating that it participated in the drought stress response and was more active in the drought resistant variety QY3. In fact, the relationship between flavonoids and drought tolerance has gradually been confirmed. Flavonoids have a strong ability to scavenge free radicals, which helps to alleviate the oxidation reaction of *Arabidopsis thaliana* and its adverse reactions under drought stress [29]. Flavonoids are secondary metabolites and can remove the singlet oxygen produced by environmental stress in plants; they can remove the reactive oxygen produced by environmental stress and prevent the oxidative damage of reactive oxygen species to plants, and they play important roles in various abiotic stresses [30, 31]. Drought stress can affect the expression of the genes of related enzymes in the pathway of flavonoid metabolism in plants, but this differential expression varies among plants. For example, in *Antirrhinum*, the expression of phenylalanine ammonia lyase, chalcone isomerase, dihydroflavonol reductase, pyrroline-5-carboxylate synthase, superoxide dismutase and peroxidase was significantly up-regulated under drought stress [32], while in grape, the flavonoid content in the leaves decreased significantly after drought stress [33]. The up-regulated or down-regulated expression of genes for related enzymes can promote or inhibit the synthesis of the corresponding flavonoids, which is a part of the self-regulatory mechanism of plants in response to drought stress. In this study, in addition to the significant enrichment of flavonoids in QY3, MapMan analysis showed that the number of up-regulated genes in the anthocyanin biosynthesis of QY3 was greater than that in QY1. As a whole, QY1 and QY3 both have a large number of up-regulated genes in the flavonoid pathway. It has been hypothesized that flavonoids could help Jerusalem artichoke resist drought stress and maintain normal metabolic activities. However, this study only analyzed the whole flavonoid-related gene expression. Therefore, the relationship between flavonoids, abiotic stress and the specific metabolic pathway changes in Jerusalem artichoke merits further study.

Transcription factors and plant drought tolerance

Drought stress can lead to changes in the content and activity of endogenous hormones in plants, thus, regulating the physiological and biochemical processes of plants, as well as the transport and distribution of assimilates. ABA, as one of the primary endogenous hormones, plays an important role in plant growth, particularly under drought stress, and is even considered to be a potential indicator to

measure crop drought resistance [34]. Transcription factors known to be associated with drought response are primarily composed of the MYB, MYC, DREB, AP2/EREBP, bZIP and NAC gene families. In this study, an analysis was conducted on the expression of the gene families that were prone to change under drought stress. Among them, ABREs in the bZIP family-controlled gene expression through the AREB and ABF transcription factors. It was up-regulated in response to drought stress and could interact with SRK2D/SnRK2.2 to enhance the expression of ABA [35]. As early as 2005, Yasunari et al. found that the overexpression of AREB1 in *A. thaliana* can regulate the ABA signaling pathway, and thus, enhance the drought tolerance of the plants [36]. Current research shows that a CHIP and electrophoretic mobility shift assay confirmed that AREB1 can combine with the ABRE promoter motif of the NAC gene and activate PtrNAC006 related to drought tolerance, PtrNAC007 and PtrNAC120 genes [37]. Although we did not study the regulation of AREB in this study, the expression of AREB1 in QY1 and QY3 increased with the increase of drought stress time, particularly in QY3, which may indirectly improve the drought tolerance of QY3. In addition, we also analyzed the expression of the NAC family and found that NAC29 had almost the same trend of expression as AREB1. Similarly, the NAC family gene was significantly correlated with the drought tolerance of plants. In maize, the level of expression of the ZmNAC55 gene was significantly up-regulated after drought stress, and the promoter region of this gene had multiple stress-related cis-elements [38]. The overexpression of GmNAC085 in soybean increased the activity of superoxide dismutase, catalase and ascorbic acid peroxidase, thus reducing the oxidative damage caused by drought [39]. The NAC transcription factor JUB1 in tomato is the drought-tolerant regulator of tomato. SlJUB1 controls the expression of SIDREB1, SIDREB2 and SIDLALA, while AtJUB1 in *A. thaliana* stimulates the expression of DREB2a and produces drought resistance by controlling the DREB genes [40, 41]. DREB2 and DREB2a were also highly expressed in both varieties of Jerusalem artichoke, particularly in QY3, in which the expression of DREB2 was extremely high. It has been proven that DREB2 was closely related to drought stress in wheat [42], *A. thaliana* [43] and mustard [44]. Compared with the DREB family, which had only a few genes, we identified a number of WRKY transcription factors in the Jerusalem artichoke transcriptome data. The high expression of WRKY primarily appeared in the QY1. Meanwhile, eight WRKY transcription factors were up-regulated in both varieties of Jerusalem artichoke. WRKY42 and WRKY4 showed the strongest responses in QY1 and QY3, respectively, suggesting that these two WRKY transcription factors may play an important role in the response of Jerusalem artichoke to drought stress. These findings also provide a theoretical basis for further studies on the role of transcription factors in the regulation of plant responses to environmental stress and provide data to support the future screening of drought-tolerant varieties of Jerusalem artichoke.

WGCNA analysis

WGCNA is an effective method for co-expression network analysis, which can specifically screen out co-expression modules with high biological significance to target traits. It has been proved to be an efficient data mining method in rice [45], *Brassica rapa* [46], and sunflower [47]. In this study, the WGCNA method was used to construct a weighted gene co-expression network based on the sequencing data of 28 drought stress transcriptomes of Jerusalem artichoke. Four modules related to drought stress were excavated, among which the dark slate blue and brown modules were positively correlated and negatively

correlated with drought stress. Through GO enrichment analysis of the genes in modules, the co-expressed genes were found to be associated with important activities, such as molecular function, cellular component, transferase activity and response to hormone stimulus. Key genes in these pathways were screened to obtain 16 genes with the highest degree of enrichment, most of which were related to histone and ABA regulation and protein kinases. There were four genes that were the most significantly related to histone in the dark red module. Research shows that histone plays an important role in plant biological and abiotic stress. The basic unit of the nucleosome of chromatin is comprised of a 146 bp sequence of DNA and a wrapped histone octamer by the DNA (two copies of H2A, H2B, H3 and H4). Chromatin tissue is easily regulated during the short term of gene expression, involving signal pathways of plant hormones, such as ABA, ethylene, jasmonic acid and brassinosteroids, which are mediators of environmental stress recognition and transcriptional factor activation [48]. In addition, Aux/IAA-ARF-dimerization in the ivory module and Protein phosphatase 2C (PP2C), Alpha tubulin and the Seven-in-absentia protein (SINA) in the brown module are also closely related to drought. Under drought stress, alpha tubulin can change the characteristics of the cell walls to resist adverse plant reactions [49]. Studies have shown that the Aux/IAA gene may be regulated by light, plant hormones and abiotic stress, and drought can significantly reduce the expression of Aux/IAA4 [50]. There are many studies on PP2C, and PP2C may play a role in maize signal transduction in response to drought stress [51]. PP2C acts as a negative regulator of the ABA signal and can dephosphorize SAPK8 and SAPK10 in vitro, and together with PYL10, SAPK8 AND SAPK10, it can form an ABA signal transduction module that is involved in the response to stress [52] [53]. SINA is the link gene between drought resistance and ABA signaling [54]. In *A. thaliana*, ABA and drought treatment significantly induced SINA, and the overexpression of SINA increased the closure of stomatal and reduced water loss, thus, improving the drought resistance of transgenic plants [55]. The important ABA regulatory genes, such as AREB1, AREB2 AND ABF3, are all induced by SINA [56] [57]. The results described above show that WGCNA can divide complex data into co-expression modules, reflect the effective information in the real biological network, better mine the core genes related to the drought resistance of Jerusalem artichoke, predict the function of potential candidate genes and provide new research ideas for the research of the gene regulatory network of different plant characteristics.

Conclusions

In this study, a comparative transcriptome analysis of the DEGs among two varieties of Jerusalem artichoke with differing degrees of response to drought tolerance was performed. A gene ontology (GO) analysis showed that there were more DEGs in QY1 than in QY3, but there were more up-regulated genes in QY3 than in QY1. The MapMan results suggested that flavonoids could help Jerusalem artichoke resist drought stress and maintain normal metabolic activities, and the ABA key metabolic pathway responds to more up-regulated genes in QY3. Further research on the genes revealed that all 16 were related to histone, ABA and protein kinase were the most significant in WGCNA module, which provide new insights for the function of drought resistance in Jerusalem artichoke and the ability to unearth related genes.

Methods

Plant materials and drought treatments

Two Jerusalem artichoke varieties "QY1" and "QY3" were selected in our laboratory and were drought sensitive and drought resistant, respectively. The experiment was conducted at the experimental base of the Institute of Horticulture, Qinghai Academy of Agricultural and Forestry Sciences, Chengbei District, Xining City, Qinghai Province (N36°43' 45.21" E101°44' 57.69"). The experimental tubers were planted in a plastic culture bowl filled with sand in April. One culture bowl represented one biological repeat. Each bowl was placed into the incubator for cultivation (14 h day 22°C/10 h night 18°C) and continued normal watering. Two water treatments were established in the experiment. For the drought treatment, each culture bowl was watered evenly and weighed to 2 kg, and then water was no longer supplied. Samples were collected at 2 d, 4 d and 10 d. For the normal water treatment, each culture bowl was watered evenly and weighed to 2 kg. The drought and normal water treatments were both established with two repetitions.

Phenotypic data determination

The contents of soil water, root water, soluble protein and chlorophyll were determined under both treatments. The formula to determine the soil moisture content was $SWC=(SFW-SW)/SFW$, in which SFW was the soil fresh weight, and SW was the soil weight. The formula to determine the root water content was $RWC=(RFW-RW)/RFW$, where RFW was the root fresh weight, and RW was the root weight. The protein content was determined by the Coomassie blue G250 method. Half a gram of Jerusalem artichoke leaves was sampled and ground with a small amount of quartz sand and 10 ml LPBS for extraction. After grinding, the leaves were centrifuged twice (12,000 g for 6 min and 20,000 g for 16 min). The supernatant was utilized as the protein extract. A volume of 0.1 ml of the protein extract was mixed with 5 ml Coomassie blue reagent, and its absorbance was measured at a wavelength of 595 nm after 2 min. The protein content of the plant materials was calculated using a standard curve. Determination of chlorophyll content: The Jerusalem artichoke leaves were removed to clean the dirt off the surfaces, cut into pieces with the midribs removed and mixed well. A total of 0.2 g of three fresh cut samples were weighed and placed into mortars. A small amount of quartz sand, calcium carbonate powder and 2-3 ml of 95% ethanol was added, and the sample was ground into a homogenous pulp. A volume of 10 ml of ethanol was added and continuously ground until the tissue turned white. The sample was incubated at room temperature for 3-5 min. A piece of filter paper was placed in a funnel and moistened with ethanol. The extract was poured into the funnel along a glass rod and filtered into a 25 ml brown volumetric flask. The mortar, rod and residue were washed several times with a small amount of ethanol, which was finally poured into the funnel together with the residue. The chloroplast pigment extract was poured into a colorimetric cup with an optical path of 1 cm. The absorbance was determined at wavelengths of 665 nm and 649 nm with 95% ethanol as a blank. The chlorophyll content was calculated using the following formula:

$$\text{Chlorophyll content (mg/g)} = \frac{C \times V \times N}{W \times 1000}$$

RNA Preparation, cDNA library preparation and Illumina sequencing.

An Omega E.Z.N.A Total RNA Kit was used to extract the total RNA from the samples, and the purity and integrity of the extracted RNA samples were determined using 1.0% agarose gel electrophoresis. Subsequent sequencing libraries were constructed for the RNA samples to conform to $1.8 < OD_{260}/280 < 2.0$, $OD_{260}/230 > 1.0$, RNA concentration $> 250 \text{ ng}/\mu\text{l}$ and RIN > 8.0 . After extracting the total RNA of the sample, the eukaryotic mRNA was enriched with magnetic beads with Oligo (dT). Fragmentation buffer was added to break the mRNA into short fragments. The first cDNA strand was synthesized by random hexamers using mRNA as a template, and the second strand was synthesized by adding buffer, dNTPs, RNase H, and DNA polymerase I. After purification with a QiaQuick PCR kit and elution with EB buffer, the synthesized RNA was repaired at the end. Poly (A) was added with a sequencing connector. The RNA was then subjected to agarose gel electrophoresis for size selection and finally amplified. The constructed sequencing library was sequenced with an Illumina Hi-Seq™ 2500 platform. Raw reads generated by sequencing were first screened and controlled for data quality. The reads with connector sequences were removed, as were the reads containing poly-N and the low-quality reads produced by sequencing. Trinity software was used for sequence assembly [58], and clean reads were finally obtained for further analysis. The contents of Q20, Q30 and GC of the clean reads were also calculated. The sequence data were uploaded to the NCBI SRA database under the number PRJNA579243.

Differential expression and GO classification analysis

In this study, sequencing samples of two genotypes of Jerusalem artichoke were compared under different watering treatments, and three differentially expressed gene sets were obtained. The volcano map tool in the OmicShare toolset was used to compare the DEGs of QY1 and QY3 under drought stress at 2 d, 4 d and 10 d (<https://www.omicshare.com/tools/>). The Venn map tool in BMKcloud was used to compare the DEGs and co-expressed gene sets of the two genotypes of Jerusalem artichoke under normal watering and drought stress (<https://console.biocloud.net/static/index.html#/drawtools/intoDrawTools/venn/input>). Cufflinks v. 2.2.1 was used to calculate the FPKM value of each unigene. DESeq v. 1.18.0 was used to analyze the differential expression of the same material under drought stress and water treatment, with the threshold of $FDR \leq 0.01$ and $|\log_2(\text{foldchange})| \geq 1$. KO-BAS 3.0 (<http://kobas.cbi.pku.edu.cn/>) and GOseq [59] were used for the functional analysis of DEGs, with Q-value < 0.05 as the threshold.

Regulation of the drought signaling pathways

Genes related to ABA synthesis were extracted from transcriptome, including 14 ABA signaling genes, 21 NAC family genes, 7 DREB family genes and 24 WRKY family genes. The expression of these QY1 and QY3 genes under normal watering and drought stress were analyzed statistically. The heatmap tool in

OmicShare was used for gene expression analysis (<https://www.omicshare.com/tools/Home/Soft/heatmap>).

MapMan analysis

Mapping Mercator software was used to match the annotation information of the unigene sequences of Jerusalem artichoke to mapping [60] by selecting the parameter of “contains DNA.” The DEG information of the Jerusalem artichoke samples was uploaded to MapMan software, which was used to create a visualized analysis of the biological function of the DEGs [61], by selecting the parameter of FPKM values of the comparison between drought stress for 10 d and normal watering for 10 d of the two Jerusalem artichoke genotypes. PageMan was used to analyze the DEGs among the samples and annotate their biological functions.

Gene co-expression network analysis

The WGCNA method was used to construct a gene co-expression network for all the drought-related genes of the two different genotypes of Jerusalem artichoke. Co-expression analysis was conducted on 12 samples treated under drought stress and 16 samples treated with normal water supplementation. Using the method in the R v. 3.5.1 package of WGCNA, the expression matrix was constructed according to the FPKM expression data of the DEGs of all the samples. The expression matrix was standardized using a Z-score, and each gene was analyzed by cluster analysis. The genes of different expression types were classified into modules through dynamic tree cut, and the minimum number of genes for each co-expression module was 50 [62]. Some similar modules were merged, and the correlation between different modules was calculated using 0.25 as the similarity threshold. Three traits were set as variety, stress and period, respectively. The correlations between the modules and variety and stress and period were calculated through correlation analysis. GO enrichment analysis was performed on the stress-related co-expression modules, and significant genes in the major enrichment pathways were selected from the enrichment results for functional prediction analysis.

Abbreviations

GO: Gene ontology; ABA: Abscisic acid; DEGs: Differentially expressed genes; SnRK2s: Sucrose non-fermenting SNF1-related protein kinase 2s; ABRE: ABA-responsive element-binding proteins; ABFs: ABRE-binding factors; RAV1: Related to ABA-Insensitive 3/VP1; NAP: Naproxen; DESeq: De novo sequence technology; FPKM: Fragments Per Kilobase per Million; DREB: Dehydration responsive element binding protein; WRKY: WRKY Transcription Factor; IAA: Indole-3-acetic acid; GA3: Gibberellin; EREBP: Ethylene responsive element binding protein; AREB1: Abscisic acid- responsive element binding protein 1; JUB1: JUBGBRUNNEN1 as known as NAC42; SAPK8/10: Serine/threonine-protein kinase 8/10

Declarations

Acknowledgments

Not applicable.

Authors' contributions

SY and LW conceived and designed this research. LW, GZ, QZ, DZ performed the experiments. SY, LW, XS analyzed the data. SY and XS wrote and revised the manuscript. All authors read and agreed to the final manuscript.

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Availability of data and materials

Raw data about our research have been uploaded to NCBI's SRA (sequence read archive) (accession nos. Are PRJNA579243), Seeds of Jerusalem artichoke "QY1" and "QY3" are available from the corresponding author upon request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1. Summary of the Illumina-generated de novo assembly statistics.

	WT (Control)	DT (Drought stress treatment)	Total/Average
Raw reads	44,534,163	45,016,588	89,550,751
Clean reads	44,602,412	44,949,553	89,551,965
Clean bases	6,734,964,250	6,773,673,635	13,508,637,885
Q20	97.59%	97.73%	97.66%
Q30	93.10%	93.43%	93.265%
GC Content	45.40%	45.77%	45.585%
Assembly Statistics			
	Genes		Transcripts
Amount	309,323		455,817
GC Content	42.236		41.474
N50[bp]	727		788
Mean Length[bp]	571		619
Assembled Bases	176,895,728		282,581,480

Table 2. Functional annotation of the core genes in the four modules

Gene ID	Annotation	Species	Module
Unigene184071	Cupredoxin	<i>Cynara cardunculus</i>	dark red
Unigene063803	Histone H4	<i>Zea mays</i>	dark red
Unigene098596	Histone H2B.1	<i>Solanum lycopersicum</i>	dark red
Unigene123539	Histone H2A.1	<i>Fragaria vesca</i> subsp. <i>vesca</i>	dark red
Unigene084340	Histone H3.2, partial	<i>Noccaea caerulea</i>	dark red
Unigene214196	Enolase, partial	<i>Micromeria varia</i>	ivory
Unigene189291	Tetratricopeptide-like helical	<i>Cynara cardunculus</i>	ivory
Unigene246133	CHASE domain containing histidine kinase protein isoform 2	<i>Theobroma cacao</i>	ivory
Unigene173687	Aux/IAA-ARF-dimerization	<i>Cynara cardunculus</i>	ivory
Unigene183521	PIN-like protein, partial	<i>Strelitzia reginae</i>	ivory
Unigene241931	Protein phosphatase 2C	<i>Cynara cardunculus</i>	brown
Unigene142825	Alpha tubulin	<i>Cynara cardunculus</i>	brown
Unigene120029	Seven-in-absentia protein (SINA)	<i>Cynara cardunculus</i>	brown
Unigene122637	Phospholipid/glycerol acyltransferase	<i>Cynara cardunculus</i>	dark slate blue
Unigene169117	Protein kinase, ATP binding site-containing protein	<i>Cynara cardunculus</i>	dark slate blue
Unigene092475	G-type lectin S-receptor-like Serine/threonine-protein kinase LECRK2 isoform	<i>Juglans regia</i>	dark slate blue

Figures

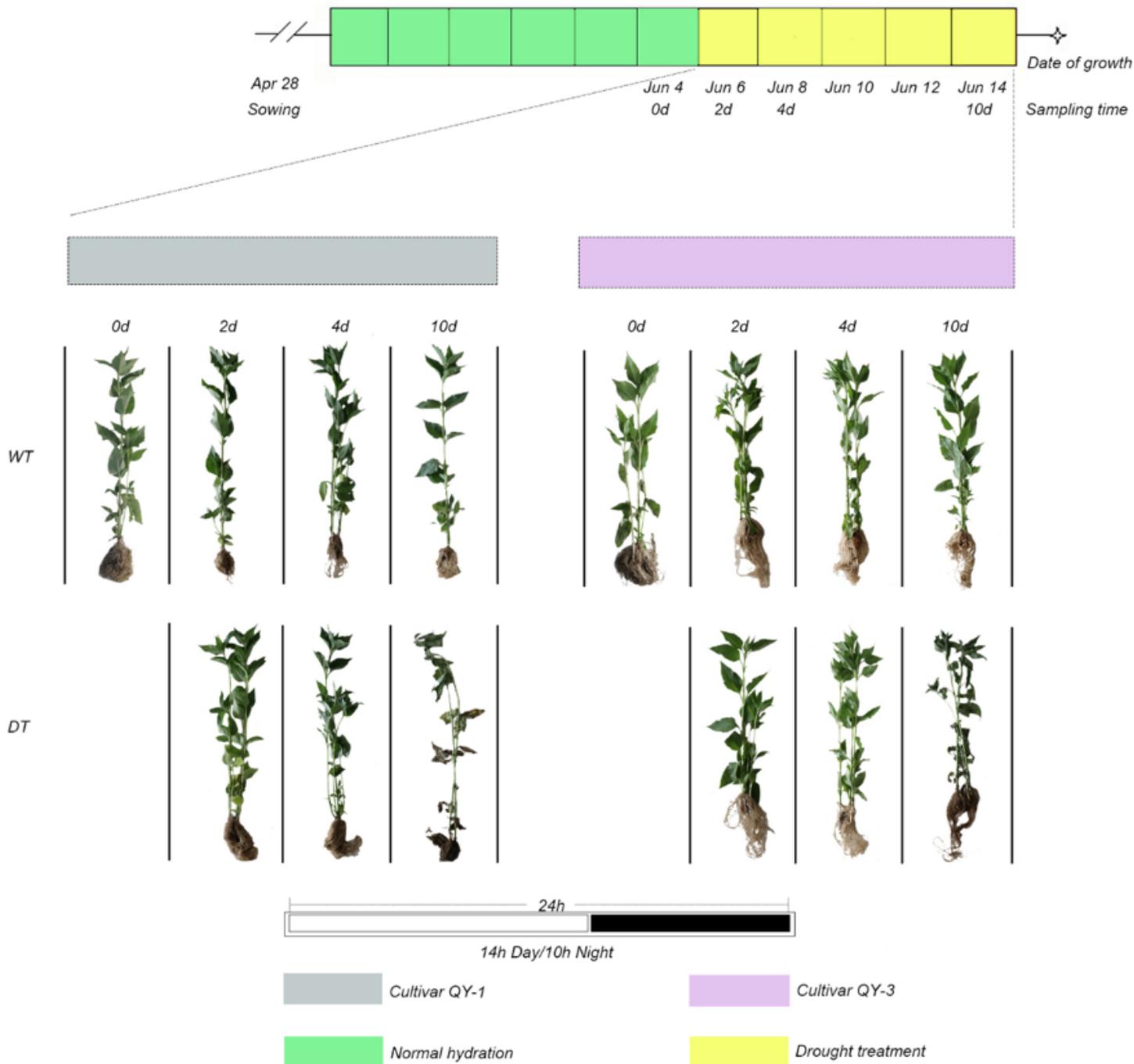


Figure 1

Morphological changes of QY1 and QY3 under water treatment (WT) and drought treatment (DT)

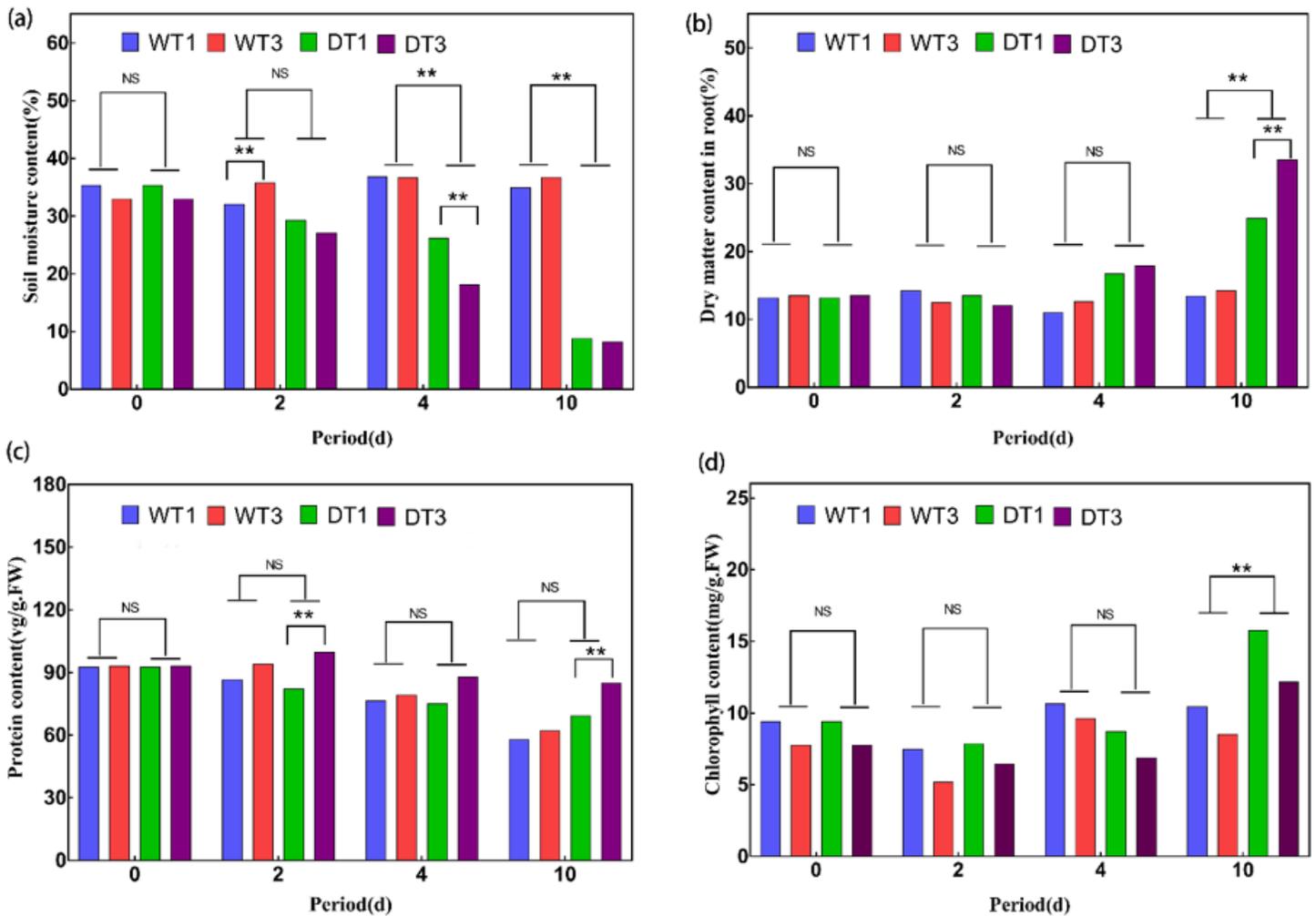


Figure 2

Physiological indicators of two genotypes of Jerusalem artichoke under drought stress. (a) Variation in the trend of soil water content; (b) Percentage of dry matter in the root; (c) Soluble protein content; (d) Variation in the trend of chlorophyll content. WT1: Normal water treatment QY1; WT3: Normal water treatment QY3; DT1: Drought treatment QY1; DT3: Drought treatment QY3. ** $P < 0.01$, NS: no significant difference.

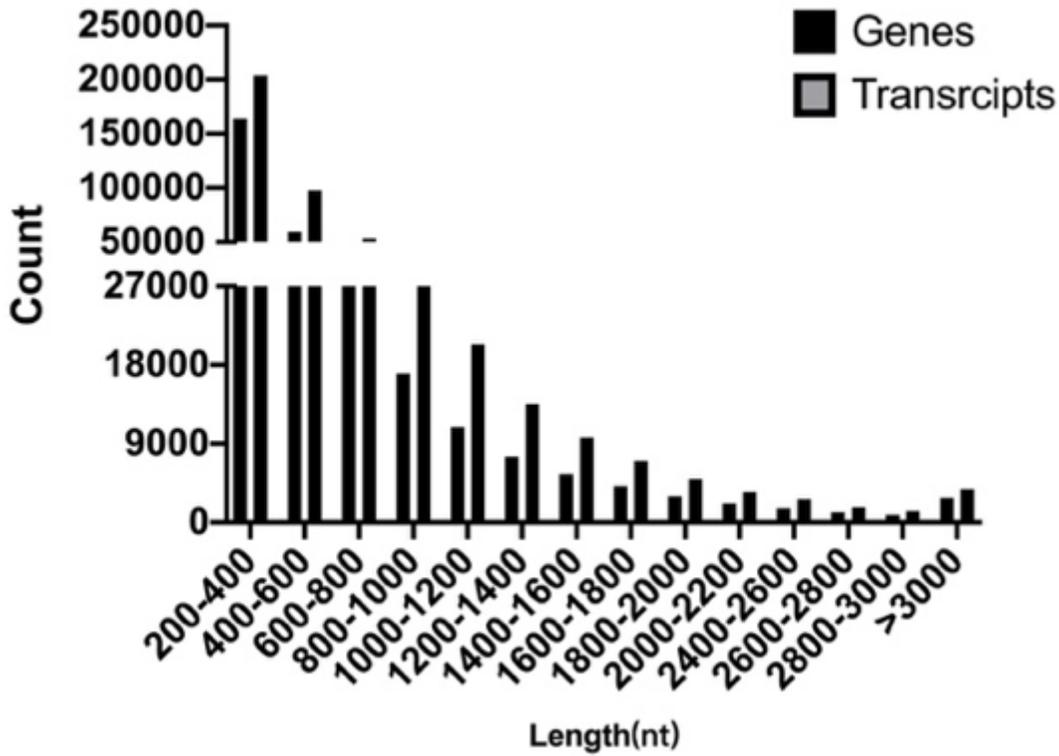


Figure 3

Length distribution of the genes and transcripts

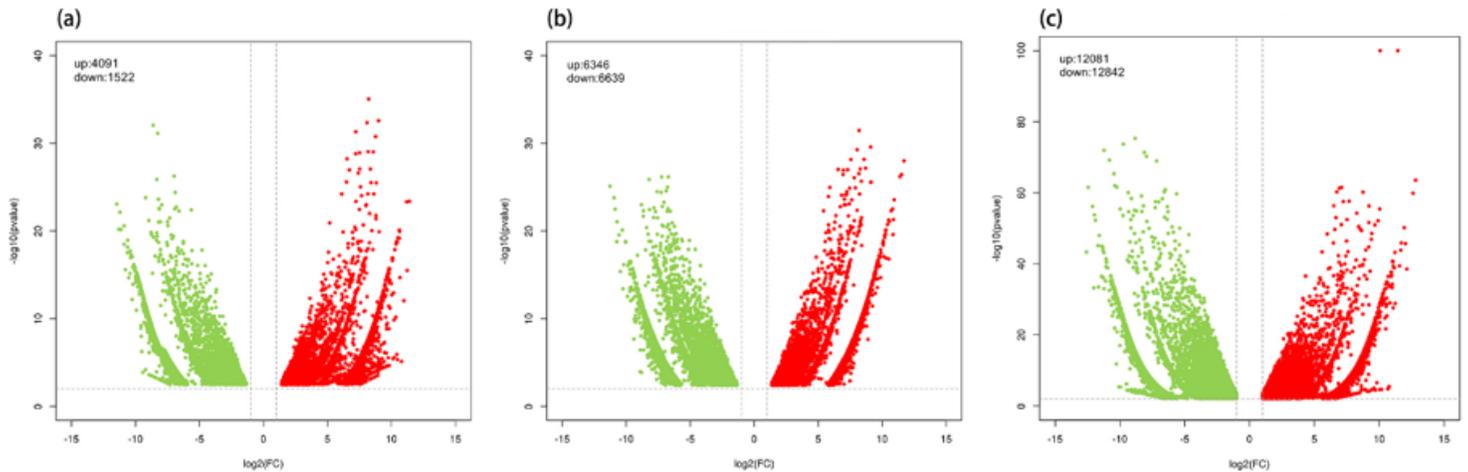


Figure 4

Volcano plots of differentially expressed genes under drought treatment, with the up-regulated and down-regulated genes represented by green and red dots, respectively. (a) DT-QY1-2d vs DT-QY3-2d; (b) DT-QY1-4d vs DT-QY3-4d; (c) DT-QY1-10d vs DT-QY3-10d

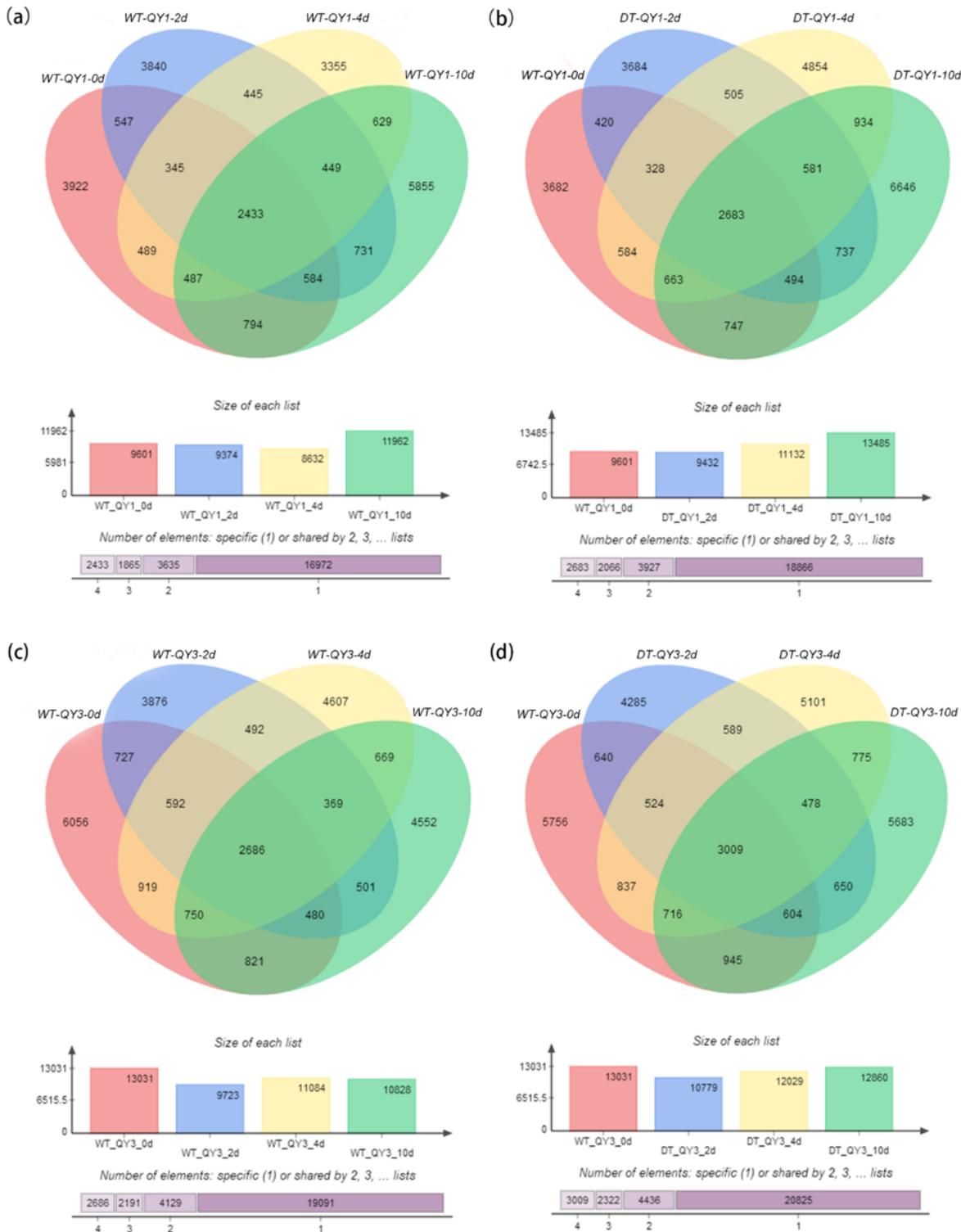


Figure 5

Venn diagram of the number of common and specific differentially expressed genes in QY1 and QY3 under water treatment (WT) and drought treatment (DT). The histogram shows the total number of elements in each sample without redundancy, and the distribution diagram shows the distribution of all the elements in each sample combination. (a) WT QY1; (b) DT QY1; (c) WT QY3; (d) DT QY3

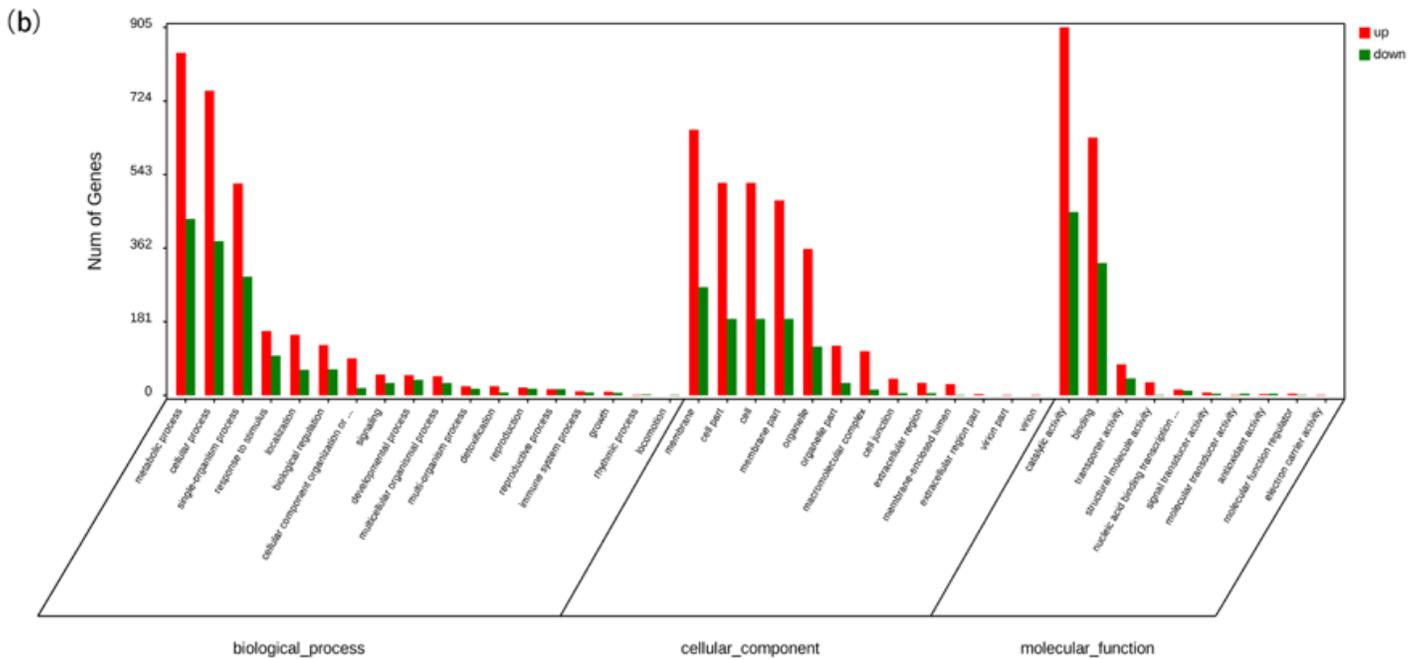
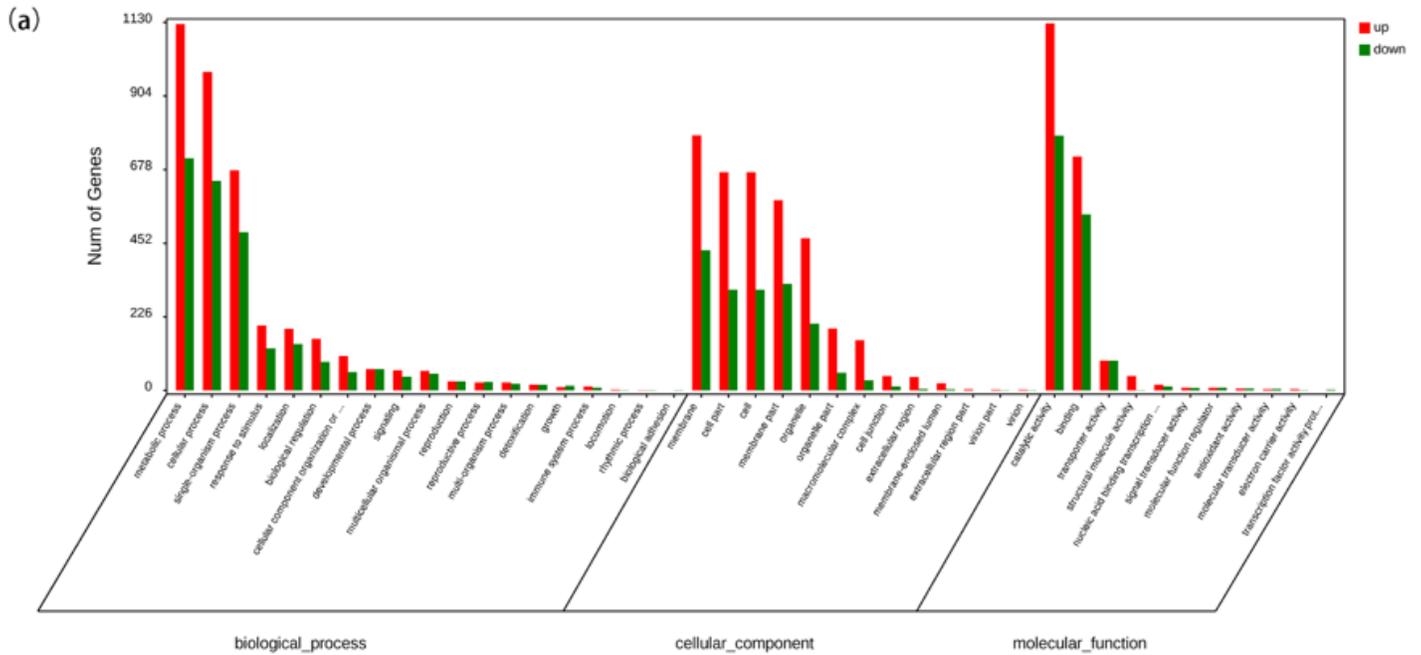


Figure 6

Gene Ontology (GO) classifications of DEGs (a) QY1 DEGs between the drought treatment (DT) and water treatment (WT) groups; (b) QY3 DEGs between the DT and WT groups

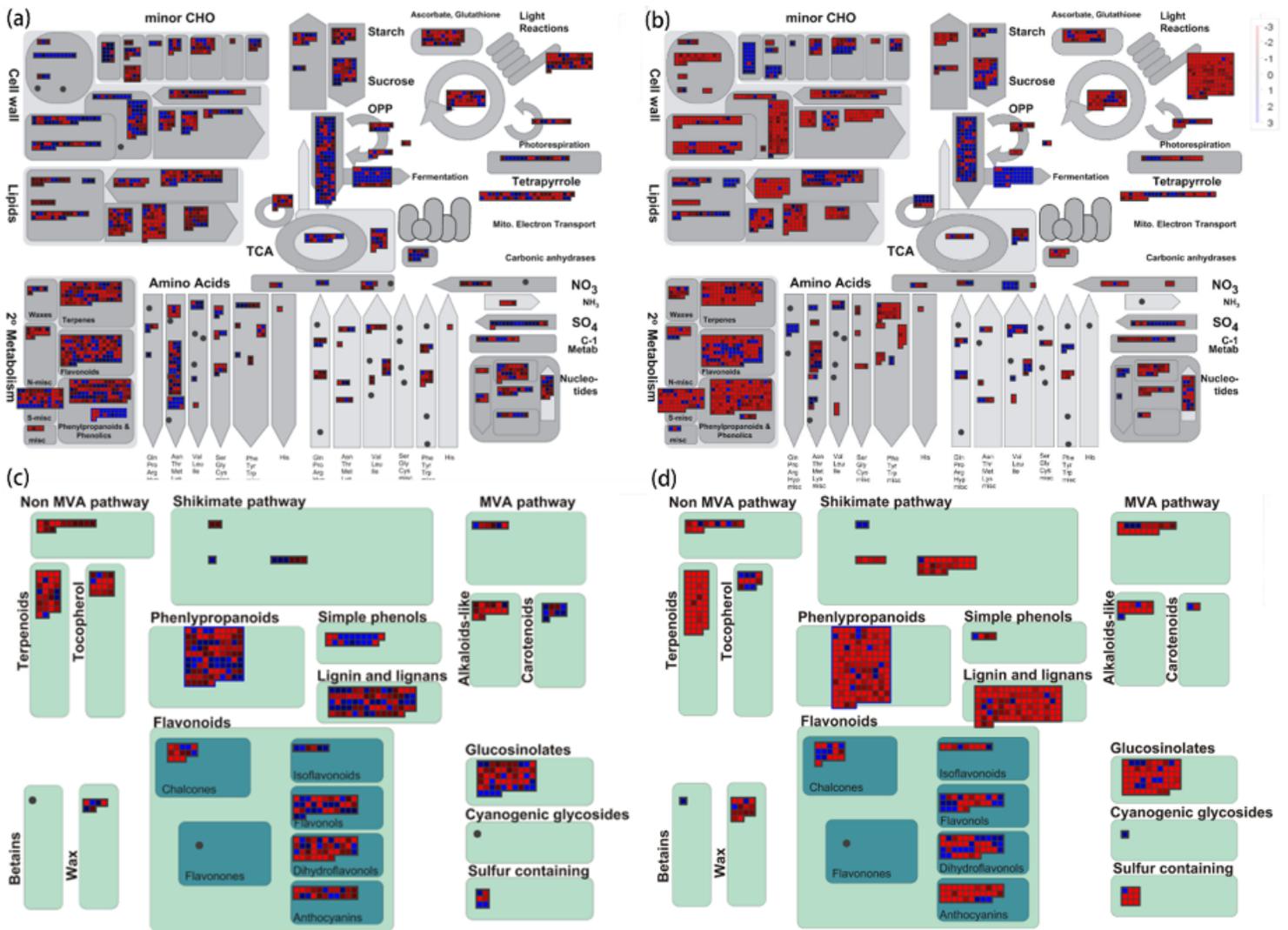


Figure 7

Function annotation of the metabolic processes of the differentially expressed genes (DEGs) of Jerusalem artichoke under drought treatment (DT). Each square represents an individual gene. (a) DEGs metabolic overview of QY1 under the DT and water treatment (WT); (b) DEGs metabolic overview of QY3 under the DT and WT; (c) DEGs secondary metabolic overview of QY1 under the DT and WT; (d) DEGs secondary metabolic overview of QY3 under the DT and WT

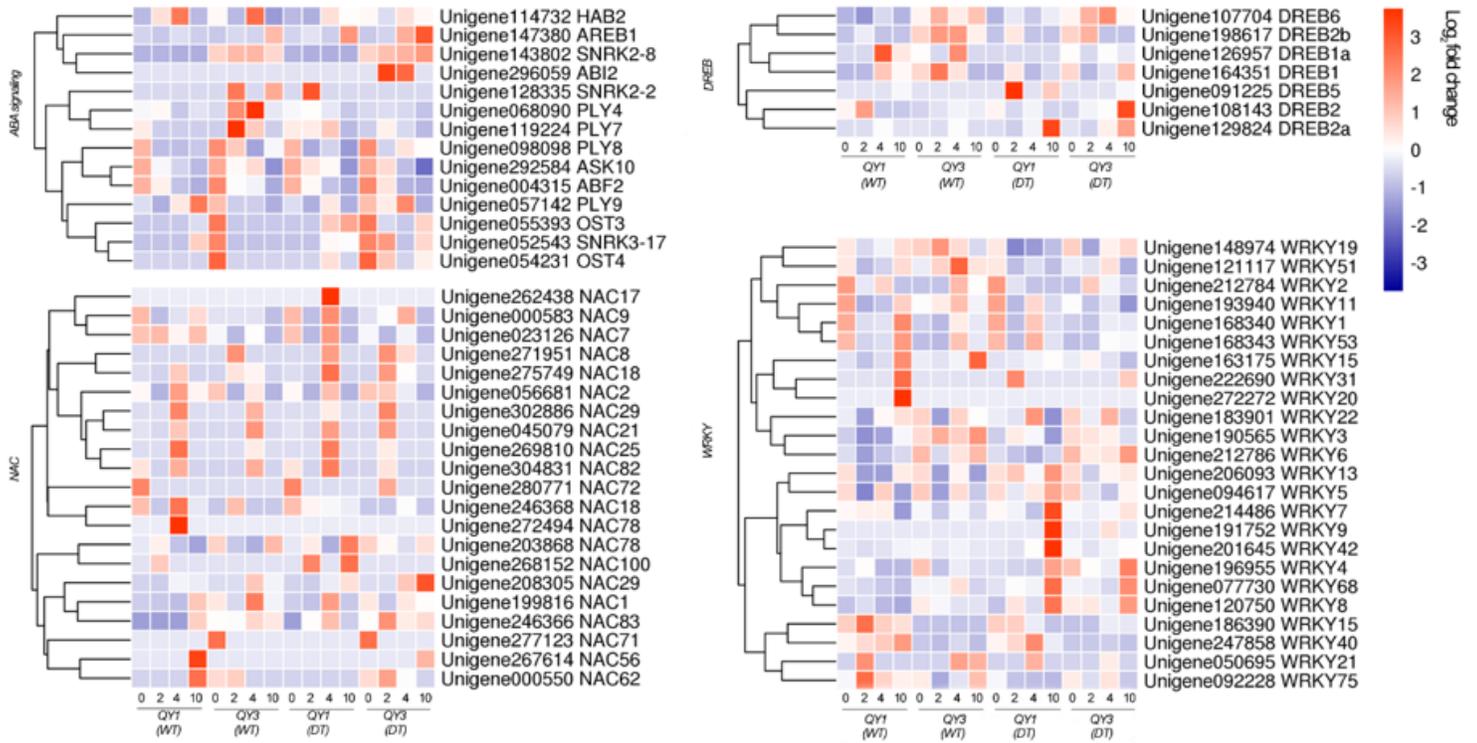


Figure 8

Expression heatmap of abscisic acid key metabolic pathway-related genes in QY1 and QY3

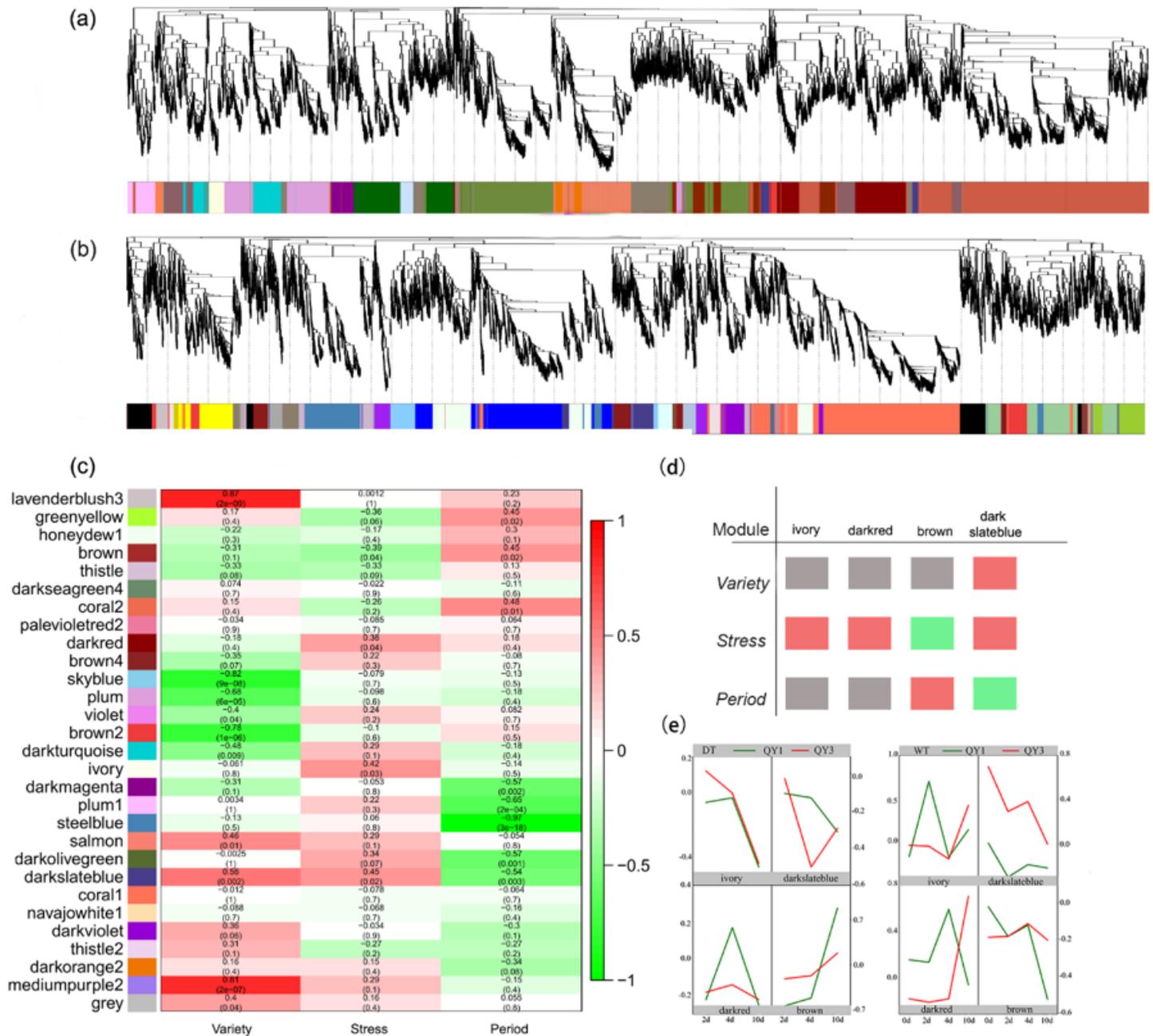


Figure 9

Analysis of the co-expression network of drought stress-related genes. Weighted gene co-expression network analysis (WGCNA) was used to analyze the co-expression network of drought stress-related genes, and the genes were clustered into different co-expression modules. (a) Gene co-expression module of QY1. (b) Gene co-expression module of QY3. (c) A correlation analysis of each gene co-expression module with variety, stress and period under drought stress was conducted. The number in the heatmap represents the Pearson correlation coefficient, and the number below represents the student asymptotic P value. (d) The correlation between the four important modules (ivory, dark red, brown and dark slate blue) and variety, stress and period. The red square represents positive correlation; the gray square represents insignificant correlation, and the green square represents negative correlation. (e) The trends of

expression of the four main modules under drought treatment (DT) and water treatment (WT) at different time periods.

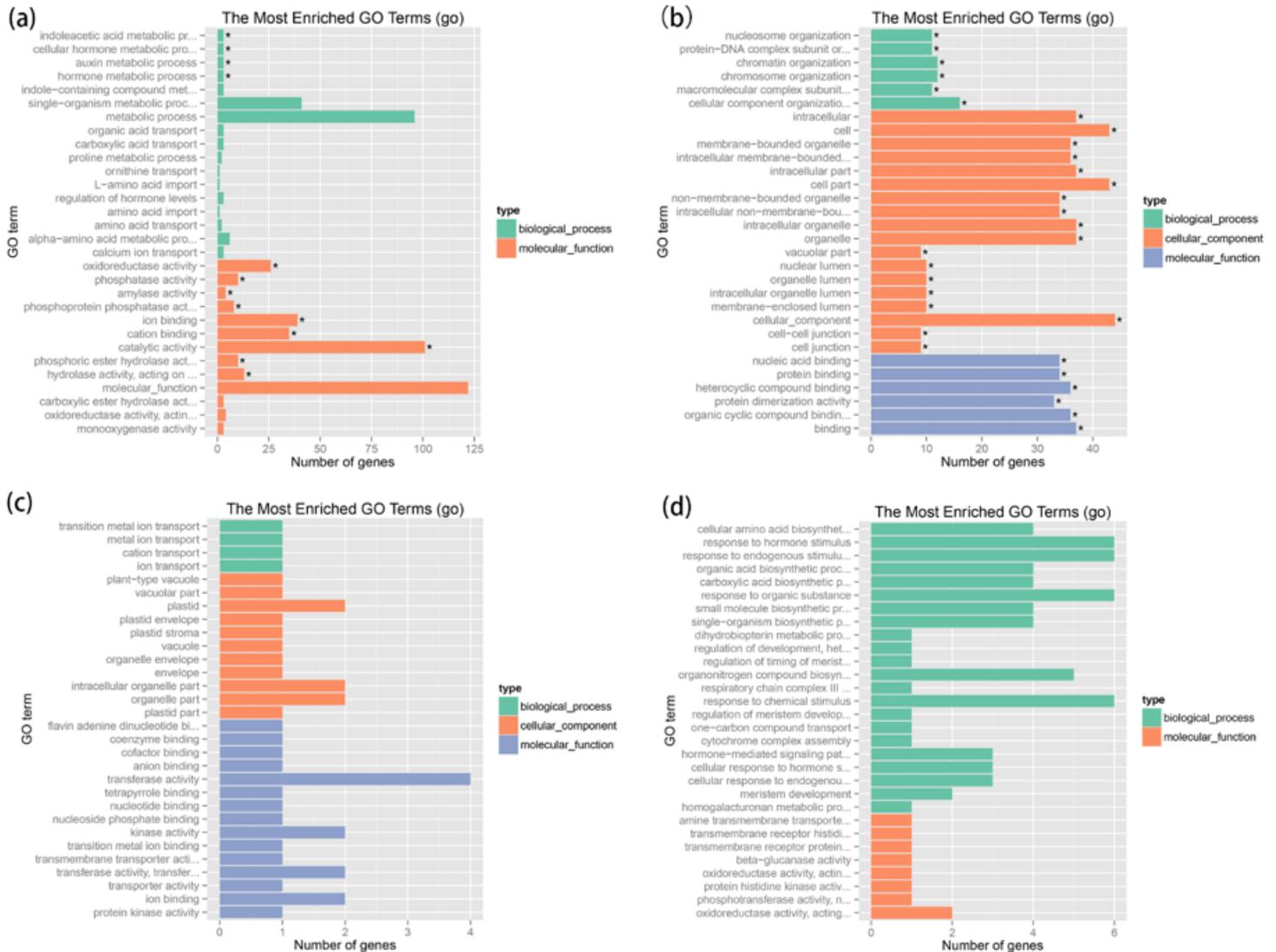


Figure 10

Gene Ontology (GO) enrichment analysis of drought resistance related co-expression module genes. (a) GO function enrichment results of the brown module. (b) GO function enrichment results of the dark red module. (c) GO function enrichment results of the dark slate blue module. (d) GO function enrichment results of the ivory module. *P<0.01