Insight of Transcriptional Regulators reveals the Tolerance Mechanism of Carpet-grass (*Axonopus compressus*) Against Drought

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Abstract

Background: Carpet grass [*Axonopus compressus* (L.)] is an important warm-season perennial grass around the world and is known for its adaptability to varied environmental conditions. However, Carpet grass lacks enough data in public data banks, which confined our comprehension on the mechanisms of environmental adaptations, gene discovery and development of molecular markers. In current study, the DEGs (differentially expressed genes) in *Axonopus compressus* under drought stress (DS) were identified and compared with CK (control) by RNA-Seq.

Results: A total of 263,835 unigenes were identified in *Axonopus compressus*, and 201,303 (also added to the numbers of the remaining 2 data bases) a sequence of unigenes significantly matched in at least one of the seven databases. A total of 153697 (58.25%) unigenes classified to 144 KEGG pathways, and 7,444 unigenes were expressed differentially between DS and CK, of which 4,249 were up-regulated and 3,195 were down-regulated unigenes. Of the 50 significantly enriched GO terms, 18, 6 and 14 items were related to BP, CC and MF respectively. Analysis of KEGG enrichment revealed 2569 DEGs involved in 143 different pathways, under drought stress. 2,747 DEGs were up-regulated and 2,502 DEGs were down-regulated. Moreover, we identified 352 transcription factors (TFs) in *Axonopus compressus*, of which 270 were differentially expressed between CK and DS. The qRT-PCR validation experiment also support the transcriptional response of *Axonopus compressus* against drought. Accuracy of transcriptome unigenes of *Axonopus compressus* was assessed with BLAST, which showed 3,300 sequences of *Axonopus compressus* in the NCBI.

Conclusion: The 7,444 unigenes were found to be between DS and CK treatments which indicate that there is a strong mechanism of drought tolerance in *Axonopus compressus*. The current findings provide the first framework for further investigations for the particular roles of these unigenes in *Axonopus compressus* in response to drought.

Background

Axonopus is a genus of the grass family (Paniceae; Poaceae; Triticeae). Approximately 100 species of Axonopus have already been identified [1], and they are generally distributed along shoreline of the China and Africa, Alaska, central Asia, eastern Asia, North Sea, South America and Oceania [2, 3]. About more than 40% (55.5×106 km^2) of the Earth's surface is occupied by Grasslands world-wide, Greenland and Antarctica are not included [4].

The social and ecological susceptibility to climate change are giant issues of modern era, the grassland gives an additional opinion about vulnerability of the ecosystem on account of alterations in policies, socio-economic elements, land use, and local climate [4]. Since the exposure to climatic hazards in various regions has become more apparent, the ecosystems are apt to have greater degree of vulnerability. Globally, the number of socio-ecological attributes of the eco-systems have changed drastically due to lack of enough soil moisture [5].

Plant growth, nutrient balance, and photosynthetic dynamics have been influenced by drought [6]. Tolerant plant species with drought responsive mechanisms at various levels such as, morphology as well as physiological and molecular basis can be used to cope with water deficit. Since the important role of carpet-grass in ecosystem protection, a number of scientists from a macro point of view have paid attention to how carpet-grass responds to global changes for instance drought, salinity, elevated temperature, and CO_2 exposure [7, 8]. However, little attention has been given to understand the genetic basis of its ecological diversifications, mainly because of the confined genomic resources in carpet-grass. Thus far, only limited ESTs and protein sequences from carpet-grass have been submitted in public online-databases [9]. The discovery of different Genes is also lagging, and just a few genes happen to be cloned and functionally authenticated [10, 11].

The high-throughput technologies, next generation sequencing (NGS) i.e., ABI/SOLiD, Roche/454, Illumina/Solexa have actually made it possible to produce genome resources at a sizable level and at a comparatively low cost [12, 13]. The next-generation sequencing have already been efficiently utilized to create large-scale transcriptome details in a number of plant species, i.e., Rice [14] Arabidopsis [15, 16], wheat [17], barley [18], and maize [19] to identify the candidate genes mechanisms.

The plants attempt to re-program their metabolic activities and growth, while confronting water deficit condition. It happens to be obvious that plants show distinct and extremely dynamic responses to limited water conditions [20]. These responses varied due to number of factors i.e., genotypes, experimental procedures, sampling technique and time [20, 21]. The molecular mechanisms in response to drought stress has been studied by many scientists and the genes associated with water uptake [22], transporter channels [23, 24], and transcription factors [25]. All these attributes are regarded as the regulators of drought tolerance in plants, although number of molecular elements and gene networks of drought tolerance mechanisms have been identified but still not fully elucidated [21].

Methods

Plant Materials and Drought Treatments

Axonopus compressus L. plants, with the age of thirty days old with uniform growth were acquired from the germplasm resource library maintained at the Hainan University, were used as experimental material. The germplasm resource library is maintained by Key Laboratory of Genetics and Germplasm Innovation of Tropical Special Forest Trees and Ornamental Plants, Ministry of Education, College of Forestry, Hainan University, Haikou 570228, P. R. China. The trial materials were from the growth chambers (27 ∘C, 16-hours day length and 60% RH). A total of 90 Axonopus compressus (L.) plants were primarily divided into two groups CK (control group) and DS (drought experiment group), 5 cutings in each pot and 20 cuttings in one replicate group, this experiment was repeated 3 times named R1, R2 and R3. The drought treatments were induced by using the Polyethylene-glycol (PEG-8000). The PEG-8000 solution was replaced after 48 hours. At the 0th, 6th and 12th, 18th, 24th, 36th, 48th and 72th hours after induction of drought treatment the functional leaves (3rd to 5th

mature leaf) were choose randomly from the plants of CK and DS for the purpose of physiological attributes. For transcriptome sequencing, sample were instantly frozen in liquid nitrogen, stored at -80°C, and finally sent to Metware Biotechnology Co., Ltd, Gaoxin Road, East Lake High-tech Zone, Wuhan, China.

Measurement of Physiological Traits

The physiological traits were recorded in different time interval of 0, 6th and 12th, 18th, 24th, 36th, 48th and 72th hour after induction of drought. At different time interval leaf water potential (LWP) in leaves was measured by Scholander chamber (SF-PRES-70, Solfranc Tecnologías SL, Vila-Seca, Spain). Electrolyte leakage (EL) was measured by estimating the electrical conductivity [26]. In the solution, the electrolyte leakage (S1) was measured using a conducti-meter after 22 h of floating at room temperature (Mettler-Toledo Instruments Co., Ltd, Shanghai, China). Total conductivity (S2) was collected after the flasks were placed in a boiling water bath for 30 min. Relative water content (RWC) was also estimated, as described in Liu et al. [27]. For each plant, three independent replicates were collected for LWP, EL, and RWC. All measurements were recorded at three biological replicates.

Extraction of RNA of Axonopus compressus, Library Preparation

Total RNA of *Axonopus compressus* was synthesized by employing the Trizol kit (Invitrogen, Carlsbad, CA, USA), and guarantee the obtained samples have been qualified for transcriptome sequencing. The RNA integrity and purity concentration were accessed by the Nanodrop method, (Qubit 2.0, Agilent 2100). Then, amount of three micro-gram of total-RNA per sample with three
biological replicates were introduced as raw materials for preparations of the RNA sample (working). The sequencing libraries of *Axonopus compressus* were created by NEBNext®Ultra™ RNA Library Prep Kit for Illumina® (New England Biolabs, Ipswich, MA, USA), as suggested by the manufacturer. The mRNAs was extracted by total RNA by Oligo (dT) and were randomly divided into short length fragments. Random hexamer primer synthesized the first strand cDNA, and buffer, DNA polymerase I, dNTPs and RNase H were used for processing the second strand cDNAs. The first strand of cDNA was extracted by random hexamer primer, afterwards, the dNTPs, buffer, DNA polymerase I and RNase H were used to generate cDNA's second strand. Finally, the AMPure XP beads were used to purify cDNAs and afterwards the end-repair and single nucleotide A (adenine) addition, the cDNA libraries (qualified) were developed by PCR technique. The Qubit 2.0 was used for primary quantitation, followed by Agilent 2100, which was employed to identify libraries insert size. Thereafter, the q-PCR technique had been used for accurate quantification of effective strength of the libraries (effective library concentration > 2 nM) to guarantee the library quality. After passing through a series of screening steps, the high-throughput sequencing had been executed by Illumina Hiseq Xten. The protein-coding region accuracy and completeness assessment in *Axonopus compressus* was performed by using BUSCO analysis (BUSCO_v2/v3) [28].

Validation of RNA-seq data by qRT-PCR

The RNA was extracted from the CK and DS groups and used to develop the cDNA library. First-strand synthesis was carried out via MonScript (Monad) according to the suggested protocol by the manufacturer. Biosystems-7500 (Thermofisher Scientific) was used to conduct the qRT-PCR and the primers pairs are listed in Additional file 1; Table S1. The gene expression was quantified through CT-method, and the Actin1 used as references for normalization.

Results

Effect of drought stress on physiological indicators in *Axonopus compressus*

In the Figure 1, it was revealed that rate of H_2O_2 (Figure 1A) production and MDA concentration (Figure 1B) increased in drought treated plants. The MDA values and H_2O_2 production rate increase with the prolonged drought stress treatment, whereas MDA values and the production rate of H_2O_2 at 36 h after drought induction were significantly increased ($p < 0.01$). Similarly, the leaf water potential and relative water content decrease significantly under drought, contrarily, the electrolyte leakage increased over the drought period in *Axonopus compressus*. As in case of MDA and H2O2 the values of EL were also increase as the time of drought stress increase. Whilst, the values of leaf water potential and relative water content decrease with the prolonged drought stress. (Figure 1C).

RNA-Seq of *Axonopus compressus* and de novo assembly

RNA-Seq of the six libraries of cDNA (DS and CK with three replicates each) resulted in 352.84 million raw reads, of which approximately 347.73 million clean reads de-novo compiled into contigs utilizing Trinity software, tend to range from 54.90 million to 62.25 million reads for each library and approximately 97% of reads with a quality score of Q20 (99% accuracy) (Additional file 1; Table S2). The contigs were assembled into 278042 unigenes with an average length of 1066 bp and an N50 length of 1430 bp. All unigenes were over 200 bp long, of which 11.77% (32718 unigenes) were 2,000 bp long. We evaluated the assembled data of transcriptome by employing Benchmarking Universal Single-Copy Orthologs (BUSCO) package. The number of total BUSCOs searched was 1440. The number of complete BUSCOs in the combined transcriptome was 1080 (75.00%), and only 198 core genes (13.75%) are fragmented in *Axonopus compressus* (Additional file 2; Figure S1).

Functional annotation and classification of unigenes

The assembly of unigenes from *Axonopus compressus* was annotated by BLASTX (E-value $<10^{-5}$) in different databases. A total of 135716 (48.81%), 188334 (67.64%), 120455 (43.32%), 189422 (68.13%), 100588 (36.18%), 153958 (55.37%) and 128626 (46.26%) unigenes would have significant levels (E-value $<10^{-5}$) against KEGG, NR, Swissprot, Trembl, KOG, GO, and Pfam, respectively (Figure 2A). Out of 278042 unique of high-quality sequences, 191893 (69.03%) unigenes complemented a sequence significantly in at least one of the seven public databases, The five key online databases (GO, KEGG, KOG, NR, and Trembl) were picked out of seven databases to draw a Venn diagram (Figure 2B), the unigenes with significant values (E-value10^{-5}) are also noticed at each junctions of the Venn diagram, in which 94 unigenes corresponds to five databases.

The alignment of sequence homology revealed that 39439 (20.94%) sequences that were found in resemblance with *Setaria italica*; 35283 (18.73%) sequences had significant hits for *Zea mays*, followed by *Sorghum bicolor* (35034, 18.6%), *Penicum hallii* (23894, 17.47%), *Dichanthelium oligosanthes* (18896, 10.03%) and *Oryza sativa japonica* group (6551, 3.48%). *Brachypodium dischyon* which included 1675 (0.89) and 9.86% of the sequences (18562) were homologous to other species (Figure 2C). The expression of genes has biological variability between different individuals, and there are differences in the degree of expression between genes. The reads per kilobase of exon model per million of aligned reads (FPKM) values were calculated as normalized expression estimates for each gene model in each sample. To evaluate the major spectral variance between CK and DS treatment samples, we performed PCA (Figure 2D). Results showed distinct separation of CK to DS sample treatments, which reveals that both groups of the treatment samples exhibit different spectral positions on PCA chart (Figure 2D).

To demonstrate the accuracy of the unigenes prediction, the nucleotide sequences from the transcriptome of *Axonopus compressus* were used as query in a BLAST search with threshold E-value $<10^{-5}$. The results showed 3,300 sequences of *Axonopus compressus* in the NCBI database. This analysis renders an assessment for the consistency of unigenes sequences in the current dataset. Based on the high-score BLASTx matches in the GO proteins database, The BLASTx high-score Predicated matches in the computer database of Go proteins were confirmed, and a total of 153958 unigenes were categorized with Blast2GO (E-value $<10^{-5}$) and were designated at least once in GO. As shown in Fig 3, the unigenes referred to three main categories of GO and 59 subcategories, namely biological processes (BP), with 28 main sub-classes (405025 unigenes); cellular compartments (CC), with 18 main sub-classes (460094 unigenes); and molecular functions (MF), with 13 main sub-classes (194989 unigenes).

Cellular processes (21.43%) were the biggest subgroups in the category of biological processes, metabolic process (19.70%), biological regulation (9.24%), response to stimulus (9.22%) and regulation of biological process (8.36%). The largest subgroups in the cellular component category were cell (22.53 %) followed by cell part (22.47 %), organelle (17.22%), and membrane (11.72%) respectively. Similarly, the main subgroups in the molecular function category were binding and catalytic activity, that contribute 77.78% and 69.28% respectively, and 115199 unigenes associated to molecular function. Within the *Axonopus compressus* unigenes, 112492 (42.64%) were classified (E-value <10-5) into twenty six KOG clusters (Figure 3B). The biggest groups which includes; 1) general function prediction, only (21955 genes, 19.52%); 2) posttranslational modification, protein turnover, and chaperones (11636 genes, 10.34%); 3) signal transduction mechanisms (11302 genes, 10.05%); 4) Function unknown (5785 genes, 5.14%); and 5) carbohydrate transport and metabolism (5616 genes, 4.99%).

Metabolic pathway analysis of *Axonopus compressus* by KEGG

A total of 135717 (51.44%) of the 263835 *Axonopus compressus* unigenes possessed significant correspondence in KO. All these unigenes have been reserved to 103 KEGG pathways and could be splited to 5 categories (Figure 3B). The pathways of KEGG which includes 4303 unigenes were the members of major group, metabolism (D), 921 associated to genetic information processing (C), 184 related to cellular processes (A), 347 involved to environmental information (B), and 151 related to category (E) of organism systems (Additional file 2; Figure S2).

CDS prediction in *Axonopus compressus*

The BLASTx protein database (NR and SwissProt database) identified 278042 unigenous CDSs, of which 23652 unigenes were larger than 500 bp, 12520 unig In addition, 43,713 unigenes were not linked to the NR and SwissProt database systems. The Estscan (Version; 3.0.3) software was used to interpret their ORF, frequency distribution, length, and related amino acid sequences of the unigene CDSs.

Differentially expressed genes (DEGs) analysis of *Axonopus compressus*

Amongst the differentially expressed unigenes, the expression of 7444 differs substantially between samples treated with drought-stress (DS) and control (CK) samples. Under drought treatment, 4249 numbers were up-regulated and 3195 numbers were down-regulated ($p<0.05$) (Figure 4A). The expression profiles of DEGs also presented through cluster analysis that showed significantly different responses of CK and DS treatments in the *Axonopus compressus* (Figure 4B).

Axonopus compressus GO pathway enrichment analysis

By employing the Gene ontology (GO) and the DEG enrichment analysis in *Axonopus compressus*, 23766 DEGs were categorized into three GO groups and 4592 numbers associated the DS with CK (number of DEGs annotated in more than one term), out of which 2766 items that were associated to BP, 477 were linked with CC and 1349 were related to MF.

The key 50 DEGs dramatically enriched in three GO categories are presented in Additional file 2; Figure S4. In the top 50 significantly enriched identities (Corrected P-Value<0.05), 18 were found to be linked with BP [carotenoid biosynthetic process GO:0016117, carotenoid metabolic process GO:0016116, cellular amine metabolic process GO:0044106, cellular biogenic amine metabolic process GO:0006576, cellular response to heat GO:0034605, cellular transition metal ion homeostasis GO:0046916, glutamine family amino acid biosynthetic process GO:0009084, Group II intron splicing GO:0000373, heat acclimation GO:0010286, oligosaccharide catabolic process GO:0009313, raffinose catabolic process GO:0034484, raffinose metabolic process GO:0033530, regulation of seed germination GO:0010029, regulation of seedling development GO:1900140 , response to high light intensity GO:0009644, response to hydrogen peroxide GO:0042542, tetraterpenoid biosynthetic process GO:0016109, tetraterpenoid metabolic process GO:0016108], and 6 items were related to CC [chloroplast nucleoid GO:0042644, DNA packaging complex GO:0044815, Nucleoid GO:0009295, Nucleosome GO:0000786, plastid nucleoid GO:0042646, protein-DNA complex GO:0032993], and 14 items were related to MF [4-coumarate-CoA ligase activity GO:0016207, alpha-galactosidase activity GO:0004557, arogenate dehydratase activity GO:0047769, carbon-nitrogen ligase activity, with glutamine as amido-N-donor GO:0016884, ferric iron binding GO:0008199, ferroxidase activity GO:0004322, galactosidase activity GO:0015925, oxidoreductase activity, acting on single donors with incorporation of molecular oxygen GO:0016701, oxidoreductase response, acting on single donors with molecular oxygen incorporation, incorporation of two atoms of oxygen GO:0016702,

oxidoreductase response, oxidizing metal ions, oxygen as acceptor GO:0016724, raffinose alpha-galactosidase activity GO:0052692, water channel activity GO:0015250, water transmembrane transporter activity GO:0005372] (Additional file 3).

Axonopus compressus KEGG pathway enrichment analysis

The KEGG pathway enrichment analysis for DEGs revealed that 2569 DEGs participated in 143 various types of pathways in *Axonopus compressus.* By comparing DS with CK, 2747 DEGs were found to be up-regulated and 2502 DEGs have been identified as down-regulated in deficit water. The twenty key pathways which were found significantly enriched by comparing DS with CK are displayed in (Figure 3C). The pathways includes Valine, leucine and isoleucine biosynthesis (215 unigenes), Stilbenoid, diarylheptanoid and gingerol biosynthesis (330 unigenes), Starch and sucrose metabolism (1642 unigenes), RNA degradation (1536 unigenes), Protein processing in endoplasmic reticulum (1628 unigenes), Porphyrin and chlorophyll metabolism (559 unigenes), Plant hormone signal transduction (1672 unigenes), Phenylalanine, tyrosine and tryptophan biosynthesis (601 unigenes), Metabolic pathways (1659 unigenes), MAPK signaling pathway plant (1661 unigenes), Linoleic acid metabolism (323 unigenes), Glyoxylate and dicarboxylate metabolism (1050 unigenes), Glycosphingolipid biosynthesis - globo and isoglobo series (103 unigenes), Galactose metabolism (735 unigenes), Carotenoid biosynthesis (506 unigenes), Carbon fixation in photosynthetic organisms (820 unigenes), Biosynthesis of secondary metabolites - unclassified (223 unigenes), Biosynthesis of secondary metabolites (1661 unigenes), Biosynthesis of amino acids (1655 unigenes), Alanine, aspartate and glutamate metabolism (639 unigenes) (Additional file 2; Figure S3).

Drought stress associated differentially expressed transcription factors (TFs)

The transcription factors play a crucial role in the growth and development of plants, and therefore can stimulate and/or suppress transcriptional gene expression to sustain normal physiological functions in deficit water stress. In current study, 352 TFs were observed in *Axonopus compressus* based on iTAK, including 270 transcription factors and 81 transcription regulatory factors that associated with 32 and 16 families, respectively (Additional file 1; Table S3, S4). Differential analysis revealed that 270 transcription factors were different as shown in comparison between DS and CK, 216 transcription factors have been noted to be up-regulated while 54 transcription factors were found down-regulated.

qRT-PCR Validation of Transcripts in *Axonopus compressus* under Drought

To check the validity of RNA-seq results, expression of six transcripts for the three biological replicates was analyzed by qRT-PCR (Figure 5). All the genes were identified as significantly different expression values (DEGs). The findings of qRT-PCR revealed that the pattern of expression of these genes was identical with the findings in the RNA-seq analysis. To validate the results, the six drought-responsive genes (NAC, MAP Kinase1, MYB2, PIP1, WRKY1, ABI5) were verified using qRT-PCR. The different genes were amplified to compare the gene expression and RNA-seq results, and the findings of this study revealed that the expression of drought-responsive genes supporting the results of the RNA-seq.

Discussion

Plants have a sessile lifestyle, and usually confront adverse environmental circumstances such as drought, cold, heat, salt, and floods. Deficit water is the most notable environmental determinant influencing crop production around the globe [29]. To improve potential tolerance mechanism against deficit water stress and also to develop drought-resistance in plants, many experts have attempted to elucidate the mechanisms of stress signaling in plants conferring the several perspectives [30, 31]. Plant defense mechanisms for abiotic stress are already being studied, but the response to drought has always been a complex event with several critical indicators to be examined.

Drought stress significantly increased ROS formation and imposes oxidative stress on the plant [26, 32]. Since lipid peroxidation is one of the earliest indicators of oxidative damage, MDA was estimated as an index for production of drought induced ROS [32]. A significant rise in MDA values and H_2O_2 concentration were noticed in *Axonopus compressus* leaves in drought conditions (Figure 1B). Electrolyte leakage increased significantly with a decline in water potential (Figure 1E), which proposed that plant systems had been damaged by drought, as studied previously [33]. Drought might have had an impact on the physical membranous structure of the lipid bilayer by inducing phase destruction [34].

To sustain under severe environmental conditions, plants have developed an obscure regularity mechanism through a series of evolutionary advancements at varied levels to respond to external signals and prompt transduction of stress signals, directing to a set of responses at different levels, i.e, physio-morphological, biochemical, and omic levels [35, 36]. As a strong drought-responsive plant, *Axonopus compressus* developed a series of morphological features to acclimate the deficit water conditions. Drought stress can drive the plant to change the metabolic status of cutin and wax, which is ultimately accomplished through the associated gene-expression regulation [37] In current study, the unigene responsible for the wax biosynthesis (Cluster-37496.124448) was enriched under DS in comparison with CK and also up-regulated at the transcript level. As a distinctive morphological feature of protection against drought stress, cutin and wax play a critical role during water deficit conditions by restricting loss of water by means of leaf epidermis (non-stomatal transpiration) [38, 39], thereby enhancing water use capability in plants. In our study, the transcription factors that belong to the family AP2/ERF-ERF, MYB-related, zinc-finger, NF-X1 were found to be up-regulated. these transcription factors were known to regulate the biosynthesis of plant cutin and wax [40].

In water deficit conditions, the plants receive stress signals first and thereafter, a number of pathways in a sequence are triggered by phyto-hormones [41]. Subsequently, plant hormones play an important role in responding to scarce water conditions, and amongst the phyto-hormones, ABA is known to be a major homeostatic controller of abiotic stresses. Under water deficit conditions, the plants increase its ABA content and closing of stomata happen to avoid the excessive water loss by transpiration across stomata [42], such a high level of water loss would sometimes continue to occur even after transient recovery of leaf water status [43].

In current study, we observed 8 unigenes which encode NCED4 (9-cis-epoxycarotenoid dioxygenase) (Cluster-37496.127826) in *Axonopus compressus*, the crucial enzyme accountable for ABA biosynthesis (GO:0009688). Amongst 8 unigenes, 4 are up-regulated, and exhibited substantially different expression levels between DS and CK. Such behavior may possibly because of drought stress; *Axonopus compressus* plants had perceived the signals, which led to ABA production, which observed to be translocated to the above-ground. The transportation mechanism system in the stem take into action and trigger short-term responses such as stomatal closure [41, 44], which will help *Axonopus compressus* to avert the dehydration and improve water holding capacity under drought stress. Accordingly, the understanding of stress signal and subsequent molecular mechanisms in *Axonopus compressus* needs to be elucidated in succeeding experiments to reveal the drought-resistance mechanism.

When plants respond to drought, the rise in amounts of ABA leads to binding with PYR/PYL, which perform a significant role in quantitative regulation of stomatal fissure and transcriptional response to ABA [45, 46]. The PYR/PYL reconstructs the PYR/PYL protein conformations, and this alteration enables the PYR/PYL to interplay with 2 C protein phosphatase (PP2C), negative regulator type, to construct a substitute complex (ABA-PYR/PYL-PP2C). The ABA-PYR/PYL-PP2C- complex may impede PP2C activity and trigger SNF1-related protein kinase 2 (SnRK2s), a positive regulator. While the PP2C also inhibits the activity of SnRK2s [47]. Such mechanism stimulate the stress-responsive genes to down-regulate its expression and support the plants to acclimate water deficit conditions [48]. 715 PP2C were found in our experiments, from which 82 genes were remarkably down-regulated, and 17 SnRK2 were also identified. This symbolises that under water deficit, the low expression of PP2C in *Axonopus compressus* rescue the suppression of SnRK, and its expression promotes the closing of stomata. The SnRK expression also triggers the stress-related genes towards downstream and support *Axonopus compressus* keeps normal growth and persistence in drought.

In plants, the stress responding genes can easily be divided into two groups i.e., functional and regulatory [49]: The functional proteins particularly with respect to small molecular osmolytes (i.e., proline, betaine, and soluble sugar), enzyme protectants (POD, SOD and CAT), later embryogenesis abundant (LEA), and aquaporins. The regulatory proteins include TFs, phosphatase, protein kinase and phospholipid metabolic enzymes, that can regulate the stress-related expression of genes under abiotic stresses.

This symbolises that during water deficit conditions, the lowest expression of PP2C in *Axonopus compressus* would rescue the SnRK suppression. The PP2C expression would induce closing of stomata and trigger the downstream drought stress-related genes and assist *the Axonopus compressus to* sustain unhampered growth under drought. These proteins will prevent the plant cells from damaging encounters of stress by managing turgor pressure, including oxygen-free radicals scavenging and the structural intracellular bio-macromolecules protection [50, 51]. The transcriptional expression of drought related genes were regulated by the TFs.

It has been confirmed that members of the TF families i.e., MYB, AP2, DREB, bHLH, PLATZ, bZIP, C2H2, NAC WRKY and HB are involved in plant stress-response mechanisms [52]. In our research, we found 270 DEGs in *Axonopus compressus* TFs, among them, 216 were up-regulated and 54 were down-regulated. It is obvious from the results that large numbers of TF expressions differed significantly under water deficit.

In the DEGs HARBI1、EEF1A、SUMO、ATXR3、SEY1、NLR、COMM、DHAR3、GUF1、SFH5、SHOC2 、MAPK's, FYVE、PIP, LSM14, PERQ、BRCA1、TIM22、STY46-like、SPN1、NEDD8、DDX21、Meis2、RAD50、MAG2、MON1、and AKT2 are all down-regulated, and RXW8, DHAR2-like, EFR3, MOCS2A, GDSL, ANP1-like, TBC1, ERCC-6, ZDHHC2, EXO84B, BAHD, SHOC2, XBAT32, RNF38, SPIRAL1, NDRG1, HESO1, NBR1, STY46, TNNI3K, PP2C26, HVA22 and DAD1 are all up-regulated. The TF families i.e., GATA, ATF/CREB, ABI, bZIP, WRKY, EREBP, AP2-like, PTI6, TRAF, ARF1, ATF/CREB, ABA responsive, RING, MYB, NAC, TUB, HSF, GRAS , C2H2 , bZIP , SET, AP2/ERF-ERF , AUX/IAA, SNF2 and PTI6 some of them were up-regulated and some of them were down-regulated. Schematic diagram of potential defense mechanism and drought tolerance involving different gene related pathways in *Axonopus compressus* are shown in Figure 6.

Several unigene belong to similar family were reported to have distinct expression models under water deficit conditions, that might symbolize various characteristic function in response to stress condition, and their behavior to drought [47, 53]. A particular transcription factor could have been associated with one and/or more than one members in the particular category and even other groups [54], which also reveals the complexity by which transcriptional categories of genes that controls the drought responses of *Axonopus compressus*. The current study provides helpful data for additional functional reviews of the TFs' functions in order to develop tolerance in *Axonopus compressus*.

During signal transduction, the post-translational protein alterations are the essential phenomenon, of phosphorylation and de-phosphorylation in plants under stress condition [55]. Phosphorylation and dephosphorylation can trigger a number of proteins and enzymes with regulatory functions to control a wide range of cellular mechanisms or signal process. Therefore, likely to halt or initiate the enzyme activity during drought, unless adjusting the concentration of resultant proteins or intracellular enzymes activity, thus it have been established that protein phosphorylation adjustment plays a significant function in the response to drought [55]. It is revealed that Arabidopsis have thousands of kinases [56], the CDPK (calcium-dependent protein kinase) and MAPK (Mitogen-activated protein kinase) are considered critical signaling mechanisms in plants under stress. The stress signals were transduced into cellular processes by MAPK, succeeding phosphorylation processes of distinct downstream proteins to on and/or off their activities [57]. As a distinctive antenna, CDPKs can immediately transform upstream $Ca2^{+}$ signals to protein phosphorylation at downstream because of its structure of multifunctional protein, which couples calcium binding, and signaling capacities in product of single gene [58].

In our study, 11089 protein kinases were observed, Of which 344 were DEGs, out of which 182 unigenes were up-regulated and 162 unigenes were found down-regulated, besides, the study identified 16 and 8 DEGs encoding MAPK and CDPK respectively, and there were 9 and 2 DEGs up-regulated and 7 and 6 DEG were down-regulated, respectively. Whilst, it suggests that the MAPK and CDPK pathways engage in the signaling of drought stress as signal transduction variables and plays an important role against osmotic stress in *Axonopus compressus*. The plants that faced water deficit conditions; osmo-tension would provoke ROS built-up in cells. Plants had previously evolved the defense mechanism to extinguish the ROS and alleviate cellular damage. This defense mechanism comprises of SOD, POD and CAT. Current study showed 5 SOD, 3 CAT and 25 POD DEGs. In addition, proteins of small-molecule such as proline, betaine and LEA (late embryogenesis abundant) likewise play significant roles in restraining the cells from the damaging effects of drought.

Since, LEA is a major osmotic regulator, LEA also belongs to a large protein group that was observed to be first accumulated while dehydration under the lateral arena of developments. In current study, we observed 370 unigenes which can encode LEA. We observed 19 DEGs in *Axonopus compressus* (DS with CK) all of which were up-regulated. **Therefore, the unigenes are crucial for developing sustainability against the water shortage for** *Axonopus compressus***. Additionally, the osmotic regulation ability of plants to hamper water loss is an additional fundamental feature to survive in drought. The bidirectional water channel AQP (aquaporin) in plants responsible for trans-membrane water mobility and long-distance water transportation.** It is established that plants have the potential to defy the numerous stress circumstances through regulating the proteins related to water channel [59]; in *Axonopus compressus*, we observed 363 unigenes coding AQP, including 31 unigenes differentially expressed, 2 unigenes were found down-regulated and none up-regulated. The AQP's showed reduced expression, demonstrated the decline in AQP's activity and/or ceased, the cessation of AQPs be able to limit moisture loss thus sustain water related homeostatic processes of plants, consequently, improving tolerance against drought in *Axonopus compressus*.

Conclusion

Under drought conditions, processing of stress signals, signal transduction, regulating gene expression and the subsequent downstream functional genes are essential variables in plant response to adversity. In current study, we observed 263835 unigenes in *Axonopus compressus* based on RNA-Seq, in which 7445 were differentially expressed unigenes (DS and CK). Overall, 2747 were up-regulated and 2502 down-regulated unigenes. In addition, 352 (TFs) were found to be differentially expressed in *Axonopus compressus* (DS and CK). Current findings indicate that these genes are involved in the campaign of resistance to drought stress and have a highly significant and complicated role. This current study provides valuable data for the molecular mechanisms underlying drought tolerance. The qRT-PCR validation of transcripts sets the basis for further investigations of the gene regulatory networks under drought stress and other abiotic stress factors in *Axonopus compressus*.

Declarations

Competing Interests

The author(s) declare no potential conflicts of interest with regards to the research, authorship, and/or publication of this manuscript.

Author Contributions

Conceptualization: MN, ZW
Data curation: MN
Formal analysis: MN, FA, AZ
Funding acquisition: MN, ZW

Investigation: MN, LL
Methodology: MN
Writing – original draft: MN, FA, YH, LL
Writing – review & editing: MN, FA, ZW, SS, UA

All authors have read and approved the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author at reasonable request.

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Abbreviations

DEGs: Differentially expressed genes

DEPs: Differentially expressed proteins

FPKM: Fragments Per Kilobase Million

GO: Gene Ontology

KO: KEGG Orthology

GST: Glutathione S-transferase

DS: Drought Stress

CK: Control

NGS: Next Generation Sequencing

KEGG: Kyoto Encyclopedia of Genes and Genomes

MAPK: Mitogen-activated protein kinase

NDEGs: No significant difference in genes

RNA-seq: RNA sequencing

ROS: Reactive oxygen species

MDA: Malondialdehyde

EL: Electrolyte Leakage

cDNA: Complementary DNA
RNA-Seq: RNA sequencing
DEGs: Differentially expressed genes

CDSs: CoDing Sequences

ORF: Open Reading Frame
ncRNAs: Non-coding RNAs

DE: Differentially expressed
TFs: Transcription factors

BP: Biological processes

CC: Cellular components

MF: Molecular functions

POD: Peroxidase

SOD: Superoxide Dismutase

CAT: Catalase

LEA: Later Embryogenesis Abundant

ABA: Abscisic acid

PYR/PYL/RCAR: Pyrabactin Resistance1/Pyr1-Like/Regulatory Components of Aba Receptors

PP2C: Protein Phosphatase type 2C

SnRK2: Serine/Threonine Kinases2

SNF1: Sucrose Non-fermentation 1

PEG: Polyethylene glycol

LWP: Leaf water potential

dNTP: Deoxynucleoside triphosphate

qRT-PCR: Real-Time Quantitative Reverse Transcription PCR

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Figures

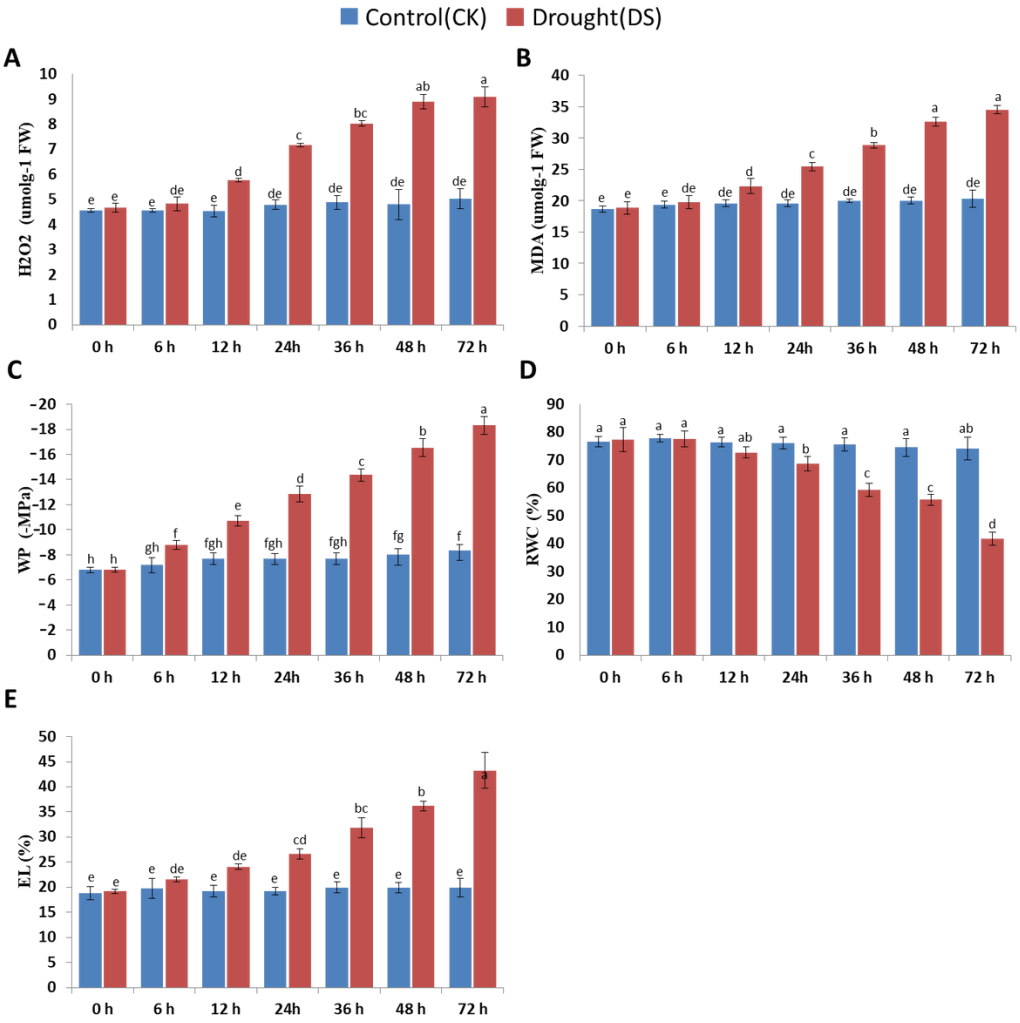

Figure 1

Effect of drought on the physiologiacal traits of the Axonopus compressus. A; effect of drought on H2O2 production, B; Effect of drought stress on MDA content, C; drought induced alteration in WP, D; Effect of drought on leaf RWC, E; drought stress effect the Electrolyte leakage(EL) in Axonopus compressus.

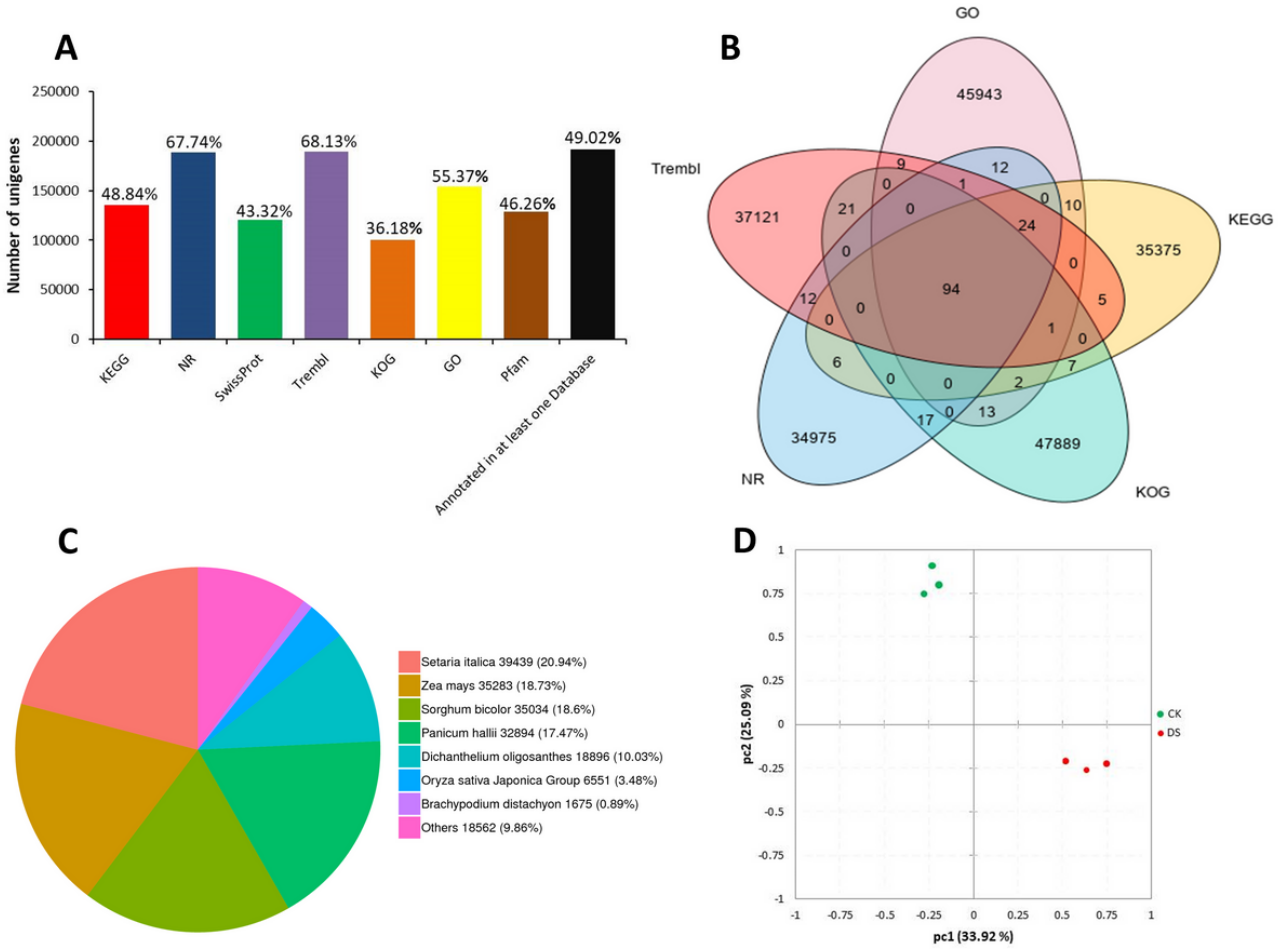

Figure 2

A; Unigenes matched in seven databases, B; Venn diagram of differential BLAST results for Axonopus compressus. The number of unigenes annotated with five databasesand the co-annotated gene number are also presented, C; Homologous species distribution of Axonopus compressus annotated in the NR database, D; Principal component analysis of transcriptome data

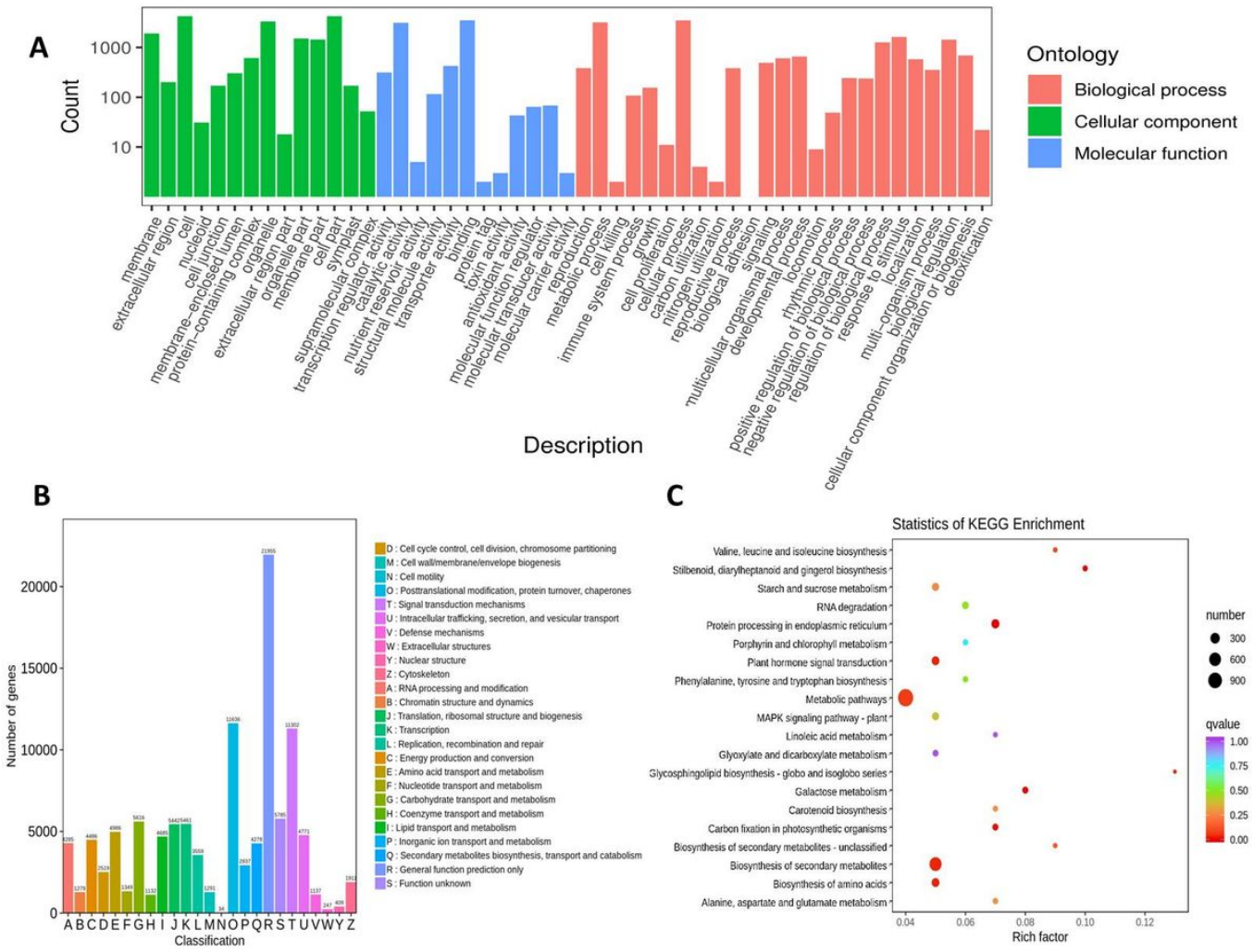

Figure 3

A; Functional classification of GO terms for Axonopus compressus. The number of genes in a specific sub-category within the main category is shown on the y-axis; the name of the sub-category is shown on the x-axis, B; KOG functional classification of the Axonopus compressus transcriptome. The unigenes with significant homologies in the KOG database (E-value <10−5) were classified into 26 KOG categories. Capital letters on the x-axis indicate KOG categories on the right side of the histogram C; KEGG pathway enrichment of DEGs in Axonopus compressus. The graph shows only the top 20 enriched pathways comparing DS with CK; different colors denotes different Q-Values, and the size of the bubble represents the number of DEGs.

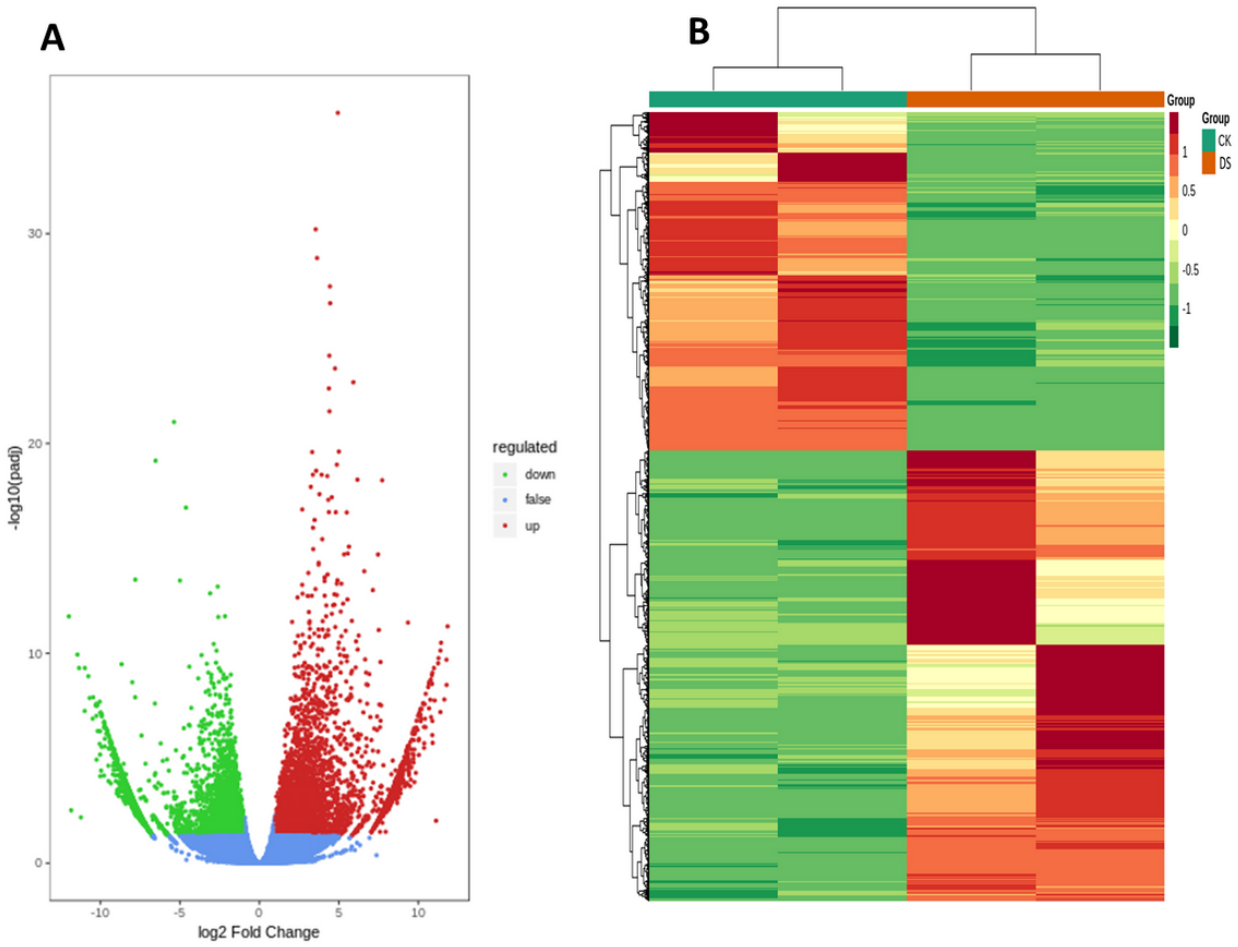

Figure 4

A; Volcano plot of differentially expressed unigenes of Axonopus compressus. The numbers of up- and down-regulated unigenes in CK and DS are shown. Red indicates up-regulated genes, green represents down-regulated unigenes, and blue represents no significant difference in gene expression, B; cluster analysis of differentially expressed unigenes of Axonopus compressus in responses to CK and DS treatments.

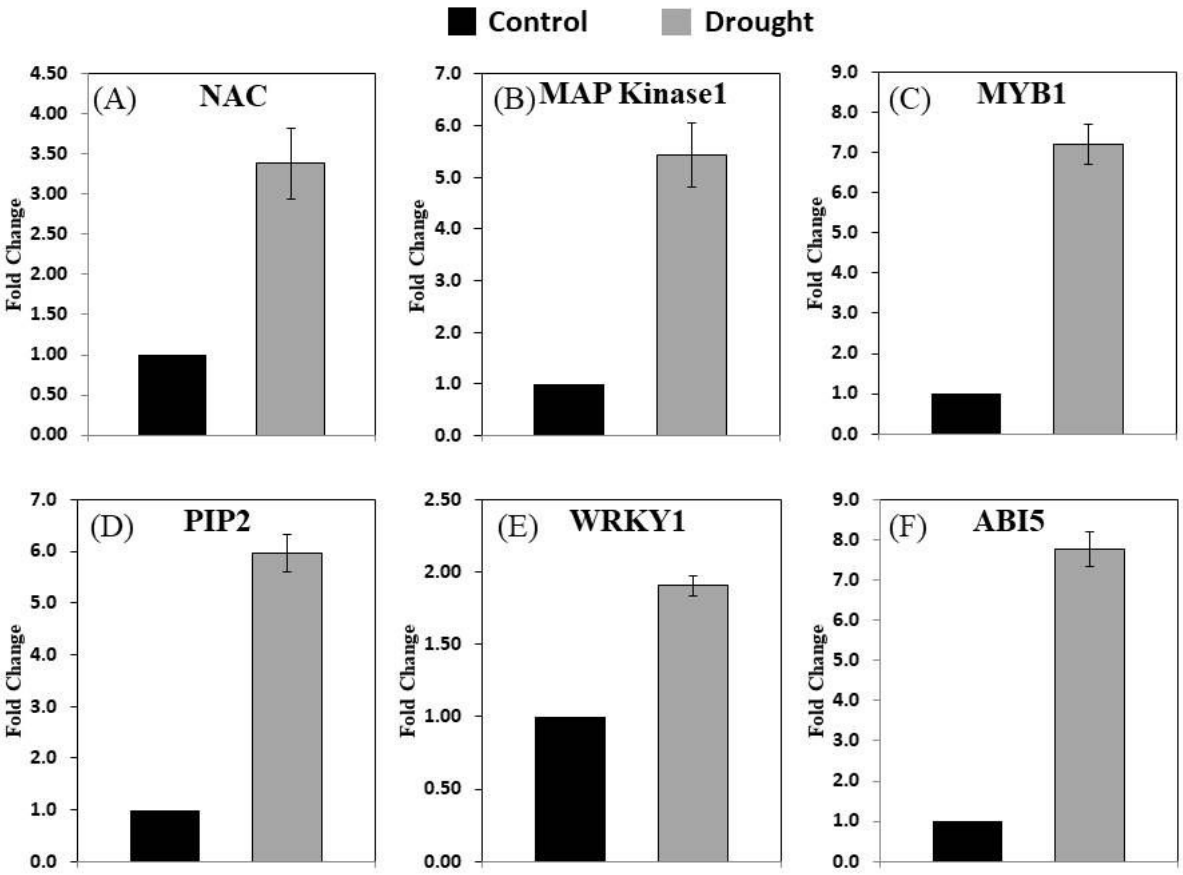

Figure 5

The qRT-PCR expression validation of drought-responsive genes in A. compressus. The bars showed relative expression of A; NAC ,B; MAP Kinase1, C; MYB1, D; PIP2, E; WRKY1, F; ABI5.

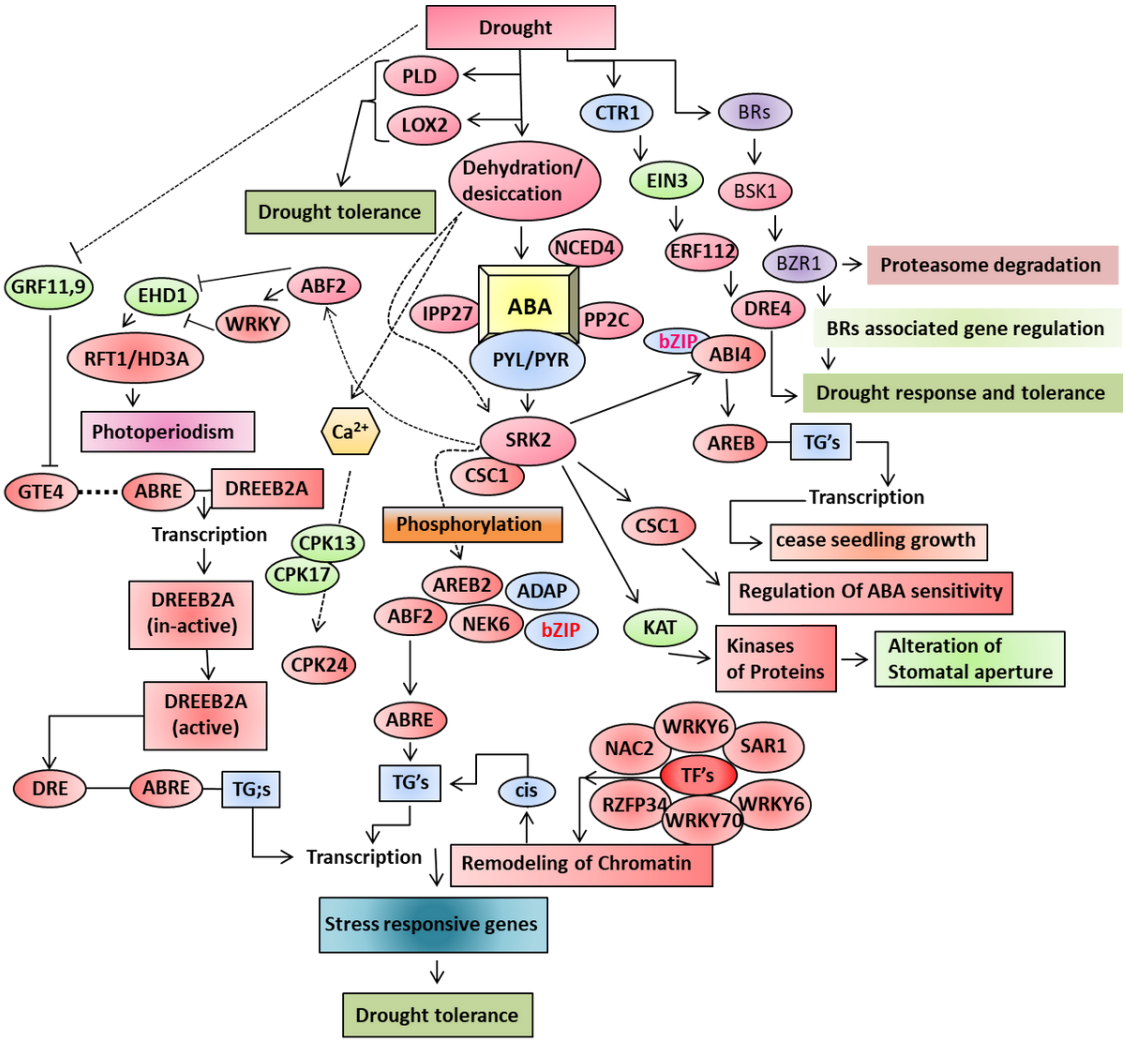

Figure 6

Schematic diagram of potential defense mechanisms for drought stress response and tolerance pathways in carpet-grass (Axonopus compressus L.).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.Metabolicpathwayanalysiskegg.xlsx
- Additionalfile2.Differentially.xlsx
- Additionalfile3.GOclassification.xlsx
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