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Variations in Protein and Gene Expression Involved in the Pathways of Carbohydrate, Abscisic Acid, and ATP-Binding Cassette Transporter in Soybean Roots under Drought Stress

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Abstract: Plant roots play crucial roles in their response to drought conditions. However, the molecular responses in soybean roots to drought stress remain unclear. We investigated the alterations in the protein expression in the roots of a drought-resistant soybean cultivar 'Jiyu 47' during the seedling phase based on tandem mass tag (TMT) proteomics analysis. The results revealed significant variations in the expression of the proteins involved in several metabolic pathways in soybean roots, including sucrose metabolism, abscisic acid (ABA) metabolism, and the ATP-binding cassette (ABC) transporter pathway. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses revealed a coordinated expression pattern of the proteins involved in the various cellular pathways responding to drought stress in soybean. The increased production of sucrose and betaine enhanced the inhibition of the damage caused by reactive oxygen species (ROS) and the tolerance of drought stress. The results of the physiological variations showed that sucrose metabolism, ABA metabolic mechanism, and the ABC transporter pathways played an important role in the antioxidant defense system in response to drought stress in soybean roots. The results of quantitative real-time PCR revealed the up-regulated expression of three genes (i.e., *GmPYR1*, *GmHO-1*, and *GmSOD*) involved in ABA biosynthesis and the signaling pathway. This study provides novel insights into the comprehension of the molecular pathways regulating the soybean root response to drought stress.

Keywords: soybean root; drought stress; proteomics; carbohydrate; abscisic acid; ATP-binding cassette transporter

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1. Introduction

During the current climate change, drought is one of the most significant factors affecting crop production [1]. Due to their immobility, plants generally develop various mechanisms in response to abiotic stresses, e.g., drought, salinity, and extreme temperatures. These abiotic stresses greatly limit the distribution of plants, alter their growth and development, and reduce crop productivity [2]. Therefore, it is important to understand the molecular mechanisms regulating the plant response to these abiotic stresses in order to perform the molecular breeding of crops with resistance and to maintain crop yield [2]. As a major crop worldwide, soybean is highly vulnerable to water deficit, which frequently causes a serious loss of production globally. Drought stress is considered to be one of the main factors severely limiting soybean growth and development [3]. Therefore, it is very important to improve the soybean resistance to drought stress [4].

Drought is generally considered to be the most ordinary environmental stress of plants under the current climate change; it has a great impact on plant growth and crop yield,

affects various morphological and physiological characteristics of crops during different developmental stages, and ultimately causes considerable agricultural and economic losses [5]. Drought stress causes a reversible decline in the water content of the leaves, photosynthetic activity, and cell membrane stability, resulting in an increased production of reactive oxygen species (ROS), lipid peroxidation, and cell membrane damage. Furthermore, the increased enzymatic activities of superoxide dismutase (SOD) and peroxidase (POD) enhance the glutathione metabolism and vitamin C production, playing a key role in the prevention of drought damage [6]. Moreover, plants regulate osmosis through the accumulation of soluble sugar, proline, and free amino acids to improve both their enzymatic and their non-enzymatic antioxidant activities [7].

To date, transcriptomic studies have usually been performed to reveal the associations between the expressions of the genes involved in photosynthesis as well as starch and sucrose metabolism during the plant's response to drought, with sucrose and trehalose playing an important role in osmotic regulation, stress protection, and ROS scavenging [8,9]. In addition, proteomics, as well as other "omics" investigations, have greatly improved our understanding of the molecular mechanisms underlying the plant stress tolerance capacity [10,11].

The ATP-binding cassette (ABC) transporters are an important class of transmembrane proteins with highly conserved structures and functions, and they are widely present in various organisms, including prokaryotes (i.e., bacteria and archaea) and eukaryotes (i.e., plants, animals, fungi, and protists) [12]. Studies have revealed that plant genomes encode a total of 120 to 140 ABC transporters, categorized into 8 subfamilies, i.e., ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, ABCG, and ABCI, based on the transmembrane domains (TMDs) and the nucleotide-binding folds (NBFs) [13–17]. In plants, ABC transporters play crucial roles in various physiological processes, including the transportation of hormones, lipids, metals, and secondary metabolites and the detoxification of xenobiotics, as well as the facilitation of the plant–microbe interactions [13]. In particular, the *Arabidopsis* genome encodes more than 100 ABC transporters [13,14], which are mainly located on the organelle membranes and are involved in the transport of a large group of substances, e.g., hormones, heavy metal complexes, lipids, and glycosides [14]. The plant ABC proteins utilize ATP binding and hydrolysis to generate energy and use conformational changes to facilitate the transmembrane transportation of diverse groups of substances, including carbohydrates, lipids, peptides, terpenes, cellular metabolites, chelates of heavy metals, and metal ions [18]. Studies have shown that ABC transporters are closely related to drought resistance in various groups of plants. For example, *AtABCG36* promotes drought resistance by reducing the content of sodium in *Arabidopsis* [19], while both *AhABCG22.1* and *AhABCG22.2* are significantly up-regulated in peanut under drought stress, resulting in the rapid accumulation of ABA in the peanut leaves and improved drought resistance [20]. In barley, *HvABCB13*, *HvABCG25*, and *HvABCB48* are significantly up-regulated under drought stress; thus, they regulate plant drought tolerance [6]. Although the ABC transporters play varied roles, each ABC protein contains at least one highly conserved ATPase domain for ATP interactions. Notably, the molecules transported by the same ABC protein may significantly differ in their chemical and structural properties [21].

Plant hormones are signaling molecules that regulate crucial aspects of growth, development, and response to environmental stresses. Abiotic stresses, such as drought, salinity, heat, cold, and flooding, have overwhelming effects on plant growth, development, and crop yield. Therefore, the plant's adaptation and tolerance to such stresses require sophisticated sensing, signaling, and stress response mechanisms [22]. Plants respond and adapt to the environmental stresses through a complex network of factors involved in the regulation of stress hormone signaling and gene expression in order to enhance their resistance and survival under the adverse conditions [23]. Among these factors, many protein kinases play an important role in stress signal perception and transduction [24]. The level of ABA in plants is enhanced by 9-cis-epoxycarotenoid dioxygenase (NCED), which in turn triggers the activation of genes involved in plant response to drought stress,

leading to the initiation of physiological processes such as stomatal closure [25,26]. Furthermore, the ABA-responsive binding factor (ABF) is involved in the expression of genes that are responsive to drought stress; thus, it preserves the plant's ability to resist drought stress [27].

To clarify the key molecular events in plant response to drought stress at the transcriptional and translational levels, we utilized the label-free quantitative proteomics analysis [28] and liquid chromatography with tandem mass spectrometry (LC-MS/MS) technologies, as well as quantitative real-time PCR (qRT-PCR) analyses, to investigate the variations in protein and gene expression as well as the physiological factors involved in the regulatory mechanisms underlying the soybean root response to drought stress. The goals of our study were to characterize the molecular and physiological variations in soybean roots under drought stress and to provide experimental evidence to support further exploration of the molecular mechanisms regulating the soybean root response to drought stress and identification of the metabolic pathways involved in the soybean root resistance to drought.

2. Materials and Methods

2.1. Plant Materials

The seeds of the drought-resistant soybean [*Glycine max* (L.) Merr.] cultivar 'Jiyu 47' were obtained from the Jilin Academy of Agricultural Sciences, Changchun, China. The selection of this soybean variety was based on its short growing season, high and stable yield, strong stress resistance, excellent quality, and wide adaptability under various environmental and ecological conditions. The seeds were first sterilized with 1% sodium hypochlorite (NaClO) and then cultured on peat moss for germination in the dark for 3 d at 25 °C. The seedlings were cultured in 1 L plastic pots containing 1/2 Hoagland nutrient solution (pH = 5.8), maintained in a controlled plant growth chamber under constant relative humidity (60%) with a photoperiod cycle of 14 h of light (light intensity 300 μmol m^{-2} s^{-1}; 25 °C) and 10 h of dark (22 °C). The fresh nutrient solution was changed every 2 d. The 7-day-old seedlings were cultured in 1/2 Hoagland nutrient solution containing 20% PEG6000, which was used to create the drought stress [29]. The soybean root apices (about 3 cm in length) of both the experimental and control groups, i.e., the groups treated with and without PEG6000, were sampled at 24 h, respectively, quickly placed in liquid nitrogen, and then stored in a freezer (−80 °C) until protein isolation. The root samples treated without PEG6000 were used as the controls.

2.2. Proteomics Analysis and Functional Enrichment Analysis of Differentially Expressed Proteins Identified in Soybean Roots

Proteomics analysis of the mass spectrometry sequencing (LC-MS/MS) and protein annotation of the soybean roots treated with 20% PEG6000 were performed as previously described [29]. Briefly, proteins were extracted from the soybean roots using phenol extraction; the resulting peptides were labeled using a Tandem Mass Tag (TMT) Systems kit (A52045; Thermo Fisher Scientific Co., Ltd., Shanghai, China) and separated using high-performance liquid chromatography (HPLC) into 60 components in 60 min. These peptides were then merged into 9 fractions for further separation based on the EASY-nLC1200 ultra-high performance liquid phase system (Semel Fisher Technology Co., Ltd., Shanghai, China), with mobile phase A (0.1% formic acid and 2% acetonitrile) and mobile phase B (0.1% formic acid and 90% acetonitrile). The settings of the liquid phase gradients in mobile phase B were as follows: 0–26 min, 6% to 25%; 26–34 min, 25% to 35%; 34–37 min, 35% to 80%; and 37–40, min 80%, with the flow rate maintained at 450.00 nL/min. Maxquant v.1.5.2.8 (https://www.maxquant.org/; accessed on 6 November 2023) was used to process the secondary mass spectrometry data. The reverse tandem mass spectrometric analysis of the soybean root protein sequences was performed based on the UniProt and the reverse bait databases (https://www.ebi.ac.uk/; accessed on 6 November 2023) to determine the quantifiable proteins. Differentially expressed proteins (DEPs) were defined

based on |fold change| > 1.2 or <0.83 and $p < 0.05$ using the EDGER package (v.3.12.1) (http://www.r-project.org/; accessed on 6 November 2023) [29] in comparison with the control groups. Correspondingly, the genes encoding the DEPs were defined as differentially expressed genes (DEGs). Based on the differentially expressed multiples, the DEPs were further categorized into four groups (i.e., Q1 to Q4), with cluster analysis performed to detect the correlation of the protein functional domains with the different expression multiples (i.e., <0.667 in Q1, in the range of 0.667–0.769 in Q2, in the range of 1.3–1.5 in Q3, and >1.5 in Q4).

Gene Ontology (GO) annotations of the DEPs were conducted using the UniProt-GOA database (http://www.ebi.ac.uk/GOA/; accessed on 6 November 2023) to identify the three categories of GO terms, i.e., cellular component (CC), molecular function (MF), and biological process (BP), involved in the soybean root response to drought stress. The enrichment analysis of the DEPs was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/; accessed on 6 November 2023) to detect the metabolic pathways involved in the soybean root response to drought stress.

2.3. Determination of Physiological Indices of Soybean Roots

To evaluate the variations in physiological indices, the soybean root apices (about 3 cm in length) were sampled at 0, 3, 6, 9, 12, and 24 h, respectively, after the treatment with 20% PEG6000, and were immediately wrapped in tin foil, placed in liquid nitrogen, and then stored in a freezer (−80 °C). The contents of sucrose (AKPL006C; Beijing Shenggong Biotechnology Co., Ltd., Beijing, China), betaine (G0122W; Suzhou Grace Biotechnology Co., Ltd., Suzhou, China), and ABA (ml077235; Shanghai Meilian Biotechnology Co., Ltd., Suzhou, China) in the soybean roots were determined using the indicated kits, respectively, to evaluate their involvement in the molecular response of the soybean root to drought stress. The root samples treated without PEG6000 were used as the controls. Each experiment was repeated with three biological replicates.

2.4. RNA Extraction and Quantitative Real-Time PCR Analysis

To perform the qRT-PCR analysis, the root apices (about 3 cm in length) were collected at 0, 3, 6, 9, 12, and 24 h, respectively, after the treatment with 20% PEG6000, and were quickly treated with liquid nitrogen and then stored in a freezer (−80 °C). The qRT-PCR analysis was performed to evaluate the transcription levels of a total of 17 DEGs involved in the soybean root response to drought stress, with the primers designed using Primer 3.0 (http://primer3.ut.ee/; accessed on 6 December 2020) (Table 1). Using the soybean tubulin gene (GenBank Gene ID 100811275) as the internal control, qRT-PCR was performed using a Mx3005P instrument (Stratagene, La Jolla, CA, USA) with a reaction volume of 25 µL, which was composed of 2 µL of cDNA template (50–100 ng), 0.5 µL each of the forward and reverse primers (10 mM), 12.5 µL of 2 × SYBR Premix ExTaq (TaKaRa, Beijing, China), and 9.5 µL of double-distilled H_2O. The melting curve analysis was completed as follows: initial denaturation at 94 °C for 30 s, followed by 40 amplification cycles of denaturation at 94 °C for 5 s and annealing at 60 °C for 15 s, and a final extension at 72 °C for 10 s. The relative expression level of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method [30]. The Statistical Investigation Data Processing System [31] was used to determine the significant differences between the treatments. The root samples that were not treated with PEG6000 were used as the controls. Each experiment was repeated with three biological replicates.

2.5. Statistical Analysis

All the measurement data were shown as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to determine the statistical significance between two groups. Multiple comparisons were conducted based on Duncan's multiple range test at a significance level of $p < 0.05$ (SPSS version 18.0). The statistical significance of the differences in the physiological indices and gene expression levels of the soybean roots

under drought stress was determined at $p < 0.05$. Graphs were generated using Microsoft Excel 2010 (Redmond, WA, USA) and TBtools (2.07) [32].

Table 1. Primers and their sequences used in the qRT-PCR analysis. "F:" and "R:" indicate the forward and reverse primers, respectively.

Primer	Sequence (5′→3′)
GmABCB1	F: AGCAGTGGAAATGGTCCGAA R: TTCTCCCCACCACCGTTACT
GmABCB20	F: TGATGGTTTCTCGGGGACTG R: CTCCATGGGCTGAGACGTG
GmABCB25	F: GAACATGGTGATGCAGTGCC R: GTGGCTGCAATCAACAGAGC
GmABCC2	F: CGAGGAATCTTTGAACCAGCG R: TGATGGAGCCTGTTCTTGGC
GmABCC4	F: AACTCAGAGCTCGACAAGCC R: AGTTTGCTTCCACGTCCCAT
GmABCF2	F: CCAAGAGAGGAGGCAAAGCA R: GGAAAGAGGATGCGAGCAGA
GmABCF4	F: CAAGCGGCTAAGAGGAGTGG R: ATCTTCCCGGTTTGGGTAGC
GmPDR12	F: AGAACCAATGACGTCTGAAGGT R: CAATGCAAAGAGAACGGCGA
GmPYR1	F: TGGACAAAACGCACAGCG R: GTCCTCGAACTCTTCCGGG
GmHO-1	F: TCCCAGTCCCAATCGCTCTA R: CATGACAGCAGCACGAACAC
GmSOD	F: GAGCCCTGTTGACCAGAAAAAC R: GTACACGTGCAACCCACGA
GmUGTA1	F: CACAACTCCTCAGAACAAC R: GAGATAGGGAATGGGAAGAA
GmUGTA2	F: CTAAGATGTTGGTGGAAGA R: CTCAGCACTGTCTCTAAC
GmSUS6	F: GAGCTTGGTGAACCTTGTGG R: GTTGCGGTAGCGATCGGTTT
GmSUS7	F: GACAGCCTTAACTCTGCTGC R: TTCATCGGGTCTGGTGCTTG
GmUDP141	F: GCTTGTGTTCTCATCTTC R: AACTGTCCAATCTGAATCT
GmUDP189	F: CTATGGTGTGGAGGAGAT R: CATTCGTAGATGAGCAGTAT
Tubulin	F: GGAAGGCTTTCTTGCATTGGTA R: AGTGGCATCCTGGTACTGC

3. Results

3.1. Proteomics Analysis and Functional Enrichment Analysis of Differentially Expressed Proteins in Soybean Roots under Drought Stress

A total of 63,286 effective spectra were defined out of 325,699 secondary spectra obtained from the soybean roots treated with or without 20% PEG6000 based on mass spectrometry analysis. A total of 39,923 peptides were detected, with 26,568 unique peptides identified. Among a total of 7875 out of 8687 proteins identified were quantifiable. A total of 468 DEPs were defined in soybean roots identified by label-free technology and LC-MS/MS based on $|\text{fold change}| > 1.2$ or < 0.83 and $p < 0.05$ (Figure 1). The proteomics data of mass spectrometry were deposited to the ProteomeXchange Consortium through the proteomics identifications database (PRIDE) under identifier PXD033092 (https://www.ebi.ac.uk/pride/; accessed on 7 April 2022).

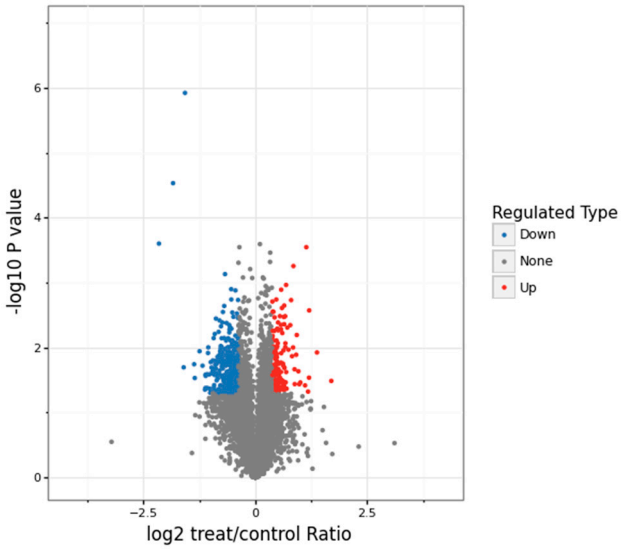

Figure 1. Volcano plot of a total of 468 differentially expressed proteins (DEPs) (144 with up-regulation and 324 with down-regulation) identified in soybean roots treated with 20% PEG6000. Red and blue dots represent up-regulated and down-regulated DEPs, respectively. X-axis and Y-axis represent the log2(fold change) and −lg(false discovery rate adjusted *p* value), respectively.

GO annotation and KEGG enrichment analysis of the DEPs were performed to evaluate the GO terms and metabolic pathways involved in the soybean root response to drought stress. The results of the GO annotation showed that among the three categories of GO terms, the top three GO terms in the category of biological processes included the cellular process, metabolic process, and response to stimulus (Figure 2); cell, organelle, and membrane were the top three most enriched GO terms in the category of cellular components; and in the category of molecular functions, both catalytic activity and binding were the top two most enriched GO terms. It is noteworthy that transporter activity was one of the significantly enriched GO terms in the category of molecular functions (Figure 2), suggesting the potential functions of these proteins in governing the soybean root response to drought stress. Both the up-regulated and down-regulated DEPs were revealed as having largely similar GO annotation patterns (Figure 2A,B).

The results of the KEGG enrichment analysis of the DEPs showed that the DEPs of the Q1 group were significantly enriched in a total of nine pathways, e.g., the methyltransferase domain, ThiF family, and cullin protein neddylation domain; the DEPs of group Q2 were mainly enriched in a total of 15 pathways, including ABC transporter and the NB-ARC domain; and groups Q3 and Q4 were each enriched in three metabolic pathways (Figure 3).

3.2. Variations in Expression Pattern of Genes Encoding ABC Transporters in Soybean Roots under Drought Stress

The qRT-PCR analysis was performed based on a total of eight ABC genes (i.e., *GmABCB1*, *GmABCB20*, *GmABCB25*, *GmABCC2*, *GmABCC4*, *GmABCF2*, *GmABCF4*, and *GmPDR12*) encoding eight differentially expressed ABC transporters based on the proteomics analysis of the soybean roots under drought stress. The results showed that most of these genes, except for *GmPDR12*, reached the highest expression levels at 9 h after the drought stress treatment; then, the expression level decreased, whereas the expression of *GmPDR12* rapidly reached the highest level at 3 h and then gradually decreased as the treatment time of the drought stress was increased (Figure 4). These results revealed that these genes encoding ABC transporters exhibited a rapid up-regulation in their expression levels up to 9 h after the drought stress treatment, thereby facilitating the enhanced production of ABC transporters and the efficient transportation of various substances. At 12 h or 24 h after the drought stress treatment, the expression of these genes was down-regulated, leading to the decreased production of ABC transporter proteins and ultimately establishing homeostasis

for the substances conferring the soybean root resistance against drought stress. These results of the qRT-PCR analysis were consistent with those of the proteomics analysis and showed the down-regulation of the proteins encoded by these genes at 24 h in soybean roots under drought stress treatment.

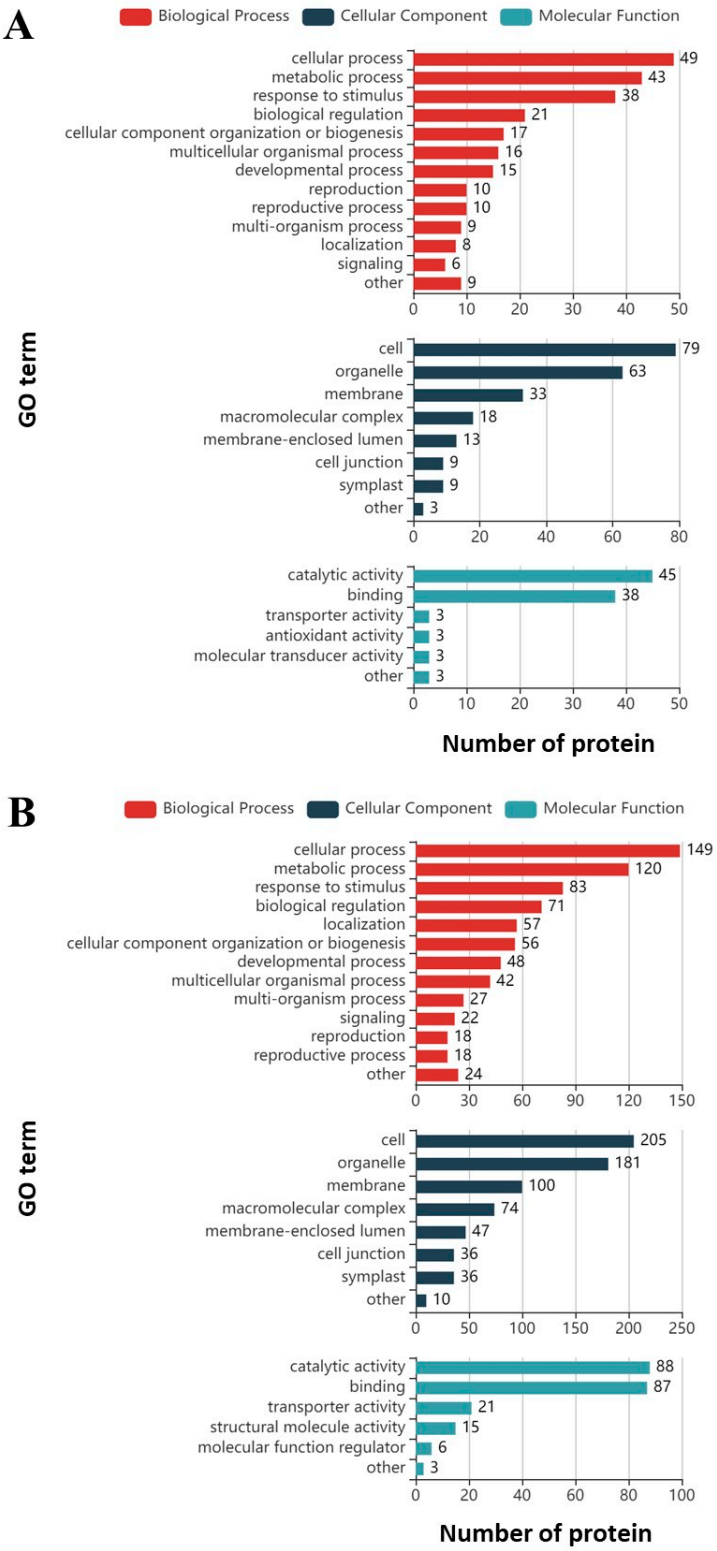

Figure 2. GO annotation of differentially expressed proteins (DEPs) showing up-regulated (**A**) and down-regulated (**B**) expression in soybean roots treated with 20% PEG6000.

Figure 3. KEGG metabolic pathway enrichment based on the differentially expressed proteins (DEPs) in groups Q1 to Q4 identified in soybean roots treated with 20% PEG6000.

3.3. Evaluation of the Expression Levels of Genes Associated with the ABA Signaling Pathway in Soybean Root Response to Drought Stress

Due to the pivotal regulatory roles that ABA plays in the plant response to drought stress, the qRT-PCR analysis was performed based on a group of three genes, i.e., *GmPYR1*, *GmHO-1*, and *GmSOD*, encoding three ABA pathway-associated proteins based on the proteomics analysis, i.e., ABA receptor pyrabactin resistance (PYR), heme oxygenase (HO), and SOD, respectively (Figure 5). The results showed that the expression level of the gene *GmPYR1* exhibited a continuously increasing trend throughout the 24 h drought stress treatment (Figure 5A), whereas the expression levels of both *GmHO-1* and *GmSOD* demonstrated a similar pattern, i.e., they increased to the highest level at 9 h after the drought stress treatment; subsequently, this was followed by a decrease at 12 h and 24 h (Figure 5B,C). These results indicated that in the soybean root response to drought treatment, a sustained up-regulation was revealed in the expression levels of the genes encoding ABA receptor proteins, resulting in an enhanced production of ABA receptors, which ultimately enhanced the soybean root resistance to drought stress by facilitating the increased binding of ABA and the subsequent activation of the ABA signaling pathway.

3.4. Evaluation of Expression Patterns of Genes Associated with Sucrose and Betaine Biosynthesis in Soybean Root Response to Drought Stress

A group of two proteins, i.e., sucrose synthase (SUS), which is involved in sucrose biosynthesis, and four proteins (UDP-glycosyltransferase and UDP-glucose 4-epimerase) that participate in the betaine biosynthetic pathway were detected based on the proteomics analysis of the soybean roots under drought stress; the expression levels of the genes encoding these proteins were quantified based on the qRT-PCR analysis. The results revealed the similar expression patterns of the two genes (*GmSUS6* and *GmSUS7*) encoding SUS, i.e., they rapidly increased to the highest levels at 3 h after the drought stress treatment and then decreased to the lowest levels at 24 h (Figure 6A,B). Two different patterns were detected in the four genes encoding the proteins involved in the betaine pathway. In particular, the expression levels of the genes *GmUGTA1* and *GmUGTA2* encoding UDP-glycosyltransferase were consistently increased to the highest levels at 24 h after the drought stress treatment (Figure 6C,D), while the expression levels of the genes *GmUDP141* and

GmUDP189 encoding UDP-glucose 4-epimerase first exhibited an increasing trend up to 3 h and 6 h, respectively, after the drought stress treatment and then decreased up to 24 h (Figure 6E,F).

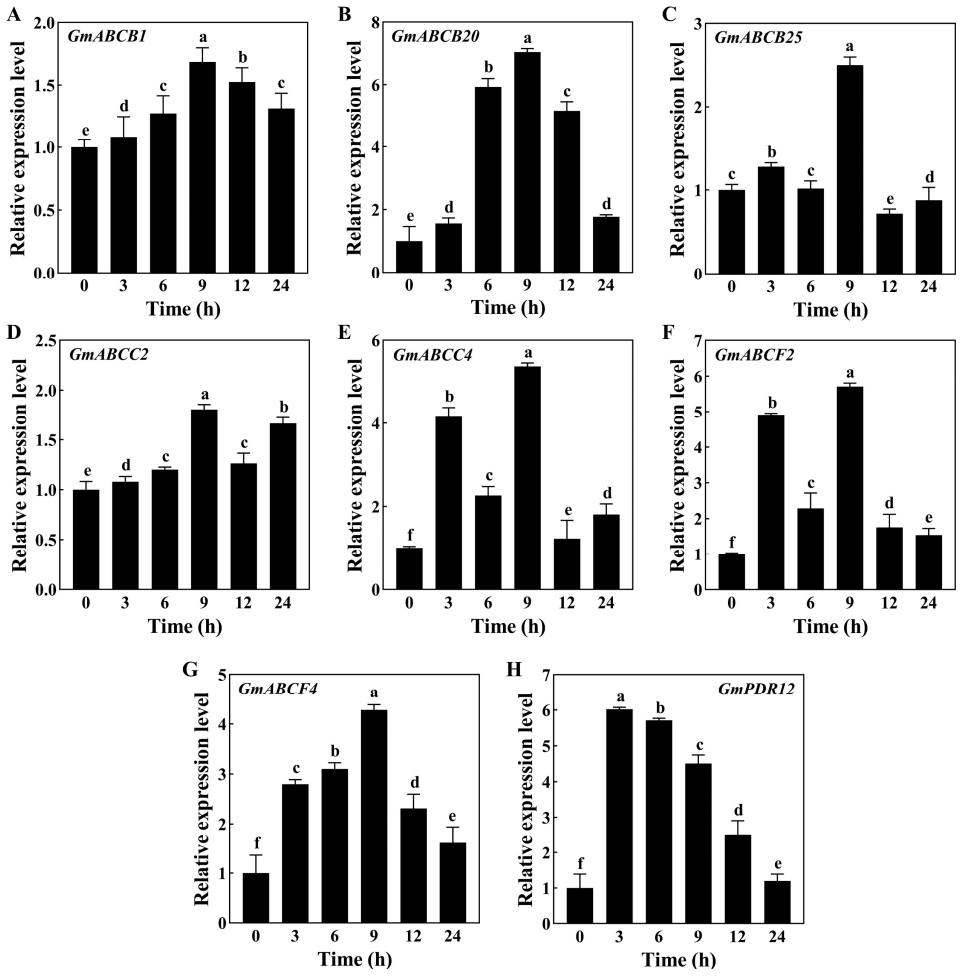

Figure 4. Relative expression levels of ABC transporter genes *GmABCB1* (**A**), *GmABCB20* (**B**), *GmABCB25* (**C**), *GmABCC2* (**D**), *GmABCC4* (**E**), *GmABCF2* (**F**), *GmABCF4* (**G**), and *GmPDR12* (**H**) in soybean roots under drought stress treatment based on qRT-PCR analysis. Data are shown as mean ± standard deviation (SD) based on three biological replicates. Different letters a, b, c, d, e, and f indicate the significant difference determined by one-way ANOVA based on $p < 0.05$.

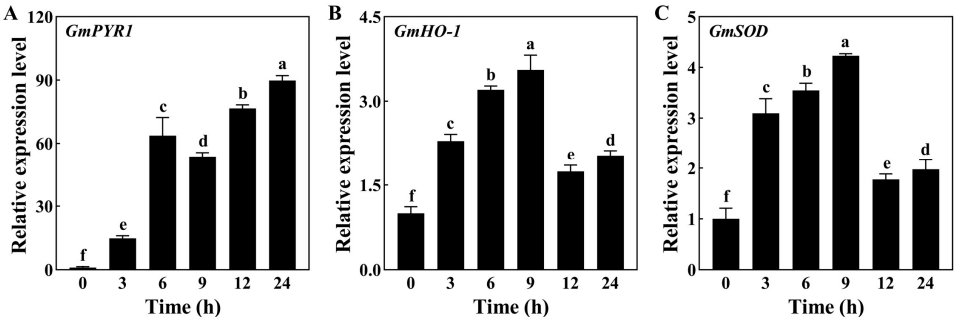

Figure 5. Relative expression levels of *GmPYR1* (**A**), *GmHO-1* (**B**), and *GmSOD* (**C**) in soybean roots under drought stress based on qRT-PCR analysis. Data are shown as mean ± standard deviation (SD) based on three biological replicates. Different letters a, b, c, d, e, and f indicate the significant difference determined by one-way ANOVA based on $p < 0.05$.

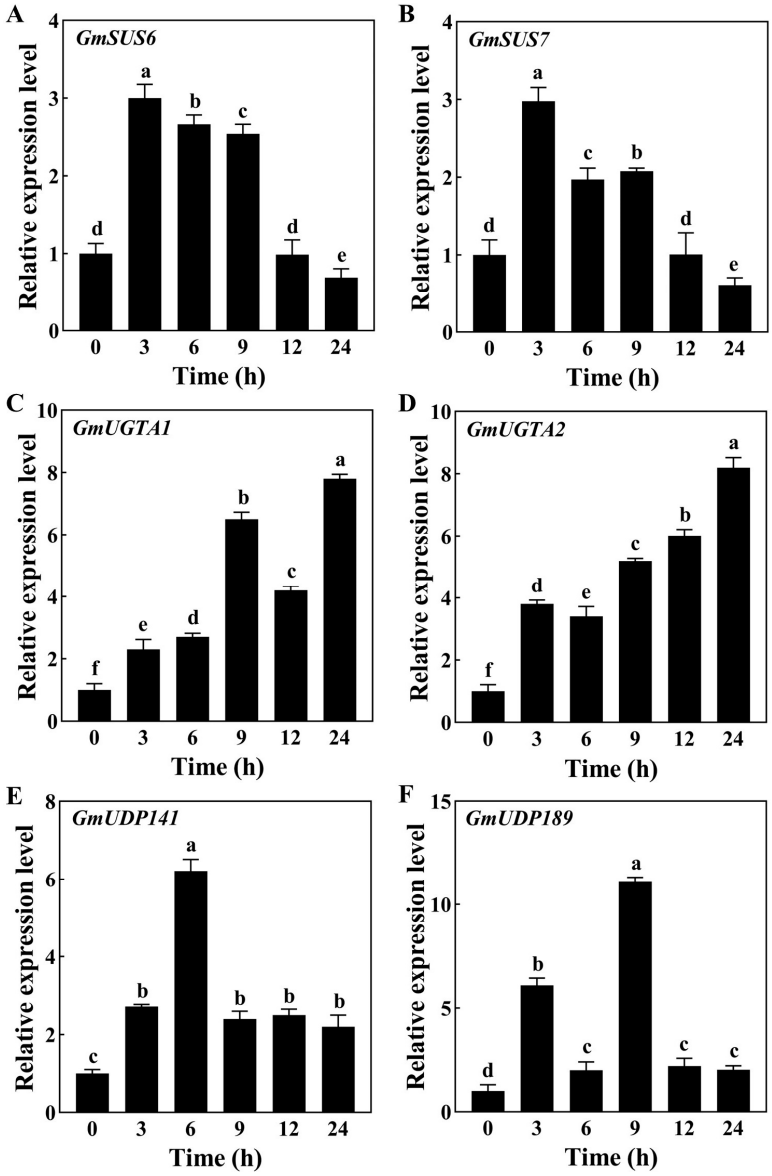

Figure 6. Relative expression levels of *GmSUS6* (**A**), *GmSUS7* (**B**), *GmUGTA1*(**C**), *GmUGTA2* (**D**), *GmUDP141* (**E**), and *GmUDP189* (**F**) in soybean roots under drought stress based on qRT-PCR analysis. Data are shown as mean ± standard deviation (SD) based on three biological replicates. Different letters a, b, c, d, e, and f indicate the significant difference determined by one-way ANOVA based on $p < 0.05$.

3.5. Determination of the Contents of Osmoregulatory Substances and ABA in Soybean Roots under Drought Stress

The levels of ABA, sucrose, and betaine were quantified in the soybean roots under drought stress. The results showed that the ABA content was first gradually increased to the highest level at 12 h after the drought stress treatment; subsequently, it decreased at 24 h (Figure 7A). The sucrose content was revealed to have an increasing trend as the treatment time of the drought stress was increased up to 24 h (Figure 7B). First, the betaine content exhibited a continuously increasing trend, reaching the highest level at 6 h after the drought stress treatment, followed by a decreasing trend up to 24 h (Figure 7C).

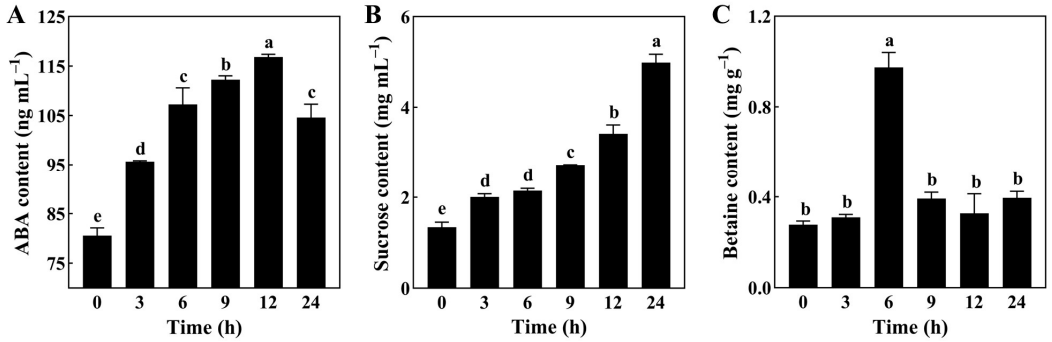

Figure 7. Physiological variations in the contents of ABA (**A**), sucrose (**B**), and betaine (**C**) in soybean roots under drought stress. Data are shown as mean ± standard deviation (SD) based on three biological replicates. Different letters a, b, c, d, and e indicate the significant difference determined by one-way ANOVA based on $p < 0.05$.

4. Discussion

As a consequence of the current global climate change, the occurrence of drought results in reduced agricultural output and amplifies annual agricultural and economic losses worldwide [33]. Drought stress has become a significant contributor to yield reduction in soybean, a globally important oilseed crop, with the potential for a significant yield decrease [34,35]. To date, a comprehensive understanding of the molecular mechanisms underlying the soybean resistance to drought stress is still lacking. Therefore, it is very important to explore the molecular mechanism regulating the soybean response to drought stress for the molecular breeding of soybean varieties with high resistance to drought. In this study, we investigated the molecular variations in protein levels and the expression of the genes involved in the metabolic pathways of carbohydrates, ABA, and ABC transporters in the roots of soybean cultivar 'Jiyu 47' under drought stress treatment, providing novel candidate proteins and genes as well as strong experimental evidence to support the molecular breeding of soybean varieties with improved resistance to drought.

4.1. DEGs Involved in ABC Biosynthesis and Signaling Pathways in Soybean Roots under Drought Stress

ABCs are a large family of transmembrane proteins, which are mainly located in the cell membrane, vacuole membrane, plastid membrane, and the membranes of other organelles. ABCs utilize the energy generated by the hydrolysis of ATP to transport substances across the membranes; the substances transported include hormones, inorganic ions, alkaloids, lipids, polysaccharides, peptides, and heavy metal chelates [14]. The transportation of these substances across membranes plays an important role in plant signal transduction and plant response to various abiotic stresses, e.g., drought. For example, studies have shown that under cadmium stress, the rice ABC transporter gene *OsABCG36* is involved in the secretion of cadmium from the root cells in the form of the cadmium ion or cadmium conjugate, thereby reducing the toxic effect of a high concentration of cadmium ions on rice [36]. In barnyard grass, EcABCC8 is located on the plasma membrane, transferring glyphosate from the cytoplasm into the exosomes and ultimately reducing the level of glyphosate in the cells and conferring glyphosate resistance in the plants [37]. Both AtABCC1 and AtABCC2 of *Arabidopsis* transport the arsenic bound to the chelating agent to the vacuole for detoxification and enhance the arsenic tolerance of *Arabidopsis* [38]. Furthermore, ABC transporters also play an important role in plant resistance to drought stress. It has been reported that under drought stress, the *Arabidopsis* ABCG transporter can transport ABA to the stomata, causing the stomatal closure and reducing the water evaporation from leaves [39]. These results are consistent with the findings revealed in the proteomics analysis of our study, which showed the down-regulation of seven soybean ABC transporters in soybean roots under drought stress treatment. These results of the proteomics analysis were consistent with those of the qRT-PCR analysis, which showed the

increased expression levels of the genes encoding these ABC proteins up to 9 h after the drought stress treatment and then a decreased expression at 24 h (Figure 4). These results indicated that in the early stage of drought stress, the expression of the genes encoding ABC transporters was increased in the soybean roots, enhancing the production of ABC transporters and the transportation of drought-resistant substances to improve the soybean root drought resistance. However, excessive substances transported into cells also have adverse effects on cells. Therefore, in the late stage of drought stress, it is necessary to reduce the amount of ABC transporters to maintain the homeostasis of drought-resistant substances in the soybean roots, as observed in the decreased expression of the genes encoding the ABC proteins in the soybean root at 24 h after the drought stress treatment.

4.2. DEGs Involved in ABA Biosynthesis and Signaling Pathways in Soybean Roots under Drought Stress

Plants mitigate drought-induced damage based on the physiological and biochemical alterations involved in the mechanisms underlying the plant response to drought stress [40]. ABA is an important stress response hormone in plants, and the level of ABA is closely related to the adaptability of plants to stress. Studies have shown that the increased level of ABA promotes stomatal closure, thereby reducing water loss due to transpiration [41]. In maize, the WRKY transcription factor ZmWRKY79 enhances the plant drought tolerance by elevating the ABA biosynthesis [42], while in rice *OsOLP1* promotes plant drought tolerance by regulating ABA biosynthesis [43]. Our study revealed the continuously increasing pattern of the gene *GmPYR1* encoding the ABA receptor PYR as well as the increased relative expression of *GmHO-1* and *GmSOD* in soybean roots under drought stress (Figure 5A). The content of ABA in soybean roots was also increased as the drought stress treatment time was increased (Figure 7A). These results were consistent with those previously reported, showing that the level of ABA in plants under stress was increased, which played an important role in the plant response to drought stress and led to the enhanced binding of ABA with PYR. For example, previous studies showed that PYR prevented the interaction between SNF1-related protein kinase 2 (SnRK2) and protein phosphatase 2C (PP2C), i.e., the PP2C-mediated dephosphorylation of SnRK2, leading to the activation of SnRK2 kinase, the phosphorylation of downstream transcription factors, and the activation of the ABA signaling pathway [44]. In our study, the relative expression of *GmSOD* and *GmHO-1* involved in the ABA signaling pathway was increased in soybean roots under drought stress, reaching the highest levels at 9 h (Figure 5B,C). These results were consistent with those previously reported. For example, in *Arabidopsis thaliana*, the mutation of the HO gene *AtHO1* (HY1) was involved in the plant response to drought resistance by changing the expression of the ABA-related genes [45]. In wheat, the expression of *TaHO1* was induced by ABA and other abiotic stresses [46]. In a recent study of durum wheat, *TdCu-ZnSOD2B-2* was significantly up-regulated in leaves treated with ABA [47], indicating that SOD was probably involved in the plant response to stress mediated by ABA. Furthermore, previous studies showed that the water loss in the srk2e/ost1 mutant of *Arabidopsis*, which exhibited impaired ABA signaling in guard cells, was further exacerbated by the *atabcg22* mutation [48]. Moreover, the *atabcg22* mutation displayed an enhanced phenotype in nced3 mutants with impaired ABA biosynthesis, suggesting the synergistic effects of *AtABCG22* in both ABA signaling and biosynthesis. Together, these studies demonstrate that ABA is an indispensable plant hormone that plays an important role in plant resistance to abiotic stress, including drought.

4.3. DEGs Involved in Sucrose and Betaine Biosynthesis and Signaling Pathways in Soybean Roots under Drought Stress

Under drought conditions, plants accumulate osmoregulatory substances to reduce the cell osmotic potential and maintain turgor pressure, ensuring their normal physiological processes [49]. For example, sucrose is revealed to regulate the osmotic potential of plant cells and to stabilize their structural components under drought stress [8]. Sugar is the primary photosynthetic product in plants, serving as a crucial source of energy and playing a vital role in regulating plant growth. Studies have shown that the growth of soybean under drought stress is primarily regulated by sugar metabolism, allocation, and transport [50]. For example, our previous studies showed that the impact of drought stress on soybean roots was demonstrated in the regulation of sugar metabolism and transport, resulting in the increased levels of soluble sugar and starch [29]. SUS is an enzyme that catalyzes the reversible reactions between the synthesis and decomposition of sucrose [51] and plays different roles during different developmental stages and environmental conditions. For example, during the early stage of drought stress, SUS primarily enhances the synthetic activity of sucrose, thereby increasing the sucrose content and reducing cellular osmotic water loss. In our study, the results showed that the expression of the genes *GmSUS6* and *GmSUS7* encoding soybean SUS was rapidly increased up to the highest levels in the soybean roots 3 h after the drought stress treatment (Figure 6A,B), and the sucrose content in the soybean roots was continuously increased with the drought stress treatment time (Figure 7B), thus alleviating the damage to plant cells caused by drought stress. These results were consistent with those recently reported, which showed the significance of increased sucrose content in the drought resistance of *ZmSUS1* transgenic maize [52]. Then, the expression of *GmSUS6* and *GmSUS7* was gradually decreased after 9 h of drought stress treatment, suggesting that in the later stage of drought stress, the intracellular environment reached a steady state and the carbohydrate-related substances were no longer needed to maintain the intracellular water retention capacity, i.e., the production of sugar was decreased. Moreover, studies have shown that the expression of the barley genes *HvSUS1* and *HvSUS3* is induced by both low temperature and drought stress [53]; the rubber tree sucrose synthase gene *HbSUS5* is involved in the response to drought stress [54]; and the expression levels of *AtSUS1* and *AtSUS3* are significantly increased in *Arabidopsis* under drought stress [55].

Betaine plays a pivotal role in the regulation of osmotic balance, serving as one of the primary nitrogen-containing compatible osmolytes identified in Poaceae; it engages in interactions with diverse molecules to safeguard the functionality of macromolecules, to preserve membrane integrity under challenging conditions, and to effectively scavenge ROS [56]. Betaine is an effective osmoprotectant, and under normal circumstances, the level of betaine in plants is generally low. However, the level of betaine is rapidly increased in response to adverse conditions. For example, previous studies showed that the accumulation of betaine in potato plants enhanced plant resistance to drought stress [57]. These results were consistent with the findings revealed in our study, which showed that the expression levels of *GmUGTA1* and *GmUGTA2* in soybean roots exhibited a continuous up-regulation following exposure to drought stress (Figure 6C,D), while the expression of *GmUDP141* and *GmUDP189* was increased to the highest levels at 6 h and 9 h, respectively, after the drought stress treatment (Figure 6E,F). Betaine generally plays its role with high efficiency, based on a small amount. For example, studies showed that the accumulation of small amounts of betaine in tomato improved its tolerance to cold stress [58]. These results were in agreement with the findings revealed in our study, which showed that the content of betaine in soybean roots was increased by about 3 times at 6 h after the drought stress treatment, followed by a subsequent decrease up to 24 h (Figure 7C). These results indicated that under drought stress, the increased production of betaine enhanced the soybean root resistance to drought stress.

4.4. A Proposed Model and the Effect of Drought Stress on Soybean Stomatal Movement

Our results showed that during the early stage of drought stress up to 9 h, the expression of both *GmSUS6* and *GmSUS7* was increased in soybean roots, thus promoting the production of sucrose and enhancing water retention in the plant cells. Then, during the later stage of drought stress, the production of sugar was decreased in soybean roots, i.e., the expression of *GmSUS6* and *GmSUS7* was decreased gradually in soybean roots at 9 h after the drought stress treatment; the intracellular environment reached a steady state, and the carbohydrate-related substances were no longer needed to maintain the intracellular water retention capacity. Furthermore, our proteomics data showed that the sucrose phosphate synthase (SPS) was down-regulated in soybean roots at 24 h after the drought stress treatment, resulting in a decrease in sucrose synthesis and transport from the leaves to the roots. Moreover, the proteomics data showed that the expression levels of UDP-galactose-4-isomerase (GalE) and 2-diphosphoglycerate mutase (2,3-BPGM) involved in sucrose metabolism in soybean roots were up-regulated, promoting the decomposition of sucrose, whereas the expression of pyruvate kinase (PK) catalyzing the conversion of phosphoenolpyruvate (PEP) to pyruvate was down-regulated. In particular, the up-regulation of GalE promoted the conversion of sucrose to galactose, and both 2,3-BPGM and PK were involved in the glycolysis, indicating that the enhanced production of sucrose in the sugar metabolic pathway elevated the level of ATP, the energy required for improved plant resistance to drought stress.

The stomata play a crucial role in plant gas metabolism, with the involvement of carbon assimilation, respiration, and transpiration. The regulation of the stomatal aperture is governed by guard cells. Under normal circumstances, guard cells do not exhibit a significant accumulation of ABA; thus, they prevent the binding of the PYR/PYR1-Like (PYL)/regulatory component of the ABA receptor (RCAR) to PP2Cs and, subsequently, the inhibition of their functions [59]. Consequently, PP2Cs dephosphorylate SnRK2 protein kinases, S-type anion channels (SLAC1), and R-type anion channels (ALMT12/QUAC1), thereby inhibiting the activities of these protein kinases and ion channels, ultimately preventing ion efflux and maintaining cellular osmotic pressure. Our results showed that the drought stress induced an up-regulation of ABA synthesis in soybean roots, ultimately leading to the transport of ABA into the guard cells via the ABC transporters. Similarly, it has been documented that in *Arabidopsis* ABCG40 actively facilitates the influx of ABA into the guard cells [60]. Furthermore, the accumulation of ABA in guard cells is perceived and bound by its receptors, i.e., PYR/PYL/RCAR. Subsequently, the ABA-bound PYR/PYL/RCAR receptors tightly bind to PP2Cs, directly inhibiting their phosphatase activity [61]. Moreover, this mechanism facilitates the activation of downstream molecular components, including SnRK2, SLAC1, and ALMT12/QUAC1, ultimately enhancing the ion transport by the guard cells. The efflux of potassium ions (K^+) and anions (A^-) induces the contraction of the guard cells, resulting in stomatal closure and, consequently, a reduction in plant transpiration [62], thereby effectively decreasing water loss in plants. Based on these findings, a model is proposed to illustrate the molecular mechanism regulating the effect of drought stress on stomatal movement in soybean (Figure 8).

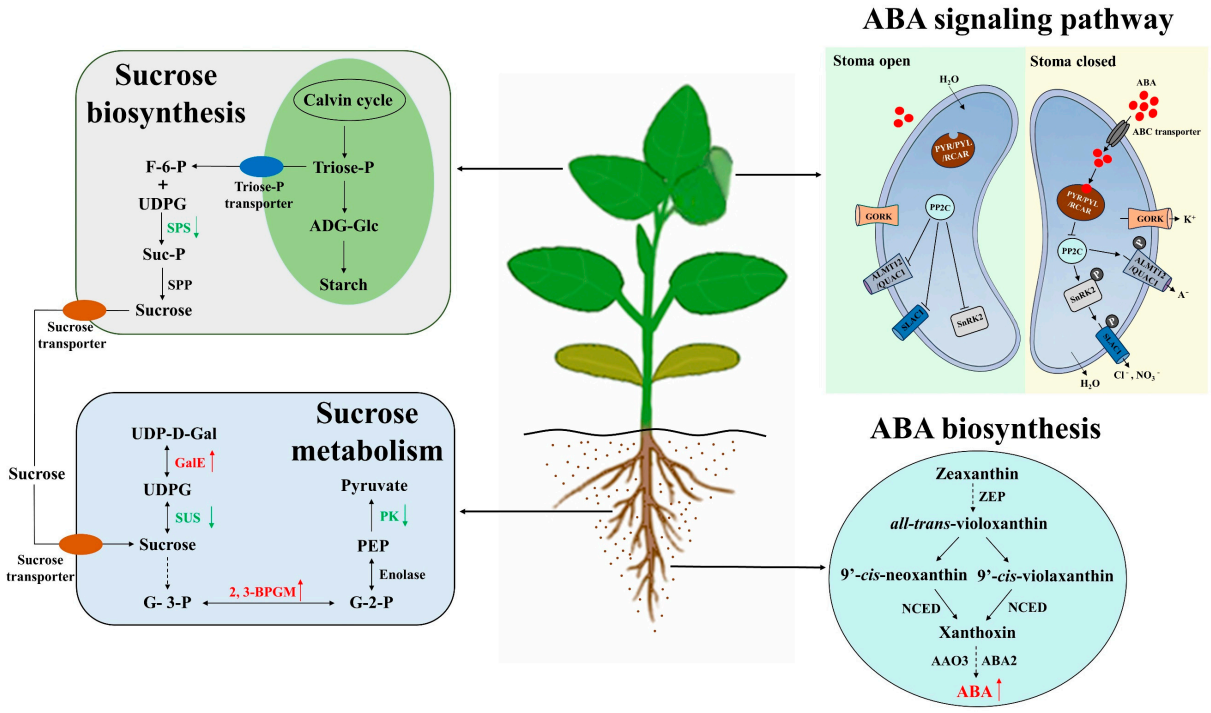

Figure 8. A proposed model illustrating the molecular mechanism underlying the effect of drought stress on soybean stomatal movement. Enzymes detected in proteomics analysis and involved in sucrose biosynthesis and metabolism with up-regulated and down-regulated expressions are indicated in red and green, respectively. Symbols "↑" and "↓" indicate up-regulation and down-regulation, respectively. The content of ABA in soybean roots is increased during the early stage (up to 12 h) after the drought stress treatment. In response to water deficit, ABA synthesized in soybean roots is transported to leaves to trigger various physiological responses to reduce water loss, e.g., stomatal closure. Triose-P, triose phosphate; F-6-P, fructose-6-phosphate; UDPG, UDP-glucose; SPS, sucrose phosphate synthase; Suc-P, sucrose phosphate; SPP, sucrose phosphorylase; UDP-D-Gal, UDP-D-galactose; GalE, UDP-galactose 4-epimerase; SUS, sucrose synthetase; G-3-P, glyceraldehyde-3-phosphate; 2,3-BPGM, 2,3-diphosphoglycerate mutase; G-2-P, glyceraldehyde-2-phosphate; PEP, phosphoenolpyruvate; PK, pyruvate kinase; ZEP, zeaxanthin epoxidase; NCED, 9-cis epoxide carotenoid dioxygenase. Cl^-, chloride ion; NO_3^-, nitrate ion; A^-, anion; K^+, potassium ion.

5. Conclusions

In this research, we characterized the molecular and physiological variations in soybean roots under drought stress and further explored the molecular mechanisms regulating the soybean root response to drought stress and identified the metabolic pathways involved in soybean root resistance to drought. The protein and gene expression in soybean roots treated with drought stress was investigated using combined methods of proteomics analysis, qRT-PCR, and metabolite profiling, revealing a group of proteins and genes involved in the metabolic pathways of ABA, carbohydrates, and ABC transporters. The biosynthesis of ABA facilitated stomatal closure via multiple metabolic pathways, thereby mitigating transpiration and enhancing soybean drought tolerance. The up-regulation of the ABC transporters in the soybean roots led to an increased transportation of drought-resistant substances; ultimately, this improved the soybean drought resistance. The accumulation of betaine enhanced the soybean resistance to drought stress. The results of the qRT-PCR and physiological analyses suggested that ABC was involved in the transport of the precursors of ABA biosynthesis as well as sugar metabolism, indicating its involvement in the metabolic pathways associated with soybean drought resistance. Consequently, the soybean modulated its response to drought stress by altering the expression of the proteins involved in plant hormone signaling, osmolyte accumulation, and antioxidant defense

mechanisms. The variations in protein and gene expression revealed in this study provided strong experimental evidence to support further exploration of the intricate molecular mechanism underlying the soybean response to drought stress. The limitations of this study are as follows: a more comprehensive study of the soybean response to drought stress is necessary to include other organs such as the stems and leaves, in addition to the roots. Furthermore, it is also necessary to characterize the molecular functions of the DEPs and DEGs involved in the soybean root response to drought stress in order to identify their functions in plant tolerance to drought stress. The identification of these proteins and genes involved in the soybean root response to drought stress provides novel candidates for further exploration of the molecular mechanisms underlying the plant response to drought stress and the molecular breeding of drought-tolerant soybean varieties; ultimately, this will improve the soybean yield in arid or semi-arid areas.

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References

1. Tian, T.; Wang, S.; Yang, S.; Yang, Z.; Liu, S.; Wang, Y.; Gao, H.; Zhang, S.; Yang, X.; Jiang, C.; et al. Genome assembly and genetic dissection of a prominent drought-resistant maize germplasm. *Nat. Genet.* **2023**, *55*, 496–506. [CrossRef]
2. Zhang, H.; Zhu, J.; Gong, Z.; Zhu, J.K. Abiotic stress responses in plants. *Nat. Rev. Genet.* **2022**, *23*, 104–119. [CrossRef]
3. Du, X.; Zhang, X.; Chen, X.; Jin, W.; Huang, Z.; Kong, L. Drought stress reduces the photosynthetic source of subtending leaves and the transit sink function of podshells, leading to reduced seed weight in soybean plants. *Front. Plant Sci.* **2024**, *15*, 1337544. [CrossRef]
4. Xu, C.; Shan, J.; Liu, T.; Wang, Q.; Ji, Y.; Zhang, Y.; Wang, M.; Xia, N.; Zhao, L. CONSTANS-LIKE 1a positively regulates salt and drought tolerance in soybean. *Plant Physiol.* **2023**, *191*, 2427–2446. [CrossRef]
5. Wei, Y.; Jin, J.; Jiang, S.; Ning, S.; Liu, L. Quantitative response of soybean development and yield to drought stress during different growth stages in the Huaibei Plain China. *Agronomy* **2018**, *8*, 97. [CrossRef]
6. Zhang, Z.; Tong, T.; Fang, Y.; Zheng, J.; Zhang, X.; Niu, C.; Li, J.; Zhang, X.; Xue, D. Genome-Wide Identification of Barley ABC Genes and Their Expression in Response to Abiotic Stress Treatment. *Plants* **2020**, *9*, 1281. [CrossRef]
7. Abid, M.; Ali, S.; Qi, L.K.; Zahoor, R.; Tian, Z.; Jiang, D.; Snider, J.L.; Dai, T. Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Sci. Rep.* **2018**, *8*, 4615. [CrossRef]
8. Wang, B.; Yang, L.; Zhang, Y.; Chen, S.; Gao, X.; Wan, C. Investigation of the dynamical expression of *Nostoc flagelliforme* proteome in response to rehydration. *J. Proteom.* **2019**, *192*, 160–168. [CrossRef]
9. Wang, X.; Song, S.; Wang, X.; Liu, J.; Dong, S. Transcriptomic and Metabolomic Analysis of Seedling-Stage Soybean Responses to PEG-Simulated Drought Stress. *Int. J. Mol. Sci.* **2022**, *23*, 6869. [CrossRef]
10. Roychowdhury, R.; Das, S.P.; Gupta, A.; Parihar, P.; Chandrasekhar, K.; Sarker, U.; Kumar, A.; Ramrao, D.P.; Sudhakar, C. Multi-omics pipeline and omics-integration approach to decipher plant's abiotic stress tolerance response. *Genes* **2023**, *14*, 1281. [CrossRef]
11. Feng, Z.; Ding, C.; Li, W.; Wang, D.; Cui, D. Applications of metabolomics in the research of soybean plant under abiotic stress. *Food Chem.* **2020**, *310*, 125914. [CrossRef] [PubMed]
12. Higgins, C.F. ABC transporters: From microorganisms to man. *Annu. Rev. Cell Biol.* **1992**, *8*, 67–113. [CrossRef] [PubMed]

13. Hwang, J.U.; Song, W.Y.; Hong, D.; Ko, D.; Yamaoka, Y.; Jang, S.; Yim, S.; Lee, E.; Khare, D.; Kim, K.; et al. Plant ABC Transporters Enable Many Unique Aspects of a Terrestrial Plant's Lifestyle. *Mol. Plant* **2016**, *9*, 338–355. [CrossRef] [PubMed]
14. Kang, J.; Park, J.; Choi, H.; Burla, B.; Kretzschmar, T.; Lee, Y.; Martinoia, E. Plant ABC Transporters. *Arab. Book* **2011**, *9*, e0153. [CrossRef] [PubMed]
15. Rea, P.A. Plant ATP-binding cassette transporters. *Annu. Rev. Plant Biol.* **2007**, *58*, 347–375. [CrossRef] [PubMed]
16. Dawson, R.J.; Hollenstein, K.; Locher, K.P. Uptake or extrusion: Crystal structures of full ABC transporters suggest a common mechanism. *Mol. Microbiol.* **2007**, *65*, 250–257. [CrossRef] [PubMed]
17. Lefèvre, F.; Boutry, M. Towards identification of the substrates of ATP-Binding cassette transporters. *Plant Physiol.* **2018**, *178*, 18–39. [CrossRef] [PubMed]
18. Theodoulou, F.L.; Kerr, L.D. ABC transporter research: Going strong 40 years on. *Biochem. Soc. Trans.* **2015**, *43*, 1033–1040. [CrossRef] [PubMed]
19. Kim, D.Y.; Jin, J.Y.; Alejandro, S.; Martinoia, E.; Lee, Y. Overexpression of *AtABCG36* improves drought and salt stress resistance in *Arabidopsis*. *Physiol. Plant.* **2010**, *139*, 170–180. [CrossRef]
20. Long, H.; Zheng, Z.; Zhang, Y.; Xing, P.; Wan, X.; Zheng, Y.; Li, L. An abscisic acid (ABA) homeostasis regulated by its production, catabolism and transport in peanut leaves in response to drought stress. *PLoS ONE* **2019**, *14*, e0213963. [CrossRef]
21. Thomas, C.; Tampé, R. Structural and Mechanistic Principles of ABC Transporters. *Annu. Rev. Biochem.* **2020**, *89*, 605–636. [CrossRef] [PubMed]
22. Waadt, R.; Seller, C.A.; Hsu, P.K.; Takahashi, Y.; Munemasa, S.; Schroeder, J.I. Plant hormone regulation of abiotic stress responses. *Nat. Rev. Mol. Cell Biol.* **2022**, *23*, 680–694. [CrossRef] [PubMed]
23. Zhu, J.K. Abiotic Stress Signaling and Responses in Plants. *Cell* **2016**, *167*, 313–324. [CrossRef] [PubMed]
24. Chen, X.; Ding, Y.; Yang, Y.; Song, C.; Wang, B.; Yang, S.; Guo, Y.; Gong, Z. Protein kinases in plant responses to drought, salt, and cold stress. *J. Integr. Plant Biol.* **2021**, *63*, 53–78. [CrossRef] [PubMed]
25. He, R.; Zhuang, Y.; Cai, Y.; Agüero, C.B.; Liu, S.; Wu, J.; Deng, S.; Walker, M.A.; Lu, J.; Zhang, Y. Overexpression of 9-cis-Epoxycarotenoid Dioxygenase Cisgene in Grapevine Increases Drought Tolerance and Results in Pleiotropic Effects. *Front. Plant Sci.* **2018**, *9*, 970. [CrossRef] [PubMed]
26. Liu, D.; Qiu, C.; Zeng, Y.; Lin, Q. Molecular and Enzymatic Characterization of 9-Cis-epoxycarotenoid Dioxygenases from Mulberry. *Protein J.* **2022**, *41*, 504–514. [CrossRef] [PubMed]
27. Hwang, K.; Susila, H.; Nasim, Z.; Jung, J.Y.; Ahn, J.H. Arabidopsis ABF3 and ABF4 Transcription Factors Act with the NF-YC Complex to Regulate SOC1 Expression and Mediate Drought-Accelerated Flowering. *Mol. Plant* **2019**, *12*, 489–505. [CrossRef]
28. Mora, L.; Bramley, P.M.; Fraser, P.D. Development and optimisation of a label-free quantitative proteomic procedure and its application in the assessment of genetically modified tomato fruit. *Proteomics* **2013**, *13*, 2016–2030. [CrossRef]
29. Zhou, Y.; Li, H.; Chen, H.; Yang, X.; Yu, T.; Wang, Y.; Wang, Y.; Jiang, K.; Wang, Y.; Chen, Z.; et al. Proteomic Investigation of Molecular Mechanisms in Response to PEG-Induced Drought Stress in Soybean Roots. *Plants* **2022**, *11*, 1173. [CrossRef]
30. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
31. Tang, K.; Zhang, Y.; Zhang, H.; Xu, P.; Liu, J.; Ma, J.; Lv, M.; Li, D.; Katirai, F.; Shen, G.X.; et al. Delivery of chemotherapeutic drugs in tumour cell-derived microparticles. *Nat. Commun.* **2012**, *3*, 1282. [CrossRef] [PubMed]
32. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef] [PubMed]
33. Mansoor, S.; Khan, T.; Farooq, I.; Shah, L.R.; Sharma, V.; Sonne, C.; Rinklebe, J.; Ahmad, P. Drought and global hunger: Biotechnological interventions in sustainability and management. *Planta* **2022**, *256*, 97. [CrossRef] [PubMed]
34. Thao, N.P.; Tran, L.S. Potentials toward genetic engineering of drought-tolerant soybean. *Crit. Rev. Biotechnol.* **2012**, *32*, 349–362. [CrossRef] [PubMed]
35. Yu, C.; Miao, R.; Khanna, M. Maladaptation of U.S. corn and soybeans to a changing climate. *Sci. Rep.* **2021**, *11*, 12351. [CrossRef] [PubMed]
36. Fu, S.; Lu, Y.; Zhang, X.; Yang, G.; Chao, D.; Wang, Z.; Shi, M.; Chen, J.; Chao, D.Y.; Li, R.; et al. The ABC transporter ABCG36 is required for cadmium tolerance in rice. *J. Exp. Bot.* **2019**, *70*, 5909–5918. [CrossRef] [PubMed]
37. Pan, L.; Yu, Q.; Wang, J.; Han, H.; Mao, L.; Nyporko, A.; Maguza, A.; Fan, L.; Bai, L.; Powles, S. An ABCC-type transporter endowing glyphosate resistance in plants. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2100136118. [CrossRef] [PubMed]
38. Song, W.Y.; Park, J.; Mendoza-Cózatl, D.G.; Suter-Grotemeyer, M.; Shim, D.; Hörtensteiner, S.; Geisler, M.; Weder, B.; Rea, P.A.; Rentsch, D.; et al. Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 21187–21192. [CrossRef]
39. Dhara, A.; Raichaudhuri, A. ABCG transporter proteins with beneficial activity on plants. *Phytochemistry* **2021**, *184*, 112663. [CrossRef]
40. Martignago, D.; Rico-Medina, A.; Blasco-Escámez, D.; Fontanet-Manzaneque, J.B.; Caño-Delgado, A.I. Drought Resistance by Engineering Plant Tissue-Specific Responses. *Front. Plant Sci.* **2020**, *10*, 1676. [CrossRef]
41. Daszkowska-Golec, A.; Szarejko, I. Open or close the gate-stomata action under the control of phytohormones in drought stress conditions. *Front. Plant Sci.* **2013**, *4*, 138. [CrossRef]

42. Gulzar, F.; Fu, J.; Zhu, C.; Yan, J.; Li, X.; Meraj, T.A.; Shen, Q.; Hassan, B.; Wang, Q. Maize WRKY Transcription Factor ZmWRKY79 Positively Regulates Drought Tolerance through Elevating ABA Biosynthesis. *Int. J. Mol. Sci.* **2021**, *22*, 10080. [CrossRef]
43. Yan, J.; Ninkuu, V.; Fu, Z.; Yang, T.; Ren, J.; Li, G.; Yang, X.; Zeng, H. OsOLP1 contributes to drought tolerance in rice by regulating ABA biosynthesis and lignin accumulation. *Front. Plant Sci.* **2023**, *14*, 1163939. [CrossRef]
44. Cutler, S.R.; Rodriguez, P.L.; Finkelstein, R.R.; Abrams, S.R. Abscisic acid: Emergence of a core signaling network. *Annu. Rev. Plant Biol.* **2010**, *61*, 651–679. [CrossRef] [PubMed]
45. Xie, Y.; Mao, Y.; Duan, X.; Zhou, H.; Lai, D.; Zhang, Y.; Shen, W. *Arabidopsis* HY1-Modulated Stomatal Movement: An Integrative Hub Is Functionally Associated with ABI4 in Dehydration-Induced ABA Responsiveness. *Plant Physiol.* **2016**, *170*, 1699–1713. [CrossRef] [PubMed]
46. Xu, D.K.; Jin, Q.J.; Xie, Y.J.; Liu, Y.H.; Lin, Y.T.; Shen, W.B.; Zhou, Y.J. Characterization of a wheat heme oxygenase-1 gene and its responses to different abiotic stresses. *Int. J. Mol. Sci.* **2011**, *12*, 7692–7707. [CrossRef]
47. Tounsi, S.; Jemli, S.; Feki, K.; Brini, F.; Najib Saïdi, M. Superoxide dismutase (SOD) family in durum wheat: Promising candidates for improving crop resilience. *Protoplasma* **2023**, *260*, 145–158. [CrossRef]
48. Kuromori, T.; Sugimoto, E.; Shinozaki, K. *Arabidopsis* mutants of *AtABCG22*, an ABC transporter gene, increase water transpiration and drought susceptibility. *Plant J.* **2011**, *67*, 885–894. [CrossRef]
49. Hong, Y.; Wang, Z.; Liu, X.; Yao, J.; Kong, X.; Shi, H.; Zhu, J.K. Two Chloroplast Proteins Negatively Regulate Plant Drought Resistance Through Separate Pathways. *Plant Physiol.* **2020**, *182*, 1007–1021. [CrossRef]
50. Du, Y.; Zhao, Q.; Chen, L.; Yao, X.; Zhang, W.; Zhang, B.; Xie, F. Effect of drought stress on sugar metabolism in leaves and roots of soybean seedlings. *Plant Physiol. Biochem.* **2020**, *146*, 1–12. [CrossRef]
51. Schäfer, W.E.; Rohwer, J.M.; Botha, F.C. Partial purification and characterisation of sucrose synthase in sugarcane. *J. Plant Physiol.* **2005**, *162*, 11–20. [CrossRef]
52. Xiao, N.; Ma, H.; Wang, W.; Sun, Z.; Li, P.; Xia, T. Overexpression of *ZmSUS1* increased drought resistance of maize (*Zea mays* L.) by regulating sucrose metabolism and soluble sugar content. *Planta* **2024**, *259*, 43. [CrossRef]
53. Barrero-Sicilia, C.; Hernando-Amado, S.; González-Melendi, P.; Carbonero, P. Structure, expression profile and subcellular localisation of four different sucrose synthase genes from barley. *Planta* **2011**, *234*, 391–403. [CrossRef]
54. Xiao, X.; Tang, C.; Fang, Y.; Yang, M.; Zhou, B.; Qi, J.; Zhang, Y. Structure and expression profile of the sucrose synthase gene family in the rubber tree: Indicative of roles in stress response and sucrose utilization in the laticifers. *FEBS J.* **2014**, *281*, 291–305. [CrossRef] [PubMed]
55. Baud, S.; Vaultier, M.N.; Rochat, C. Structure and expression profile of the sucrose synthase multigene family in *Arabidopsis*. *J. Exp. Bot.* **2004**, *55*, 397–409. [CrossRef]
56. Annunziata, M.G.; Ciarmiello, L.F.; Woodrow, P.; Dell'Aversana, E.; Carillo, P. Spatial and Temporal Profile of Glycine Betaine Accumulation in Plants Under Abiotic Stresses. *Front. Plant Sci.* **2019**, *10*, 230. [CrossRef]
57. You, L.; Song, Q.; Wu, Y.; Li, S.; Jiang, C.; Chang, L.; Yang, X.; Zhang, J. Accumulation of glycine betaine in transplastomic potato plants expressing choline oxidase confers improved drought tolerance. *Planta* **2019**, *249*, 1963–1975. [CrossRef]
58. Park, E.J.; Jeknić, Z.; Sakamoto, A.; DeNoma, J.; Yuwansiri, R.; Murata, N.; Chen, T.H. Genetic engineering of glycine betaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. *Plant J.* **2004**, *40*, 474–487. [CrossRef]
59. Lee, S.C.; Lan, W.; Buchanan, B.B.; Luan, S. A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21419–21424. [CrossRef] [PubMed]
60. Kang, J.; Hwang, J.U.; Lee, M.; Kim, Y.Y.; Assmann, S.M.; Martinoia, E.; Lee, Y. PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 2355–2360. [CrossRef]
61. Ma, Y.; Szostkiewicz, I.; Korte, A.; Moes, D.; Yang, Y.; Christmann, A.; Grill, E. Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* **2009**, *324*, 1064–1068. [CrossRef] [PubMed]
62. Lee, S.C.; Lim, C.W.; Lan, W.; He, K.; Luan, S. ABA signaling in guard cells entails a dynamic protein-protein interaction relay from the PYL-RCAR family receptors to ion channels. *Mol. Plant* **2013**, *6*, 528–538. [CrossRef] [PubMed]