



Comparative ABA-Responsive Transcriptome in Soybean Cultivars Submitted to Different Levels of Drought

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Abstract

Background The drought condition is responsible for considerable losses in soybean production, which in turn may result in billion-dollar losses. After drought perception, plants activate a cascade of protecting genes against water deficit (WD), many of which are responsive to abscisic acid, the most important phytohormone to plants' adaptation. This work aimed to recover abscisic acid (ABA)-responsive differentially expressed genes (DEG) from an RNA-Seq, carried out from leaves and roots of drought-sensitive (BR16) and tolerant (Embrapa 48) soybean cultivars under mild (MiWD), moderate (MoWD), and severe (SWD) water-deficit treatments. Enriched ABA-responsive pathways important to drought tolerance in soybean were also identified.

Results In drought-sensitive and tolerant soybean cultivars, approximately 75% of genes were identified as ABA-responsive by containing more than two ABRE (ABA-responsive elements) in the promoter region. Most of these genes were positively regulated. Roots were the tissue with more ABA-responsive genes and pathways triggered in response to WD in both cultivars, although, on the tolerant cultivar, these pathways were higher expressed. The most important enriched ABA pathways observed in the roots of both cultivars were involved in sugar and sulfur amino acid biosynthesis, osmoregulation, and crosstalk among ABA and ethylene, jasmonate, auxin, and cytokinin. Other pathways enriched were involved in phytoalexin production, ROS homeostasis, and membrane stability by glycerolipid and glycerophospholipid production. ABA-responsive genes were also ordered based on their expression profile in tissue and cultivar, and nine confidence groups could be observed. More than 80% of these clustered genes showed the same regulation profile under MiWD, MoWD, and SWD treatments. Activation of ABA biosynthesis under water deficit was validated by RT-qPCR by increasing the expression level of *NCED3*, an important enzyme in this pathway, and *GOLS*, a known ABA-responsive gene.

Conclusions A robust catalog of ABA-responsive genes was made available in this work. Considering ABA's role in drought-response mechanisms, the genes in the groups pointed out in this study would be reliable candidates to be used in strategies to develop soybean lines more tolerant to drought. This paper, presented for the first time, ABA-responsive genes and ABA-enriched pathways in contrasting soybean cultivars for drought tolerance.

Keywords Glycine max · Abscisic acid · ABRE motif · Water deficit · RNA-Seq

Background

Soybean is one of the most important commodity worldwide, showing high socio-economic relevance by participating actively in the economy of a lot of countries such as Brazil,

Argentina, and the US. Periods of drought can severely damage the crop, implying billion-dollar losses (Ferreira 2016; Fuganti-Pagliarini et al. 2017; Bijalwan et al. 2022).

Unfortunately, given the current climate change, for the next few years, it is expected that the average temperature of the planet will increase and extreme weather conditions, such as drought, will intensify, jeopardizing agriculture and the global food supply in the future (Intergovernmental Panel on Climate Change (2022)). To minimize expected production losses, intensive research efforts around the world are being made to develop more drought-tolerant soybean cultivars

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(Fuganti-Pagliarini et al. 2017; Honna et al. 2016a; Ortiz 2019; Molinari et al. 2020, 2021a; Suhartina et al. 2022).

Among the most important pathways of plant adaptability to drought is the ABA-responsive (Shinozaki and Yamaguchi-Shinozaki 2007; Yoshida et al. 2014; Aslam et al. 2022). This phytohormone is responsible for triggering the first defensive mechanisms and controlling a variety of different molecular, physiologic, and metabolic responses under drought conditions (Munemasa et al. 2015; Bulgakov et al. 2019; Pizzio 2022). ABA-responsive genes can be identified by containing in their promoter region at least 2 conserved sequences named ABRE motifs (ACGT core) that are required for gene transcription. Usually, this motif is proximally located within the -1 to -2000 bp upstream transcription start site (TSS) (Hobo et al. 1999; Uno et al. 2000; Maruyama et al. 2012; Duarte et al. 2019; Liu et al. 2020).

The present study allowed the identification of ABA-responsive genes and the most expressed pathways in drought-tolerant and drought-sensitive soybean cultivars. Understanding the mechanism of action of the hub genes involved in these ABA-responsive pathways can provide essential information for the development of breeding strategies using biotechnological tools aiming to cope with the lack of water and, ultimately, reduce production losses.

Methods

Plant Materials and WD Experiment

Two soybean cultivars contrasting for drought response were used to determine the gene expression profile under different WD levels. BR16 cultivar is considered drought-sensitive and Embrapa 48 (E48) drought-tolerant (Oya et al. 2004; Rodrigues et al. 2012). Soybean plants were grown in a greenhouse under a hydroponic system following the protocol established by Martins et al. (Martins et al. 2008). Briefly, seeds were pre-germinated on filter paper for four days in a growth chamber at 25 ± 1 °C and 100% relative humidity. Seedlings were placed in plastic boxes in a way that roots were completely immersed in 50% of Hoagland's solution (Hoagland and Arnon 1950), which was continuously aerated and weekly changed. Boxes were kept in a greenhouse under a natural photoperiod of 12/12 h light/dark cycle with a temperature of 30 ± 5 °C and 60 ± 10 % relative humidity.

The experimental design was randomized blocks with nine biological replicates. When plants reached the V3 developmental stage (Fehr et al. 1971), roots and leaves of both soybean cultivars were collected following WD treatments levels: 0 min (T0—control), 25 min (T25), 50 min (T50), 75 min (T75), 100 min (T100), 125 min (T125), and 150 min (T150). Treatments were applied by removing

plants from the hydroponic solution and placing them in empty boxes for the different water deprivation periods (minutes). Roots and leaf samples from all treatments and soybean cultivars were collected, deep in liquid nitrogen, and stored in a freezer until nucleic acids extraction.

mRNA-Seq Libraries Sequencing

Total RNA was extracted from leaves and root tissues from both soybean cultivars using TRIzol® reagent (Invitrogen, California, USA). Removal of remaining DNA was carried out using DNase I kit (Invitrogen, California, EUA), and the removal of rRNA was performed with RiboMinus™ plant kit (Invitrogen, California, EUA), following manufacturer's instructions. RNA concentration and purity were measured using BioAnalyzer software (Agilent California, EUA), and the integrity was analyzed on a 1% agarose gel. Quantification of the mRNA was carried out by Qubit (ThermoFisher, Massachusetts, EUA). Samples with RNA Integrity Number (RIN) ≥ 8.0 were used for sequencing.

RNA from all water deficit treatments were equimolar pooled into four categories as follows: T0—control condition; T25–50 min, considered mild water deficit—MiWD; T75–100 min, considered moderate water deficit—MoWD; T125–150 min, considered severe water deficit—SWD. WD levels were chosen based on physiological analyses performed by Rodrigues et al. (Rodrigues et al. 2012) and Martins et al. (Martins et al. 2008), which showed that within prolonged exposure to the lack of water, photosynthesis of soybean cultivars was maximum at time zero but affected and strongly inhibited after 100 min.

The libraries were prepared using Illumina TruSeq™ SBS v5 kit with 200 ng of mRNA. Single-end libraries of 100-bp size were synthesized by Fasteris company (Switzerland) and sequenced at Illumina HiSeq 2000 platform. A total of sixteen libraries (4 WD treatment levels \times 2 soybean cultivars \times 2 tissues) were synthesized, sequenced, and analyzed.

Bioinformatics Analysis

The quality of reads before and after the trimming process was evaluated using FastQC software version 0.11.5 (Andrews 2010). Removal of adapters, low-quality sequences (Phred quality score ≥ 30), and sequences shorter than 40 bp were carried out using Trimmomatic software version 0.36 (Bolger et al. 2014). The soybean reference genome (Gmax_Wm82.a2.v1) used was downloaded from Phytozome v13 (Goodstein et al. 2012). Alignment of reads in the reference genome was performed using HISAT2 software version 2.1.0, retrieving unique alignments with higher quality (Kim et al. 2015). PCR artifacts (unnatural read duplications) were removed by Samtools rmdup software version 1.5 to improve mapping

quality (Li et al. 2009). Assembly of transcripts was performed by StringTie software version 1.3.3 (Pertea et al. 2015). The StringTie output was used in GFOLD software version 1.1.4 (Feng et al. 2012) to obtain DEGs. For each soybean cultivar and tissue, DGEs were obtained by comparing control and WD treatment levels. Only genes that presented \log_2 fold-change ($\text{Log}_2 \text{FC}$) ≤ -1 (down-regulated) and ≥ 1 (up-regulated) were considered differentially expressed. This means that an expression level of at least 2 times between the control and WD levels was considered. The adoption of good practices of RNA-Seq (RNA sequencing) analyses was performed according to experimental design (Conesa et al. 2016; Molinari et al. 2021b).

In Silico Recovery of ABA-Responsive DEGs and Ontology

The promoter region of all DEGs from each WD treatment (MiWD, MoWD, and SWD) and plant tissue were separately recovered using the RSAT plant (Contreras-Moreira et al. 2016). To recover only ABA-responsive genes among all DEGs, a script in shell language was applied to the data. Genes with at least two cis-elements ACGT core between -1 to -2000 bp within the promoter region were considered (Maruyama et al. 2012; Narusaka et al. 2003; Shen et al. 2004; Nakashima et al. 2006, 2014; Yoshida et al. 2010; Wang et al. 2019). The biological annotation of ABA-responsive DEGs was performed using the Phytomine tool available at Phytozome (Goodstein et al. 2012).

To retrieve genes expressed in all levels of WD treatments, a Venn diagram was executed (Bardou et al. 2014). This group of genes represents 100% of expression reproducible along with the WD treatments and was highly accurate to evaluate ABA responses, minimizing sequencing biases. ABA-responsive DEGs identified in all three levels of WD levels (MiWD, MoWD, and SWD) were used to generate 09 genic groups according to their expression profile in tissues and cultivars. The KEGG pathways were evaluated using ShinyGO software version 0.66 (p -value ≥ 0.05) (Ge et al. 2020).

RT-qPCR

To validate the expression of *NCED3* (9-cis-epoxycarotenoid dioxygenase) and *GOLS* (galactinol synthase), known drought-responsive genes, identified in the RNA-Seq, an RT-qPCR (reverse transcriptase quantitative polymerase chain reaction) was performed. Total RNA was extracted from soybean leaves and root samples from both soybean cultivars using TRIzol[®] reagent and treated with DNase I kit (Invitrogen, Carlsbad,

CA) to remove possible remaining DNA. After that, cDNA was synthesized using SuperScript[®] III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Sets of primers for *GOLS* (Glyma.10G145300—primer F 5'GCTACCCGATGTTCCCAAG3'/ primer R 5'GGGGTACACGGGCTCAATCT3'), *NCED* (Glyma.15G250100—primer F 5'CCAGAAGCCTTACCTCAAAT3'/primer R 5'TCATGGTGGGTTCTTTCAA3') genes were designed. Sequences were obtained from Phytozome v13, and specific primers were designed using Primer3Plus software (Primer3Plus software. 2022). Homo and heterodimers were checked using Multiple Primer Analyze software (Multiple Primer Analyze software 2022).

RT-qPCR reactions were composed of cDNAs, 0.2 μM F and R primers, and 1 \times reaction buffer Platinum[®] SYBR Green[®] qPCR SuperMix UDG (Invitrogen, Carlsbad, CA). PCR primer efficiencies were estimated using LinRegPCR software v.2012.0 (Ruijter et al. 2019), considering efficient primers displaying values $\geq 90\%$. Gene expression relative quantification was performed using three random biologicals and three technical replicates ($n=9$). Reactions were made through 7900HT thermocycler equipment (Applied Biosystems). Cycling conditions used were denaturation at 95 °C for 20 s, followed by 40 cycles of 95 °C for 3 s, 60 °C for 26 s, and 1 cycle for the Melting curve at 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s.

Gene expression calibration was performed using β -actin (Glyma.15G050200—primer F 5'GAGCTATGAATTGCC TGATGG3'/primer R 5'CGTTTCATGAATTCCAGTAGC3') and Fyve (Glyma.13G114700—primer F 5'TTCTGTCTT CTGCAAGTGGTG3'/primer R 5'GATCCCTCATCCATA CATTTCAG3') genes, as described by Marcolino-Gomes et al. (Marcolino-Gomes et al. 2015). The expression level was determined using the formula $2^{-\Delta\Delta C_t}$ adapted according to the primer's efficiencies (Livak and Schmittgen 2001).

Results

ABA-Responsive DEG in Three Levels of WD Treatment

Results showed that the total number of differentially expressed genes identified in leaves were 5256 and 5279, respectively, in BR16 (sensitive cultivar) and Embrapa 48 (tolerant cultivar). In roots, for BR16 and Embrapa 48, the total number of DEG was 4957 and 5665, respectively (Fig. 1). In Fig. 1, it can also be observed the number of ABA-responsive genes identified in each dataset (3875; 3753; 3875; 4256). Those numbers indicated that an average of 75% of the DEG were identified as ABA-responsive, once at least two cis-elements ACGT core were found between -1 to -2000 bp within their

promoter region. Among the ABA-responsive genes, approximately 40% were differentially expressed in all three levels of water deficit treatments (MiWD; MoWD; SWD). This subset of genes is important for both soybean cultivars as the drought intensifies and has 100% reproducibility within the independent libraries of the present study (756; 1892; 865; 2535 genes identified in all treatments, in leaves and roots of BR16 and Embrapa 48), representing an accurate gene set to study the ABA-enriched pathways among soybean cultivars (Fig. 1). The individual expression of these data sets' genes is available in Additional file 1 and Additional file 2.

Groups of ABA-Responsive Genes

The intersection of ABA-responsive genes (756; 1892; 865; 2535) was clustered into 09 ABA-responsive groups (Fig. 2). In group 01, 105 genes were observed in leaves,

mostly being upregulated (61). Forty-one genes showed downregulation in all WD treatments and soybean cultivars, and 3 genes presented different expression profiles, varying accordingly with WD treatments (MiWD, MoWD, and SWD) (Additional file 3, sheet: group 01). Group 02 comprised 1071 genes expressed in the roots, with 888 and 182 genes showing, respectively, upregulation and downregulation. Only one gene showed expression profile variation among WD treatments (Additional file 3, sheet: group 02).

Groups 03 and 04 clustered ABA-responsive genes classified as cultivar-specific. In group 03, 16 genes were identified as being expressed in the leaves and roots of drought-sensitive cultivar BR16. Among these genes, 12 were upregulated and 1 downregulated, with 3 other genes showing different expression profiles, varying accordingly with treatments (additional file 3, sheet: group 03). Group 04 comprised genes expressed in leaves and roots of

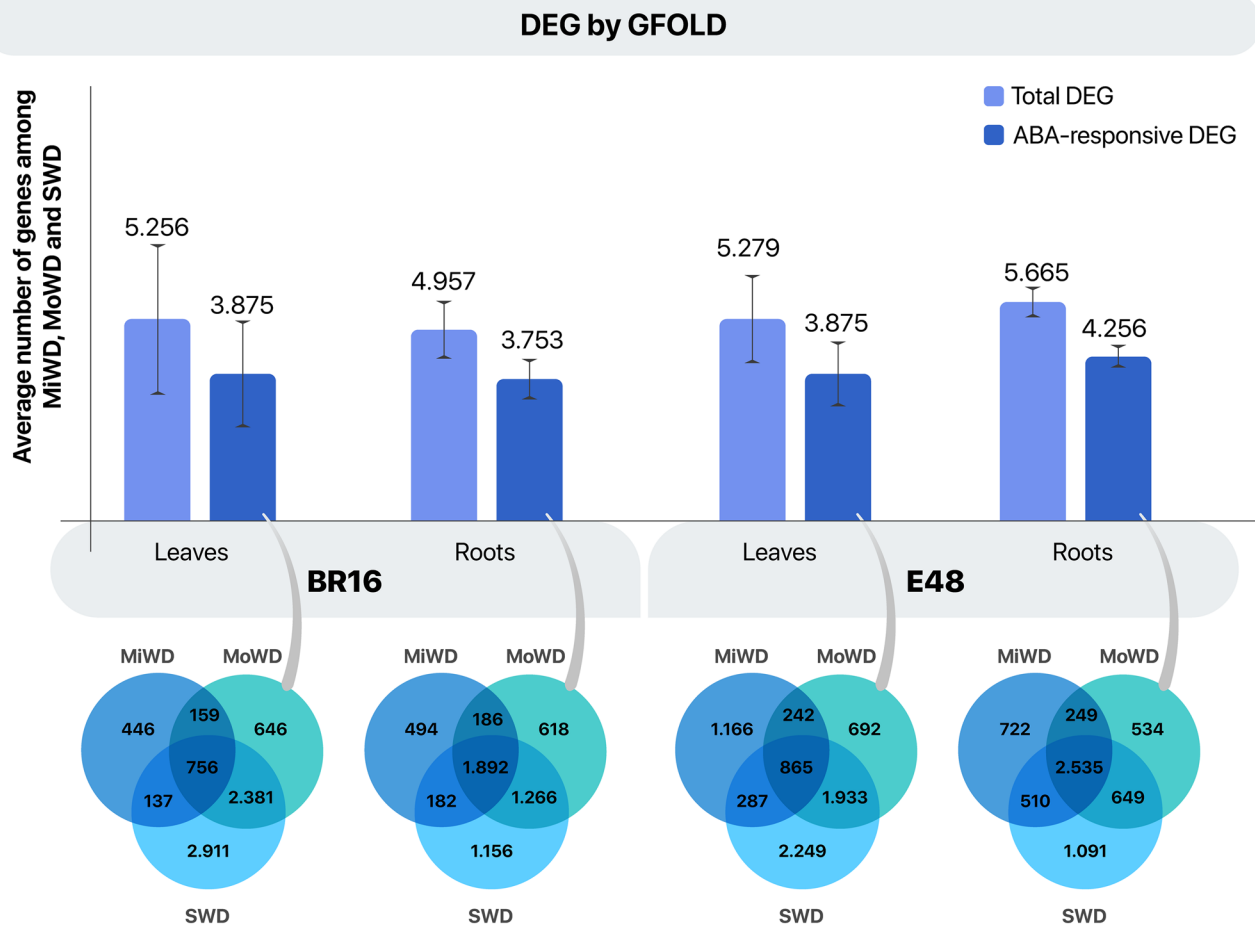


Fig. 1 The total set of differentially expressed genes (DEGs) and the subset of ABA-responsive genes in BR16 (drought-sensitive) and Embrapa 48 (drought-tolerant) soybean cultivars during mild (MiWD—T25–50 min), moderate (MoWD—T75–100 min), and severe water deficit (SWD—T125–150 min) treatments compared

to the control (no WD). The error bar represents the standard error among the number of the average genes in the three water-deficit levels. Venn diagrams represent the intersection of ABA-responsive genes expressed in the three water-deficit levels (756; 1892; 865; 2535)

drought-tolerant cultivar E48. In this group, 40 genes were observed, 13 being upregulated, 19 downregulated, and 8 genes presented expression profiles discrepant among WD treatments (Additional file 3, sheet: group 04).

Groups 05 and 06 included genes expressed, respectively, in leaves and roots of sensitive cultivar BR16. Thus, in group 05, a total of 187 genes were identified, with 129 and 43 showing upregulation and downregulation, respectively, in all three WD treatments. Fifteen genes presented expression profiles differing among MiWD, MoWD, and SWD treatments (Additional file 3, sheet: group 05). In group 06, 381 genes expressed only in the roots of BR16 were identified. Among them, 179 were upregulated, 201 were downregulated, and one gene showed an expression profile varying accordingly with WD treatments (Additional file 3, sheet: group 06). In these groups, most of the genes were downregulated, opposing previous groups.

Groups 07 and 08 included genes expressed, respectively, in leaves and roots of drought-tolerant cultivar E48. Again, in these groups, most of the genes were downregulated. In group 07, from a total of 488 genes, 152 and 301 genes showed, respectively, upregulation and downregulation. Thirty-five genes presented expression profiles varying accordingly with WD treatments (additional file 3, sheet: group 07). In group 08, genes expressed only in roots of E48 were gathered. From a total of 933 genes, 533 were upregulated and 400 were downregulated. No genes showing expression profile variation among WD treatments were identified.

Finally, in group 09, ABA-responsive DEGs were identified in both soybean cultivars (E48 and BR16), and tissues (leaves and roots) in all levels of WD treatments (MiWD, MoWD, and SWD) were collected (Additional file 3, sheet: group 09). In this group, 134 genes were observed, with 125 being upregulated and 9 downregulated. No genes showing expression profile variation among WD treatments were identified.

ABA-Enriched Responsive Pathways in Three Levels of Drought (MiWD; MoWD; SWD)

KEGG analyses indicated 06 ABA-enriched pathways in leaves of BR16. These were involved in sugar, camalexin, glycerophospholipid, ethylene, jasmonate biosynthesis, and reactive oxygen species (ROS) homeostasis. In BR16 roots, 10 enriched pathways were observed, being 06 of them the same as observed in leaves. The remaining four were identified as involved in glycolipid, amines, auxin, and cytokinin biosynthesis (Fig. 3).

In leaves of drought-tolerant Embrapa 48, 07 ABA-enriched pathways were observed. They were glycerolipid, sulfur amino acid, sugar, auxin, camalexin, ethylene, and jasmonate biosynthesis. In Embrapa 48 roots, 11 enriched

pathways were found, 7 of them as identified in leaves, plus pathways related to amines, glycerophospholipid, auxin, and cytokinin biosynthesis.

Despite the similarities among the ABA responses between soybean cultivars, the number of compounds in each pathway was higher in the tolerant cultivar than in the sensitive one. This was seen mainly for membrane components like glycerolipid and glycerophospholipid in the roots, suggesting that ABA responses may be more intense in Embrapa 48 (tolerant cultivar) (Fig. 3).

RT-qPCR of Known ABA-Responsive Genes

RT-qPCR and RNA-Seq showed the same expression profile of *GmNCED3* and *GmGOLS* genes. Both genes were upregulated in both techniques (Fig. 4). These genes presented higher expression on the roots of both soybean cultivars. Also, more than two ABRE motifs were observed in the promoters of the *GmNCED3* (5 ABRE motifs identified) and *GmGOLS* (6 ABRE motifs identified) genes, confirming the ABA biosynthesis activation under water deficit conditions (Fig. 4).

Discussion

ABA-Responsive DEGs and Enriched Pathways Under WD Treatments

Most of the drought and ABA-related genes identified here are greatly known in literature, increasing the reliability of this approach to identify genes with a real profile under drought *in silico*. Besides that, the same expression profile (mostly upregulation) of genes, showing slight modulation differences, reinforces the involvement of these genes in WD defense mechanisms and the accuracy of the pipeline employed. This is possible to infer due to all libraries' being completely independent but presenting similar responses to the conditions applied.

In general, a higher number of differential expressed genes were identified in both leaves and roots for both soybean cultivars, with a high percentage being ABA-responsive as well. Most DEGs and ABA-responsive genes identified in root-specific groups (Additional file 2; Additional final 3/group 02/group 06 and group 08) were drought-response genes such as heat shock proteins and heat shock TFs (transcription factors), genes related to osmotic adjustment (proline, oligosaccharides, trehalose, and glucose), hormone-related (ethylene, auxin, and gibberellin), LEA (late embryogenesis abundant) and chaperones, water channels (aquaporins) and other WD-response related TFs such

Venn diagrams showing 9 clusters of ABA-responsive DEGs identified in all levels of water deficit treatments, in both soybean cultivars and plant tissues

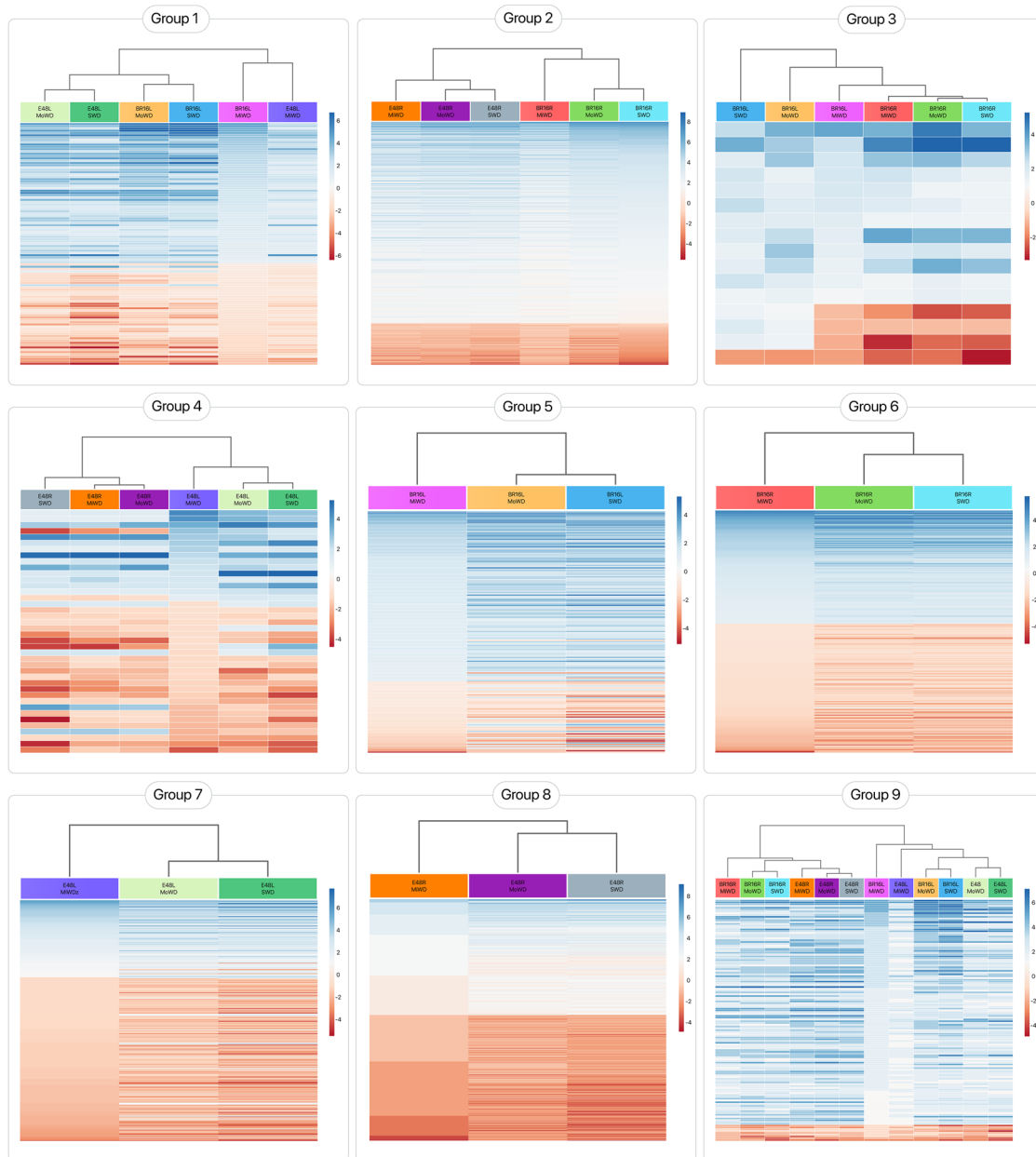
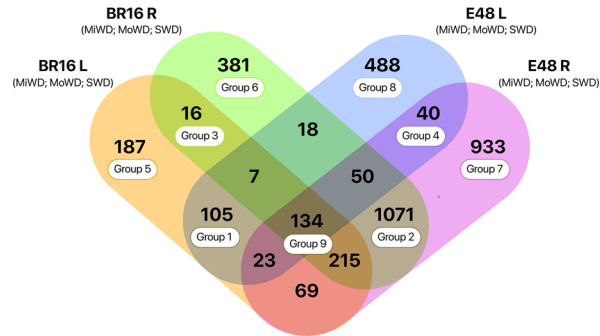


Fig. 2 Venn diagram of 09 clusters of ABA-responsive DEGs identified in all three levels of water-deficit (MiWD—25–50 min, MoWD—75–100 min, and SWD—125–150 min) treatments, in drought-sensitive (BR16) and drought-tolerant (E48) soybean cultivars and tissues (roots and leaves). Legend: L, leaves; R, roots; orange, BR16L, leaves; green, BR16R, roots; blue, E48L, leaves; pink, E48R, roots. Graphics show the total of genes identified in each soybean cultivar, tissue, and WD treatment level

as DREB (dehydration-responsive element-binding protein), NAC (NAM—no apical meristem, Δ TAF1-2-Arabidopsis transcription activation factor, and CUC2—cup-shaped cotyledon), MYB and WRKY. As reported here, these genes were also described to be associated with drought responses in roots of different plant species (Janiak et al. 2016) and recently identified in RNA-Seq analyses carried out in roots of rice (Zhang et al. 2017), sorghum (Zhang et al. 2019), chickpea (Mashaki et al. 2018), and wheat (Chaichi et al. 2019; Iquebal et al. 2019).

As an organ in direct contact with the soil, roots are capable of precepting and signaling soil status toward the shoot, which triggers changes in the regulation of gene expression that afterward controls molecular mechanisms of drought responses (Janiak et al. 2016). In this role, ABA plays an important part in regulating drought sensing by roots. The increase in ABA of roots in response to drought was correlated with an increase in foliar-ABA concentrations, suggesting that drought-induced ABA plays a significant role in controlling leaf water potential (Zegada-Lizarazu and Monti 2019). ABA accumulated in roots is transported to trigger stomatal closure, limiting transpirational water loss while up-regulating genes to promote osmotic adjustment in leaves (Haworth et al. 2018; Bharath et al. 2021). This data corroborates and reinforces the expression of the ABA-responsive genes and their role in leaves' response to water deficit.

Among the 134 genes clustered as commonly expressed in both soybean cultivars, tissues, and in all WD levels (Additional final 2/group 09), drought response genes showing upregulation profile were also identified, such as NAC and WRKY TFs, heat shock TFs and heat shock proteins, and NCED. The NAC TF family is involved in various stress responses. High-throughput transcriptome studies of plants submitted to drought showed that NAC-encoding genes were differentially expressed in roots of rice (Moumeni et al. 2011), pine (Lorenz et al. 2011), cotton (Ranjan and Sawant 2014), and common bean (Recchia et al. 2013). In poplar and soybean, differential expression of NAC genes was identified specifically or preferentially in roots (Cohen et al. 2010; Le et al. 2011), corroborating results found here. Many reports have shown the involvement of WRKYs and ABA, mainly in the stomata closure response (Finatto et al. 2018). In Arabidopsis, *AtWRKY* genes are involved in increasing salt and osmotic stress sensibility (Chen et al. 2010). In tobacco, data showed that ABA participated in WRKY-induced stomatal

closure (Chu et al. 2015). In addition, overexpression of *WRKY* genes increased drought tolerance in *Vitis vinifera* (Wang et al. 2014), cotton (*Gossypium hirsutum*) (Yan et al. 2015), rice (Wu et al. 2009), and tobacco (Ding et al. 2015).

Heat shock factors (HSFs) are important transcription factors that are frequently induced by heat along with other abiotic stresses like salt, drought, and cold (Manna et al. 2020). The involvement of HSFs in drought responses has been reported in many crop species, such as chickpea (Ma et al. 2016), maize (Li et al. 2015), wheat (Huang et al. 2016), sorghum (Tashi et al. 2018), and rice (Yoshida et al. 2011).

ABA-enriched pathways were identified in both tissues and soybean cultivars under all three levels of WD treatments. Sugar-related, camalexin, glycerophospholipid, and pathways for ethylene and jasmonate hormones biosynthesis were identified in both leaves and tissues. In addition to these pathways, amines, auxin, and cytokinin pathways were root specific (Fig. 3). Cellular sugar status is essentially maintained during normal growth conditions but is adversely affected during various environmental perturbations. Drought presents such an unfavorable environmental condition that it hinders the photosynthetic fixation of carbon in sugars and affects their transport, decreasing the cellular osmotic potential (Kaur et al. 2021). However, the tolerant cultivar was able to produce greater amounts of these compounds under drought and guarantee their osmoprotection and thus, adaptation to lack of water. Besides, drought reprograms the distribution of sugar in cellular and subcellular compartments. Usually, plants more tolerant to drought present higher levels of sugars, guaranteeing osmotic potential maintenance, protection of photosynthetic apparatus, maintenance of ROS, positive feedback in ABA biosynthesis, biosynthesis of heat shock proteins, increase in numbers of polyamines, proline accumulation, and prevention of water loss by lignin deposition (Kaur et al. 2021) (Fig. 3).

In addition to the sugar content, drought induces changes in glycerolipid metabolism and glycerophospholipid metabolism (Liu et al. 2021a). In general, lipids play important roles in cellular functions, including serving as structural molecules in membranes, affecting membrane proteins' function, and enhancing their protection. As drought induces an increase in fatty acid saturation of plasma membrane lipids and ROS accumulation, this leads to membrane stiffness (Liu et al. 2021a). However, the accumulation of lipids leads to the protection of these membranes in dry conditions. The presence of these pathways in the Embrapa 48 cultivar suggested that, besides many other defense mechanisms triggered by water deficit, this could be an additional one to cope with drought in tolerant cultivars (Fig. 3).

Furthermore, the presence of enriched camalexin pathways in both soybean cultivars shows the well-known

Enriched ABA-responsive pathways

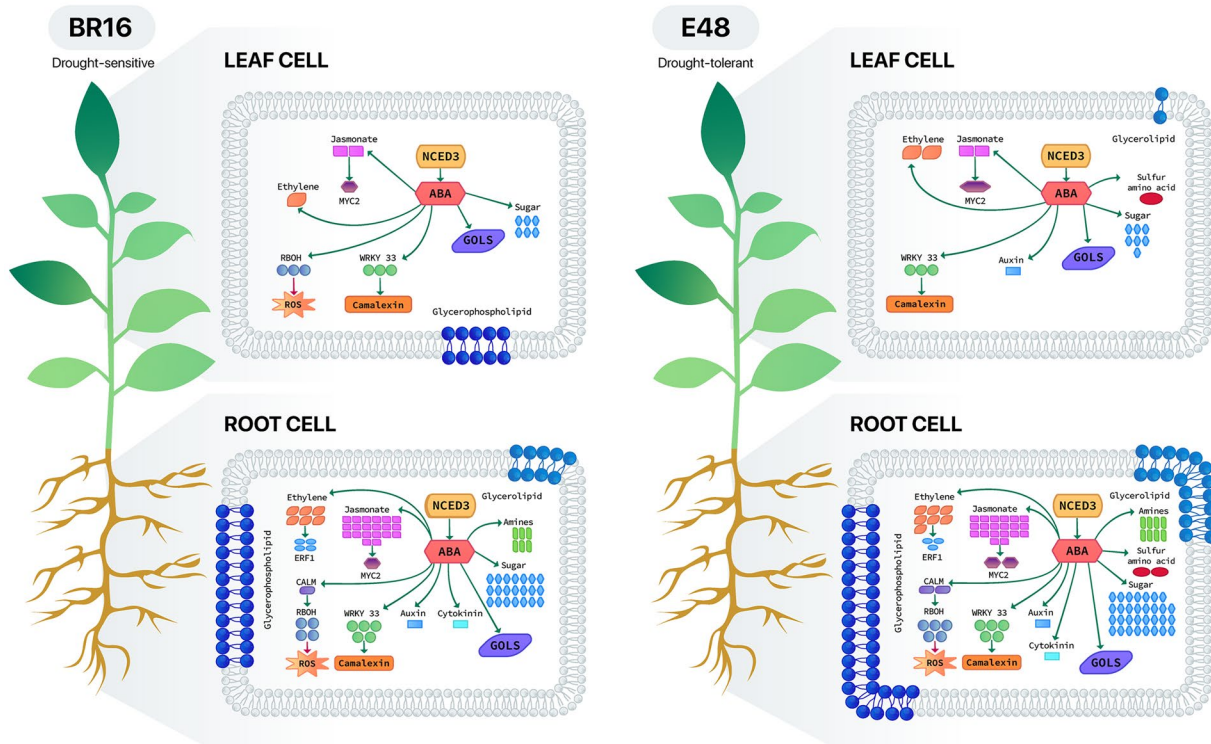


Fig. 3 ABA-enriched responsive pathways under all levels of water deficit treatments (mild, MiWD; moderate, MoWD; severe, SWD) in drought-sensitive (BR16) and tolerant E48 (Embrapa 48) soybean cultivars. Green arrows represent activation; red arrow represents modulation

crosstalk between biotic and abiotic stress responses. The phytoalexin camalexin plays a crucial role in defense against fungal and bacterial pathogens (Mewis et al. 2012), and high levels of camalexin in plants subjected to drought can potentially reduce infection by pathogens (Mewis et al. 2012; Zhang et al. 2014).

The ROS homeostasis pathway was only enriched in leaves of BR16, a drought-sensitive cultivar. Some of the secondary messengers triggered by ABA can participate in plants' adaptation to abiotic and biotic stress. Examples are ROS (enriched pathway identified in leaves of BR16), nitric oxide (NO), and cytosolic-free Ca^{2+} (León et al. 2014; Huang et al. 2019). The generation of ROS in plants under water deficit is due to ABA binding to its receptor, which inactivates protein phosphatase 2C, resulting in the activation of OST1 (open stomata 1) kinase, which stimulates NADPH oxidase (due to phosphorylation) enzyme to generate ROS and then the production of NO. Both ROS and NO can elevate levels of cytosolic Ca^{2+} . The high levels of ROS, NO, and Ca^{2+} act either directly or together to activate anion/cation efflux channels while inhibiting the influx channels. The result is the loss of cations/anions from guard

cells, resulting in turgor loss and stomatal closure (Agurla et al. 2018), a well-known response triggered by water deficit conditions.

Besides ABA, several other hormones increase when plants are exposed to stress, which closes stomata and helps plant defense responses. As pathways enriched here, auxins, cytokinins, jasmonate, and ethylene usually complement ABA's role in drought conditions responses (Iqbal et al. 2022). Auxins are involved in many plants' developmental processes as well as in a dynamic role in mediating and improving plant tolerance to noninfectious stresses, such as deficiency conditions (Kazan 2013). When plants are exposed to drought, different modulations in the synthesis, metabolism, transport, and activity of auxins take place (Ljung 2013). In tobacco seedlings, auxins promote root branching and have a potential role in drought tolerance mechanisms (Verma et al. 2016; Wang et al. 2018). Molecularly, according to Jung and co-workers (Jung et al. 2015), among auxin-coding genes recognized in rice plants, some genes were activated by drought stress. In addition, the overexpression of auxin-related genes also resulted in enhanced drought tolerance

overexpression of *CKX1*, *CKX2*, *CKX3*, and *CKX4* genes resulted in transgenic lines with reduced CK levels and subsequently greater drought tolerance (Nishiyama et al. 2011). Ethylene is strongly involved in the response to dehydration. In addition to acting on osmoregulation, it involves the activation of transcription factors of the ERF (responsive factors of ethylene) family, involved in drought tolerance (Kaur et al. 2021). In Arabidopsis, the overexpression of an ERF gene resulted in lines showing drought tolerance by lessening transpiration, squeezing the aperture of the stomata, and thinning the cuticle (Scarpecci et al. 2017). In cotton, ethylene-responsive genes or binding protein elements such as AP2 (apetala2), EREBPs (ethylene-responsive element binding proteins), and Apetala2 were identified in response to heat and drought conditions (Liu and Zhang 2017). Furthermore, in soybean, *GmERF3* was induced by drought, ABA, salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), and its overexpression in tobacco plants lead to lines exhibiting more tolerance to drought due to high contents of proline and solubilized sugar (Zhang et al. 2009). It is worth emphasizing that in the present study, the ERF1 gene was upregulated in the roots of both soybean cultivars, corroborating ethylene's role in activating drought responses in plants.

Jasmonate also plays a substantial role in water stress tolerance. Its role is well established in crops such as peanuts, rice, soybeans, and broccoli (Kaur et al. 2021). When this phytohormone is applied exogenously, it leads to the accumulation of soluble sugars among other metabolites, in addition to improving the activity of genes that eliminate ROS, thus improving drought tolerance (Abdelgawad et al. 2014; Shan et al. 2015). This data corroborates our data on sugar and ROS homeostasis-enriched pathways in both BR 16 and Embrapa 48 soybean cultivars. Under normal conditions, the proteins JAI3/JAZ bind to various transcription factors, including MYC2, and limit their activity. However, during drought, the degradation of JAZ proteins occurs, resulting in active transcription factors that upregulate genes of JA, enhancing tolerance (Ollas and Dodd 2016; Ullah et al. 2018).

It is important to highlight that all these hormones normally do not work in exclusive pathways but depend on each other at different stages to control responses to environmental factors (Ullah et al. 2018), such as drought. Thus, via jasmonate, the MYC2 transcription factor was enriched, mainly in the roots of both soybean cultivars assayed here. As a result of ABA and jasmonate activation, soybean exhibit pivoting roots with a great ability to penetrate deeper soils in search of water (Kaur et al. 2021). This strong hormonal relationship in the roots could have led the tolerant cultivar to better adapt to the condition of lack of water, as it is observed that these pathways were more expressive in the tolerant cultivar in a number of genes (Fig. 4). Other

examples of these hormones' crosstalk are described below. Exogenous application of auxin enhanced drought tolerance and levels of ABA and JA in *Trifolium repens* (Zhang et al. 2020b). Moreover, JA signaling pathways interact with ABA signaling pathways, suggesting their role in response to drought stress. It has been recently revealed that JA enhanced the hydraulic conductivity of plant roots under drought stress by interacting with calcium and ABA-dependent and independent signaling pathways (De Ollas and Dodd 2016).

Drought-Responsive Genes Expressed Under WD Treatments

ABA increased synthesis is a well-known mechanism triggered by plants to cope with water deficit conditions. The NCED enzyme is a key point control in ABA biosynthesis (Bhaskara et al. 2012; Behnam et al. 2013) and the overexpression of *NCED* genes have improved drought tolerance in many plants such as *Arabidopsis* (Iuchi et al. 2001; Tong et al. 2017), avocado (*Persea americana*) (Chernys and Zeevaart 2000), citrus (*Citrus sinensis*) (Neves et al. 2013; Pedrosa et al. 2015; Rodrigo et al. 2006), common bean (*Phaseolus vulgaris*) (Qin and Zeevaart 1999), tobacco (Qin and Zeevaart 2002; Pedrosa et al. 2017), rice (*Oriza sativa*) (Sultana et al. 2014; Huang et al. 2018), petunia (Estrada-Melo et al. 2015), cotton (*Gossypium hirsutum*) (Souza et al. 2016), cowpea (*Vigna unguiculata*) (Iuchi et al. 2000), grape (*Vitis vinifera*) (Soar et al. 2004), peanuts (*Arachis hypogaea*) (Wan and Li 2006), tomato (*Solanum lycopersicum*) (Burbidge et al. 1999; Thompson et al. 2000), and turmeric (*Curcuma longa*) (Ahrazem et al. 2012). In soybean lines overexpressing the *NCED3* gene, plants showed higher concentrations of ABA under WD (Molinari et al. 2020), reinforcing the role of this phytohormone in drought-response mechanisms as well as corroborating data found here for soybean cultivars BR16 and Embrapa 48.

Galactinol synthase (GOLs) is a key enzyme in raffinose family oligosaccharide (RFO) biosynthesis. In different environmental stresses such as cold, heat, and dehydration drought, RFOs regulate the osmotic potential and protect enzymes and membranes (Crowe et al. 1984; Wang et al. 2004). The expression of some *GOLS* genes was induced by drought, heat shock, salinity, and osmotic shock (Taji et al. 2002; dos Santos et al. 2015). In addition, the involvement of *GOLS* genes in drought responses, as well as the overexpression of these in successfully increasing drought tolerance, has been reported for many plants, such as Arabidopsis (Taji et al. 2002), *Brachypodium distachyon* (Himuro et al. 2014), poplar (Liu et al. 2021b), rice (Takahashi et al. 1994; Shimosaka and Ozawa 2015), maize (Gu et al. 2016), coffee (Santos et al. 2015, 2011), tomato (*Lycopersicon*

esculentum Mill. cv MoneyMaker) (Downie et al. 2003), grape (*Vitis vinifera*) (Pillet et al. 2012), and *Salvia miltiorrhiza* (Wang et al. 2012).

Corroborating our findings here, that the *GmGOLS* gene participates closely in drought defense responses in soybean, Marcolino and co-workers (Marcolino-Gomes et al. 2014) described a diurnal oscillation and induced expression for *GmGOLS*-like gene in lines submitted to water deficit. Gene expression quantification for soybean *GOLS* (Glyma19g40680) confirmed the expression induction in plants submitted to water deficit (Rodrigues et al. 2015). In addition, soybean lines overexpressing *AtGOLS2* showed increased galactinol transcripts, which may have acted as osmoprotectants, leading to higher drought tolerance and survival rate. In field conditions, these plants presented higher yields, probably due to the increased synthesis of RFOs under well-watered conditions (Honna et al. 2016b).

Conclusions

Gene group 09, a hub of drought and ABA-responsive genes, are reliable candidates to be used in projects to develop more drought-tolerant lines, which, in the long-term, can positively impact the soybean crop, minimizing losses due to water scarcity.

Enriched pathways confirmed the complexity of responses triggered in both tolerant and sensitive soybean cultivars by water deficit conditions as well as the large-scale hormonal crosstalk responsible and involved in these defense mechanisms.

Abbreviations ABA: Abscisic acid; ABRE: Abscisic acid-responsive element; ACS: 1-Aminocyclopropane-1-carboxylate synthase; AP2: Apetala2; ARFs: Auxin response factors; At: *Arabidopsis thaliana*; ATAF1-2: Arabidopsis transcription activation factor 1–2; cDNA: Complementary deoxyribonucleic acid; CKK: Cytokinin dehydrogenase; CUC2: Cup-shaped cotyledon; DEGs: Differentially expressed genes; DNA: Deoxyribonucleic acid; DNase: Deoxyribonuclease; DREB: Dehydration-responsive element-binding protein; E48: Embrapa 48; EREBPs: Ethylene-responsive element binding proteins; ERF: Responsive factors of ethylene; ET: Ethylene; Gm: Glycine max; GOLS: Galactinol synthase; HSFs: Heat shock factors; JA: Jasmonic acid; JAI3/JAZ: Jasmonate-insensitive/jasmonate-zim; KEGG: Kyoto Encyclopedia of Genes and Genomes; LEA: Late embryogenesis abundant; Log₂ FC: Log₂ fold-change; MiWD: Mild water-deficit treatment; MoWD: Moderate water-deficit treatment; mRNA: Messenger ribonucleic acid; NAC: No apical meristem; NADPH: Nicotinamide adenine dinucleotide phosphate; NCED3: 9-Cis-epoxycarotenoid dioxygenase; NO: Nitric oxide; OST1: Open stomata 1; RFO: Raffinose family oligosaccharide; RIN: RNA integrity number; RNA: Ribonucleic acid; RNA-Seq: RNA sequencing; ROS: Reactive oxygen species; rRNA: Ribosomal ribonucleic acid; RT-qPCR: Reverse transcriptase quantitative polymerase chain reaction; SA: Salicylic acid; SWD: Severe water-deficit treatment; TFs: Transcription factors; TSS: Transcription start site; WD: Water deficit.

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Author Contribution MDCM conceived and designed the study, performed data analysis and interpretation, and wrote the manuscript. RFP also conceived and designed the study, performed data analysis and results compilation, reviewed the manuscript, and provided scientific editing and language proofreading. EGB reviewed the manuscript and provided scientific editing and language proofreading. DAB, DRM, and SRRM were involved in the experiments and collecting of biological material in a greenhouse. LMMH and ALN are the principal researchers in the project and reviewed the final version of the manuscript. All authors have read and approved the final version of the article.

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Declarations

Ethical Approval and Consent to Participate Not applicable.

Consent for Publication All authors gave their consent for the publication of the research results.

Conflict of Interest The authors declare no competing interests.

References

- Abdelgawad ZA, Khalafaallah AA, Abdallah MM (2014) Impact of methyl jasmonate on antioxidant activity and some biochemical aspects of maize plant grown under water stress condition. *Agric Sci* 5(12):1077–1088. <https://doi.org/10.4236/as.2014.512117>
- Agurla S, Gahir S, Munemasa S, Murata Y, Raghavendra AS (2018) Mechanism of stomatal closure in plants exposed to drought and cold stress. In: Iwaya-Inoue M, Sakurai M, Uemura M (eds) *Survival strategies in extreme cold and desiccation. Advances in experimental medicine and biology*, vol 1081. Springer, Singapore, pp 215–232
- Ahrazem O, Rubio-Moraga A, Trapero A, Gómez-Gómez L (2012) Developmental and stress regulation of gene expression for a 9-cis-epoxycarotenoid dioxygenase, CstNCED, isolated from *Crocus sativus* stigmas. *J Exp Bot* 63(2):681–694. <https://doi.org/10.1093/jxb/err293>
- Andrews S (2010) FastQC: a quality control tool for high throughput sequence data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>. Accessed 28 Mar 2022
- Aslam MM, Waseem M, Jakada BH, Okal EJ, Lei Z, Saqib HSA, Yuan W, Xu W, Zhang Q (2022) Mechanisms of abscisic acid-mediated drought stress responses in plants. *Int J Mol Sci* 23(3):1084. <https://doi.org/10.3390/ijms23031084>

- Bardou P, Mariette J, Escudé F, Djemiel C, Klopp C (2014) jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics* 15(1):1–7. <https://doi.org/10.1186/1471-2105-15-293>
- Behnam B, Iuchi S, Fujita M, Fujita Y, Takasaki H, Osakabe Y, Shinozaki K (2013) Characterization of the promoter region of an *Arabidopsis* gene for 9-cis-epoxycarotenoid dioxygenase involved in dehydration-inducible transcription. *DNA Res* 20(4):315–324. <https://doi.org/10.1093/dnares/dst012>
- Bharath P, Gahir S, Raghavendra AS (2021) Abscisic acid-induced stomatal closure: an important component of plant defense against abiotic and biotic stress. *Front Plant Sci* 12:615114. <https://doi.org/10.3389/fpls.2021.615114>
- Bhaskara GB, Nguyen TT, Verslues PE (2012) Unique drought resistance functions of the highly ABA-responsive clade A protein phosphatase 2Cs. *Plant Physiol* 160(1):379–395. <https://doi.org/10.1104/pp.112.202408>
- Bijalwan P, Sharma M, Kaushik P (2022) Review of the effects of drought stress on plants: a systematic approach. *Preprints* 2022020014:1–21. <https://doi.org/10.20944/preprints202202.0014.v1>
- Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15):2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Bouzroud S, Gouiaa S, Hu N, Bernadac A, Mila I, Bendaou N, Smouni A, Bouzayen M, Zouine M (2018) Auxin response factors (ARFs) are potential mediators of auxin action in tomato response to biotic and abiotic stress (*Solanum lycopersicum*). *PLoS ONE* 13(2):e0193517. <https://doi.org/10.1371/journal.pone.0193517>
- Bulgakov VP, Wu HC, Jinn TL (2019) Coordination of ABA and chaperone signaling in plant stress responses. *Trends Plant Sci* 24(7):636–651. <https://doi.org/10.1016/j.tplants.2019.04.004>
- Burbidge A, Grieve TM, Jackson A, Thompson A, McCarty DR, Taylor IB (1999) Characterization of the ABA-deficient tomato mutant *notabilis* and its relationship with maize *Vp14*. *Plant J* 17(4):427–431. <https://doi.org/10.1046/j.1365-313X.1999.00386.x>
- Chaichi M, Sanjarian F, Razavi K, Gonzalez-Hernandez JL (2019) Analysis of transcriptional responses in root tissue of bread wheat (*Triticum aestivum* L.) reveals drought avoidance mechanisms under water scarcity. *PLoS ONE* 14(3):e0212671. <https://doi.org/10.1371/journal.pone.0212671>
- Chen H, Lai Z, Shi J, Xiao Y, Chen Z, Xu X (2010) Roles of *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. *BMC Plant Biol* 10:281. <https://doi.org/10.1186/1471-2229-10-281>
- Chernys JT, Zeevaart JA (2000) Characterization of the 9-cis-epoxycarotenoid dioxygenase gene family and the regulation of abscisic acid biosynthesis in avocado. *Plant Physiol* 124(1):343–354. <https://doi.org/10.1104/pp.124.1.343>
- Chu X, Wang C, Chen X, Lu W, Li H, Wang X, Hao L, Guo X (2015) The cotton WRKY gene GhWRKY41 positively regulates salt and drought stress tolerance in transgenic *Nicotiana benthamiana*. *PLoS ONE* 10(11):e0143022. <https://doi.org/10.1371/journal.pone.0143022>
- Cohen D, Bogeat-Triboulot MB, Tisserant E, Balzergue S, Martin-Magniette M-L, Lelandais G, Ningre N, Renou JP, Tamby JP, Thiec DL, Hummel I (2010) Comparative transcriptomics of drought responses in *Populus*: a meta-analysis of genome-wide expression profiling in mature leaves and root apices across two genotypes. *BMC Genomics* 11:630. <https://doi.org/10.1186/1471-2164-11-630>
- Colebrook EH, Thomas SG, Phillips AL, Hedden P (2014) The role of gibberellin signalling in plant responses to abiotic stress. *J Exp Biol* 217:67–75. <https://doi.org/10.1242/jeb.089938>
- Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szczesniak MW, Wojciech M, Gaffney DJ, Elo LL, Zhang X, Mortazavi A (2016) A survey of best practices for RNA-seq data analysis. *Genome Biol* 17(1):1–19. <https://doi.org/10.1186/s13059-016-0881-8>
- Contreras-Moreira B, Castro-Mondragon JA, Rioualen C, Cantalapiedra CP, Van Helden J (2016) RSAT:Plants: Motif discovery within clusters of upstream sequences in plant genomes. In: Hehl R (ed) *Plant Synthetic Promoters*, vol 1482. Humana Press, New York, pp 279–295
- Crowe JH, Crowe LM, Chapman D (1984) Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science* 223:701–703. <https://doi.org/10.1126/science.223.4637.701>
- De Ollas C, Dodd IC (2016) Physiological impacts of ABA-JA interactions under water-limitation. *Plant Mol Biol* 91:641–650. <https://doi.org/10.1007/s11103-016-0503-6>
- Ding ZJ, Yan JY, Li CX, Li GX, Wu YR, Zheng SJ (2015) Transcription factor WRKY46 modulates the development of *Arabidopsis* lateral roots in osmotic/salt stress conditions via regulation of ABA signaling and auxin homeostasis. *Plant J* 84(1):56–69. <https://doi.org/10.1111/tpj.12958>
- dos Santos TB, Budzinski IGF, Marura CJ, Petkowicz CLO, Pereira LFP, Vieira LGE (2011) Expression of three galactinol synthase isoforms in *Coffea arabica* L. and accumulation of raffinose and stachyose in response to abiotic stresses. *Plant Physiol Biochem* 49:441–448. <https://doi.org/10.1016/j.plaphy.2011.01.023>
- dos Santos TB, Lima RB, Nagashima GT, Petkowicz CLO, Carpentieri-Pípelo V, Pereira LFP, Domingues DS, Vieira LGE (2015) Galactinol synthase transcriptional profile in two genotypes of *Coffea canephora* with contrasting tolerance to drought. *Genet Mol Biol* 38(2):182–190. <https://doi.org/10.1590/S1415-475738220140171>
- Downie B, Gurusinge S, Dahal P, Thacker RR, Snyder JC, Nonogaki H, Yim K, Fukunaga K, Alvarado V, Bradford KJ (2003) Expression of a galactinol synthase gene in tomato seeds is up-regulated before maturation desiccation and again after imbibition whenever radicle protrusion is prevented. *Plant Physiol* 131:1347–1359. <https://doi.org/10.1104/pp.016386>
- Duarte KE, de Souza WR, Santiago TR, Sampaio BL, Ribeiro AP, Cotta MG, Molinari HBC (2019) Identification and characterization of core abscisic acid (ABA) signaling components and their gene expression profile in response to abiotic stresses in *Setaria viridis*. *Sci Rep* 9(1):4028. <https://doi.org/10.1038/s41598-019-40623-5>
- Estrada-Melo AC, Chao C, Reid MS, Jiang CZ (2015) Overexpression of an ABA biosynthesis gene using a stress-inducible promoter enhances drought resistance in petunia. *Hortic Res* 2:1–9. <https://doi.org/10.1038/hortres.2015.13>
- Fehr WR, Caviness CE, Burmood DT, Perennigton JS (1971) Stage of development description for soybeans [*Glycine max* (L.) Merrill]. *Crop Sci* 11(6):929–931. <https://doi.org/10.2135/cropsci1971.0011183X001100060051x>
- Feng J, Meyer CA, Wang Q, Liu JS, Liu X, Zhang Y (2012) GFOLD: a generalized fold change for ranking differentially expressed genes from RNA-seq data. *Bioinformatics* 28(21):2782–2788. <https://doi.org/10.1093/bioinformatics/bts515>
- Ferreira RC (2016) Quantification of drought losses in soybean crop in Brazil. State University of Londrina, Thesis
- Finatto T, Viana VE, Woyann LG, Busanello C, da Maia LC, de Oliveira AC (2018) Can WRKY transcription factors help plants to overcome environmental challenges? *Genet Mol Biol* 41(3):533–544. <https://doi.org/10.1590/1678-4685-GMB-2017-0232>
- Fuganti-Pagliarini R, Ferreira LC, Rodrigues FA, Molinari HBC, Marin SRR, Molinari MDC, Marcolino-Gomes J, Mertz-Henning LM, Farias JRB, de Oliveira MCN, Neumaier N, Kanamori N, Fujita Y, Mizoi J, Nakashima K, Yamaguchi-Shinozaki K, Nepomuceno AL (2017) Characterization of soybean genetically modified for drought tolerance in field conditions. *Front Plant Sci* 8:448. <https://doi.org/10.3389/fpls.2017.00448>

- Ge SX, Jung D, Yao R (2020) ShinyGO: a graphical gene-set enrichment tool for animals and plants. *Bioinformatics* 36(8):2628–2629. <https://doi.org/10.1093/bioinformatics/btz931>
- Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytosome: a comparative platform for green plant genomics. *Nucleic Acids Res* 40(D1):D1178–D1186. <https://doi.org/10.1093/nar/gkr944>
- Gu L, Zhang Y, Zhang M, Li T, Dirk LMA, Downie B, Zhao T (2016) ZmGOLS2, a target of transcription factor ZmDREB2A, offers similar protection against abiotic stress as ZmDREB2A. *Plant Mol Biol* 90:157–170. <https://doi.org/10.1007/s11103-015-0403-1>
- Haworth M, Marino G, Cosentino SL, Brunetti C, De Carlo A, Avola G, Riggia E, Loreto F, Centritto M (2018) Increased free abscisic acid during drought enhances stomatal sensitivity and modifies stomatal behaviour in fast growing giant reed (*Arundo donax* L.). *Environ Exp Bot* 147:116–124. <https://doi.org/10.1016/j.envexpbot.2017.11.002>
- Himuro Y, Ishiyama K, Mori F, Gondo T, Takahashi F, Shinozaki K, Kobayashi M, Akashi R (2014) Arabidopsis galactinol synthase AtGolS2 improves drought tolerance in the monocot model *Brachypodium distachyon*. *J Plant Physiol* 171(13):1127–1131. <https://doi.org/10.1016/j.jplph.2014.04.007>
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Second ed. Circular. Cal Agric Exp Sta Cir 347(2):1–32.
- Hobo T, Asada M, Kowyama Y, Hattori T (1999) ACGT-containing abscisic acid response element (ABRE) and coupling element 3 (CE3) are functionally equivalent. *Plant J* 19(6):679–689. <https://doi.org/10.1046/j.1365-313x.1999.00565.x>
- Honna PT, Fuganti-Pagliarini R, Ferreira LC, Molinari MDC, Marin SRR, Oliveira MCN, Farias JRB, Neumaier N, Mertz-Henning LM, Kanamori N, Nakashima K, Takasaki H, Urano K, Shinozaki K, Yamaguchi-Shinozaki K, Desidério JA, Nepomuceno AL (2016a) Molecular, physiological, and agronomical characterization, in greenhouse and in field conditions, of soybean plants genetically modified with AtGolS2 gene for drought tolerance. *Mol Breeding* 36:157. <https://doi.org/10.1007/s11032-016-0570-z>
- Honna PT, Fuganti-Pagliarini R, Ferreira LC, Molinari MD, Marin SR, de Oliveira MC, Nepomuceno AL (2016b) Molecular, physiological, and agronomical characterization, in greenhouse and in field conditions, of soybean plants genetically modified with AtGolS2 gene for drought tolerance. *Mol Breeding* 36(11):1–17. <https://doi.org/10.1007/s11032-016-0570-z>
- Huang YC, Niu CY, Yang CR, Jinn TL (2016) The heat stress factor HSFA6b connects ABA signaling and ABA-mediated heat responses. *Plant Physiol* 172:1182–1199. <https://doi.org/10.1104/pp.16.00860>
- Huang Y, Guo Y, Liu Y, Zhang F, Wang Z, Wang H, Wang F, Li D, Mao D, Luan S, Liang M, Chen L (2018) 9-Cis-epoxycarotenoid dioxygenase 3 regulates plant growth and enhances multi-abiotic stress tolerance in rice. *Front Plant Sci* 9:162. <https://doi.org/10.3389/fpls.2018.00162>
- Huang S, Waadt R, Nuhkat M, Kollist H, Hedrich R, Roelfsema MRG (2019) Calcium signals in guard cells enhance the efficiency by which abscisic acid triggers stomatal closure. *New Phytol* 224(1):177–187. <https://doi.org/10.1111/nph.15985>
- Iqbal S, Wang X, Mubeen I, Kamran M, Kanwal I, Díaz GA, Abbas A, Parveen A, Atiq MN, Alshaya H, Zin El-Abedin TK, Fahad S (2022) Phytohormones trigger drought tolerance in crop plants: outlook and future perspectives. *Front Plant Sci* 12:799318. <https://doi.org/10.3389/fpls.2021.799318>
- Iqbal MA, Sharma P, Jasrotia RS, Jaiswal S, Kaur A, Saroha M, Rai A (2019) RNA-Seq analysis reveals drought-responsive molecular pathways with candidate genes and putative molecular markers in root tissue of wheat. *Sci Rep* 9:1–18. <https://doi.org/10.1038/s41598-019-49915-2>
- Intergovernmental Panel on Climate Change (2022) Climate change 2022: impacts, adaptation and vulnerability <https://www.ipcc.ch/report/ar6/wg2/> Accessed 05 Jun 2022
- Iuchi S, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2000) A stress-inducible gene for 9-cis-epoxycarotenoid dioxygenase involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. *Plant Physiol* 123(2):553–562. <https://doi.org/10.1104/pp.123.2.553>
- Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. *Plant J* 27:325–333. <https://doi.org/10.1046/j.1365-313x.2001.01096.x>
- Janiak A, Kwaśniewski M, Szarejko I (2016) Gene expression regulation in roots under drought. *J Exp Bot* 67(4):1003–1014. <https://doi.org/10.1093/jxb/erv512>
- Jung H, Lee D-K, Do Choi Y, Kim J-K (2015) OsIAA6, a member of the rice Aux/IAA gene family, is involved in drought tolerance and tiller outgrowth. *Plant Sci* 236:304–312. <https://doi.org/10.1016/j.plantsci.2015.04.018>
- Kaur H, Manna M, Thakur T, Gautam V, Salvi P (2021) Imperative role of sugar signaling and transport during drought stress responses in plants. *Physiol Plant* 171(4):833–848. <https://doi.org/10.1111/ppl.13364>
- Kazan K (2013) Auxin and the integration of environmental signals into plant root development. *Ann Bot* 112:1655–1665. <https://doi.org/10.1093/aob/mct229>
- Ke M, Zheng Y, Zhu Z (2015) Rethinking the origin of auxin biosynthesis in plants. *Front Plant Sci* 6:1093. <https://doi.org/10.3389/fpls.2015.01093>
- Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast-spliced aligner with low memory requirements. *Nat Methods* 12(4):357–360. <https://doi.org/10.1038/nmeth.3317>
- Le DT, Nishiyama R, Watanabe Y, Mochida K, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS (2011) Genome-wide survey and expression analysis of the plant-specific NAC transcription factor family in soybean during development and dehydration stress. *DNA Res* 18(4):263–276. <https://doi.org/10.1093/dnares/dsr015>
- León J, Castillo MC, Coego A, Lozano-Juste J, Mir R (2014) Diverse functional interactions between nitric oxide and abscisic acid in plant development and responses to stress. *J Exp Bot* 65(4):907–921. <https://doi.org/10.1093/jxb/ert454>
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Durbin R (2009) The sequence alignment/map format and SAMtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
- Li HC, Zhang HN, Li GL, Liu ZH, Zhang YM, Zhang HM, Guo XL (2015) Expression of maize heat shock transcription factor gene ZmHsf06 enhances the thermotolerance and drought-stress tolerance of transgenic Arabidopsis. *Funct Plant Biol* 42:1080–1091. <https://doi.org/10.1071/FP15080>
- Li W, Herrera-Estrella L, Tran LP (2016) The Yin-Yang of cytokinin homeostasis and drought acclimation/adaptation. *Trends Plant Sci* 21:548–550. <https://doi.org/10.1016/j.tplants.2016.05.006>
- Liu C, Zhang T (2017) Expansion and stress responses of the AP2/EREBP superfamily in cotton. *BMC Genomics* 18:118. <https://doi.org/10.1186/s12864-017-3517-9>
- Liu W, Zhang Y, Li W, Lin Y, Wang C, Xu R, Zhang L (2020) Genome-wide characterization and expression analysis of soybean trihelix gene family. *PeerJ* 8:e8753. <https://doi.org/10.7717/peerj.8753>
- Liu L, Long X, Wua W, Xiang S, Yu X, Demura T, Lia D, Zhuge Q (2021a) Galactinol synthase confers salt-stress tolerance by

- regulating the synthesis of galactinol and raffinose family oligosaccharides in poplar. *Industrial Crops Prod* 165:113432. <https://doi.org/10.1016/j.indcrop.2021.113432>
- Liu L, Wu X, Sun W, Yu X, Demura T, Li D, Zhuge Q (2021b) Galactinol synthase confers salt-stress tolerance by regulating the synthesis of galactinol and raffinose family oligosaccharides in poplar. *Ind Crops Prod* 165:1–16. <https://doi.org/10.1016/j.indcrop.2021.113432>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25(4):402–408. <https://doi.org/10.1006/meth.2001.1262>
- Ljung K (2013) Auxin metabolism and homeostasis during plant development. *Development* 140:943–950. <https://doi.org/10.1242/dev.086363>
- Lorenz WW, Alba R, Yu YS, Bordeaux JM, Simões M, Dean JF (2011) Microarray analysis and scale-free gene networks identify candidate regulators in drought-stressed roots of loblolly pine (*P. taeda* L.). *BMC Genomics* 12:264. <https://doi.org/10.1186/1471-2164-12-264>
- Ma H, Wang C, Yang B, Cheng H, Wang Z, Mijiti A, Ren C, Qu G, Zhang H, Ma L (2016) CarHSFB2, a class B heat shock transcription factor, is involved in different developmental processes and various stress responses in chickpea (*Cicer Arietinum* L.). *Plant Mol Biol Report* 34:1–14. <https://doi.org/10.1007/s11105-015-0892-8>
- Manna M, Thakur T, Chirom O, Mandlik R, Deshmukh R, Salvi P (2020) Transcription factors as key molecular target to strengthen the drought stress tolerance in plants. *Physiol Plant* 172(2):847–868. <https://doi.org/10.1111/ppl.13268>
- Marcolino-Gomes J, Rodrigues FA, Fuganti-Pagliarini R, Bendix C, Nakayama TJ, Celaya B, Hugo Molinari BC, Oliveira MCN, Harmon FG, Nepomuceno AL (2014) Diurnal oscillations of soybean circadian clock and drought responsive genes. *PLoS ONE* 9(1):e86402. <https://doi.org/10.1371/journal.pone.0086402>
- Marcolino-Gomes J, Rodrigues FA, Fuganti-Pagliarini R, Nakayama TJ, Reis RR, Farias JRB, Harmon F, Molinari HBC, Molinari MDC, Nepomuceno A (2015) Transcriptome-wide identification of reference genes for expression analysis of soybean responses to drought stress along the day. *PLoS ONE* 10(9):e0139051. <https://doi.org/10.1371/journal.pone.0139051>
- Martins PK, Jordão BQ, Yamanaka N, Farias JR, Beneventi MA, Binneck E, Nepomuceno AL (2008) Differential gene expression and mitotic cell analysis of the drought tolerant soybean (*Glycine max* L. Merrill Fabales, Fabaceae) cultivar MG/BR46 (Conquista) under two water deficit induction systems. *Genet Mol Biol* 31(2):512–521
- Maruyama K, Todaka D, Mizoi J, Yoshida T, Kidokoro S, Matsukura S, Kojima M (2012) Identification of cis-acting promoter elements in cold-and dehydration-responsive transcriptional pathways in Arabidopsis, rice, and soybean. *DNA Res* 19(1):37–49. <https://doi.org/10.1093/dnares/dsr040>
- Mashaki KM, Garg V, Ghomi AAN, Kudapa H, Chitikineni A, Nezhad KZ, Thudi M (2018) RNA-Seq analysis revealed genes associated with drought stress response in kabuli chickpea (*Cicer arietinum* L.). *PLoS ONE* 13(6): e0199774. <https://doi.org/10.1371/journal.pone.0199774>
- Mewis I, Khan MA, Glawischnig E, Schreiner M, Ulrichs C (2012) Water stress and aphid feeding differentially influence metabolite composition in *Arabidopsis thaliana* (L.). *PLoS ONE* 7(11):e48661. <https://doi.org/10.1371/journal.pone.0048661>
- Molinari MDC, Fuganti-Pagliarini R, Marin SRR, Ferreira LC, Barbosa DA, Marcolino-Gomes J, Oliveira MCN, Mertz-Henning LM, Kanamori N, Nakashima K, Takasaki H, Urano K, Shinozaki K, Yamaguchi-Shinozaki K, Nepomuceno AL (2020) Overexpression of AtNCED3 gene improved drought tolerance in soybean in greenhouse and field. *Genet Mol Biol* 43(3):e20190292. <https://doi.org/10.1590/1678-4685-GMB-2019-0292>
- Molinari MDC, Fuganti-Pagliarini R, Marcolino-Gomes J, de Amorim BD, Rockenbach Marin SR, Mertz-Henning LM, Rech Filho EL (2021a) Flower and pod genes involved in soybean sensitivity to drought. *J Plant Interact* 16:187–200. <https://doi.org/10.1080/17429145.2021.1921293>
- Molinari MDC, Fuganti-Pagliarini R, Mendonça JA, de Amorim BD, Marin DR, Mertz-Henning L, Nepomuceno AL (2021b) Transcriptome analysis using RNA-Seq from experiments with and without biological replicates: a review. *Rev Ciênc Agrar* 64:1–13
- Moumeni A, Satoh K, Kondoh H, Asano T, Hosaka A, Venuprasad R, Serraj R, Kumar A, Leung H, Kikuchi S (2011) Comparative analysis of root transcriptome profiles of two pairs of drought-tolerant and susceptible rice near-isogenic lines under different drought stress. *BMC Plant Biol* 11:174. <https://doi.org/10.1186/1471-2229-11-174>
- Multiple Primer Analyze software. <https://www.thermofisher.com/br/pt/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html> Accessed 06 Jun 2022
- Munemasa S, Hauser F, Park J, Waadt R, Brandt B, Schroeder JI (2015) Mechanisms of abscisic acid-mediated control of stomatal aperture. *Curr Opin Plant Biol* 28:154–162. <https://doi.org/10.1016/j.pbi.2015.10.010>
- Nakashima K, Fujita Y, Katsura K, Maruyama K, Narusaka Y, Seki M, Yamaguchi-Shinozaki K (2006) Transcriptional regulation of ABI3- and ABA-responsive genes including RD29B and RD29A in seeds, germinating embryos, and seedlings of Arabidopsis. *Plant Mol Biol* 60(1):51–68. <https://doi.org/10.1007/s11103-005-2418-5>
- Nakashima K, Jan A, Todaka D, Maruyama K, Goto S, Shinozaki K, Yamaguchi-Shinozaki K (2014) Comparative functional analysis of six drought-responsive promoters in transgenic rice. *Planta* 239(1):47–60. <https://doi.org/10.1007/s00425-013-1960-7>
- Narusaka Y, Nakashima K, Shinwari ZK, Sakuma Y, Furihata T, Abe H, Yamaguchi-Shinozaki K (2003) Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high-salinity stresses. *Plant J* 34(2):137–148. <https://doi.org/10.1046/j.1365-3113x.2003.01708.x>
- Neves DM, Coelho Filho MA, Bellele BS, Silva MFGF, Souza DT, Soares Filho WDS, Gesteira AS (2013) Comparative study of putative 9-cis-epoxycarotenoid dioxygenase and abscisic acid accumulation in the responses of Sunki mandarin and Rangpur lime to water deficit. *Mol Biol Rep* 40:5339–5349. <https://doi.org/10.1007/s11033-013-2634-z>
- Nishiyama R, Watanabe Y, Fujita Y, Dung TL, Kojima M, Werner T, Vankova R, Yamaguchi-Shinozaki K, Shinozaki K, Kakimoto T, Sakakibara H, Schmölling T, Tran L-SP (2011) Analysis of cytokinin mutants and regulation of cytokinin metabolic genes reveals important regulatory roles of cytokinins in drought, salt and abscisic acid responses, and abscisic acid biosynthesis. *Plant Cell* 23:2169–2183. <https://doi.org/10.1105/tpc.111.087395>
- Ortiz R (2019). Role of plant breeding to sustain food security under climate change. In: Yadav SS, Redden RJ, Hatfield JL, Ebert AW, Hunter D (eds) Food security and climate change, first ed. John Wiley & Sons Ltd, Hoboken, pp 145–158
- Oya T, Nepomuceno AL, Neumaier N, Farias JRB, Tobita S, Ito O (2004) Drought tolerance characteristics of Brazilian soybean cultivars. *Plant Prod Sci* 7(2):129–137. <https://doi.org/10.1626/pps.7.129>
- Pedrosa AM, Cidade LC, Martins CPS, Macedo AF, Neves DM, Gomes FP, Floh EIS, Costa MGC (2017) Effect of overexpression of citrus 9-cis-epoxycarotenoid dioxygenase 3 (CsNCED3)

- on the physiological response to drought stress in transgenic tobacco. *Genet Mol Res* 16(1):1–10. <https://doi.org/10.4238/gmr16019292>
- Pedrosa AM, Martins CDPS, Goncalves LP, Costa MGC (2015) Late embryogenesis abundant (LEA) constitutes a large and diverse family of proteins involved in development and abiotic stress responses in sweet orange (*Citrus sinensis* L. Osb.). *PloS ONE* 10(12):e0145785. <https://doi.org/10.1371/journal.pone.0145785>
- Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL (2015) StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol* 33(3):290–295. <https://doi.org/10.1038/nbt.3122>
- Pillet J, Egert A, Pieri P, Lecourieux F, Kappel C, Charon J, Gomes E, Keller F, Delrot S, Lecourieux D (2012) VvGOLS1 and VvHsfA2 are involved in the heat stress responses in grapevine berries. *Plant Cell Physiol* 53:1776–1792. <https://doi.org/10.1093/pcp/pcs121>
- Pizzio GA (2022) Abscisic acid machinery is under circadian clock regulation at multiple levels. *Stresses* 2(1):65–78. <https://doi.org/10.3390/stresses2010006>
- Primer3Plus software. <https://primer3plus.com/cgi-bin/dev/primer3plus.cgi> Accessed 06 Jun 2022.
- Qin X, Zeevaart JA (1999) The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. *Proc Natl Acad Sci* 96:15354–15361. <https://doi.org/10.1073/pnas.96.26.15354>
- Qin X, Zeevaart JA (2002) Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. *Plant Physiol* 128:544–551. <https://doi.org/10.1104/pp.010663>
- Ranjan A, Sawant S (2014) Genome-wide transcriptomic comparison of cotton (*Gossypium herbaceum*) leaf and root under drought stress. *3 Biotech* 5:585–596. <https://doi.org/10.1007/s13205-014-0257-2>
- Recchia GH, Gomes Caldas DG, Ahern Beraldo AL, da Silva MJ, Tsai SM (2013) Transcriptional analysis of drought-induced genes in the roots of a tolerant genotype of the common bean (*Phaseolus vulgaris* L.). *Int J Mol Sci* 14(4):7155–7179. <https://doi.org/10.3390/ijms14047155>
- Rodrigo MJ, Alquezar B, Zacarías L (2006) Cloning and characterization of two 9-cis-epoxycarotenoid dioxygenase genes, differentially regulated during fruit maturation and under stress conditions, from orange (*Citrus sinensis* L. Osbeck). *J Exp Bot* 57:633–643. <https://doi.org/10.1093/jxb/erj048>
- Rodrigues FA, Marcolino-Gomes J, Carvalho JD, Nascimento LCD, Neumaier N, Farias JRB, Nepomuceno AL (2012) Subtractive libraries for prospecting differentially expressed genes in the soybean under water deficit. *Genet Mol Biol* 35:304–314. <https://doi.org/10.1590/S1415-47572012000200011>
- Rodrigues FA, Fuganti-Pagliarini R, Marcolino-Gomes J, Nakayama TJ, Molinari HBC, Lobo FP, Harmon FG, Nepomuceno AL (2015) Daytime soybean transcriptome fluctuations during water deficit stress. *BMC Genomics* 16:505. <https://doi.org/10.1186/s12864-015-1731-x>
- Ruijter J, Van Der Velden S, Ilgun A (2019) LinRegPCR: analysis of quantitative RT-PCR data. https://www.gene-quantification.de/LinRegPCR_help_manual_v11.0.pdf. Accessed 28 Mar 2022
- Scarpeci TE, Freia VS, Zanol MI, Valle EM (2017) Overexpression of AtERF019 delays plant growth and senescence and improves drought tolerance in *Arabidopsis*. *J Exp Bot* 68:673–685. <https://doi.org/10.1093/jxb/erw429>
- Shan C, Zhou Y, Liu M (2015) Nitric oxide participates in the regulation of the ascorbate-glutathione cycle by exogenous jasmonic acid in the leaves of wheat seedlings under drought stress. *Protoplasma* 252:1397–1405. <https://doi.org/10.1007/s00709-015-0756-y>
- Shen QJ, Casaretto JA, Zhang P, Ho THD (2004) Functional definition of ABA-response complexes: the promoter units necessary and sufficient for ABA induction of gene expression in barley (*Hordeum vulgare* L.). *Plant Mol Biol* 54(1):111–124. <https://doi.org/10.1023/B:PLAN.0000028773.94595.e8>
- Shimosaka E, Ozawa K (2015) Overexpression of cold-inducible wheat galactinol synthase confers tolerance to chilling stress in transgenic rice. *Breeding Sci* 65:363–371. <https://doi.org/10.1270/jsbbs.65.363>
- Shinozaki K, Yamaguchi-Shinozaki K (2007) Gene networks involved in drought stress response and tolerance. *J Exp Bot* 58:221–227. <https://doi.org/10.1093/jxb/erl164>
- Singh PK, Indoliya Y, Agrawal L, Awasthi S, Deeba F, Dwivedi S, Chakrabarty D, Shirke PA, Pandey V, Singh N, Dhankher OP, Barik SK, Tripathi RD (2022) Genomic and proteomic responses to drought stress and biotechnological interventions for enhanced drought tolerance in plants. *Curr Plant Biol* 29:100239. <https://doi.org/10.1016/j.cpb.2022.100239>
- Soar CJ, Speirs J, Maffei SM, Loveys BR (2004) Gradients in stomatal conductance, xylem sap ABA and bulk leaf ABA along canes of *Vitis vinifera* cv. Shiraz: molecular and physiological studies investigating their source. *Funct Plant Biol* 31:659–669. <https://doi.org/10.1071/FP03238>
- Souza A, Batista VG, Pinheiro MP, Suassuna JF, Lima LMD, Fernandes PD (2016) Expression of NCED gene in colored cotton genotypes subjected to water stress. *Rev Bras Eng Agríc Ambient* 20:692–696. <https://doi.org/10.1590/1807-1929/agriambi.v20n8p692-696>
- Suhartina, Purwantoro, Nugrahaeni N, Taufiq A, Mejaya MJ (2022) Yield stability performance of soybean (*Glycine max* [L.] Merrill) lines tolerant to drought. In: AIP Conference Proceedings, AIP Publishing LLC, 2462(1):020004 Jan 2022
- Sultana S, Turečková V, Ho CL, Napis S, Namasivayam P (2014) Molecular cloning of a putative *Acanthus ebracteatus*-9-cis-epoxycarotenoid dioxygenase (AeNCED) and its overexpression in rice. *J Crop Sci Biotechnol* 17(4):239–246. <https://doi.org/10.1007/s12892-014-0006-4>
- Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K (2002) Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *Plant J* 29:417–426. <https://doi.org/10.1046/j.0960-7412.2001.01227.x>
- Takahashi R, Joshee N, Kitagawa Y (1994) Induction of chilling resistance by water stress, and cDNA sequence analysis and expression of water stress regulated genes in rice. *Plant Mol Biol* 26:339–352. <https://doi.org/10.1007/BF00039544>
- Tashi G, Zhan H, Xing G, Chang X, Zhang H, Nie X, Ji W (2018) Genome-wide identification and expression analysis of heat shock transcription factor family in *Chenopodium quinoa* Willd. *Agronomy* 8(7):103. <https://doi.org/10.3390/agronomy8070103>
- Thompson AJ, Jackson AC, Symonds RC, Mulholland BJ, Dadswell AR, Blake PS, Burbidge A, Taylor IB (2000) Ectopic expression of a tomato 9-cis-epoxycarotenoid dioxygenase gene causes overproduction of abscisic acid. *Plant J* 23:363–374. <https://doi.org/10.1046/j.1365-3113x.2000.00789.x>
- Tong SM, Xi HX, Ai KJ, Hou HS (2017) Overexpression of wheat TaNCED gene in *Arabidopsis* enhances tolerance to drought stress and delays seed germination. *Biol Plant* 61:64–72. <https://doi.org/10.1007/s10535-016-0692-5>
- Ullah A, Manghwar H, Shaban M, Khan AH, Akbar A, Ali U, Fahad S (2018) Phytohormones enhanced drought tolerance in plants: a coping strategy. *Environ Sci Pollut Res* 25(33):33103–33118. <https://doi.org/10.1007/s11356-018-3364-5>

- Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. *Proc Natl Acad Sci* 97(21):11632–11637. <https://doi.org/10.1073/pnas.190309197>
- Verma V, Ravindran P, Kumar PP (2016) Plant hormone-mediated regulation of stress responses. *BMC Plant Biol* 16:1–10. <https://doi.org/10.1186/s12870-016-0771-y>
- Wan XR, Li L (2006) Regulation of ABA level and water-stress tolerance of Arabidopsis by ectopic expression of a peanut 9-cis-epoxycarotenoid dioxygenase gene. *Biochem Biophys Res Commun* 347:1030–1038. <https://doi.org/10.1016/j.bbrc.2006.07.026>
- Wang S, Wan C, Wang Y, Chen H, Zhou Z, Fu H, Sosebee RE (2004) The characteristics of Na⁺, K⁺ and free proline distribution in several drought-resistant plants of the Alxa Desert, China. *J Arid Environ* 56:525–539. [https://doi.org/10.1016/S0140-1963\(03\)00063-6](https://doi.org/10.1016/S0140-1963(03)00063-6)
- Wang D, Yao W, Song Y, Liu W, Wang Z (2012) Molecular characterization and expression of three galactinol synthase genes that confer stress tolerance in *Salvia miltiorrhiza*. *J Plant Physiol* 169:1838–1848. <https://doi.org/10.1016/j.jplph.2012.07.015>
- Wang C, Zhao Y, Gu P, Zou F, Meng L, Song W, Yang Y, Wang S, Zhang Y (2018) Auxin is involved in lateral root formation induced by drought stress in tobacco seedlings. *J Plant Growth Regul* 37:539–549. <https://doi.org/10.1007/s00344-017-9752-0>
- Wang X, Guo C, Peng J, Li C, Wan F, Zhang S, Yang S (2019) ABRE-binding factors play a role in the feedback regulation of ABA signaling by mediating rapid ABA induction of ABA co-receptor genes. *New Phytol* 221:341–355. <https://doi.org/10.1111/nph.15345>
- Wang M, Vannozzi A, Wang G, Liang YH, Tomielli GB, Zenoni S, Cavallini E, Pezzotti M, Cheng ZM (2014) Genome and transcriptome analysis of the grapevine (*Vitis vinifera* L.) WRKY gene family. *Hortic Res* 1:14106. <https://doi.org/10.1038/hortres.2014.16>
- Wu X, Shiroto Y, Kishitani S, Ito Y, Toriyama K (2009) Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing OsWRKY11 under the control of HSP101 promoter. *Plant Cell Rep* 28:21–30. <https://doi.org/10.1007/s00299-008-0614-x>
- Yan Y, Jia H, Wang F, Wang C, Shuchang L, Xingqi G (2015) Overexpression of GhWRKY27a reduces tolerance to drought stress and resistance to *Rhizoctonia solani* infection in transgenic *Nicotiana benthamiana*. *Front Physiol* 6:265. <https://doi.org/10.3389/fphys.2015.00265>
- Yoshida T, Fujita Y, Sayama H, Kidokoro S, Maruyama K, Mizoi J, Yamaguchi-Shinozaki K (2010) AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. *Plant J* 61:672–685. <https://doi.org/10.1111/j.1365-313X.2009.04092.x>
- Yoshida T, Ohama N, Nakajima J, Kidokoro S, Mizoi J, Nakashima K, Maruyama K, Kim J-M, Seki M, Todaka D (2011) Arabidopsis HsfA1 transcription factors function as the main positive regulators in heat shock-responsive gene expression. *Mol Genet Genomics* 286:321–332. <https://doi.org/10.1007/s00438-011-0647-7>
- Yoshida T, Mogami J, Yamaguchi-Shinozaki K (2014) ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. *Curr Opin Biotechnol* 21:133–139. <https://doi.org/10.1016/j.pbi.2014.07.009>
- Zegada-Lizarazu W, Monti A (2019) Deep root growth, ABA adjustments and root water uptake response to soil water deficit in giant reed. *Ann Bot* 124:605–616. <https://doi.org/10.1093/aob/mcz001>
- Zhang G, Chen M, Li L, Xu Z, Chen X, Guo J, Ma Y (2009) Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. *J Exp Bot* 60(13):3781–3796. <https://doi.org/10.1093/jxb/erp214>
- Zhang N, Lariviere A, Tonsor SJ, Traw MB (2014) Constitutive camalexin production and environmental stress response variation in *Arabidopsis* populations from the Iberian Peninsula. *Plant Sci* 225:77–85. <https://doi.org/10.1016/j.plantsci.2014.05.020>
- Zhang F, Zhou Y, Zhang M, Luo X, Xie J (2017) Effects of drought stress on global gene expression profile in leaf and root samples of Dongxiang wild rice (*Oryza rufipogon*). *Biosci Rep* 37(3):1–11. <https://doi.org/10.1042/BSR20160509>
- Zhang DF, Zeng TR, Liu XY, Gao CX, Li YX, Li CH, Song YC, Shi YS, Wang TY, Yu LI (2019) Transcriptomic profiling of sorghum leaves and roots responsive to drought stress at the seedling stage. *J Integr Agri* 18(9):1980–1995. [https://doi.org/10.1016/S2095-3119\(18\)62119-7](https://doi.org/10.1016/S2095-3119(18)62119-7)
- Zhang Y, Li Y, Hassan MJ, Li Z, Peng Y (2020b) Indole-3-acetic acid improves drought tolerance of white clover via activating auxin, abscisic acid and jasmonic acid related genes and inhibiting senescence genes. *BMC Plant Biol* 20:1–12. <https://doi.org/10.1186/s12870-020-02354-y>
- Zhang Y, Li Y, Hassan MJ, Li Z, Peng Y (2020a) Indole-3-acetic acid improves drought tolerance of white clover via activating auxin, abscisic acid and jasmonic acid related genes and inhibiting senescence genes. *BMC Plant Biol* 20(1):1–12. <https://doi.org/10.1186/s12870-020-02354-y>
- Zwack PJ, Rashotte AM (2015) Interactions between cytokinin signalling and abiotic stress responses. *J Exp Bot* 66:4863–4871. <https://doi.org/10.1093/jxb/erv172>

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