Physiological and transcriptomic analyses of the effects of SlBRI1 expression levels on drought tolerance in tomato seedlings

Shuming Nie  
China West Normal University

Zaijun Yang  
China West Normal University

Dan Wang (✉ wangdd0310@163.com)  
China West Normal University

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Abstract

**Background:** Brassinosteroids (BRs) not only influence plant growth and development but also regulate the various stress responses of plants. BRASSINOSTEROID-INSENSITIVE 1 (BRI1) acts as a BR receptor, sensing the BRs and then activating BR signaling. In this study, how *SlBRI1* regulate the drought resistance of tomato have further been researched at physiological and transcriptomic level.

**Results:** We obtained *SlBRI1*-overexpressing and *SlBRI1* weak mutant (*abs*) plants in the same background to research the underlying mechanism in drought resistance. In this study, physiological analyses revealed that *abs* plants had a higher net photosynthetic rate and less wilting than MM plants; *abs* plants also had lower H$_2$O$_2$ and O$_2^-$ accumulation through higher antioxidant enzyme activities under drought conditions. RNA-Seq analysis showed that 768 (53.9%) of 1425 drought-induced genes and 418 (49.8%) of 840 drought-repressed genes were regulated by *abs* in the same direction under normal conditions. Moreover, 158 drought-induced and 43 drought-repressed genes are further upregulated and downregulated in *abs* plants under drought conditions, respectively. An in-depth analysis of these DEGs revealed that *abs* regulated the expression of genes related to ABA metabolism and polyamine biosynthesis as well as oxidoreductase activity genes under normal and drought conditions. Furthermore, analysis of transcription factor expression suggested that *abs* affected drought tolerance mainly through the regulation of WRKY, ERF, bHLH and MYB transcription factors. However, the expression of most of these genes was the same or opposite in *SlBRI1OE* plants compared to the MM group.

**Conclusion:** Our results establish that *SlBRI1* expression level was negatively involved in drought responses of tomato. Furthermore, our study provides valuable information for future breeding to appropriately reduce the expression of *SlBRI1* and improve drought resistance without affecting plant growth.

**Background**

Water availability is one of the most important environmental factors limiting plant species distribution and agricultural production [1]. When a plant suffers mild water stress, its photosynthesis and respiration rates decrease, and a series of physiological and biochemical responses also change to adapt to the drought stress [2]. More severe water stress can inhibit growth and damage the ultrastructures of cells and organelles, which results in cellular dehydration, the accumulation of toxic substances, the loss of cell membrane permeability, the inactivation of enzymes, and changes in protein structure. All of these factors eventually lead to metabolic disturbances and even the death of the plant [3–6]. Furthermore, drought stress can also induce the enhanced production of reactive oxygen species (ROS), which leads to oxidative stress [7]. To resist drought-induced oxidative stress, plants can eliminate excess ROS by upregulating the activity of enzymatic and nonenzymatic antioxidants [8]. Increased antioxidant enzyme activities play an important role in plant drought resistance [9].
Brassinosteroids (BRs) are steroid hormones that exist in all plant tissues. BRs play important roles in plant growth and development processes, such as promoting seed germination, cell elongation and division, pollen fertility, vascular differentiation, fruit set, and seed setting rate \cite{10-15}. In addition, BRs can enhance plant photosynthesis and chlorophyll content, delay ageing, and elevate stress resistance\cite{16}. To date, well-developed BR signal transduction models have been established in Arabidopsis. BRs first bind to the plasma membrane-localized receptor kinase BRASSINOSTEROID INSENSITIVE1 (BRI1) and the coreceptor BR1-ASSOCIATED RECEPTOR KINASE1 (BAK1) \cite{17-20}. BRI1 interacts with BAK1 and activates BR signalling, after which the BR signals are transduced to downstream components. Finally, dephosphorylated BES1 (bri1-EMS-suppressor 1) and BZR1 (Brassinazole-resistant 1) accumulate in the nucleus to regulate the expression of thousands of BR response genes \cite{21,22}.

Brassinosteroid (BR) signalling intensity not only influences plant growth and development but also regulates the various stress responses of plants. OsGSK1 (\textit{Oryza sativa} glycogen synthase kinase 3-like gene 1) is an orthologue of Arabidopsis brassinosteroid insensitive 2 (BIN2), and knockout of OsGSK1 enhances tolerance to cold, heat, salt, and drought stresses\cite{23}. The Arabidopsis elongated-D mutant, a BAK1 single base gain-of-function mutant, increases BR signalling and is more vulnerable to a bacterial pathogen and salinity stress\cite{24}. The Arabidopsis transcription factor RESPONSIVE TO DESICCATION26 (RD26) inhibits BR-regulated growth but increases drought tolerance by upregulating the expression of drought-induced genes. RD26 mediates crosstalk between drought and BR signalling\cite{25}. WRKY46, WRKY54, and WRKY70 positively regulate BR signalling and plant growth, while WRKYs negatively regulate drought tolerance by inhibiting dehydration-inducible gene expression\cite{26}. The AP2/ERF transcription factor TINY negatively regulates plant growth and compromises BR-responsive gene expression. TINY positively regulates drought resistance by promoting drought-responsive gene expression\cite{27}. Wheat brassinazole-resistant 2 (TaBZR2) positively regulates BR signalling and drought responses. TaBZR2 directly activates the expression of the \textit{T. aestivum} glutathione-transferase-1 (TaGST1) gene, which can scavenge drought-induced superoxide anions\cite{28}.

The expression level of the BR receptor BRI1 directly influences BR signal intensity and drought resistance. RNAi of a BRI1 homologue in \textit{Brachypodium distachyon} decreases the growth potential of plants while increasing their tolerance to drought and drought-responsive gene expression\cite{29}. Arabidopsis gain-of-function BR mutants (bes1-D) negatively regulate drought tolerance by repressing the expression of drought response genes, while the BR weak mutant (bri1-5) increases drought tolerance by upregulating the expression of these genes \cite{25}. Overexpression of the vascular BR receptor BRL3 increases drought tolerance and the accumulation of osmoprotectant metabolites without affecting plant growth\cite{30}.

In a previous study, we used \textit{SIBRI1} overexpressing plants of the tomato cultivar Micro-Tom to increase BR signalling and decrease drought resistance\cite{31}. We further found that \textit{SIBRI1} weak mutants in the tomato cultivar Money Maker (MM) had altered brassinolide sensitivity (abs) with a missense mutation in the kinase domain, delayed growth and enhanced drought resistance\cite{31,32}. To further understand the
relationship between the SIBRI1 expression level and drought resistance, we obtained SIBRI1 overexpressing and SIBRI1 weak mutant (abs) plants from the same background to research the underlying mechanism in drought resistance. Recently, various advanced RNA-Seq methods have been applied in numerous organisms, enabling this technique to be widely used in identifying key stress metabolism pathways (Lu et al., 2014; Wang et al., 2009). Therefore, RNA-Seq was used to analyse the effects of different SIBRI1 expression levels on drought tolerance in tomato.

**Results**

**Physiological analysis**

**SIBRI1 transgenic plants have increased SIBRI1 expression levels**

To investigate the associations of drought tolerance with the SIBRI1 expression level, we generated transgenic tomato plants in the tomato cultivar ‘Money Maker’ background in which SIBRI1, driven by the constitutive CaMV 35S promoter, was overexpressed. The transcript levels of SIBRI1 in the two transgenic lines, SIBRI1-OE-6 and SIBRI1-OE-7, were 5.4 and 16.5 times higher, respectively, than those in MM plants (Fig. 1B). Moreover, the SIBRI1 protein levels in the SIBRI1-OE-6 and SIBRI1-OE-7 lines were confirmed by Western blot analysis (Fig. 1A). The expression levels of the BR biosynthetic genes DWARF and CPD were significantly lower than those in MM plants (Fig. 1C and D). These results showed that transgenic plants had increased SIBRI1 expression levels and BR signaling intensity.

**SIBRI1 expression level alters plant growth, leaf relative water content and electrolyte leakage under drought stress**

To investigate whether the SIBRI1 expression level is related to the drought resistance of tomato seedlings, SIBRI1 overexpressing, MM and abs (SIBRI1 weak mutants) tomato seedlings were all subjected to drought stress (water withheld) for 12 d. After 12 d of drought stress, all plants showed different degrees of leaf wilting. The abs plants showed slight wilting, while the wilting was the most serious in the SIBRI1-OE-6 and SIBRI1-OE-7 plants (Fig. 2A, B).

Drought stress decreased leaf relative water content (RWC), and the leaf RWC in SIBRI1 overexpressing lines was significantly lower than that in MM plants, while the RWC of abs plants was significantly higher than that of MM plants at 10 d of drought stress, and was 24.5%, 30.6% and 35.6% higher than those of MM, SIBRI1-OE-6 and SIBRI1-OE-7 plants, respectively (Fig. 2C). Drought stress resulted in enhanced electrolyte leakage levels in all plants. The leaf electrolyte leakage levels of SIBRI1 overexpressing lines were significantly higher than those of MM plants, while the electrolyte leakage levels of abs plants were significantly lower than those of MM plants at 10 d of drought stress and were 19.1%, 33.6% and 40.5% lower than those of MM, SIBRI1-OE-6 and SIBRI1-OE-7 plants, respectively (Fig. 2D). These results indicated that SIBRI1 expression levels negatively regulated the drought tolerance of tomato seedlings.

**SIBRI1 expression level affects gas exchange under drought stress**
Compared with the control, SlBRI1 overexpressing plants had slightly increased net photosynthetic rate (Pn) and stomatal conductance (Gs) before drought stress. However, drought stress obviously decreased the Pn, Gs and transpiration rate (Tr) but increased the intercellular CO₂ concentration (Ci) in all plants (Fig. 3A-D). At 10 d of drought stress, the Pn and Tr of abs plants were significantly higher, and Ci was obviously lower, than those of other plants, while in SlBRI1 overexpressing plants, Pn and Tr were obviously lower, and Ci was markedly higher, than in other plants (Fig. 3A-D). For example, the Pn of abs plants was 27.1%, 49.2% and 56.5% higher than those of MM, SlBRI1-OE-6 and SlBRI1-OE-7 plants, respectively. There were no significant differences in Gs among all plants (Fig. 3A).

**SlBRI1 expression level affects accumulation of H₂O₂, O₂⁻ and antioxidant enzyme activities under drought stress**

Histochemical observations were used to assess the accumulation of hydrogen peroxide (H₂O₂) and superoxide (O₂⁻) in tomato leaves. Before drought stress, there were no significant differences in H₂O₂ and O₂⁻ accumulation among all plants (Fig. 4A, B). After 10 d of drought stress, the accumulation of H₂O₂ and O₂⁻ in abs leaves was lower than that in MM leaves, but SlBRI1-overexpressing leaves accumulated much higher levels than MM (Fig. 4A, B). Therefore, abs plants had lower ROS levels, and SlBRI1 overexpressing plants had higher ROS levels than MM plants under drought stress.

Drought stress obviously increased the antioxidant enzyme activities of all plants. The SOD, POD and CAT activities of abs plants were significantly higher than those of other plants at 10 d of drought stress and were 39.9%, 47.9% and 29.8% higher than those of SlBRI1-OE-6 plants, respectively. The APX activities of MM and abs plants were significantly higher than those of SlBRI1 overexpressing plants after drought stress (Fig. 4C-F). These results showed that BR signalling was negatively related to the antioxidant enzyme activities of tomato seedlings under drought stress.

**Transcriptome Analysis**

**Sequencing of different SlBRI1 expression level plants RNA-Seq**

To further understand how SlBRI1 negatively regulates drought responses, we performed global gene expression studies with MM, SlBRI1-OE-7 and abs plants by high-throughput RNA sequencing (RNA-seq). As shown in Table 1, six cDNA libraries (three replicates per library) were constructed. RNA-Seq of these libraries generated approximately 47–62 million total reads, with an average of 98.9% clean reads obtained after quality filtering. Clean reads from every library showed a match rate of approximately 95% to the tomato genome, indicating that the sequencing data could be used for subsequent transcriptome analysis (Table 1).

Table 1
Summary of RNA-Seq datasets.
**SlBRI1 negatively regulates drought responsive genes expression**

Analysis of DEGs was performed between every pair of groups (MMDS/SlBRI1OEDS, MMDS/absDS, MM/abs, abs/absDS, MM/MMDS, SlBRI1OE/SlBRI1OEDS and MM/SlBRI1OE) based on FPKM with thresholds FDR < 0.05 and FC > 2. There were 3144 DEGs (2096 up- and 1048 downregulated) between MMDS and absDS, 6162 DEGs (3873 up- and 2289 downregulated) between MM and abs, and 2265 DEGs (1425 up- and 840 downregulated) between MM and MMDS. There were only 85 DEGs between MM and SlBRI1OE, comprising 59 upregulated and 26 downregulated DEGs (Fig. 5). We selected the above 4 groups for comprehensive analysis. The number of upregulated DEGs was higher than that of downregulated DEGs across all four comparisons (Fig. 5). However, we further found that 768 (53.9%) of 1425 drought-induced and 418 (49.8%) of 840 drought-repressed genes were regulated by abs in the same direction under normal conditions (Figs. 6A, B). There were 158 drought-induced and 43 drought-repressed genes that were further upregulated and downregulated in absDS, respectively (Fig. 6A, B). These results indicated that abs may regulate the expression of a number of drought-related genes, which results in the inhibition of plant growth under normal conditions, consistent with the growth phenotype of abs.

To further investigate how SlBRI1-OE and abs affect drought-related gene expression, we performed clustering analysis of the genes that were upregulated and downregulated in each treatment. Under normal conditions, MM upregulated genes and downregulated genes were repressed and induced under drought conditions, respectively (Fig. 6C). However, many drought stress-induced genes and repressed genes were already upregulated and downregulated in abs under normal conditions, respectively (cluster b). Many drought stress-induced genes had higher expression in absDS, while many drought stress-repressed genes had lower expression in absDS under drought conditions (cluster a). Overall, our transcriptome analyses support a role of the SlBRI1 expression level in modulating drought-responsive gene expression, largely in an antagonistic manner.
Gene Ontology (GO) and GO enrichment analyses of different *SIBRI1* levels under normal conditions

To explore the effects of *SIBRI1* overexpression under normal conditions, we performed GO analyses, focusing on differences between the MM group and the *SIBRI1OE* group. There were 71 DEGs, 72 DEGs and 74 DEGs enriched in “biological process”, “cellular component” and “molecular function”, respectively (Fig. 7, Table S2). The main biological process categories were “metabolic process” and “cellular process”. DEGs in the molecular function category were related to “catalytic activity” and “binding”. The most DEGs were assigned to the “cell” and “membrane part” cellular component categories (Fig. 7, Table S2). These results highlighted the involvement of *SIBRI1-OE* in cellular processes and metabolic processes, consistent with the regulation of growth by *SIBRI1-OE* under normal conditions.

To explore how *abs* functions under normal conditions, we performed GO enrichment analyses, focusing on differences between the MM group and *abs* group. The top 20 most obviously enriched pathways are shown in Fig. 8. The DEGs were enriched for “photosynthesis, light harvesting in photosystem I”, “photosynthesis, light harvesting”, “protein phosphorylation”, “phosphorylation”, “plastoglobule”, “cellular protein modification process”, “protein modification process”, “response to abiotic stimulus”, “lipid metabolic process”, “phosphorus metabolic process”, “phosphate-containing compound metabolic process”, “carbohydrate metabolic process” and “intrinsic component of membrane” (Fig. 8, Table S3). In addition, the most enriched category for DEGs in this study was “catalytic activity”, with a total of 2445 DEGs. A total of 148 DEGs were annotated as “response to abiotic stimulus” (Fig. 8, Table S3). These results indicated that *abs* upregulated the expression of a number of stress response-related genes under normal conditions.

Differences in stress response gene expression between different treatments

To further explore how *abs* increased drought resistance, we selected stress-related metabolic pathways from GO enrichment analyses. Four ABA biosynthesis genes, 9-cis-epoxycarotenoid dioxygenase 2 (Solyc08g016720.1), notabilis 9-cis-epoxycarotenoid dioxygenase (Solyc07g056570.1), beta-carotene, Pfam: PF05834 (Solyc06g074240.3), and zeaxanthin epoxidase (Solyc02g090890.4), were upregulated in MM under drought stress (Table S4). These genes were also induced in *abs* compared with MM under normal conditions. However, the expression of these genes was not obviously changed in *SIBRI1OE* plants compared with MM plants. Solyc08g016720.1 and Solyc06g074240.3 were upregulated between MMDS and *abs*DS (Table S4). Eleven of 13 polyamine biosynthetic processes were upregulated in MM under drought stress. All 13 genes were induced in *abs* compared with MM under normal conditions. However, the expression of these genes was not obviously changed in *SIBRI1OE* plants compared with MM plants. Eight of 13 were upregulated between MMDS and *abs*DS. These results indicated that *abs* may increase ABA and polyamine contents by inducing ABA and polyamine biosynthetic gene expression under normal and drought conditions (Table S5). Seven of 11 oxidoreductase activity genes were upregulated in MM under drought stress. All 11 genes were induced in *abs* compared with MM under normal conditions. However, the expression of most of these genes was not obviously changed in *SIBRI1OE* plants compared with MM plants. Nine of 11 genes were upregulated between MMDS and
absDS, whereas 2 of 11 genes were downregulated (Fig. 9A; Table S6). These results indicated that abs may decrease reactive oxygen content by inducing oxidoreductase activity gene expression and further enhance antioxidant enzyme activities under normal and drought conditions.

Transcription factors (TFs) play a critical role in abiotic stress via gene regulatory networks. We further selected 25 TFs in 7 different families from the MMDS group, 18 upregulated and 7 downregulated. Most of the differentially expressed TFs participate in the drought stress response, and the majority are derived from the WRKY, ERF, bHLH and MYB families (Fig. 9B; Table S7). Eighteen drought stress-induced TFs were also upregulated, and 7 of the drought-downregulated TFs were also downregulated in abs under normal conditions. Seventeen TFs did not show obvious changes in expression in SIBRI1OE plants compared with MM plants; 4 TFs, ERF (Solyc06g068360.3), AP2-ERF (Solyc10g084340.2), MYB75 (Solyc10g086250.2) and bHLH079 (Solyc02g078130.3), had the same expression direction, while 4 TFs, WRKY33 (Solyc09g014990.4), WRKY46 (Solyc08g067340.4), MYB76 (Solyc05g008250.2) and bHLH150 (Solyc09g065100.3), had opposite expression changes in SIBRI1OE plants and drought-exposed plants (Fig. 8B; Table S5). Eighteen of 25 TFs had the same expression direction in absDS compared with MMDS, and 7 of 25 TFs were not obviously changed (Fig. 9B; Table S7). In abs plants, reduced BR signalling may upregulate or downregulate drought-related TFs, which further regulate drought stress-related gene expression and improve drought resistance under both normal conditions and drought conditions.

Validation of RNA-Seq data by qRT-PCR analysis
To validate the RNA-seq data, we selected 15 DEGs of MM vs abs for qRT-PCR determination. We further compared the results obtained from qRT-PCR with those generated from the RNA-seq data. The trends of expression were consistent for all transcripts in both analyses, with a correlation coefficient of $R^2 = 0.883$ (Fig. 10). These results confirmed the reliability of the RNA-seq data.

Discussion
Plants frequently encounter various environmental stresses, such as drought, salt and extreme temperature, which severely affect plant growth and yield. In many previous studies, it has been reported that the application of BRs can improve the drought resistance of plants. In recent years, we found that SIBRI1-overexpressing plants from the tomato cultivar Micro-Tom have increased BR signaling but decreased drought resistance. We further found that SIBRI1 weak mutants in the tomato cultivar Money Maker (MM) background (altered brassinolide sensitivity, abs) exhibited delayed growth and enhanced drought resistance[31]. To further understand the relationship between the SIBRI1 expression level and drought resistance in tomato, we obtained SIBRI1 overexpressing and SIBRI1 weak mutant plants in the same background to examine their differences in drought resistance. The expression levels of SIBRI1 in transgenic plants were significantly higher than those in MM plants (Figs. 1A, B). Furthermore, the transgenic plants already exhibited SIBRI1 protein expression. In addition, the expression of the BR biosynthetic genes DWARF and CPD was significantly inhibited in the transgenic plants (Figs. 1C, D). All results indicated that the SIBRI1 transgenic plants had obviously increased BR signaling.
Abiotic stress can lead to the reduction of photosynthesis through stomata-dependent and stomata-independent pathways. If Ci and Gs are reduced simultaneously, the reduction of Pn can be considered to be primarily due to stomatal factors. In contrast, if Gs is reduced but Ci does not change or is increased, the reduction in Pn can be considered to be due to nonstomatal factors [37–40]. In our study, drought stress decreased the Pn of all plants. Furthermore, the Pn and Tr of abs plants were significantly higher than those of other plants, while the Ci of abs plants was significantly lower than that of other plants under drought conditions (Figs. 3A-D). These results were consistent with BRI1 RNAi plants having higher photosynthetic capacity than wild-type plants in Brachypodium distachyon under drought stress [29]. In contrast, the Pn and Tr of SIBRI1-overexpressing plants were significantly lower than those of other plants, while the Ci was significantly higher than that of other plants under drought conditions (Figs. 3A, C, D). Therefore, the photosynthetic capacity of abs plants was less affected by drought stress. These results indicated that the reduction in Pn in all plants could be attributed to stomatal factors and nonstomatal factors. Nonstomatal factors may include the reduced activity and efficiency of partial enzymes of the Calvin cycle, destroyed photosynthetic apparatus and other organelles and slowed transport of photosynthetic products [41].

Normally, the formation and elimination of ROS are balanced and steady, but this balance is destroyed when plants are subjected to drought stress. In this study, drought stress obviously increased the accumulation of H$_2$O$_2$ and O$_2^-$ in all plants, as determined by NBT and DAB staining (Fig. 4A, B). The accumulation of H$_2$O$_2$ and O$_2^-$ in abs leaves was lower than that in MM leaves, but SIBRI1-overexpressing leaves accumulated much more H$_2$O$_2$ and O$_2^-$ than MM leaves (Fig. 4A, B). Therefore, abs plants had lower ROS levels, and SIBRI1 overexpressing plants had higher ROS levels, than MM plants under drought stress. These results were consistent with the observation that the SOD, POD, CAT and APX activities of abs plants were significantly higher than those of other plants under drought stress (Fig. 4C-F). The activities of these enzymes in SIBRI1-overexpressing plants were significantly lower than those in MM and abs plants after drought stress. Furthermore, our RNA-seq data indicated that 11 oxidoreductase activity genes were induced in abs plants under normal conditions. Seven of 11 genes were upregulated in MM plants under drought stress. However, most of these genes were not obviously changed in SIBRI1OE plants. In addition, the expression of 9 of these same 11 genes was higher in absDS plants than in MMDS plants (Fig. 9A). These results indicated that abs plants may decrease reactive oxygen content by inducing oxidoreductase activity gene expression and further enhance antioxidant enzyme activities under drought conditions.

The pleiotropic roles of BR are complex, including developmental processes and multiple types of stress tolerance in plants. To further understand the relationship between the SIBRI1 expression level and drought resistance in tomato, we used RNA-Seq to characterize the influence of the SIBRI1 expression level on gene expression under normal and drought conditions. We focused on comparing the differences in gene expression between MM and MMDS, MM and abs, MM and SIBRI1OE and MMDS and absDS. We found that 768 (53.9%) of 1425 drought-induced and 418 (49.8%) of 840 drought-repressed genes were regulated by abs in the same direction under normal conditions (Fig. 6A, B). There were 158 drought-
induced and 43 drought-repressed genes that were further upregulated and downregulated in absDS, respectively (Fig. 6A, B). This result was consistent with the observation that RNAi of a BRI1 homologue in Brachypodium distachyon decreased the growth potential of plants while increasing their tolerance to drought and drought-responsive gene expression [29]. ABA plays a crucial role in drought resistance. Drought stress usually induces the expression of ABA biosynthesis genes, which further increases the ABA content and eventually enhances the drought resistance of plants [31]. Therefore, we compared stress-related metabolic pathways identified in GO enrichment analyses. Our results indicated that drought stress upregulated 4 ABA biosynthesis genes that were already induced in abs plants under normal conditions. Additionally, the expression of 2 ABA biosynthesis genes was higher in absDS plants than in MMDS plants (Table S4). These results indicated that abs may increase ABA contents and further improve drought resistance by inducing ABA biosynthetic gene expression under normal and drought conditions.

Dopamine also plays an important role in the drought resistance of plants [42]. A previous study showed that dopamine could regulate photosynthetic oxygen reduction and the photophosphorylation of chloroplasts [43]. Furthermore, dopamine could increase the drought resistance of apple seedlings by improving the photosynthetic capacity and antioxidant enzyme activity of the plants [44]. Our results indicated that 13 polyamine biosynthetic genes were upregulated in abs plants under normal conditions. Eleven of these 13 were induced in MM under drought stress. Furthermore, the expression of 8 genes was higher in absDS plants than in MMDS plants. However, most of these genes did not show obvious changes in expression in SIBRI1OE plants compared with MM plants (Table S5). Therefore, these results indicated that abs may increase polyamine contents and further improve drought resistance by inducing polyamine biosynthetic gene expression under normal and drought conditions.

TFs play an important role in drought stress signalling. Some TFs are major components in signalling networks, including WRKY, NAC, ERF, bHLH, bZIP and MYB. In our study, 25 transcription factor-encoding genes were differentially expressed between different treatments, mainly from the WRKY, ERF, bHLH and MYB families. Furthermore, the most abundant TF members were from the WRKY transcription factor family (Fig. 9B, C; Table S7). Previous studies found that WRKY38 participates in the drought stress response and that WRKY40 is involved in ABA signalling [45, 46]. The overexpression of OsWRKY30 obviously improved drought tolerance in rice. Compared to the MM group, the MMDS group showed upregulation of drought-related TFs such as WRKY81, WRKY33 and WRKY46. However, in abs plants, the expression of these genes was already upregulated under normal conditions. Moreover, the expression of these genes in absDS plants was higher than that in MMDS plants, while the expression of these genes was downregulated in SIBRI1OE plants compared with MM plants (Fig. 9B; Table S7). Therefore, drought-related WRKY TFs may be upregulated in abs plants and further improve drought resistance under both normal conditions and drought conditions.

AP2/ERF TFs are involved in the regulation of plant drought responses and plant growth (Phukan et al., 2017; Xie et al., 2019). The overexpression of stress-inducible AP2/ERF TFs inhibited plant growth but improved the drought resistance of transgenic plants. TINY positively regulates drought responses by
inducing drought-responsive genes and promoting abscisic acid–regulated stomatal closure. BR signalling negatively regulates TINY through BIN2 phosphorylation, and TINY inhibits BR-mediated growth through antagonistic TINY-BES1 interactions. Three AP2/ERF TFs each were upregulated and downregulated in the MMDS group compared with the MM group. However, these same genes were already upregulated and downregulated under normal conditions in abs plants (Fig. 9B). However, the expression of most of the same genes was not different in SIBRI1OE plants compared to MM plants. We found similar results for some bHLH and MYB family TFs in abs plants and SIBRI1OE plants (Fig. 9B). Therefore, in abs plants, bri1 mutation leads to reduced BR signalling and may upregulate or downregulate drought-related TFs, which further regulate drought stress-related gene expression and ultimately improve drought resistance both under normal conditions and drought conditions. These results were consistent with the growth phenotype of abs.

Conclusions

In conclusion, physiological analyses revealed that abs plants were more drought resistant than MM plants through two mechanisms. First, abs plants had a higher net photosynthetic rate and relative water content as well as lower wilting and electrolyte leakage; second, abs plants had lower H$_2$O$_2$ and O$_2^-$ accumulation through higher antioxidant enzyme activities under drought conditions. RNA-Seq was used to characterize the influence of SIBRI1 expression level on drought tolerance in tomato. In total, 768 (53.9%) of 1425 drought-induced genes and 418 (49.8%) of 840 drought-repressed genes were regulated in abs in the same direction under normal conditions. Moreover, 158 drought-induced and 43 drought-repressed genes were further upregulated and downregulated in abs plants under drought conditions, respectively. We further found that abs regulated the expression of genes related to the metabolism of ABA and polyamine biosynthesis, as well as oxidoreductase activity genes under normal and drought conditions, by GO enrichment analyses. Furthermore, abs may affect tomato drought tolerance by regulating the expression of WRKY, ERF, bHLH and MYB transcription factors. Therefore, our study provides valuable information for future breeding to reduce the expression of SIBRI1 and improve drought resistance without affecting plant growth.

Methods

Plant materials, growth conditions and plant transformation

Seeds of tomato cultivar Money Maker (MM), abs (SIBRI1 weak mutant) and T2-generation transgenic tomato plants were germinated at 28°C in Petri plates lined with two layers of filter paper moistened with deionized water. Tomato MM and abs seeds were obtained from Northwest A&F University Xiaofeng Wang laboratory. The germinated seeds were then sown in plastic pots (8 cm × 8 cm × 9 cm) filled with 70 g of a mixture of peat and vermiculite (v/v = 7:3) at one seed per pot. Seedlings were grown in a controlled environment room with a temperature of 25°C, photosynthetic photon flux density (PPFD) of 500 μmol m$^{-2}$ s$^{-1}$, and a photoperiod of 16/8 h (day/night).
The overexpression of 35S:SIBRI1 transgenic tomato was obtained using the method of Nie et al. [33, 34]. Two independent homozygous SIBRI1 overexpression plants (SIBRI1-OE-6 and SIBRI1-OE-7) were used for drought experiments because of high expression levels of SIBRI1. abs were sown 30 d earlier than other tomato plants because abs plants slowly grow.

Drought experiments

abs seeds were sown 30 d earlier than other tomato seeds because abs plants slowly grow. All plants were grown to the six-leaf stage. Plants of the same size were selected for subsequent experiments. The selected plants were unwatered for 12 days. There were 40 seedlings in each group.

Measurements of relative water content (RWC) and electrolyte leakage

Fresh fully expanded leaves were harvested for measuring the RWC and electrolyte leakage. RWC and electrolyte leakage were measured according to Liu et al. [35].

Measurement of photosynthetic parameters

The net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO₂ concentration (Ci), and transpiration rate (Tr) of the seedlings were measured by using a portable photosynthesis system (LI-6400-40, Li-Cor, Lincoln, NE, USA). All measurements were carried out at 400 µmol mol⁻¹ CO₂, 25°C, and 500 µmol m⁻² s⁻¹ light intensity. For each measurement, 8 leaves were measured from different seedlings per treatment.

Antioxidant enzyme activities and the accumulation of ROS

Antioxidant enzyme activities and the accumulation of ROS were performed in accordance with the method of Nie et al.[31].

Western blot analysis

Total proteins were extracted from the young leaves (0.2g) of 25-day-old different tomato plants. The procedure was performed as previously described [36].

RNA extraction and Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted and reverse-transcribed according to Nie et al. [33]. qRT-PCR was performed following the three-step protocol of BioEasy Master Mix (Bioer Technology) in a CFX96 real-time system (Bio-Rad) as described in the manufacturer's instructions. Each assay consisted of three biological replicates. We used UBI3 gene as the internal control. All specific primer sequences are listed in Table S1.

RNA-Seq and transcriptomic analysis

Total RNA samples were collected from the meristem and young leaves by using the RNAprep Pure Plant Kit (Tiangen Biotech) following the manufacturer's instructions. RNA quality and concentration were assessed by using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). For the transcriptome analyses, RNA-seq libraries were prepared from cDNA by Majorbio and sequenced on an Illumina HiSeq 4000. Raw sequences were quality-filtered by SeqPrep (https://github.com/jstjohn/SeqPrep) and Sickle.
and mapped to the *Solanum lycopersicum* SL4.0 reference genome (https://www.solgenomics.net/organism/Solanumlycopersicum/genome/) by using TopHat2 (http://ccb.jhu.edu/software/tophat/index.shtml). DESeq2 was used for the differential expression analysis of RNA-Seq expression profiles. Absolute values of log2 (fold change) \( \geq 1 \) and p-adjusted < 0.05 were set as thresholds. The genes that were expressed at significantly different levels were subjected to KEGG, GO function analysis and GO enrichment analysis. The metabolic pathway heatmap was generated using Multiple Array Viewer.

The RNA-Seq samples were divided into six groups: (1) Money Maker normal sample before drought stress (MM); (2) *abs* normal sample before drought stress (*abs*); (3) *SIBRI1-OE-7* normal sample before drought stress (*SIBRI1OE*); (4) Money Maker drought-stressed 8 d sample (MMDS); (5) *abs* drought-stressed 8 d sample (*absDS*); and (6) *SIBRI1-OE-7* drought-stressed 8 d sample (*SIBRI1OEDS*).

**Statistical Analysis**

All data in this study were analyzed using SPSS version 17.0 and the LSD test. The means and standard errors were calculated, and P < 0.05 was considered statistically significant in comparisons with MM plants.

**Abbreviations**

BRs: Brassinosteroids; ABA, Abscisic acid; *abs*: altered brassinolide sensitivity; MM: Money Maker; 35S: Constitutive cauliflower mosaic virus 35S promoter; BRI1: BRASSINOSTEROID-INSENSITIVE 1; ROS: reactive oxygen species; BAK1: BRI1-ASSOCIATED RECEPTOR KINASE1; BES1: BRI1-EMS SUPPRESSOR1; BZR1: BRASSINAZOLERESISTANT1; CBB: Coomassie brilliant blue; CPD: CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARF; DWARF: 6-DEOXOCASTASTERONE OXIDASE; BIN2: brassinosteroid insensitive 2; RD26: RESPONSIVE TO DESICCATION26; TaBZR2: Wheat brassinazole-resistant 2; TaGST1: *T. aestivum* glutathione-transferase-1; RWC: relative water content; Pn: net photosynthetic rate; Gs: stomatal conductance; Tr: transpiration rate; Ci: intercellular CO\(_2\) concentration; H\(_2\)O\(_2\): hydrogen peroxide; O\(_2\)^{−}: superoxide; SOD, Superoxide dismutase; CAT: Catalase; POD: Peroxidase; APX: Ascorbate peroxidase; RNA-seq: RNA sequencing;

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not Applicable.

**Availability of data and materials**
All data generated or analyzed during this study are included in this published article and its supplementary information files. The datasets and plant materials used during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Author contributions**

WD and NS planned and designed the research. WD and NS performed the experiments. WD, YZ and NS analyzed the data. WD and NS wrote the manuscript. All authors read and approved the final manuscript.

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**Authors' Information**

Key Laboratory of Southwest China Wildlife Resources Conservation (Ministry of Education), College of Life Science, China West Normal University, Nanchong 637009, Sichuan, China

Shu-Ming Nie, Zai-Jun Yang & Dan Wang

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