Physiological and transcriptomic analyses of the effects of *SLBRI1* expression levels on drought tolerance in tomato seedlings

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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 *SIBRI1* regulate the drought resistance of tomato have further been researched at physiological and transcriptomic level.

Results: We obtained *SIBRI1*-overexpressing and *SIBRI1* 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 H₂O₂ and O₂⁻ 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 *SIBRI1OE* plants compared to the MM group.

Conclusion: Our results establish that *SIBRI1* 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 *SIBRI1* 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 [10–15]. In addition, BRs can enhance plant photosynthesis and chlorophyll content, delay ageing, and elevate stress resistance[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 BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) [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 [21, 22].

Brassinosteroid (BR) signalling intensity not only influences plant growth and development but also regulates the various stress responses of plants. OsGSK1 (*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[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[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[25]. WRKY46, WRKY54, and WRKY70 positively regulate BR signalling and plant growth, while WRKYs negatively regulate drought tolerance by inhibiting dehydration-inducible gene expression[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[27]. Wheat brassinazole-resistant 2 (TaBZR2) positively regulates BR signalling and drought responses. TaBZR2 directly activates the expression of the *T. aestivum* glutathione-transferase-1 (TaGST1) gene, which can scavenge drought-induced superoxide anions[28].

The expression level of the BR receptor BRI1 directly influences BR signal intensity and drought resistance. RNAi of a BRI1 homologue in *Brachypodium distachyon* decreases the growth potential of plants while increasing their tolerance to drought and drought-responsive gene expression[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 [25]. Overexpression of the vascular BR receptor BRL3 increases drought tolerance and the accumulation of osmoprotectant metabolites without affecting plant growth[30].

In a previous study, we used *S/BRI1* overexpressing plants of the tomato cultivar Micro-Tom to increase BR signalling and decrease drought resistance[31]. We further found that *S/BRI1* 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[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, *SIBR11* 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 *SIBR11* 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, *SIBR11-OE-6* and *SIBR11-OE-7* plants, respectively. There were no significant differences in Gs among all plants (Fig. 3A).

***SIBR11* 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 *SIBR11*-overexpressing leaves accumulated much higher levels than MM (Fig. 4A, B). Therefore, *abs* plants had lower ROS levels, and *SIBR11* 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 *SIBR11-OE-6* plants, respectively. The APX activities of MM and *abs* plants were significantly higher than those of *SIBR11* 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 *SIBR11* expression level plants RNA-Seq

To further understand how *SIBR11* negatively regulates drought responses, we performed global gene expression studies with MM, *SIBR11-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**.

Sample	Total reads	Clean reads	Multiple mapped	Uniquely mapped	Q30(%)
MM_1	49508234	49000446	1682647(3.43%)	45330385(92.51%)	95.14
MM_2	51088508	50546354	2757649(5.46%)	44715920(88.47%)	95.33
MM_3	47788844	47336100	3387809(7.16%)	38838001(82.05%)	95.45
<i>SIBRI1</i> OE_1	51510028	51003574	1536162(3.01%)	47370570(92.88%)	95.02
<i>SIBRI1</i> OE_2	50321154	49823380	1743341(3.5%)	46222183(92.77%)	95.24
<i>SIBRI1</i> OE_3	48124782	47623938	2051124(4.31%)	43224455(90.76%)	95.14
<i>abs</i> _1	50356448	50014982	1731053(3.46%)	46186727(92.35%)	95.73
<i>abs</i> _2	55847692	55342986	2315453(4.18%)	51076174(92.29%)	95.43
<i>abs</i> _3	54333414	53803982	1946159(3.62%)	49851557(92.65%)	94.82
MMDS_1	48958602	48519080	1373243(2.83%)	45016374(92.78%)	95.35
MMDS_2	48481508	47966526	1658663(3.46%)	44716200(93.22%)	95.34
MMDS_3	57482010	56987708	2183676(3.83%)	52715415(92.5%)	95.59
<i>SIBRI1</i> OE DS_1	52315202	51751238	1574749(3.04%)	48059300(92.87%)	95.35
<i>SIBRI1</i> OE DS_2	49916312	49379104	1304795(2.64%)	46550624(94.27%)	95.22
<i>SIBRI1</i> OE DS_3	50834194	50350288	2526321(5.02%)	44485042(88.35%)	95.27
<i>abs</i> DS_1	59879032	59361416	2887893(4.86%)	54465020(91.75%)	95.11
<i>abs</i> DS_2	62685918	62142522	1947751(3.13%)	56526086(90.96%)	95.43
<i>abs</i> DS_3	58976216	58470256	2040186(3.49%)	53175772(90.94%)	95.42

SIBRI1 negatively regulates drought responsive genes expression

Analysis of DEGs was performed between every pair of groups (MMDS/ *SIBRI1*OEDS, MMDS/ *abs*DS, MM/ *abs*, *abs*/ *abs*DS, MM/MMDS, *SIBRI1*OE/ *SIBRI1*OEDS and MM/ *SIBRI1*OE) based on FPKM with thresholds FDR < 0.05 and FC > 2. There were 3144 DEGs (2096 up- and 1048 downregulated) between MMDS and *abs*DS, 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 *SIBRI1*OE, 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 (Fig. 6A, B). There were 158 drought-induced and 43 drought-repressed genes that were further upregulated and downregulated in *abs*DS, 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 *SIBRI1*-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 *abs*DS, while many drought stress-repressed genes had lower expression in *abs*DS under drought conditions (cluster a). Overall, our transcriptome analyses support a role of the *SIBRI1* 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 C_i and G_s are reduced simultaneously, the reduction of P_n can be considered to be primarily due to stomatal factors. In contrast, if G_s is reduced but C_i does not change or is increased, the reduction in P_n can be considered to be due to nonstomatal factors [37–40]. In our study, drought stress decreased the P_n of all plants. Furthermore, the P_n and T_r of *abs* plants were significantly higher than those of other plants, while the C_i 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 P_n and T_r of *SIBR1*-overexpressing plants were significantly lower than those of other plants, while the C_i 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 P_n 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_2O_2 and O_2^- in all plants, as determined by NBT and DAB staining (Fig. 4A, B). The accumulation of H_2O_2 and O_2^- in *abs* leaves was lower than that in MM leaves, but *SIBR1*-overexpressing leaves accumulated much more H_2O_2 and O_2^- than MM leaves (Fig. 4A, B). Therefore, *abs* plants had lower ROS levels, and *SIBR1* 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 *SIBR1*-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 *SIBR1OE* 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 *SIBR1* expression level and drought resistance in tomato, we used RNA-Seq to characterize the influence of the *SIBR1* 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 *SIBR1OE* 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_2O_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 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and a photoperiod of 16/8 h (day/night).

The overexpression of *35S:SIBR1* transgenic tomato was obtained using the method of Nie et al. [33, 34]. Two independent homozygous *SIBR1* overexpression plants (*SIBR1-OE-6* and *SIBR1-OE-7*) were used for drought experiments because of high expression levels of *SIBR1*. *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 $\mu\text{mol mol}^{-1}$ CO₂, 25°C, and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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

(<https://github.com/najoshi/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) ≥ 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, Absciscic 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-DEOXYCASTASTERONE 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 CO₂ concentration; H₂O₂: hydrogen peroxide; O₂⁻: 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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Figures

Figure 1

SIBRI1, DWARF and CPD expression in Money Maker (MM) and SIBRI1-overexpressing plants. (A) Transgenic BRI1 protein levels examined by immunoblotting with anti-Flag antibodies. CBB, Coomassie brilliant blue. The Western blot has been cropped and uncropped blot have be included in the additional files. (B) The relative transcript levels of SIBRI1. (C) The relative transcript levels of DWARF. (D) The relative transcript levels of CPD. Data values are the means $\pm$ SD of three independent biological samples. Different letters indicate significant differences according to Tukey's test ($P < 0.05$). The different samples were derived from the same experiment.

Figure 2

MM, SIBRI1 overexpression and abs plants under drought stress. (A) and (B) Phenotypes of MM, SIBRI1-OE-6, SIBRI1-OE-7, and abs plants after 0 and 12 d of drought stress, respectively. (C) Leaf relative water content (RWC) of WT, SIBRI1-OE-6, and SIBRI1-OE-7 plants after 0 and 10 d of drought stress. (D) Electrolyte leakage. Data values are the means $\pm$ SD of three independent biological samples. Different letters indicate significant differences according to Tukey's test ($P < 0.05$).

Figure 3

Effects of different SIBRI1 expression levels on gas exchange parameters in tomato seedlings under drought stress. (A) Photosynthetic rate (P_n), (B) Stomatal conductance (G_s), (C) Intercellular CO_2 concentration (C_i), and (D) Transpiration rate. Data values are the means $\pm$ SD of three independent biological samples. Different letters indicate significant differences according to Tukey's test ($P < 0.05$).

Figure 4

Effects of different *SIBRI1* expression levels on reactive oxygen species (ROS) accumulation and antioxidant enzyme activity in tomato seedlings under drought stress. (A) The accumulation of hydrogen peroxide (H_2O_2) in MM, *SIBRI1*-OE-6, *SIBRI1*-OE-7, and *abs* plants after 0 and 10 d of drought stress. (B) The accumulation of superoxide (O_2^-). (C) Superoxide dismutase (SOD) activity in MM, *SIBRI1*-OE-6, *SIBRI1*-OE-7, and *abs* plants after 0 and 10 days of drought stress. (D) Peroxidase (POD) activity. (E)

Catalase (CAT) activity. (F) Ascorbate peroxidase (APX) activity. Data values are the means $\pm$ SD of three independent biological samples. Different letters indicate significant differences according to Tukey's test ($P < 0.05$).

Figure 5

Number of DEGs among the seven comparison groups in tomato seedlings.

Figure 6

The number of DEGs among the four comparison groups in the Venn diagram showed overlap of up-regulated (A) and downregulated (B) DEGs in tomato seedlings. (C) Heatmap of DEGs in each sample.

GO annotations analysis

Figure 7

GO annotation analysis of DEGs between MM and SIBRI10E. The x-axis indicates the number of genes in each classification, and the Y-axis indicates the GO classifications.

GO enrichment analysis (MM_vs_abs)

Figure 8

GO enrichment analysis of DEGs between the MM and abs. The X-axis shows the rich factor. Blue represents a high adjusted P value, and red represents a low adjusted P value. The Y-axis shows the top 20 KEGG pathways. The bigger size of spot, the more DEGs enriched.

Figure 9

A heatmap heat map was constructed based on log2-transformed FPKM expression values of oxidoreductase activity genes (A) and each TF (B). (C) The numbers of differentially expressed TFs between MM and MMDS, MM and abs, MM and SIBRI10E, MMDS and absDS.

Figure 10

Validation of the RNA-Seq results by qRT-PCR. Data are presented as the means of three replicates.

Supplementary Files

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