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Sorghum WRKY transcription factor SbWRKY45 enhanced seed germination under drought stress in transgenic Arabidopsis

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Abstract: The WRKY transcription factors (TFs) family is an important family of plant-specific TFs, playing vital roles in various abiotic and biotic stress responses. WRKY TFs are gaining considerable attention due to their significant roles in stress responses. However, their functions in sorghum (Sorghum bicolor) are lagging behind. In this study, a WRKY gene designated as SbWRKY45 was isolated and characterized from sorghum. SbWRKY45, belonging to Group IIa, consists of one intron and two exons and encodes 430 amino acids. SbWRKY45 is located in chromosome 4. The cis-element prediction analysis showed that the promoter region of SbWRKY45 has several abiotic stress-associated elements. The qRT-PCR results showed that SbWRKY45 was significantly up-regulated in response to drought and cold under salt treatments, was notably changed, and was induced weakly under heat stress. SbWRKY45 exhibited a response to stress in different sorghum tissues, including leaves, stems, and roots. A tissue-specific expression pattern showed that SbWRKY45 was highly expressed in roots compared with leaves and stems, suggesting that SbWRKY45 may play an important role in roots. Overexpression of SbWRKY45 increased germination rates and promoted root growth in transgenic Arabidopsis under drought stress. Taken together, our findings indicate that SbWRKY45 may be involved in mediating the response to drought stress and play a vital role in the abiotic stress response of sorghum.

Key words: WRKY, sorghum, transcription factors, abiotic stress, gene, overexpression

1. Introduction
The WRKY is a plant-specific transcription factor (TF) family, which plays essential roles in plant responses to environmental stresses (Eulgem et al., 2000). The WRKY domain is about 60 amino acid residues in length, consisting of a highly conserved heptapeptide sequence WRKYGQK at the N-terminus and zinc-finger-like motif (C-X4+5-C-X22-23-H-X7-H) or (C-X7-C-X23-H-X1-C) at the C-terminus (Eulgem et al., 2000; Chen et al., 2019a). The conservation of the WRKY domain is determined by its cognate binding site. WRKY TFs bind to the cis-acting elements W box (TTGACC/T), by which WRKY TFs activate or repress the downstream genes. It was demonstrated that WRKY TFs play a vital role in regulating several plant processes, including regulation of transcriptional reprogramming associated with the plant stress response. WRKY TFs are diversely distributed in plants and respond to different abiotic and biotic stresses (Erpen et al., 2018). In the last few years, increasing genome sequencing data from diverse plant species with the development of novel sequencing technologies and bioinformatics provided an excellent platform for the genome-wide analysis of WRKY family genes. Tremendous progress has been made in this regard, and genome-wide identification and functional characterization of WRKY genes family have been done in several crop plants (Chen et al., 2019b). Recently, several WRKY genes have been identified, for example, 56 putative WRKYs have been found in tea, 49 in coffee, 70 in chickpea, and 61 in Chinese jujube (Wang et al., 2018; Dong et al., 2019; Waqas et al., 2019; Chen et al., 2019b).

Previous studies indicated that WRKY genes induced a response to abiotic stress and improved stress tolerance. For example, ZmWRKY40-overexpressing transgenic Arabidopsis exhibited significant drought tolerance, through controlling different processes, including the up-regulation of stress-associated genes and lowering reactive oxygen species (ROS), which led to enhancement of the activities of other process, including peroxide dismutase (POD) and...
catalase (CAT) in response to drought stress treatment (Wang et al., 2018a). Furthermore, ZmWRKY106 was induced by drought, heat, and exogenous ABA; transgenic Arabidopsis plants overexpressing ZmWRKY106 showed improved tolerance to drought by regulating stress-related genes via the ABA-signaling pathway (Wang et al., 2018b). In wheat, TaWRKY1 and TaWRKY33 in wheat are other examples; both were involved in response to drought and heat stress by activating several stress-related downstream genes, promoted root growth, and increased germination rate in transgenic Arabidopsis; also, the transgenic lines showed a lower rate of water loss comparing to wild-type plants during hydration (He et al., 2016).

The functional characterization of many WRKY genes has been reported in Arabidopsis; however, in nonmodel plants, few studies have been reported. For instance, overexpression of Arabidopsis WRKY30 exhibited enhanced drought and heat tolerance in transgenic wheat. Furthermore, the expression analysis showed up-regulation of stress-related genes and enzyme-encoding genes (El-Esawi et al., 2019). In cotton, overexpression of GhWRKY33 in transgenic Arabidopsis plants exhibited reduced tolerance to drought stress and participation in ABA signaling pathway. Moreover, the transgenic lines showed increased expression of drought-related genes and ABA-responsive genes (Wang et al., 2019). Transgenic wheat plants overexpressing AtWRKY30 showed enhanced heat and drought stress tolerance. The proline content, relative water content, soluble protein, soluble sugar content, and antioxidant enzymes such as peroxidase (POX) and superoxide dismutase (SOD) were increased in transgenic plants overexpressing AtWRKY30 (El-Esawi et al., 2019). Previous studies have indicated that WRKY genes are involved in response to low temperature. For instance, VaWRKY12 overexpression enhanced cold tolerance in transgenic Arabidopsis and grapevine plants. The antioxidant enzymes encoding genes (glutathione S-transferases and peroxidases) were up-regulated under cold stress treatment (Zhang et al., 2019). Moreover, overexpression of cucumber (Cucumis sativus) WRK gene CsWRKY46 enhanced seedling survival under freezing stress in transgenic Arabidopsis plants. Furthermore, higher proline accumulation was observed whereas less electrolyte leakage and lower MDA levels were found. The study concluded that cucumber CsWRKY46 involved and enhanced cold stress tolerance in the transgenic plants (Zhang et al., 2016).

Sorghum (Sorghum bicolor) is the fifth most important cereal crop; although many WRKY TFs have been identified in various species such as Arabidopsis, maize, wheat, and rice, only a few or no functional studies have been reported in sorghum. Our previous report indicated that there were 94 WRKY genes in sorghum genome; to elucidate the roles of SbWRKYs, we found that SbWRKY45 was strongly induced by drought, and its homolog from maize ZmWRKY40 improved drought tolerance when overexpressed in Arabidopsis; therefore, we wish to know whether a WRKY homolog in sorghum can function similarly as in maize; if so, the gene could be a candidate for further genetic modification and engineering in sorghum. In this study, we isolated and characterized SbWRKY45 gene from sorghum and determined its response to abiotic stress. The overexpression of SbWRKY45 conferred drought tolerance and enhanced seed germination in Arabidopsis.

2. Materials and methods

RNA was extracted from 2-week-old sorghum seedlings using TRIzol reagent (TransGen Biotech, Beijing, China) following the manufacturer's instructions. The DNA was removed using RNase-free DNase I (TransGen Biotech, Beijing, China); then, the RNA concentration was checked using a ThermoScientific NanoDrop™ 2000c Spectrophotometer. The first-strand cDNA was synthesized from 1 µg of total RNA using First-Strand Synthesis following the manufacturer's protocol (TransGen Biotech, Beijing, China) and used as the template for PCR reaction.

2.1. Cloning of SbWRKY45 and sequence analysis

The primers of SbWRKY45 (XM_002451621.1), also known as (SORBI_3004G065900), was designed based on the sequence obtained from NCBI (https://www.ncbi.nlm.gov/ 11/9/2019) to clone the gene from sorghum. The PCR product was cloned into a pMD18-T vector (TAKARA BIO, Beijing, China) and sequenced. The sequence of SbWRKY45 was analyzed using blast search, and other orthologs were obtained from the NCBI database. Multiple sequence alignment and phylogenetic tree analysis were performed using MEGA7.0 with neighbor-joining (NJ) method using default values for all the parameter, and the confidence levels were estimated with bootstrap analysis of 1000 replicates (Kumar et al., 2016). The functional domain of SbWRKY45 was predicted by SMART (http://coot.embl-heidelberg.de/SMART/ 16/10/2019) (Letunic and Bork, 2018). The protein structure analysis was conducted by (http://genome.cbs.dtu.dk/services/CPHmodels-2.0Server-3D.htm, 16/10/2019). The cis-acting elements in the promoter region of SbWRKY45 were predicted using plant cis-acting regulatory element (PlantCARE, http://bioinformatics.psb.ugent.be/webtools/plantcare/html, 18/4/2020) as described by (Gao et al., 2018). Additionally, the gene structure of SbWRKY45 was identified by using the Gene Structure Display Server GSDSv2.0 (Hu et al., 2015).

2.2. Stress treatment and samples collection

Seeds of Sorghum bicolor genotype (SX44B) used in this study were provided by Professor Zhang Fuyao (Sorghum
theoretical isoelectric point (PI) 8.45. The CDS of SbWRKY45 was amplified with gene-specific primers; the sequence alignment of SbWRKY45 presented that the gene was open reading frame (ORF) containing about 340 amino acid residues and the reverse primer 5′ ATTCAGAATT CACTGTTGA TA and the forward primer 5′ CACTGTGTTA TATATGACCTTCT CGAG CTTTGACC’ and the reverse primer 5′ ATTCCAGAATT TG AGTCGTGTCGCCITTG TCACGGCTACTCG3 (NdeI and SaCl site underlines, respectively). The fragment of SbWRKY45 was ligated into pRI201vector, which was digested with NdeI and SaCl under the control of the Cauliflower mosaic virus (CaMV) 35S promoter with kanamycin as the selectable marker. The recombinant plasmid was confirmed by sequencing. The recombinant vector pRI201-SbWRKY45 was introduced into Agrobacterium tumefaciens EHA 105 for Arabidopsis transformation using the floral dip method (Niu et al., 2012).

2.5. Drought tolerance analysis of SbWRKY45 transgenic Arabidopsis plants

Further investigation of the biological functions of transgenic Arabidopsis plants overexpressing SbWRKY45 was created. Germination and growth assay were conducted to identify the phenotype differences between wild-type and transgenic lines under drought stress. The germination assay was performed by culturing the seed of transgenic lines and wild type on 1/2 MS basal medium plates supplemented with or without 4% PEG6000. The media plates were incubated at 4 °C for 3 days and then moved to 22 °C with a photoperiod of 16 h light/8 h dark. Seeds were considered to be germinated when radicals emerged from the seed coat.

For root growth assay, 1-week-old T2 transgenic Arabidopsis lines grown on the 1/2 MS medium were transferred to 1/2 MS media supplemented with or without 8% PEG6000 and kept in growth chamber vertically. The root lengths were photographed and measured (Ma et al., 2014).

2.6. Statistical analysis

In this study, three biological replicates were used throughout the experiment. When appropriate, the analysis of variance (ANOVA) was used for quantitative real-time PCR to determine the statistical significance of the differential expression patterns between treatments and/or between tissues. For the germination and growth assay statistical analysis was performed using Student's t test to determine the statistical differences relative to WT. Mean values of three independent experiments were used to plot figures, the error bars represent the standard deviation.

3. Results

3.1. Gene cloning of the SbWRKY45 and sequencing

SbWRKY45 with (accession number: XM_002451621.2) was successfully isolated from sorghum using reverse transcription PCR (RT-PCR), the gene designated as SbWRKY45. The gene selected based on the similarity to the ortholog counterpart ZmWRKY40 (GRMZM2G120320) in maize was induced by abiotic stress and improved drought stress tolerance in transgenic plants (Wang et al., 2018a); therefore, we are interested to know whether the ortholog from sorghum can have the same role in response to drought stress. The SbWRKY45 gene was amplified with gene-specific primers; the sequence analysis of SbWRKY45 gene presented that the gene was 1040 bp in length and coded a deduced protein sequence of SbWRKY45 containing about 340 amino acid residues along with a predicted molecular weight (MW) of 38.9 and theoretical isoelectric point (PI) 8.45.
The SbWRKY45 protein domains analysis confirmed that SbWRKY45 belongs to group IIa of the WRKY family in sorghum, according to our classification based on the method used by (Eulgem et al., 2000). Furthermore, the results showed that SbWRKY45 contained WRKYGQK domain, which is 60 amino acid residues at the N-terminus, and contained an extended zinc finger motif (C-X_{2-23}H-X_{2-3}C) at the C-terminus compared with the typical WRKY domain as in Arabidopsis WRKY. There are 10 chromosomes in the sorghum genome, and SbWRKY45 is located in the long arm of chromosome 4 with one copy (Baillo et al., 2020).

The protein domain analysis showed that SbWRKY45 has WRKY domain and zinc finger domain. Sequence alignment showed that SbWRKY45 is highly similar to orthologous genes, including Zea mays ZmWRKY40 (GRMZM2G120320), Arabidopsis thaliana AtWRKY40 (AT1G80840), Setaria italica SiWRKY71 (XP_004951681.1), Panicum miliaceum WRKY40 (RLM80886.1), and Triticum aestivum TaWRKY80 (AFW98256.1) (Figure 1). All sequences used were found to have highly conserved domain WRKYGQK sequence as well as nucleus localization signal (NLS).

3.2. Phylogenetic relationship of SbWRKY45 with other WRKY transcription factors

For the phylogenetic analysis of SbWRKY45, similar protein members from other species were retrieved and used. Eighteen available sequences from different species with high similarity were downloaded from NCBI. SbWRKY45 was classified in one clade with ZmWRKY40, ZmWRKY71, AtWRKY40, and SsWRKY; all members shared the highest genetic similarities, suggesting a possible functional similarity (Figure 2). Furthermore, phylogenetic analysis showed some divergence of mono and dicots orthologous genes.

3.3. Structure and promoter analysis of SbWRKY45

The structure analysis conducted by using Gene Structure Display Server (GSDS2.0) revealed that SbWRKY45 contained two exons and one intron besides untranslated region upstream. To detect the abiotic stress that induces SbWRKY45 and elucidate the underlying biological functions of the gene, 1000 bp of the upstream was used to predict the cis-acting element in the promoter region of SbWRKY45 using online cis-element prediction software PlantCARE. We found that the promoter region of SbWRKY45 has several abiotic stress-associated elements such as abscisic acid-responsive element (ABRE), TGACG-motif, light-responsive element (C-box), MYB, and W-box, which are binding sites for WRKYs that might indicate SbWRKY45 being involved in WRKY transcription factor interactions that activate or deactivate stress-related genes (Table 1). A four-stranded B-sheet with a zinc-binding pocket was formed by conserved Cys/His residues in the WRKY domain in the structure of SbWRKY45.

3.4. Expression of SbWRKY45 gene under abiotic stresses and different tissues

The expression patterns of SbWRKY45 under various abiotic stresses and different tissues were investigated by quantitative real-time PCR (qRT-PCR). The results showed that SbERKY45 was differentially induced by drought, salt, and cold but was induced weakly by heat stress treatment (Figure 3). SbWRKY45 expression was induced gradually to reach a maximum at 6 h with 12.2-fold change after drought treatment and then declined to a level similar to the control (Figure 3A). In tissue-specific expression, SbWRKY45 was highly induced within 6 h in roots in response to drought, but the gene was lower in leaves in comparison to other tested tissues. When treated with cold stress, the transcript of SbWRKY45 was induced within 3 h, reached its peak (5.45-fold) at 6 h (Figure 3B), and decreased gradually after 12 and 24 h. SbWRKY45 exhibited the highest response in roots in comparison with leaves and stems in a cold condition. Under salt treatment, the transcript of SbWRKY45 began significantly increasing after 3 h treatment (1.12-fold) and 6 h (1.14-fold) compared to the control (0 h) (Figure 3C). The highest expression level was recorded at 6 h in all tested tissues; however, in leaves the gene was stable at different time points and then decreased after 24 h. When treated with heat stress, the expression of SbWRKY45 was down-regulated, although a slight increase was observed in leaves at 3 h (Figure 3D). SbWRKY45 had a low response to heat stress compared with drought, cold, and salt stress. SbWRKY45 was found to be differentially expressed in leaves, stems, and roots; however, SbWRKY45 was more abundant in roots than that in leaves and stems, especially under drought and cold. The results revealed that the transcript level of SbWRKY45 was increased gradually in roots under all treatments.

The expression pattern of SbWRKY45 under abiotic stress was similar to its ortholog ZmWRKY40 (Wang et al., 2018a), in particular under drought stress; however, they showed different expression patterns under cold and salt stress, and the time point for the begging expression was different. Furthermore, the expression pattern of tested tissues was similar and the highest expression was in root. These indicated that SbWRKY45 and ZmWRKY40 might play a similar role in response to abiotic stress, especially drought. These results suggested that the SbWRKY45 may play a fundamental role in response to different abiotic stress.

3.5. Overexpression of SbWRKY45 improved seed germination under drought stress in transgenic Arabidopsis

The expression analysis showed that the SbWRKY45 expression was increased significantly by drought; therefore, to further investigate the biological function of
SbWRKY45, transgenic Arabidopsis plants overexpressing SbWRKY45 under the control of cauliflower mosaic virus 35S promoter were generated. From the transformation, we obtained 51 independent T₀ lines; specific primers were used for positive lines screening. The qRT-PCR analysis results of T₀ showed that among six Arabidopsis transformants lines (L2, L3, L7, L9, L20, and L39) overexpressing SbWRKY45, two lines (L2 and L3) revealed high SbWRKY45 expression level; therefore, they were selected for further study after confirmation of the positive transgenic plants and their transcription level were advanced for a further generation to select T₂. The RT-PCR result conferred that the exogenous SbWRKY45 was successfully expressed in transgenic Arabidopsis. Two lines of the T2 transgenic Arabidopsis overexpressing SbWRKY45 were selected for germination analysis. The seed germination rate of the SbWRKY45-overexpressing Arabidopsis and wild-type (WT) were observed in normal MS medium (control) and MS medium supplemented with 4% PEG6000 for drought stress. The germination rate of the transgenic Arabidopsis plants overexpressing SbWRKY45 was significantly higher than WT (Figure 4). Radical emergence was scored daily until no further germination occurred; the transgenic plants germinated better at each time point after being transferred to drought stress condition (MS + PEG) (Figure 4). When under normal condition (MS without PEG), there was no visible morphological, developmental, or cotyledon greening differences of the transgenic Arabidopsis plants compared with the WT.

SbWRKY45-overexpressing Arabidopsis plants were observed to have higher greening degree comparing
to WT (Figure 4) under drought stress; however, some transgenic plants have shown up-normal germination. The plants were photographed 2 weeks after transfer. The total root lengths of the transgenic and WT seedlings were measured after growing 7-day-old seedlings on 1/2 MS medium supplemented with or without 8% PEG6000 for 2 weeks. No differences have been observed in total root length under normal conditions; however, under drought stress, the root length of the transgenic line was slightly higher than WT. The seedling’s growth was observed, and the 7-day-old seedlings of the transgenic Arabidopsis plants and WT were transferred to the MS supplemented with 8% PEG. Figure 5 shows that inhibition of root elongation by PEG stress was aggravated in WT plants in comparison to transgenic plants. The total root length of WT was one third that of transgenic Arabidopsis overexpressing SbWRKY45. The transgenic Arabidopsis plants overexpressing SbWRKY45 showed slight differences compared to wild-type; the plants were somewhat greener than WT under PEG treatment and the root was observed to be slightly longer than WT, suggesting that the SbWRKY45 transgenic lines can tolerate drought stress treatment.

### 4. Discussion

Transcription factors such as NAC, bZIP, and WRKY are involved in response to different environmental stresses due to their major roles in plant growth and physiology (Ali et al., 2018; Lata et al., 2011; Baloglu et al., 2014). Among TF, WRKY TF has played an important role in the regulation of transcription reprogramming related to plant abiotic stress responses (Baillo et al., 2019). In sorghum, 94 WRKY genes provided basic information for functional studies of WRKYS in sorghum (Baillo et al., 2020). In the current study, it was elucidated that the
functions of SbWRKY45 in response to abiotic stresses in particular drought. SbWRKY45 was the ortholog of maize ZmWRKY40, which has improved drought stress tolerance in Arabidopsis and provided the basis for cloning functional WRKY genes.

Our results confirmed that SbWRKY45 is a member of sorghum Iia subgroup according to the classification of WRKYs. Sequence analysis of the SbWRKY45 transcription factor revealed it consists of one WRKY domain and a C_{2}H_{2} zinc finger motif. Generally, WRKY TFs consist of WRKY domain and C_{2}H_{2} or C_{2}HC zinc-finger motif, which is used for WRKY TFs classification in all plant species (Chen et al., 2012). The structure of SbWRKY45 was similar to ZmWRKY40 and AtWRKY40; ZmWRKY40 was isolated from maize and conferred improved drought tolerance in Arabidopsis (Wang et al., 2018a).

The sequence alignment and phylogenetic analysis demonstrated that SbWRKY45 was clustered into Iia subgroup, which was more closely related to ZmWRKY40, SsWRKY, and AtWRKY40. The results indicated that SbWKY45 is an important member of the WRKY TF family in sorghum. The predicted cis-element was found to contain several stress response elements besides W-box, such as ABRE (ABA and drought-responsive elements), the CGTCA motif MeJA, MYB (ABA and drought-responsive elements), and C-box (regulatory element involved in light response). All these elements may play a vital role in helping the promoter drive the expression of several downstream genes (Jiang et al., 2017). It is worth mentioning that WRKYs family members have been reported to bind directly to the W-box in the distance in the promoter of the target genes to activate or suppress the response to the stresses (Ciolkowski et al., 2008). The findings above suggest that SbWRKY45 might activate the expression of the stress-responsive genes via binding to the W-box in their promoter region.

Previous studies reported that WRKY genes can be induced by several abiotic stresses (Chen et al., 2019b). The expression of SbWRKY45 under drought, cold, salt, and heat was enriched mostly in the roots compared with the leaves and stems. Under drought and cold stress treatments, the transcript level of SbWRKY45 in roots was remarkably increased, especially in drought and was the highest among all tested tissues (Figure 3A). It has been
reported that sorghum roots showed a larger number of differentially expressed genes in response to drought. The authors highlighted WRKY transcription factors earlier as representing the majority of genes under drought (Varoquaux et al., 2019). This finding supports our results, which showed that the expression of SbWRKY45 was higher in the roots under drought stress conditions. Furthermore, previous studies have proved that various WRKYs were induced in response to drought in several crops, for example, several WRKY genes were reported in maize (Kimotho et al., 2019). Additionally, under cold stress treatment, SbWRKY45 showed increased expression level. Our results are inconsistent with previous studies, which confirmed the involvement of WRKYs in cold stress. For example, in rice, OsWRKY71 showed increased expression level and had a positive function in cold tolerance by regulating downstream genes (Kimotho et al., 2019). Additionally, under cold stress treatment, SbWRKY45 showed increased expression level. Salt treatment. Additionally, exogenous expression of ZmWRKY33 in Arabidopsis improved salt stress tolerance under stress conditions (Li et al., 2013). Interestingly, in this study, SbWRKY45 under heat stress condition was found to be down-regulated. However, this result disagrees with previous results; for example, TaWRKY1 and TaWRKY33 from wheat were induced by heat stress and confer heat resistance in transgenic Arabidopsis plants (He et al., 2016). We mentioned many pieces of evidence that SbWRKY45 was involved in a positive response to stress. However, SbWRKY45 was reported to have the opposite function in response to pathogen infection, for example, the expression of SbWRKY45 was repressed during red stripe disease infection caused by Herbaspirillum rubrisubalbicans in sorghum (Tuleski et al., 2020). The repression was attributed to the bacteria’s ability to modulate plant immunity. Moreover, the orthologous gene of SbWRKY45 in melon (CmWRKY15) was down-regulated in response to powdery mildew infection, implying that SbWRKY45 might play negative roles in response to pathogens (Jiao et al., 2018). Moreover,
SbWRKY45 orthologous in Arabidopsis AtWRKY18 and AtWRKY40 negatively regulate the expression of defense response genes which enhanced the pathogen invasion (Brotman et al., 2013). Furthermore, AtWRKY18 and AtWRKY40 both negatively regulate Golovinomyces orontii infection (Schön et al., 2013). These orthologous studies suggest that SbWRKY45 might act as a negative regulator in response to biotic stresses. In this study, our analysis of SbWRKY45 showed that the gene was up-regulated under the abiotic stress except under heat. This suggested that SbWRKY45 gene had participated in drought, cold, and salt stress, possibly related to different cis-acting elements in the SbWRKY45 promoter (Table 1). Transcriptome-based reports have proved that multiple pathways respond independently to abiotic stressors (Jiang et al., 2015). Our results showed that SbWRKY45 exhibited drought tolerance.

5. Conclusion
We isolated and identified SbWRKY45 gene from sorghum. SbWRKY45 was differentially up-regulated by drought, cold, and salt treatments; however, it was weakly induced by heat. Overexpression of SbWRKY45 improved drought tolerance in transgenic Arabidopsis plants. These results demonstrate that the SbWRKY45 gene plays important roles in drought stress response; moreover, SbWRKY45 enhanced seed germination and root development. SbWRKY45 might be a potential candidate for molecular genetic modification of sorghum. These findings provide a basis for further functional analysis of sorghum WRKYs in response to abiotic stress.

Supplementary material
Supplementary material is available online at www.mdpi.com/xxx/s1, Table S1: SbWRKY45 specific primers used in qRT-PCR analysis.

Author contributions
E.B. designed and performed the experiment and prepared the manuscript. P.X and Z.Z. reviewed the results and revised the manuscript. M.H. analyzed the results. All authors made substantial intellectual contributions to the work and approved it for publication. All authors read and agreed to the published version of the manuscript.

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Conflicts of interest
The authors declare no conflicts of interest.

References


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33