The transcription factor HcERF4 confers salt and drought tolerance in kenaf (Hibiscus cannabinus L.)

Jiao Yue1 · Meiqiong Tang1 · Hui Zhang1 · Dengjie Luo1 · Shan Cao1 · Yali Hu1 · Zhen Huang1 · Qijing Wu1 · Xia Wu1 · Jiao Pan1 · Canni Chen1 · Caijin Wang1 · Peng Chen1

Received: 24 November 2021 / Accepted: 20 February 2022 / Published online: 4 March 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
Ethylene response factors (ERF) are members of the APETALA2/ERF transcription factor family, and they play an important role in plant growth, development, and response to various environmental stresses. In this study, an ERF transcription factor HcERF4 was isolated and characterized from kenaf. The results showed HcERF4 contained an open reading frame of 702 bp, which comprised of single AP2 domain. Subcellular localization analysis revealed that the HcERF4 gene was localized in the nucleus and cytoplasm. Transactivation assays in yeast demonstrated that HcERF4 functions as a transcriptional activator. Its expression profile was investigated by real-time PCR analysis in different tissues and under salt as well as drought stress condition. The result revealed that HcERF4 could be markedly induced by salt and drought in a time and concentration dependent manner. Moreover, silencing of HcERF4 by using the VIGS technique, caused a significant growth inhibition under salt or drought stress condition, with lower plant height, stem diameter, and fresh weight, indicating HcERF4 plays key roles response abiotic stress. In addition, physiological index analyses showed that the contents of MDA, O$_2^-$, H$_2$O$_2$, and free proline significantly increased, while the activities of antioxidant enzymes were significantly restrained in HcERF4 silenced plants. Furthermore, the expression level of eight important genes involved in plant growth regulation, including HcERF3, HcNAC29, HcSOD, HcP5CS, HcbZIP43, HcWRKY31, HcMYB44, and HcCBP25 were investigated, and the result showed that all eight genes were highly expressed in the leaves of the wild kenaf, but were significantly downregulated in the leaves of the VIGS kenaf plants under drought and salt stress conditions; Consequently, the present results indicate HcERF4 plays a positive role in regulating salt and drought stress in kenaf.

Key message
HcERF4 transcription factor modulates drought and salinity stress in kenaf (Hibiscus cannabinus L.).

Keywords Kenaf (Hibiscus cannabinus L.) · HcERF4 · Salinity stress · Drought stress · Virus induced gene silencing (VIGS)

Introduction
Salinity and drought stress are the main abiotic stress factors, with highly negative effects on plant growth and development (Suzuki et al. 2014). Due to ever-changing environmental conditions and a high rate of pollution, plants have evolved various survival strategies to cope with water deficit and salinity stress (Meena et al. 2017). The evolutionary strategy adopted by plants as a result of sessile living is through the regulation of the concentration levels of various phytohormones and the induction of physiological and/or molecular processes of stress-responsive genes (Udawat et al., 2016, 2017). Transcription factors (TFs) are the most important regulators in all abiotic stress responses (Do et al. 2020). To date, numerous transcription factors have been identified and implicated in the regulation of stress responses, for instance, MYB, NAC, AP2/ERF and WRKY families (Xie et al. 2019). Among these TFs, the AP2/ERF
Ethylene transcription factors (ERFs) are a large family of transcription factors unique to plants with conserved AP2/ERF domains (Faraji et al. 2020). They play a transcriptional regulatory role by combining ethylene response sequence motifs such as GCC-box or DRE/CRT motifs. Many previous studies have found that ERF TFs play a role in regulating plant growth and development, plant organ development, cell division, differentiation, flower development and fruit maturation (Feng et al. 2020; Zhang et al. 2020a; Chen et al. 2021a). In addition, ERFs have also been reported in abiotic stress regulation, including drought, salt, extreme temperature, and so on (Gao et al. 2020; Han et al. 2020; Kavas et al. 2020). The AP2/ERF family has been identified in Arabidopsis (Xing et al. 2019), rice (Jisha et al. 2015; Neogy et al. 2020). The AP2/ERF family has been proven to be indispensable for responding to environmental stresses (Do et al. 2020).

In the present work, a stress-responsive AP2/ERF transcription factor, HcERF4, was isolated from kenaf. The expression profiles were revealed in different tissues and under salt and drought stress condition. The subcellular localization and transcriptional activity were analyzed by transient expression experiments in tobacco leaf cells and transactivation assay in yeast. Virus-induced gene silencing (VIGS) of HcERF4 caused the reduced salt or drought tolerance in kenaf. Our study shows that HcERF4 functions as a stress-responsive transcription factor and confers salt and drought tolerance in kenaf.

Materials and methods

Plant materials and growth conditions

Kenaf cultivar SF192 were used in this study. Healthy and plump seeds were initially soaked in distilled water for 1 h, then surface sterilized with 3% H2O2 for 10 min, and finally washed with distilled water for three times. The sterilized seeds were put into a plastic nursery pot (27 cm × 18 cm × 9 cm) lined with double-layer paper towels. Then place them in a light incubator at 27 °C for 14/10 h light/dark (280 μmol m−2 s−1), with a relative humidity of 60–65%. After 4 days, the seedlings that grew evenly were selected and transferred to a seedling tray containing 0.5 × Hoagland solution for hydroponic cultivation.

Cloning and bioinformatics analysis of HcERF4

According to our previous transcriptome data (Chen et al. 2021b) of kenaf, the HcERF4 gene sequence was obtained to design the primer for HcERF4 cloning (Table S1), and the RNA of healthy growing kenaf leaves was extracted by the TRIzol method. cDNA was synthesized using HiScript®III 1st Strand cDNA Synthesis Kit (Vazyme Biotech Co., Ltd, Nanjing, China), and used as template to amplify the full length of the gene. The reaction program is 95 °C 5 min, 95 °C 30 s, 60 °C 30 s, 72 °C 30 s, 35 cycles, 72 °C 5 min, 12 °C storage. The amplified target fragments were recovered from gel, and ligated with 1ul pEASY-Blunt (TransGen Biotech, Beijing, China) to transform into E. coli DH5α (TransGen Biotech, Beijing,
China), screened with LB (Luria–Bertani) medium plates containing kanamycin, and sent positive clones to Tsingke Biotechnology Co., Ltd, China for sequencing, and verifying its sequence, the vector was named Blunt-HcERF4.

The DNAMAN8.0 and Jalview software was used to predict the amino acid sequence of the gene. Conserved domain analysis and homology comparison analysis was performed by using the Blast function of NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi); The molecular weight, isoelectric point of the encoded protein amino acid sequence, basic physical and chemical properties were predicted with the ProtParam tool in ExPASy (https://web.expasy.org/protparam/); The three-dimensional structures and transmembrane regions were predicted by the Phyre2 server (http://www.sbg.bio.ic.ac.uk/~phyre2/html/page.cgi?id=index); The phylogenetic tree was constructed by using MEGA7.0 software; HcERF4 subcellular localization was predicted use the online software Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/).

Subcellular localization analysis of HcERF4

The Open reading frame (ORF) of HcERF4 cDNA sequence was amplified by specific primers designed by Primer 5 (GFP-HcERF4-F/R (EGFP vector construct) in Table S1). Then, the HcERF4 was inserted into pBI121-EGFP at BamH I enzyme site. The recombinant and empty vector were introduced into Agrobacterium GV3101 separately. The Agrobacterium was cultured in LB liquid medium. the bacteria were collected, and re-suspended in a 10 mM MgCl2 (containing 120 μM AS) suspension, when the OD600 reached about 0.6. 1 mL of Agrobacterium solution was injected the epidermis of tobacco leaves when the OD600 reached about 0.6. 1 mL of Agrobacterium strain were collected by centrifugation at 8000 rpm for 10 min, and re-suspend in a resuspension solution (pH 7.2) containing 200 mM NaCl, and sent positive clones to Tsingke Biotechnology Co., Ltd, China for sequencing, and verifying its sequence, the vector was named Blunt-HcERF4.

Transactivation assay of HcERF4 in yeast

The coding sequence of HcERF4 was sub-cloned into the GAL4-binding domain of the pGBK7 vector to produce the plasmid of pGBK7-HcERF4. Afterwards, the pGBK7-HcERF4 recombinant was introduced into the yeast strain Y2H and plated on SD/-Trp medium. Empty pGBK7 vector was used as a negative control. After PCR detection, 3ul of the positive transformants were spotted on SD/-Trp/His/-Leu and SD/-Trp/His/-Leu X-α-gal plates to observe yeast growth at 30 °C for 3 days.

Expression analysis of HcERF4 in kenaf plant

Kenaf seedlings were hydroponic cultured in 1/4 Hoagland nutrient solution condition. 2 weeks later, the seedlings were exposed to drought (10 and 20% w/v, PEG 6000) and salt (100 and 200 mM NaCl) stress, respectively. All treatments involved three biological replicates, and leaf samples were harvested after stress treatment at 0, 1, 3, 6, 12, 24 and 48 h, and unstressed seedlings were used as control. Afterward, the collected plant materials were directly frozen in liquid nitrogen and stored at − 80 °C for total RNA extraction and qRT-PCR analysis.

qRT-PCR was used to detect the expression of HcERF4 under stress conditions. Total RNA was extracted from seedlings leaves under different stress conditions suing TRIzol reagent according to the manufacturer’s instructions (Vazyme Biotech Co., Ltd, Nanjing, China). The RNAs were transcribed to cDNA using HiScript II QRT SuperMix for qPCR (Vazyme Biotech Co., Ltd, Nanjing, China) following the manufacturer’s instructions. For qRT-PCR analysis, a total of 20 μl reaction system containing 1 μl cDNA template, each of 0.4 μl 10μm forward primer and reverse primer, 10 μl ChamQ SYBR Color qPCR Master Mix (Vazyme Biotech Co., Ltd, Nanjing, China), and 8.2 μl ddH2O, was used with a Bio-Rad CFX96 (Bio-Rad, USA). The PCR conditions are set as follows: 95 °C 30 s; 95 °C 10 s, 60 °C 30 s, 40 cycles; 95 °C 15 s, 60 °C 60 s, 95 °C 15 s. The 2−ΔΔCT method was used to determine the expression abundance of HcERF4, and Actin was used as an internal reference gene to calculate the relative change of HcERF4 expression in the qRT-PCR experiment. Each group performed 3 biological replicates, and each reaction performed 3 technical replicates (See Table S1 for primers for HcERF4 and Actin expression analysis).

VIGS-induced gene silencing vector construction and transformation

The online software SGN VIGS Tool (https://vigs.solgennomics.net/) was used to predict the gene silencing region, and primer premier5.0 was used to design gene-specific primers name of TRV-HcERF4-F/R (Table S1). HcERF4 fragment (227 bp) was amplified from kenaf leaf cDNA. The resulting product was cloned into pTRV2 by seamless cloning BamH I digestion, and the recombinant vector pTRV2-HcERF4 was produced. The pTRV1(Helper plasmid), pTRV2(Empty plasmid) and pTRV2-HcERF4 recombinant vectors were introduced into Agrobacterium strain GV3101, and cultured in YEB medium containing kanamycin, rifampicin, and streptomycin at 180 rpm for 12 h until the OD600 reached 1.5. After shaking for 12 h, Agrobacterium strain were collected by centrifugation at 8000 rpm for 10 min, and re-suspend in a resuspension solution (pH 7.2) containing 200 mM NaCl

expression analysis of HcERF4 in kenaf plant

Kenaf seedlings were hydroponic cultured in 1/4 Hoagland nutrient solution condition. 2 weeks later, the seedlings were exposed to drought (10 and 20% w/v, PEG 6000) and salt (100 and 200 mM NaCl) stress, respectively. All treatments involved three biological replicates, and leaf samples were harvested after stress treatment at 0, 1, 3, 6, 12, 24 and 48 h, and unstressed seedlings were used as control. Afterward, the collected plant materials were directly frozen in liquid nitrogen and stored at − 80 °C for total RNA extraction and qRT-PCR analysis.

qRT-PCR was used to detect the expression of HcERF4 under stress conditions. Total RNA was extracted from seedlings leaves under different stress conditions suing TRIzol reagent according to the manufacturer's instructions (Vazyme Biotech Co., Ltd, Nanjing, China). The RNAs were transcribed to cDNA using HiScript II QRT SuperMix for qPCR (Vazyme Biotech Co., Ltd, Nanjing, China) following the manufacturer’s instructions. For qRT-PCR analysis, a total of 20 μl reaction system containing 1 μl cDNA template, each of 0.4 μl 10μm forward primer and reverse primer, 10 μl ChamQ SYBR Color qPCR Master Mix (Vazyme Biotech Co., Ltd, Nanjing, China), and 8.2 μl ddH2O, was used with a Bio-Rad CFX96 (Bio-Rad, USA). The PCR conditions are set as follows: 95 °C 30 s; 95 °C 10 s, 60 °C 30 s, 40 cycles; 95 °C 15 s, 60 °C 60 s, 95 °C 15 s. The 2−ΔΔCT method was used to determine the expression abundance of HcERF4, and Actin was used as an internal reference gene to calculate the relative change of HcERF4 expression in the qRT-PCR experiment. Each group performed 3 biological replicates, and each reaction performed 3 technical replicates (See Table S1 for primers for HcERF4 and Actin expression analysis).

VIGS-induced gene silencing vector construction and transformation

The online software SGN VIGS Tool (https://vigs.solgennomics.net/) was used to predict the gene silencing region, and primer premier5.0 was used to design gene-specific primers name of TRV-HcERF4-F/R (Table S1). HcERF4 fragment (227 bp) was amplified from kenaf leaf cDNA. The resulting product was cloned into pTRV2 by seamless cloning BamH I digestion, and the recombinant vector pTRV2-HcERF4 was produced. The pTRV1(Helper plasmid), pTRV2(Empty plasmid) and pTRV2-HcERF4 recombinant vectors were introduced into Agrobacterium strain GV3101, and cultured in YEB medium containing kanamycin, rifampicin, and streptomycin at 180 rpm for 12 h until the OD600 reached 1.5. After shaking for 12 h, Agrobacterium strain were collected by centrifugation at 8000 rpm for 10 min, and re-suspend in a resuspension solution (pH 7.2) containing 200 mM NaCl
5.6) containing 10 mM 3-(N-morpholino) ethane sulfonic acid, 10 mM magnesium chloride, and 200 mM AS (acetosyringeone) until the OD600 reached about 0.8–1.0. The resuspension of pTRV1 was mixed with pTRV2 and pTRV2-HcERF4 at a ratio of 1:1, and allowed to lay at room temperature for 3 h in the dark before injection. Before the first true leaf appearance, approximately 1 ml of Agrobacterium-containing osmotic medium was infiltrated into the kenaf cotyledons. Seedlings infiltrated with pTRV1 and pTRV2 were used as negative controls. Each treatment has three seeding trays, and each seeding tray was infiltrated with 21 seedlings. After 2 weeks, newly grown leaves after injection were randomly selected and detected with HcERF4 specific primers (Table S1) to determine the silenced seedlings.

Salt and drought treatments for VIGS silenced-plants

After VIGS silencing for 15 days, the wild type plants, pTRV2 plants and pTRV2-HcERF4 silenced plants were treated with 1/4 Hoagland solution containing 150 mM NaCl or 15%PEG, respectively, and the treatment solution was changed every 2 days. After 7 day of stress treatment, the phenotype of each treated plant was photographed and recorded. The plant height, stem diameter, fresh weight and relative leaf water content (RWC) were measured respectively.

Physiological indexes detection

The leaves were collected after 7 days under salt and drought stress. Use the hydroxylamine oxidation method (Li et al. 2021b) to measure the MDA content, O$_2^-$ and H$_2$O$_2$ in VIGS silent and wild-type plants. In addition, the activities of SOD, POD, and CAT were measured according to the protocol described by Chen et al. (2020). The proline content was measured according to the spectrophotometric method (Jimenez-Bremont et al. 2006). All these experiments were carried with three biological repetitions.

Histochemical detection of O$_2^-$ and H$_2$O$_2$

Superoxide radical (O$_2^-$) detection was performed using the nitro blue tetrazolium (NBT) (Liu and Friesen 2012) in situ staining method, and histochemical detection of H$_2$O$_2$ was carried out by means of the 3,3′-diaminobenzidine (DAB) staining method (Daudi and O’Brien 2012). Seedlings after VIGS silencing were treated with 150 mM NaCl or 15%PEG for 7 days. Leaves were soaked in freshly prepared NBT (2 mg/ml NBT in 50 mM sodium phosphate buffer, pH 7.5) or DAB (1 mg/ml DAB, pH 3.8) solution, and stored overnight at room temperature. After that, the leaves were soaked in absolute ethanol and further boiled for 10 min, then the decolorized and stained leaves were photographed.

Expression analysis of stress responsive genes

In order to investigate the knockdown effect of the HcERF4 gene in kenaf, eight stress-responsive genes were used to evaluate their expression levels in leaves of the wild type, the pTRV2 empty vector and HcERF4-silenced plants under salt and drought stress conditions. The leaf samples were obtained after 7 days of stress exposure. The RNA extraction and cDNA transcription were same as mentioned above. Gene-specific primers were designed for HcERF3, HcNAC29, HcSOD, HcP5CS, HcbZIP43, HcWRKY31 HcMYB44, and HcCBP25 for qRT-PCR are shown in Table S1. qRT-PCR was conducted using the SYBR® Premix Ex Taq™ (Tli RNaseH) kit with a CFX96 Real-Time PCR Machine (Bio-Rad, U.S.A). The PCR program was: 95 °C for 20 s, 39 cycles of 95 °C for 15 s and 60 °C for 25 s. The qRT-PCR assay was measured with three repeats.

Statistical analysis

All of the above experiments were repeated at least three times independently, and the data shown are the mean ± SD. In this research, statistical analyses were performed using the statistical tools (Student’s t-test) of Excel 2010 software (Microsoft Corp., Albuquerque, NM, USA). The significance level was defined as *(P < 0.05), **(P < 0.01) and ***(P < 0.001).

Results

Cloning and bioinformatics analysis of HcERF4

The ERF transcriptional factor, HcERF4, was isolated from kenaf according to the transcriptome data (Chen et al. 2020). The open reading framer (ORF) is 702 bp in length, encoding 233 amino acids, containing a typical AP2 motif from 25 to 57 (Fig. 1A). The molecular weight of the protein is 25.53 KDa and the theoretical pI value is 8.89. The average hydrophilic coefficient (GRAVY) of HcERF4 is -0.640, indicating it is a hydrophilic protein. The instability coefficient of this protein is 65.71, which means it is an unstable protein. To understand the phylogenetic relationships between HcERF4 and its homologous proteins, multiple sequence alignment was carried out using the Jalview program, the result showed that they shared high sequence similarity, especially the AP2/ERF domain (Fig. 1B). The three-dimensional structure predicted by Phyre2 showed that HcERF4 has one transmembrane α-helices (Fig. 1C). Phylogenetic analysis based on amino acid sequence of HcERF4 and homologs from...
other species revealed that the \textit{HcERF4} gene has the closest homology relationship with two ERF4 proteins in \textit{Hibiscus syriacus} and \textit{Gossypium hirsutum} which belong to the same family of \textit{Malvaceae} (Fig. 1D).

\textbf{HcERF4 localizes in the nucleus and cytoplasm and with transcriptional activity}

Firstly, the online software WoLF PSORT was used to predict subcellular localization, and the results showed that \textit{HcERF4} is localized in the nucleus. In order to further determine whether \textit{HcERF4}, as an ERF-type transcription factor, is localized in the nucleus, we conducted transient expression experiments in tobacco leaf cells using an \textit{HcERF4} (green fluorescent protein) fusion vector. Compared with the epidermal cells transformed with an empty GFP vector alone, the detected GFP fluorescence suggests that \textit{HcERF4} can function in the nucleus and cytoplasm (Fig. 2A). These results indicated that the subcellular localization of \textit{HcERF4} protein was not solely nucleus-targeted.

The yeast test was used to study the transcriptional activity of the \textit{HcERF4} transcription factor (Fig. 2B). The yeast transformants containing either pGBK7 (negative control) or pGBK7-\textit{HcERF4} grew normally on SD/Trp (single dropout supplements) medium. However, only the yeast transformed with pGBK7-\textit{HcERF4} grew normally on SD/-Trp-His-Leu (triple dropout supplements) medium. When added with X-\textalpha-gal on the medium, the yeast transformed with pGBK7-\textit{HcERF4} showed blue, the yeast transformed with pGBK7 could not. These results indicate that the \textit{HcERF4} transcription factor has transcriptional activation activity.
Expression profile analysis of HcERF4 response to stress

qRT-PCR was employed to analyze the HcERF4 expression profile. Firstly, the HcERF4 expression level in different tissues was investigated, and the result indicated that HcERF4 was expressed extensively in all tested tissues including leaves, roots and stems under normal condition. HcERF4 has the highest expression level in leaves, followed by in roots, and the lowest in stems (Fig. 3A).

To further reveal its detailed expression model to salt or drought stress, kenaf leaves samples from different stress condition and different time were selected for qRT-PCR analysis. Under salt stress conditions, the expression of HcERF4 in kenaf leaves increased with the increase of salt concentration (Fig. 3B). With the duration of high salt concentration, there is a trend of first increasing and then decreasing. The expression level of HcERF4 under 100 mM NaCl treatment reached the highest at 3 h, which was 4.77 folds that of the control, and under 200 mM NaCl stress, the expression level reached the highest at 1 h, which was 17.05 folds that of the control, and the expression level gradually recovered after 12 h. Under drought stress, with the increase of PEG concentration, the gene expression level showed a trend of first increasing and then decreasing (Fig. 3C). With the increase of stress time, under 10% PEG stress, the expression level reached its peak at 1 h, which was 19.55 folds that of the control, and then increased with time. It showed a gradual downward trend, and the expression level showed an upward trend in 24 h. Under 20%PEG treatment, the expression level reached the highest at 1 h, and was 6.43 folds that of the control, and then gradually decreased with the increase of time. Our results show that the expression pattern of HcERF4 is different under different stress treatments and could be induced by salt and drought stress. These results indicate that HcERF4 may be involved in salt and drought signal response pathways.

Virus-induced gene silencing (VIGS) analysis of HcERF4

To verify the role of it’s in response to salt and drought stress in kenaf, HcERF4 was selected for VIGS analysis. After 14 days, the leaf tissue of silenced plants was quantitatively detected by qRT-PCR. As a represent example shown in Fig. 4, the result showed that the expression of HcERF4 in 7 out of 10 VIGS seedlings (2#, 5#, 6#, 7#, 8#, 9# and 10#) was significantly lower than that of the pTRV2, indicating that the HcERF4 was successfully silenced in these 7 kenaf seedlings. The authentic silenced plant seedlings were used in subsequent phenotype and physiological analyses.

HcERF4 VIGS silenced kenaf reduced the tolerance to salt stress

In order to study the role of HcERF4 in response to salt stress, the HcERF4 silenced kenaf seedlings were subjected
to stress treatments under 150 mM NaCl stress for 7 days. As shown in Fig. 5, there was no significant difference in appearance of CK and pTRV2, while \(HcERF4\) silenced plants showed significantly lower plant height, smaller leaf area, and less developed root system compare with CK and pTRV2 (Fig. 5A). The agronomic characteristics of VIGS plants including plant height, stem diameter, fresh weight, and relative water content (RWC) were measured under salt stress condition. As shown in Fig. 5, the plant height (Fig. 5B), stem diameter (Fig. 5C), fresh weight (Fig. 5D), and RWC (Fig. 5E) of \(HcERF4\) silenced plants were significantly lower than those of CK and pTRV2 plants. The results showed that the silencing of \(HcERF4\) inhibited the growth of kenaf seedlings and reduced its salt tolerance.

The physiological indexes were analyzed and the results showed that the contents of MDA, \(O_2^-\) and \(H_2O_2\) in pTRV2-\(HcERF4\) plants were significantly increased by 64.3, 43.9 and 21.6% under salt stress compared with wild type seedlings (Fig. 5F–H). The activity of CAT and SOD were significantly reduced by 65.6 and 91.3% (Fig. 5K, I), with the exception of POD activity, it significantly increased by 69.8% (Fig. 5J). Salt stress also significantly increased the proline content in silent plants (Fig. 5L). These results indicated that silencing of \(HcERF4\) caused kenaf sensitive to salt stress.

Fig. 3  Time-course expression level of \(HcERF4\) in kenaf SF192 using qRT-PCR method. A: Expression patterns of \(HcERF4\) in root, stem and leaf in normal condition. B and C: Expression patterns of \(HcERF4\) in control, salt (100 mM NaCl, 200 mM NaCl) and drought (10% PEG, 20% PEG) in leaf at the following time point: 0, 1, 3, 6, 12, 24, and 48 h. The reference genes \(HcACTIN\) were used as controls in this study. The error bar display is based on three repeated SDs. *Represents the significance level at \(P < 0.05\); **represents the significance level at \(P = 0.01\); *** represents the significance level at \(P < 0.001\).
Silencing of HcERF4 in kenaf reduced the tolerance to drought stress

In order to study the role on the drought tolerance, HcERF4 VIGS silenced seedlings were treated with 15% PEG6000 for 7 days. By observing the phenotypes, it was found that there was no significant difference between CK and pTRV2 plants. However, HcERF4 silenced plants were significantly lower than CK and pTRV2 plants, and the leaves are curled and wilted in shape, the root system is under developed (Fig. 6A). This phenomenon indicates that the silence of VIGS reduces the drought tolerance of kenaf. As shown in Fig. 6, after the drought stress treatment, the agronomic indicators of the treated plants were determined. Among them, CK and pTRV2 had no significant differences in plant height (Fig. 6B), stem diameter (Fig. 6C), fresh weight (Fig. 6D), and relative water content (Fig. 6E). However, after the VIGS silencing, the indicators decreased with exception of stem diameter, indicating that the HcERF4 may positively regulates the drought resistance in kenaf.

To study the effect of HcERF4 on drought tolerance, the content of MDA content and antioxidant activity were determined. Under drought stress, the MDA content of silenced plants was significantly reduced (Fig. 6F). After drought stress, the \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) content of silent plants increased by 1.28 and 1.31 folds, respectively, compared with the control (Fig. 6G, H). Further study the oxidative stress under drought stress by analyzing the activity of antioxidant enzymes. The results showed that drought stress had a significant effect on the antioxidant enzyme activity of silenced plants. SOD is the first line of defense against ROS-mediated oxidative stress. After drought stress, the SOD and POD activities of silent plants were significantly reduced, about 5.98 folds and 1.48 folds lower than that of the control, respectively under 15% PEG stress (Fig. 6I, J). CAT activity showed similar trends, compared with the control, the CAT activity of silent plants under drought stress was significantly reduced by 3.08 folds (Fig. 6K). However, the proline contents of silenced plants under drought stress were not significantly different from those of control plants (Fig. 6L). These results indicated that the decline of antioxidant enzymes activity after HcERF4 silencing led to the increase in ROS levels, thereby aggravating membrane damage and reducing the drought tolerance in kenaf.

Histochemical detection of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \)

Additionally, in order to analyze the accumulation of ROS in the HcERF4-silenced and control plants after salt stress, NBT and DAB staining were used to detect \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) levels. As shown in Fig. 7, after 7 days of salt stress or drought stress, the NBT and DAB stained area of silenced plants was significantly larger than that of wild-type plants. In addition, the \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) content in the silenced plants was significantly higher than that of wild-type plants (Figs. 5G, H and 6 G, H). These results indicated that HcERF4 silenced plants aggravated its susceptibility to salt and drought stress.

Expression of stress-response genes in HcERF4 silenced plants

Analysis of various stress-responsive genes indicated the possibility of the silenced gene’s involvement in enhancing stress tolerance. To further clarify its mechanism, we monitored the transcriptional level of several stress-responsive genes in the WT, pTRV2 empty vector, and pTRV2-HcERF4 lines, including ethylene responsive element binding factor 3 (ERF3), NAC transcription factor 29-like (NAC29), superoxide dismutase (SOD), 1-Pyrroline-5-Carboxylate Synthetase (P5CS), basic leucine-zipper 43 (bZIP43), WRKY DNA-binding protein 31 (WRKY31), MYB domain protein 108 (MYB108), and cap
Binding Protein 25 (CBP25). All eight genes showed significantly reduced expression levels in the leaves of the *HcERF4*-silencing kenaf plants under salt and drought stress conditions (Fig. 8). These results suggest that *HcERF4* may regulate these genes involved pathways at the transcription level to response salt and drought stress.

**Discussion**

Adverse environmental conditions such as salinity and drought severely affect plant growth and development, posing a global threat to the continuous growth and yield
of most crops. In order to survive and maintain the integrity of the entire plant, plants have evolved certain adaptive mechanisms to deal with these adversities, have a certain ability to perceive stress signals, and quickly adjust their biological responses (Zhao et al. 2021). Thus, the expression of stress-related genes and many involved metabolic pathways were finely regulated through multifarious manners. Transcription factors interact with cis-acting elements in the promoter regions of various stress-related genes and have been found to be important components for regulating gene expression. APETAL2/Ethylene responsive factor (AP2/ERF) TF family is one of the largest TF families in the plant kingdom. ERFs play important roles throughout the plant life cycle and contribute to the ability of plants to withstand various abiotic and biotic stresses. Although these functions of the AP2/ERF genes have been
investigated to some extent in other plants, the function of AP2/ERF genes in kenaf, especially in abiotic stress has not been studied so far.

In the present study, a kenaf ERF gene, HcERF4, was functionally characterized. Sequence homologous analysis showed that HcERF4 is a member of the ERF family (Fig. 1A). Phylogenetic analysis showed that HcERF4 with a closest phylogenetic relationship to ERF4 of Hibiscus syriacus and Gossypium hirsutum which belong to the same family of Malvaceae (Fig. 1D). Subcellular localization assay suggested that HcERF4 was localized in the nucleus and cytoplasm (Fig. 2A). The transcription factors were mainly reported targeted to the nuclei (Li et al. 2019, 2021a; Yang et al. 2020; Ma et al. 2021), however, some of them acts as nucleocytoplasmic shuttling proteins, such as BZR1 (Ryu et al. 2007) and AaERF3 (Lu et al. 2012). In the subcellular localization of the HcERF4 protein in vivo, the results indicated that the HcERF4 protein was not only targeted to the nuclei, but also to the cytoplasm. The protein of HcERF4 should be a nucleocytoplasmic shuttling protein, just like AaERF3 and BZR1. Transcriptional activation assay showed that HcERF4 exhibited transcriptional activation activity.

**Fig. 8** Stress-responsive genes (A–H: HcERF3, HcNAC29, HcSOD, HcP5CS, HcZIP43, HcWRKY31, HcMYB44, and HcCBP25) expression profiling in HcERF4-silencing kenaf plants. HcActin was used as the reference gene. The letters a/b indicate statistically significant differences (two-tailed, P < 0.01). Error bars of the gene expression levels represent the standard deviation of three biological replicates. CK: untreated. Salt: 150 mM NaCl treatment. Drought: 15% PEG-6000 treatment.
like many other ERF factors, such as *HuERF1* (Qu et al. 2020), *OsERF71* (Yu et al. 2017), and *ThERF1* (Wang et al. 2014). These findings demonstrate that *HcERF4* has the basic characteristics of a TF and functions as a transcriptional activator.

Generally, TFs are induced rapidly in response to abiotic stresses, reach a peak within several hours, and subsequently decline in their transcription level (Wang et al. 2020). Although numerous studies have shown that plant AP2/ERFs play considerable roles in abiotic stress tolerance, including drought, salt, and even cold, the expression pattern of AP2/ERFs should provide the most immediate evidence (Bahieldin et al. 2016; Killi et al. 2020; Zhou and Yarra 2021). In the present study, *HcERF4* is expressed in different tissues at different expression levels in kenaf (Fig. 3A). We mimicked some abiotic stress, such as high salinity (100 mM NaCl, 200 mM NaCl), drought (10%PEG, 20%PEG). Under different salt concentration treatments, the expression level of *HcERF4* reached the highest at 3 h and 1, 3, and 48 h, respectively (Fig. 3B). Under different concentrations of drought stress, the expression level of *HcERF4* reached the highest at 1, 24 h and 1, 3 h, respectively (Fig. 3C). The expression profile indicated that the expression level of *HcERF4* was induced by salt and drought stress and was closely related to the stress concentration.

To verify that the role of *HcERF4* in kenaf in response to abiotic stress, the *HcERF4* VIGS knocked down kenaf seedlings were treated with high salt or PEG imitated drought stress. The morphology of *HcERF4* silenced plants was significantly affected by salt or drought stress (Figs. 5A, 6A), in addition, the plant height, biomass, and relative water content (RWC) were significantly lower than those of WT plants, indicating that silencing of *HcERF4* reduced the salt and drought tolerance of kenaf. Moreover, oxidant and antioxidant enzyme assays showed that the VIGS plants registered higher levels of oxidant enzymes and a significant reduction of the antioxidant enzymes under salt and drought stress conditions compared with their wild types (Magwanga et al. 2019). The MDA is a measure of lipid peroxidation or degradation levels; the levels of MDA and H$_2$O$_2$ indicate free oxygen radical reactions occurring in the stressed tissue (Mayne 2013). Thus, higher concentration levels in the leaves of the VIGS plant showed that the plants suffered more oxidative stress compared with the wild types, which further revealed the vital role of *HcERF4* gene in enhancing drought and salt stress tolerance in kenaf. The activity of proline, malondialdehyde (MDA) and antioxidant enzymes can be used to indicate the degree of damage caused by stress (Jin et al. 2018). The higher the MDA content, the higher the degree of membrane peroxidation of plant cells and the more severe the damage to the cell membrane (Sun et al. 2014). Salt stress increased the proline content of silent kenaf plants. Plant antioxidant enzyme system plays an important role in resisting external environmental stress. They can inhibit the accumulation of free radicals, thereby reducing the occurrence and lethal effects of oxidative damage. The silencing of *HcERF4* reduces the tolerance of kenaf plants to salt and drought stress, and leads to a decrease in the activities of SOD and CAT, and an increase in the content of MDA, O$_2$- and H$_2$O$_2$. Knock down of *HcERF4* may reduce its salt tolerance and drought tolerance by changing these physiological indicators under adversity.

Accumulating evidence suggests that activator-type ERF transcription factors regulate multiple stress responses through the activation of defense- and stress-related genes following interacting with various cis-acting elements including the GCC-box or even through interacting with other transcription factor (Trujillo et al. 2008; Tian et al. 2015). Our present study showed that the *HcERF4* is an ERF transcription activator. We supposed that *HcERF4* possibly activates the expression of some stress-related genes following interaction with the GCC-box or other cis-elements. To investigate this notion, the eight stress-related genes (*HcERF3*, *HcNAC29*, *HcSOD*, *HcP5CS*, *HcbZIP43*, *HcWRKY31* *HcMYB44*, and *HcCBP25*) were subjected to qRT-PCR analysis in *HcERF4*-silencing and WT kenaf plants under salt and drought conditions. ERF3, NAC29 and bZIP TFs positively contribute to plant tolerances to salt and drought stresses in other plants (Rong et al. 2014; Huang et al. 2015; Kang et al. 2019; Zhang et al. 2020b). Previous studies have shown that AP2/ERF TFs were activated or upregulated by the bZIP, MYB, WRKY, and NAC TFs (Ritonga et al. 2021). Extensive studies had shown that members of AP2/ERF superfamily and R2R3-MYB family TFs were associated with the regulation of lipid metabolism in higher plants (Xing et al. 2021). SOD affect the secondary cell wall biosynthesis during salt stress which tolerance to the transgenic plants (Shaﬁ et al. 2015). Tomato ERF2 was involved in salt tolerance in transgenic rice via activation of cold-related genes, including OsMyb, and OsSODB (Nakano et al. 2006). Abscisic acid (ABA) mediates various abiotic stress responses, and ethylene responsive factors (ERFs) play vital role in resisting stresses (Zhao et al. 2019), CBP is also involved in the response to ABA and abiotic stress (Daszkowska-Golec et al. 2013). In *HcERF4*-silencing plants, the expression of *HcCBP25* was inhibited. It is speculated that there is a certain interaction between *HcERF4* and *HcCBP25*. The P5CS gene is known to play an integral role in the proline biosynthesis pathway because it encodes for a bi-functional enzyme that catalyzes the rate-limiting reaction in proline biosynthesis in living organisms (Stein et al. 2011). P5CSs are involved in the proline glutamate biosynthesis pathway, which can increase the accumulation of proline and improve salt and drought tolerance in various plants along with a ROS scavenger that attenuates oxidative stress under high salinity conditions (Dai et al. 2018; Yang...
et al. 2021). Here, the suppress expression of *HcERF4* in VIGS plants caused the downregulation of *HcP5CS* may hence lead to proline less accumulation in VIGS plants under salt/drought stress conditions. In this study, the transcript levels of these stress-related genes were suppressed in *HcERF4*-silencing kenaf plants as compared with WT control under salt and drought stresses (Fig. 8). These results suggested that *HcERF4* may function as an integrator of the responses of these stress-related genes to salt and drought stresses. Therefore, it could be induced *HcERF4* may interaction with these stress-responsive related genes and metabolic pathways in salt and drought tolerance. However, the mechanism of *HcERF4* in regulating of these stress-related genes and the specific regulatory pathways in kenaf abiotic stress response remains to be further revealed.

**Conclusions**

The present study characterized an ethylene transcription factors (ERFs) *HcERF4* from kenaf. *HcERF4* contained a typical AP2 conserved domain, located in the nuclei and cytoplasm, and with transcriptional activation activity. The expression profile of *HcERF4* has been characterized in different tissues and both salt and drought stress conditions. Virus-induced gene silencing (VIGS) knocked down plants of *HcERF4* showed more sensitive to salt or drought stress, indicating that *HcERF4* plays an important role in the resistance of kenaf to abiotic stress. Moreover, eight stress-responsive genes were all down-regulated in the leaf of the *HcERF4*-silencing kenaf plants as compared with WT control under salt and drought stresses condition. This study is of great significance for understanding the regulation mechanism of *HcERF4* in kenaf salt and drought stress, and also provides useful information for improving the salt and drought tolerance of kenaf.

**Supplementary Information** The online version contains supplementary material available at [https://doi.org/10.1007/s11240-022-02260-1](https://doi.org/10.1007/s11240-022-02260-1).

**Acknowledgements** This research work was supported by the National Natural Science Foundation of China (Grant No. 31960368), and the China Agriculture Research System of MOF and MARA (CARS-16-E14); and the Innovation Project of Guangxi Graduate Education (Grant No. YCBZ2020015).

**Author contributions** JY: Data curation; Methodology, Formal analysis, Roles/Writing—original draft. MT: Investigation, Formal analysis. HZ: CC: Investigation, Data curation, Formal analysis. DL: SC: YH: ZH: Software, Methodology, Formal analysis. QW: XW: JP: Formal analysis, Validation. PC: Conceptualization, Writing—review & editing, Funding acquisition, Project administration.

**Funding** Funding was provided by National Natural Science Foundation of China (Grant No. 31960368), (China Agriculture Research System of MOF and MARA (Grant No. CARS-16-E14) and Innovation Project of Guangxi Graduate Education (Grant No. YCBZ2020015)).

**Data availability** The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Declarations**

**Conflict of interest** The authors declare that they have no competing financial interests.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**References**

An X, Jin G, Luo X, Chen C, Li W, Zhu G (2020) Transcriptome analysis and transcription factors responsive to drought stress in *Hibiscus cannabinus* L. PeerJ 8:e8470. [https://doi.org/10.7717/peerj.8470](https://doi.org/10.7717/peerj.8470)

Bahieldin A, Atef A, Edris S, Gadalla NO, Ali HM, Hassan SM, Al-Kordy MA, Ramadan AM, Makki RM, Al-Hajar AS, El-Domyati FM (2016) Ethylene responsive transcription factor ERF109 retards PCD and improves salt tolerance in plant. BMC Plant Biol 16(1):216. [https://doi.org/10.1186/s12870-016-0908-z](https://doi.org/10.1186/s12870-016-0908-z)

Chen P, Chen T, Li QZ, Jia RX, Luo DJ, Tang MQ, Lu H, Hu YL, Yue J, Huang Z (2020) Transcriptome analysis revealed key genes and pathways related to cadmium-stress tolerance in Kenaf (*Hibiscus cannabinus* L.). Ind Crop Prod 158. [https://doi.org/10.1016/j.indcrop.2020.112970](https://doi.org/10.1016/j.indcrop.2020.112970)

Chen HC, Chien TC, Chen TY, Chiang MH, Lai MH, Chang MC (2021a) Overexpression of a novel ERF-X-type transcription factor, OsERF106MZ, reduces shoot growth and tolerance to salinity stress in rice. Rice 14(1):82. [https://doi.org/10.1186/s12284-021-00525-5](https://doi.org/10.1186/s12284-021-00525-5)

Chen P, Li QZ, Luo DJ, Jia RX, Lu H, Tang MQ, Hu YL, Yue J, Huang Z (2021b) Comparative transcriptomic analysis reveals key genes and pathways in two different cadmium tolerance kenaf (*Hibiscus cannabinus* L.) cultivars. Chemosphere 263. [https://doi.org/10.1016/j.chemosphere.2020.128211](https://doi.org/10.1016/j.chemosphere.2020.128211)

Dai W, Wang M, Gong X, Liu JH (2018) The transcription factor FcWRKY40 of *Fortunella* crassifolia functions positively in salt tolerance through modulation of ion homeostasis and proline biosynthesis by directly regulating SOS2 and P5CS1 homologs. New Phytol 219(3):972–989. [https://doi.org/10.1111/nph.15240](https://doi.org/10.1111/nph.15240)

Daszkowska-Golec A, Wojnar W, Rosikiewicz M, Szarejko I, Maluszynski M, Szweykowska-Kulinska Z, Jarmolowski A (2013) *Arabidopsis* suppressor mutant of abh1 shows a new face of the already known players: ABH1 (CBP80) and ABH4 in response to ABA and abiotic stresses during seed germination. Plant Mol Biol 81(1–2):189–209. [https://doi.org/10.1007/s11103-012-9991-1](https://doi.org/10.1007/s11103-012-9991-1)

Daudi A, O’Brien JA (2012) Detection of hydrogen peroxide by DAB staining in *Arabidopsis* leaves. Bio Protoc 2(18). [https://doi.org/10.21769/BioProtoc.263](https://doi.org/10.21769/BioProtoc.263)

Ding A, Tang X, Yang D, Wang M, Ren A, Xu Z, Hu R, Zhou G, O’Neill M, Kong Y (2021) *ERF4* and MYBS2 transcription factors play antagonistic roles in regulating homogalacturonan demethylesterification in *Arabidopsis* seed coat mucilage. Plant Cell 33(2):381–403. [https://doi.org/10.1093/plcell/koa031](https://doi.org/10.1093/plcell/koa031)

Do TH, Pongthai P, Ariyaratne M, Teh OK, Fujita T (2020) AP2/ERF transcription factors regulate salt-induced chloroplast division...
in the moss Physcomitrella patens. J Plant Res 133(4):537–548. https://doi.org/10.1007/s10265-020-01195-y

Duan SW, Cheng LF, Feng XY, Yang Q, Liu ZY, Zheng K, Peng Y (2020) Insights on bio-degrading of kenaf bast based on metamatogen and proteomics. BMC Genomics 21(1):121. https://doi.org/10.1186/s12864-020-6531-2

Faraji S, Fizile E, Kazemitabar SK, Vanozzoi A, Palumbo F, Barcaccia G, Heidari P (2020) The AP2/ERF gene family in Triticum durum: genome-wide identification and expression analysis under drought and salinity stresses. Genes 11(12). https://doi.org/10.3390 GENES11121464

Feng K, Hou XL, Xing GM, Liu JX, Duan AQ, Xu ZS, Li MY, Zhuang Q, Yang G, He G (2015) Feng K, Hou XL, Xing GM, Liu JX, Duan AQ, Xu ZS, Li MY, Zhuang Q, Yang G, He G (2015) Fos1, an ERF transcription factor, enhances salt tolerance in transgenic Arabidopsis thaliana. BMC Genomics 16:240–435. https://doi.org/10.1186/j.plaphy.2020.09.027

Giwa Ibrahim S, Karim R, Saari N, Wan Abdullah WZ, Zawawi N, Ab Razak AF, Hamim NA, Umar RA (2019) Kenaf (Hibiscus cannabinus L.) seed and its potential food applications: a review. J Food Sci Technol 54(8):2015–2023. https://doi.org/10.1175/1750-3841.2020.1768509

Gao Y, Han D, Jia W, Ma X, Yang Y, Xu Z (2020) Molecular characterization and systematic analysis of NaTAP2/ERF in tobacco and functional determination of NaTARV-4 under drought stress. Plant Physiol Biochem 156:420–435. https://doi.org/10.1016/j.plaphy.2020.09.027

Jimenez-Bremont JF, Becerra-Flora A, Hernandez-Lucero E, Rodriguez-Kessler M, Acosta-Gallegos JA, Ramirez-Pimentel JG (2006) Proline accumulation in two bean cultivars under salt stress: the effect of polyamines and ornithine. Biol Plant 50(4):763–766. https://doi.org/10.1007/s10035-006-0126-x

Jin Y, Pan WY, Zheng XF, Cheng X, Liu MM, Ma H, Ge XC (2018) OsERF101, an ERF family transcription factor, regulates drought stress response in reproductive tissues. Plant Mol Biol 98(1–2):51–65. https://doi.org/10.1007/s11103-018-0762-5

Jisha V, Dampanaboina L, Vadassery J, Mithefat A, Kappara S, Ramanan R (2015) Overexpression of an AP2/ERF type transcription factor OsEREFP1 confers biotic and abiotic stress tolerance in rice. PLoS ONE 10(6):e0127831. https://doi.org/10.1371/journal.pone.0127831

Kang C, Zhai H, He S, Zhao N, Liu Q (2019) A novel sweetpotato bZIP transcription factor gene, IbBPZIP1, is involved in salt and drought tolerance in transgenic Gossypium hirsutum. Plant Cell Physiol 60(10):2343–2355. https://doi.org/10.1111/pcp.13717

Kasivisvanathan A, Ponnusamy V, Velmurugan R, Manickam R, Thangavelu A, Deshpande S, Rawat S (2020) Knockdown of cytochrome P450 genes Gh_D07G1197 and Gh_A13G2057 on chromosomes D07 and A13 reveals their putative role in enhancing drought and salt stress tolerance in Gossypium hirsutum. Genes (Basel) 10(3). https://doi.org/10.3390/genes10030226

Mayne ST (2013) Oxidative stress, dietary antioxidant supplements, and health: is the glass half full or half empty? Cancer Epidemiol Biomarkers Prev 22(12):2145–2147. https://doi.org/10.1158/1055-9965.EPI-13-1026

Meena KK, Sorty AM, Bitta UM, Choudhary K, Gupta P, Pareek A, Singh DP, Prabha R, Sahu PK, Gupta VK, Singh HB, Krishanani KK, Minhas PS (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Front Plant Sci 8:172. https://doi.org/10.3389/fpls.2017.00172

Nakano T, Suzuki K, Fujimura T, Shinshii H (2006) Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol 140(2):411–432. https://doi.org/10.1104/pp.105.073783

Neogy A, Garg T, Kumar A, Dwiwedi AK, Singh H, Singh U, Singh Z, Prasad K, Jain M, Yadav SR (2019) Genome-wide transcript profiling reveals an auxin-responsive transcription factor, OsAP2/ERF-40, promoting rice adventitious root development. Plant Cell Physiol 60(10):2343–2355. https://doi.org/10.1111/pcp.13717

Ritonga FN, Ngaita G, Wang Y, Khoso MA, Farooq U, Chen S (2021) AP2/ERF, an important cold stress-related transcription factor family in plants: a review. Physiol Mol Biol Plants 27(9):1953–1968. https://doi.org/10.1007/s12298-021-01061-8

Wong W, Qi L, Wang A, Ye X, Du L, Liang H, Xin Z, Zhang Z (2014) The ERF transcription factor TaERF3 promotes tolerance to salt and drought stresses in wheat. Plant Biotechnol J 12(4):468–479. https://doi.org/10.1111/pbi.12153
Ryu H, Kim K, Cho H, Park J, Choe S, Hwang I (2007) Nucleocytoplastic shuttling of BZR1 mediated by phosphorylation is essential in *Arabidopsis* brassinosteroid signaling. Plant Cell 19(9):2749–2762. https://doi.org/10.1105/tpc.107.053728

Seo YJ, Park JB, Cho YJ, Jung C, Seo HS, Park SK, Nahm BH, Song JT (2010) Overexpression of the ethylene-responsive factor gene BrERF4 from Brassica rapa increases tolerance to salt and drought in *Arabidopsis* plants. Mol Cells 30(3):271–277. https://doi.org/10.1007/s11033-010-0114-z

Shaﬁ A, Chauhan R, Gill T, Swarnkar MK, Sreenivasulu Y, Kumar S, Kumar N, Shankar R, Ahuja PS, Singh AK (2015) Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in *Arabidopsis* under salt stress. Plant Mol Biol 87(6):615–631. https://doi.org/10.1007/s11103-015-0301-6

Stein H, Honig A, Miller G, Erster O, Eilenberg H, Csonka LN, Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R (2014) Abiotic stress and regulatory networks in hormone and abiotic stress responses in *Arabidopsis*. Front Plant Sci 10:228. https://doi.org/10.3389/fpls.2019.00228

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.