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ORIGINAL ARTICLE

A *CkDREB1* gene isolated from *Caragana korshinskii* Kom. enhances *Arabidopsis* drought and cold tolerance

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Abstract

Caragana korshinskii Kom., an arbuscular legume with important economic and ecological value in feed, processing industry, and environmental protection, also has great tolerance potential to abiotic stress conditions. An AP2 domain-containing gene was isolated from the suppression subtractive hybridization library of *C. korshinskii* under drought stress. In addition, the isolated gene was also found to be responsive to cold and ABA treatment. Phylogenetic analysis indicates that the deduced protein belongs to the DREB A-1 subfamily and is designated as CkDREB1. Overexpression of *CkDREB1* in *Arabidopsis thaliana* (L.) Heynh increased drought and cold tolerance compared with the wild type. The drought responsive genes *RD29A*, *RD29B*, *KIN1*, and *KIN2*, as well as cold-responsive marker genes *COR15A* and *COR47*, were also highly induced in the overexpression lines under drought and cold conditions. These results should shed light on our understanding on the mechanisms of abiotic resistance of *C. korshinskii*.

Keywords AP2 DNA-binding motif · DREB · Drought stress · Transcription activator

1 Introduction

Plants have developed multiple mechanisms to adapt to stresses such as cold, drought, salinity, and heat (Zou et al. 2010; Abu-Romman 2016; Zandalinas et al. 2018). Freezing and drought tolerance might have certain protective mechanisms in common (Siminovitch and Cloutier 1983). For instance, COR15a, a member of cold-regulated (COR) genes and responsive to dehydration genes (RD29A) of *Arabidopsis thaliana* (L.) Heynh, was induced during both cold acclimation and water-deficit condition (Liu et al. 1998). Later, a cis-acting DNA element, the C-repeat/dehydration responsive element (DRE), was identified from the promoters of many cold-regulated genes, including COR15a (Baker et al. 1994), COR78/RD29A (Yamaguchi-Shinozaki and Shinozaki 1994), and BN115 (Jiang et al. 1996). In 1997, the first transcription activator that binds to the C-repeat/DRE, namely the CBF1 (C-repeat/DRE binding factor 1 or DRE binding protein, DREB), was isolated by yeast-one-hybrid screening from *Arabidopsis* (Stockinger et al. 1997). Soon afterward, DREB1A-C and DREB2A-B were cloned by Shinozaki's group, and DREB1B was found to be identical to previously reported CBF1 (Stockinger et al. 1997; Liu et al. 1998).

Both CBFs/DREBs and EREBPs (for ethylene responsive element binding protein) contain a typical AP2 (for APETALA2) DNA-binding motif and were divided into two groups based on the number of the AP2 domains (Jofuku et al. 1994, 2005; Klucher et al. 1996; Okamuro et al. 1997). One class encodes proteins containing two AP2 domains such as Glossy15 from maize (Bowman et al. 1989; Drews et al. 1991; Moose and Sisco 1996; Lee et al. 2005), and APETALA2, and AINTGUMENTA from *Arabdopsis*,

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which play a central role in defining of floral organ identity. The other class of proteins contains only one AP2 domain, including EREBPs, TINY, CBF1, AtEBP, DREBs, and Ptis from tomato (Ohme-Takagi and Shinshi 1995; Wilson et al. 1996; Buttner and Singh 1997; Stockinger et al. 1997; Liu et al. 1998).

Among these AP2 domain-containing proteins, DREBs form a large multigene family and play crucial roles as key regulators in plants responding to various abiotic stresses. So far, DREBs were isolated from over 31 species such as *Arabidopsis*, tomato, rice, and wheat, and the transgenic plants were confirmed to confer cold, drought, and salinity tolerance. These DREBs were clustered to six subfamilies from A1 to A6. *Arabidopsis* DREB1s/CBFs belong to the A-1 subfamily.

Caragana is the common name of the legume shrub and tree, including more than 80 species (Perveen et al. 2014). The nature of a tree or subshrub limits its study at the molecular level. Up to date, there were several genes from *Caragana* cloned in full length with their transcripts confirmed to be induced by various abiotic stresses and phytohormones (Wang et al. 2009, 2010, 2011; Wu et al. 2009; Bhardwaj et al. 2010, 2011). *Caragana korshinskii* Kom., a perennial, deciduous shrub growing in semiarid and desert area of northwest China and Mongolia, is valuable for its key roles in vegetation restoration and feed industry. It is believed to be highly stress-tolerant (Wang et al. 2007; Wu et al. 2009) since it could survive − 40 °C and with a water precipitation of only 100 mm/year. However, the molecular mechanisms underlying the abiotic stress tolerance properties of *C. korshinskii* are unknown. Recently, it has been reported that transgenic tobacco overexpressing CkDREB from *C. korshinskii* conferred salt and osmotic tolerance (Wang et al. 2011), while in rice and *Arabidopsis*, in total, 4 and 3 DREB1 family members were identified so far respectively (Liu et al. 1998; Dubouzet et al. 2003a).

In order to uncover the abiotic stress tolerance mechanisms of *C. korshinskii*, a suppression subtractive hybridization (SSH) library was constructed using 30-day-old seedlings with or without drought stress. Among the 822 ESTs annotated, a fragment encoding an AP2 domain-containing protein was identified and named as CkDREB1. In this paper, we focus on the expression pattern of *CkDREB1*, and its function in tolerance to drought, cold, and salinity was also characterized.

2 Materials and methods

Plant materials and growth conditions – *C. korshinskii* and *A. thaliana* which is the wild-type and in the Columbia ecotype (Col-0) background were used in this study. Before sowing, the seeds were sterilized. Seeds of *C. korshinskii* from Erdos Forestry Bureau, Inner Mongolia, China, were sterilized in 75% EtOH solution for 10 min and then soaked in 6% NaClO for 30 min. Seeds of *A. thaliana* were sterilized in 70% EtOH for 10 min and then were with 100% EtOH containing 0.05% Tween-20 for another 10 min.

After sterilization, the seeds of *C. korshinskii* were washed with sterile water for 5 ~ 6 times, and then excess water was blotted away by sterilized filter paper. The seeds were first sown in petri dishes with Murashige and Skoog (MS) agar medium (0.7% agar, 3% sucrose, pH 5.8) at 4 °C for 3 days and then transferred into a growth chamber at 25 °C under 16-h light/8-h dark cycle for another 2 weeks. *C. korshinskii* was also grown in the rich soil mixed with vermiculite (1:1 (v/v)). The sterilized seeds of *C. korshinskii* were sown in the pots, and then the top of seeds was covered with about 0.5 cm soil. The pots were covered with a plastic lid to retain moisture and then placed in a growth chamber at 22 °C with a 12-h daily photoperiod.

Similarly, seeds of *A. thaliana* were sown in petri dishes with half-strength MS agar medium (0.7% agar, 3% sucrose, pH 5.7) and incubated at 4 °C for 3 days before being transferred to 22 °C under 14-h light cycle. At day 10 after growing in petri dishes, the seedlings were transferred to pots containing soil mixed with vermiculite (1:1 (v/v)) and were grown in a growth chamber at 22 °C for a 12-h daily photoperiod.

Plasmid construction and *Arabidopsis* transformation – For overexpression of *CkDREB1*, the genomic DNA extracted from *C. korshinskii* seedlings was used as template and the *CkDREB1* gene fragment was PCR amplified with the primers 5′-GCGGTACCTTAACACATGAACAACCAC-3′ and 5′-GTGGATCCCTCTCCATTCTAAAATCCA-3′.

The resulting PCR product was subcloned into *Kpn* I and *Bam*H I sites of the binary vector pCHF3 (Borevitz et al. 2000) under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The constructs were introduced into *Agrobacterium tumefaciens* (Smith & Townsend, 1907) (strain GV3101) by electroporation and were then used to transform *Arabidopsis* wild-type plants by the floral dipping method (Clough and Bent 1998). Transgenic lines used in this study were homozygous plants in T4 generation with a single copy insertion unless indicated otherwise.

RNA isolation and Northern blotting – Total RNA was extracted from 12-day-old seedlings with or without stress treatment with Trizol regent, and total RNA samples (10 μg) were used for Northern blotting. Gene-specific probes were labeled with DIG (digoxigenin; 11636090910, Roche). The

primers used for Northern blotting are listed in Supplementary Table S1.

Quantitative real-time PCR analysis – Total RNA, extracted from various samples, was treated with RNase-free DNase (Ambion). Total RNA (0.5 μg) underwent reverse transcription into cDNA with the M-MLV reverse transcriptase kit (TaKaRa) using oligo (dT) 18 primers (TaKaRa) according to the manufacturer's instructions. The cDNA was amplified using SYBR Premix Ex Taq (TaKaRa), with a Roche Light Cycler 480 Real-Time PCR system. The thermal cycling program was 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 15 s. The relative expression level of each transcript was calculated after normalization to *EF1α* (*At5G60390*). The comparative $2^{-\Delta\Delta CT}$ method was used to evaluate the relative quantities of each amplified product in the samples. For all the quantitative real-time PCR (qRT-PCR) analysis, three technical replicates were performed for each experiment, and at least three independent repetitions of the biological experiments were performed. The primers used for qRT-PCR are listed in Supplementary Table S1.

Drought and freezing tolerance test – Drought tolerance test was performed as described before (Zou et al. 2010). Plants were grown in soil for 4 weeks with sufficient watering. Each pot had four plants, and for each genotype at least six pots of plants were grown. The plants were then subjected to drought treatment by halting irrigation. Plants under a normal watering regime were used as control. To avoid the "position effect," all pots were placed under the same conditions, and the position of each pot was changed randomly every other day. After 12 days without watering, wild-type plants exhibited lethal effects of dehydration, watering was resumed, and the plants were allowed to grow for an additional 3 days. Pictures were taken at different time points after drought treatment or 3 days after re-watering, and the survival rate was calculated. The experiments were repeated three times with similar results.

For water loss measurement, rosette leaves were detached from 4-week-old plants, weighed immediately on a piece of weighing paper, and then placed on the laboratory bench (the aerial relative humidity was about 50% and the temperature was about 25 °C) for 5 h. The weight loss of the leaves was measured at different time points. The water loss rate was calculated on the basis of the initial fresh weight of the leaves (Ren et al. 2010).

The freezing tolerance assay was performed as described before (Shi et al. 2012), with some modifications. Briefly, 2-week-old seedlings grown at 22 °C on 0.8% agar plates were treated with or without cold acclimation at 4 °C for 6 days and then were placed in a freezing chamber set to − 20 °C for 1.5 h. After the freezing treatment, the plants were transferred to fresh half-strength MS agar medium at 22 °C for 48 h. The phenotype of the seedlings was observed 48 h after the transfer.

Another method used to test the ability of the cold resistance was like the following: Two-week-old plants were treated at − 11 °C for 2 h, then were transferred to the incubator, and cultured in dark at 4 °C for 12 h. Finally, the cold-treated plants were recovered for 72 h in a growth chamber at 22 °C under a 16-h daily light period.

Subcellular localization – For subcellular localization, the coding region of *CkDREB1* cDNA was amplified with the forward primer: CATGCCATGGACATGAACAACCAC TCTTTCTATCC and the reverse primer: CGGACTAGT AATAGAAAAACTCCACAGTGACACA and the fragment was ligated into the pCambia 1302 vector to obtain the *CkDREB1* N-terminal fused green fluorescent protein (GFP) construct, *35S::GFP-CkDREB1*. *Arabidopsis* leaf protoplasts were prepared and transfected as described before (Yoo et al. 2007). Nuclear localization was observed under a fluorescence microscope (Zeiss Axio imager A1).

Statistical analysis – The two-sample Students *t* test was carried out to analyze the statistical significance of the expression level of genes, ** indicates a statistically significant difference at *P* values lower than 0.01 compared with the WT; * indicates a significant difference at *P* values lower than 0.05 compared with the WT.

3 Results

***CkDREB1* was induced by drought, cold and ABA treatment –** Full-length cDNA was amplified by rapid amplification of cDNA end technique. It contains a 615 bp open reading frame, a 128 bp 5′-untranslated region, and a 279 bp 3′-untranslated region with a poly (A) tail composed of 12. The deduced polypeptide is composed by 204 amino acids with a calculated molecular mass of 23.15 kDa. The sequence of CkDREB exhibited 55% identities to the CBF1 from *Arabidopsis*. Phylogenetic analysis showed that the protein was grouped with the DREB A-1 subfamily (Supplementary Fig. S1), and its closest relative was from *Lycopersicon esculentum* Mill. (LeCBF1, AY034473). This gene was designated as *CkDREB1*.

To further confirm whether it is really involved in drought stress or not, a probe was synthesized using the isolated sequence and the transcript level was monitored in dehydration treated *C. korshinskii* seedling samples. The gene

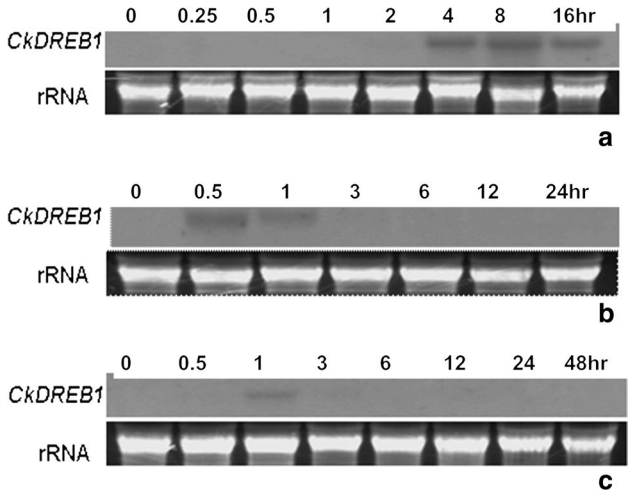

Fig. 1 Northern blot analyses of the *CkDREB1* transcript induced by various abiotic stresses. **a** The expression of *CkDREB1* after 0 °C treatment. **b** The expression of *CkDREB1* under drought treatment. **c** The expression of *CkDREB1* under 200 μM ABA treatment

has a very low basal level expression and was even undetectable by Northern blot (Fig. 1a–c). However, it was indeed induced 0.5 h after dehydration and gradually dropped thereafter (Fig. 1a). In addition, it was also induced by treatments under 0 °C or with 200 μM ABA (Fig. 1b, c).

CkDREB1 localized into nuclei – To examine the subcellular localization, the fragments of *CkDREB1* were fused to the *GFP* gene and then transferred into *Arabidopsis* leaf protoplasts. In the control, signal of the GFP protein was distributed throughout the cell, as expected (Fig. 2b, upper panel). However, imaging of GFP fluorescence revealed that the *GFP-CkDREB1* fusion protein was exclusively localized in the nuclei (Fig. 2a, upper panel), which is consistent with its role as a transcription factor (Zhang et al. 2010).

Overexpression of *CkDREB1* in *Arabidopsis* conferred drought tolerance – To further reveal the role of *CkDREB1* in responding to abiotic stress, transgenic *Arabidopsis* expressing the *CkDREB1* under the control of the *Cauliflower mosaic virus 35S* promoter was generated. Two overexpression (*OE*) lines, *OE18* and *OE20* with different levels of *CkDREB1* expression, were used for further analyses unless stated otherwise (Supplementary Fig. S2).

Dwarfism and stunted growth were frequently observed due to the overexpression of *DREBs* in other transgenic plants (Liu et al. 1998; Dubouzet et al. 2003a), but in our study, there were no obvious morphological differences between wild-type and the OE lines (Figs. 3a, 4b). Then the phenotype between the two genotypes was compared under drought stress treatment. Most wild-type plants were wilt after drought treatment, whereas the OE lines appeared relatively healthy (Fig. 3a). After re-watering for 3 days, 85.0% and 65.0% of the two OE lines were able to resume their growth and survived, respectively, whereas only 45.0% of the wild-type plants survived (Fig. 3b). The assays of water loss from detached leaves showed that the wild-type plants lost water much more quickly than the OE plants (Fig. 3c). After 5 h of dehydration, the wild-type leaves lost about 55% water, but the leaves from two OE

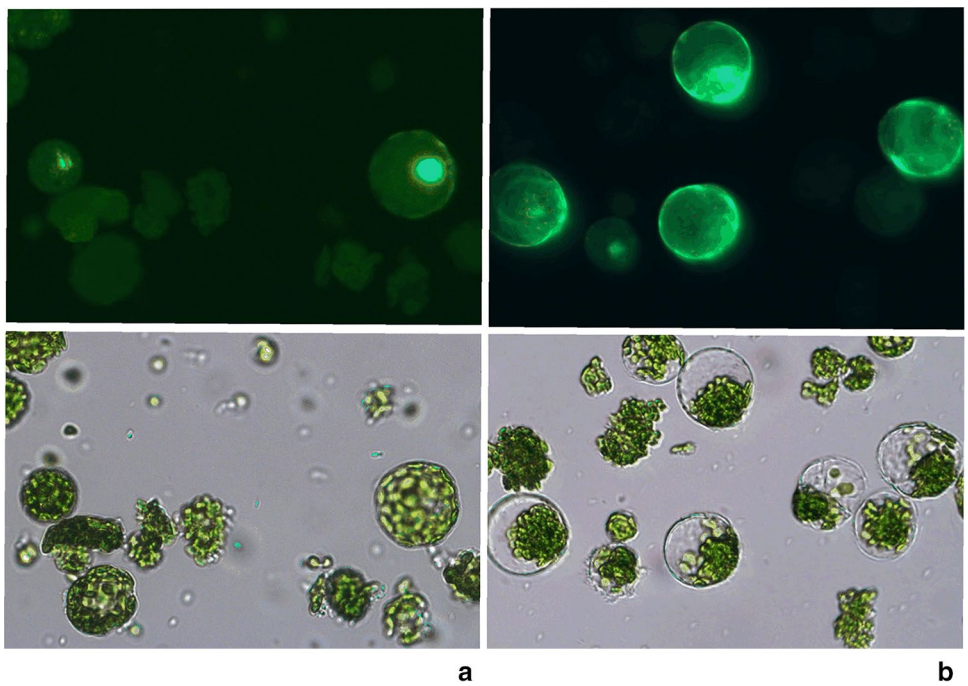

Fig. 2 Subcellular localization of CkDREB1. **a** Plasmids containing 35S::GFP-CkDREB were introduced into *Arabidopsis* leaf protoplasts. **b** Plasmids containing 35S::GFP were introduced into *Arabidopsis* leaf protoplasts. Nuclear localization was observed under a fluorescence microscope (Zeiss Axio imager A1)

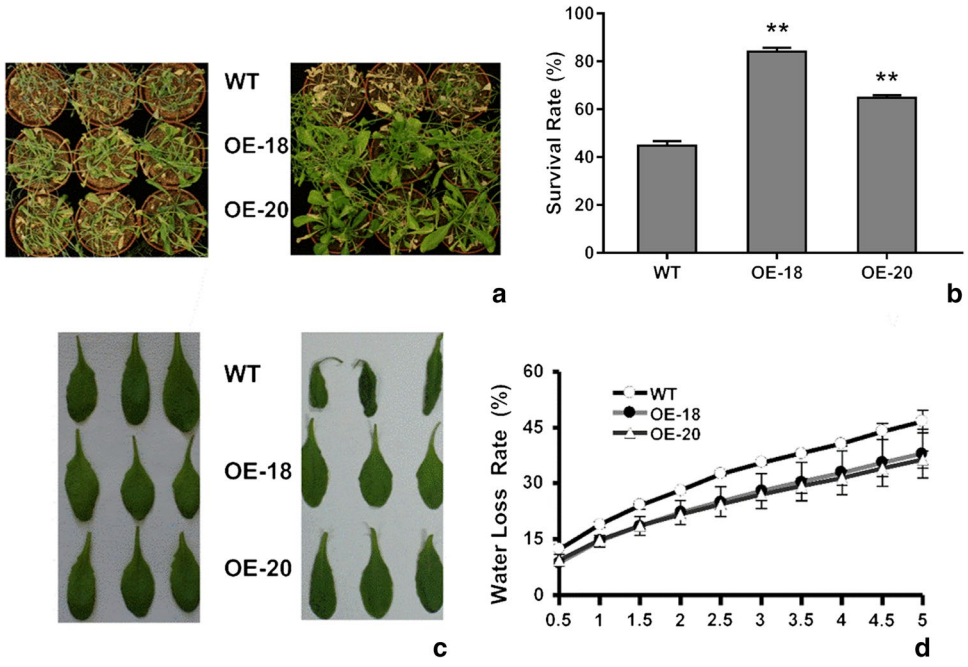

Fig. 3 The phenotype of *CkDREB1* overexpression *Arabidopsis* and WT under drought and cold stresses. **a** Four-week-old wild-type (WT), *CkDREB1* overexpression (OE) plants were exposed to drought stress by halting watering for 12 days, followed by re-watering. The photographs were taken immediately after drought treatment or 2 days after re-watering. **b** The survival rate after re-watering. **c** Leafs of four-week-old WT, OE plants were dehydrated at RT for 5 h. (**d**) The water loss rate of the detached leaves of different genotypes. Water loss is expressed as a percentage of the initial fresh weight. The experiments were repeated three times with similar results. ** Indicates $P < 0.01$

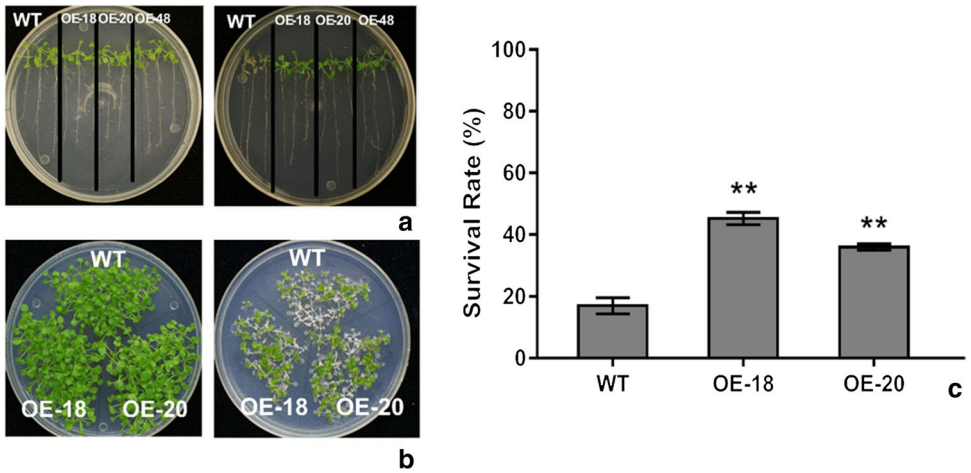

Fig. 4 Cold tolerance of the *CkDREB1* overexpression *Arabidopsis* seedlings was enhanced. **a** Recovery growth of transgenic and wild-type *Arabidopsis*. Both two-week-old WT and OE plants were acclimated at 4 °C for 6 days, then were frozen at − 20 °C for 1.5 h, and recovered at 22 °C for 48 h. **b** Two-week-old plants were treated at − 11 °C for 2 h and then transferred to 4 °C dark incubator for 12 h. Finally, all plants were recovered in a growth chamber at 22 °C in a 16-h daily light period. **c** The survival rate of transgenic and wild-type *Arabidopsis* after cold treatment. ** Indicates $P < 0.01$

lines lost about 41% and 42% water, respectively (Fig. 3d). The results confirmed that the transgenic plants have the better leaf water-holding capacity. These data suggest that CkDREB1 functions as a positive regulator in plant responding to drought stress.

Overexpression of *CkDREB1* in *Arabidopsis* confers cold tolerance – To check whether CkDREB1 is involved in cold tolerance, both wild-type and the OE lines were treated by freezing at − 20 °C for 1.5 h. After the freezing treatment, the plants were transferred to fresh half-strength MS agar medium. Two days later, the wild-type plants displayed less tolerance to freezing than the wild type (Fig. 4a). In addition, two-week-old plants were treated with cold stress (− 11 °C) for 2 h in a plate, and the survival rate of OE lines is higher than that of WT (Fig. 4b, c). These results also showed that the resistance of OE lines to cold is stronger than that of wild-type.

***CkDREB1* overexpression enhanced drought- and cold-responsive genes expression –** To identify the possible molecular mechanisms underlying the function of CkDREB1 in drought and cold response, we used qRT-PCR analysis to examine the expression level of several marker genes involved in drought and cold stress, including RD (responsive to dehydration) family members, such as *RD29A* and *RD29B*; *KIN* genes, such as *KIN1* and *KIN2*, as well as COR (cold-regulated) family members, *COR15A* and *COR47*. The transcript of the examined genes was constitutively higher in the OE lines without any treatment; after dehydration, it increased significantly in the OE lines compared with that in the wild type (Fig. 5a). After cold treatment, the examined genes increased significantly in the OE lines compared with the wild type also (Fig. 5b).

4 Discussion

DREB proteins are important transcription factors in plant stress responses and signal transduction. The DREB proteins contain an ERF/AP2 DNA-binding domain. The ERF/AP2 domain is quite conserved, and the transcription factor(s) containing it are widely found in many plants (Agarwal et al. 2006).

A novel DREB gene, designated *CkDREB1*, was isolated from the desert-grown bush, *C. korshinskii* and demonstrated that it is a functional gene by overexpression assay in *Arabidopsis*. Based on multiple sequence alignment and phylogenetic characterization, like the known AtDREB1A, OsDREB1A, BnCBP, and LeCBF1, it was classified into the A-1 group of DREB transcription factors, while in the previous study, another CkDREB reported by Wang et al. belongs to the A-2 group (Wang et al. 2010).

Members of the DREB transcription factors from different subfamilies play different roles in responding to various abiotic stresses. The A-1 group of DREBs are induced by

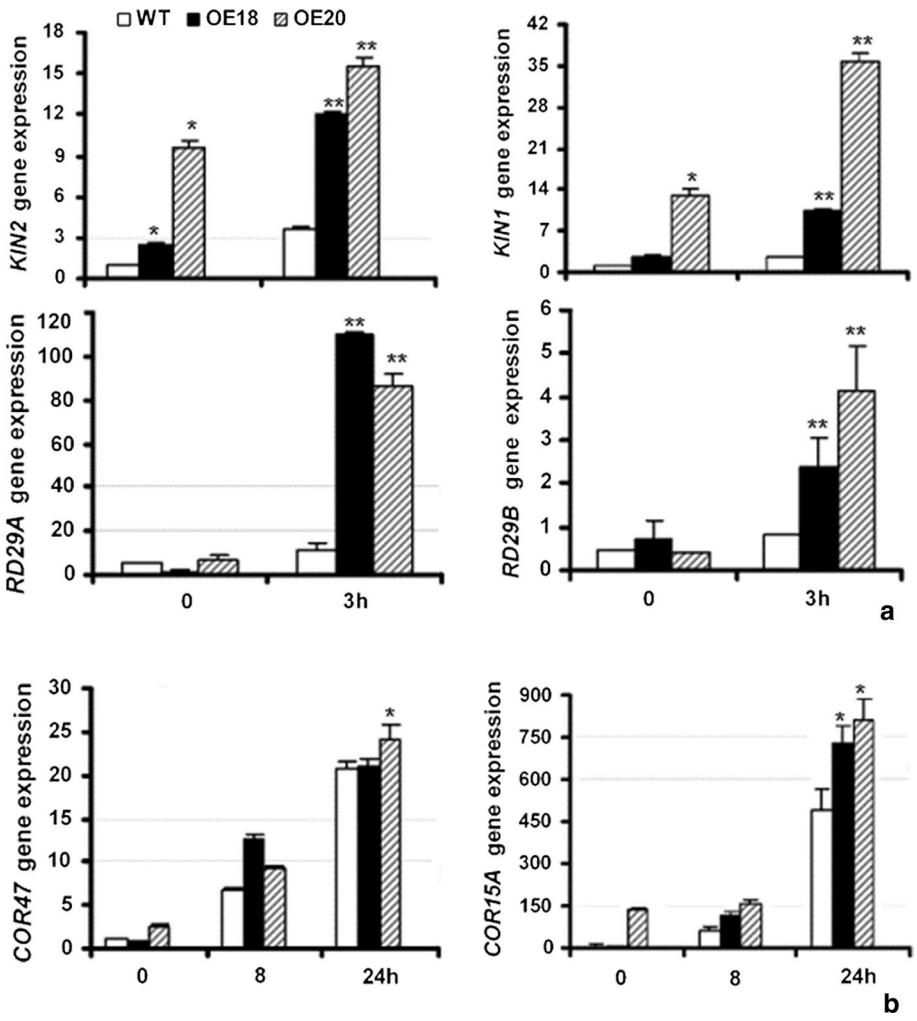

Fig. 5 The transcript level of several drought or cold-responsive genes in the *CkDREB1* overexpression lines was altered after drought or cold treatment. **a** Two-week-old WT and OE plants were dehydrated for the indicated times, and the mRNA levels of *KIN1*, *KIN2*, *RD29A*, and *RD29B* were detected by qRT-PCR. **b** Two-week-old WT and OE plants were treated at 4 °C at the indicated time points, and the mRNA levels of *COR15A* and *COR47* were detected by qRT-PCR. ** Indicates $P < 0.01$, and * Indicates $P < 0.05$

low temperature and are not induced by drought and high salt (Shinwari et al. 1998), and the A-2 group of DREBs are induced by high salt and drought, but not by cold treatment (Nakashima et al. 2000; Dubouzet et al. 2003b). The AP2 domain of CkDREB1 contains two conserved sequences, "PKK/RPAGRXKFXETRHP" and "DSAWR"; both of them are called tag sequences of DREB1 and are associated with low temperature stress (Gilmour et al. 2000). To investigate the response mechanisms to stress conditions, the expression level of *CkDREB1* was monitored under different abiotic stresses. The results showed that *CkDREB1* expression was up-regulated in response to low temperature and drought (Fig. 1), consistent with other A-1 groups of *DREBs*, indicating that it may function under cold stress like the DREB members of other species. However, recent studies have shown that some A-1 group of DREBs are also induced by ABA and cold, indicating that there is cross-talk between different groups (Xu et al. 2008; Jinhuan et al. 2009). For example, CkDREB is not only induced by salt, drought, and ABA, but also induced by cold, although it belongs to A-2 group (Wang et al. 2010).

Overexpression *DREBs* can improve various tolerance of plant, such as drought, salt, cold, and heat (Khedr et al. 2011; Lata and Prasad 2011; Navarro et al. 2011; Zhou et al. 2012). In order to study the function of CkDREB1, we generated *CkDREB1* overexpression lines in *Arabidopsis* and found that overexpression of *CkDREB1* enhanced drought and cold tolerance (Figs. 4, 5). This supports the previous findings that members of DREB1 family act as positive regulators in abiotic stress tolerance (Agarwal et al. 2006). For example, transgenic tomato plants overexpressing *CBF1/DREB1B* from *Arabidopsis* were tolerant to low temperature and drought (Hsieh et al. 2002). Overexpression of *OsDREB1A* in *Arabidopsis* and rice has been shown to increase tolerance to low temperature, high salinity, and drought of the transgenic plants (Dubouzet et al. 2003a; Ito et al. 2006).

A cDNA microarray analysis of transgenic *Arabidopsis* plants overexpressing *AtDREB1A* revealed that 6 stress-related genes, such as *RD29A*, KIN*1*, *COR6.6*/*KIN2*, *COR15a*, *COR47*/*RD17*, and *ERD10*, expressed twofold higher than in the wild-type (Liu et al. 1998). A similar study on transgenic *Arabidopsis* overexpressing *CBF* showed induction of *cor* expression and an increase in freezing tolerance (Agarwal et al. 2006). In our study, the transcript of *RD29A*, *RD29B*, *KIN1*, *KIN2*, *COR15A*, and *COR47* was constitutively higher in the OE lines than WT (Fig. 5a, b). The activation and accumulation of these genes improve the tolerance of transgenic *Arabidopsis* to drought and cold.

Many studies have shown that overexpression *DREBs* can improve tolerance of salt (Xu et al. 2009; Bouaziz et al. 2012) and heat of transgenic plants. In our study, the resistance of transgenic plants to salt and heat was also detected, but there was no significant difference between WT and transgenic lines (Fig. S3, 4). The relative root length of WT and *CkDREB1* overexpressing lines under NaCl and ABA treatment was also detected, and there was no difference between them either (Fig. S5). These results indicate that CkDREB1 has no function in response to salt, heat, and ABA in *C. korshinskii*.

In transgenic tobacco, overexpressing *CkDREB* improves salt and osmotic tolerance (Wang et al. 2011). But our transgenic plants analysis revealed that overexpression of *CkDREB1* in *Arabidopsis* significantly improved their tolerance to water deficit and cold stresses, suggesting that CkDREBs might participate in the stress response pathway in different manners.

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Author contributions RW and XL conceived of the research, and RW designed the study and wrote the manuscript. ZZ, QY, and CZ conducted the most of the experiments. LW performed all of the plasmid construction. RY completed the plant transformation and provide plant materials. GL did bioinformatics analysis.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Abu-Romman S (2016) Genotypic response to heat stress in durum wheat and the expression of small HSP genes. Rend Lincei-Sci Fis 27:261–267. https://doi.org/10.1007/s12210-015-0471-9

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:1263–1274. https://doi.org/10.1007/s00299-006-0204-8

Baker SS, Wilhelm KS, Thomashow MF (1994) The 5′-region of *Arabidopsis thaliana* cor15a has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol Biol 24:701–713

Bhardwaj PK, Ahuja PS, Kumar S (2010) Characterization of gene expression of QM from *Caragana jubata*, a plant species that grows under extreme cold. Mol Biol Rep 37:1003–1010. https://doi.org/10.1007/s11033-009-9791-0

Bhardwaj PK, Kaur J, Sobti RC, Ahuja PS, Kumar S (2011) Lipoxygenase in *Caragana jubata* responds to low temperature, abscisic acid, methyl jasmonate and salicylic acid. Gene 483:49–53. https://doi.org/10.1016/j.gene.2011.05.014

Borevitz JO, Xia Y, Blount J, Dixon RA, Lamb C (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12:2383–2394

Bouaziz D et al (2012) Ectopic expression of dehydration responsive element binding proteins (StDREB2) confers higher tolerance to

salt stress in potato. Plant Physiol Biochem 60:98–108. https://doi.org/10.1016/j.plaphy.2012.07.029
Bowman JL, Smyth DR, Meyerowitz EM (1989) Genes directing flower development in Arabidopsis. Plant Cell 1:37–52. https://doi.org/10.1105/tpc.1.1.37
Buttner M, Singh KB (1997) *Arabidopsis thaliana* ethylene-responsive element binding protein (AtEBP), an ethylene-inducible, GCC box DNA-binding protein interacts with an ocs element binding protein. Proc Natl Acad Sci USA 94:5961–5966
Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743
Drews GN, Bowman JL, Meyerowitz EM (1991) Negative regulation of the Arabidopsis homeotic gene AGAMOUS by the APETALA2 product. Cell 65:991–1002
Dubouzet JG et al (2003a) OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J 33:751–763. https://doi.org/10.1046/j.1365-313x.2003.01661.x
Dubouzet JG et al (2003b) OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J 33:751–763
Gilmour SJ, Sebolt AM, Salazar MP, Everard JD, Thomashow MF (2000) Overexpression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol 124:1854–1865
Hsieh TH, Lee JT, Yang PT, Chiu LH, Charng YY, Wang YC, Chan MT (2002) Heterology expression of the Arabidopsis C-repeat/dehydration response element binding factor 1 gene confers elevated tolerance to chilling and oxidative stresses in transgenic tomato. Plant Physiol 129:1086–1094. https://doi.org/10.1104/pp.003442
Ito Y et al (2006) Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol 47:141–153. https://doi.org/10.1093/pcp/pci230
Jiang C, Iu B, Singh J (1996) Requirement of a CCGAC cis-acting element for cold induction of the BN115 gene from winter Brassica napus. Plant Mol Biol 30:679–684
Jinhuan C, Xinli X, Weilun Y (2009) Expression profiling and functional characterization of a DREB2-type gene from *Populus euphratica*. Biochem Biophys Res Commun 378:483–487
Jofuku KD, den Boer BG, Van Montagu M, Okamuro JK (1994) Control of Arabidopsis flower and seed development by the homeotic gene APETALA2. Plant Cell 6:1211–1225
Jofuku KD, Omidyar PK, Gee Z, Okamuro JK (2005) Control of seed mass and seed yield by the floral homeotic gene APETALA2. Proc Natl Acad Sci USA 102:3117–3122. https://doi.org/10.1073/pnas.0409893102
Khedr AHA, Serag MS, Nemat-Alla MM, Abo-Elnaga AZ, Nada RM, Quick WP, Abogadallah GM (2011) A DREB gene from the xero-halophyte *Atriplex halimus* is induced by osmotic but not ionic stress and shows distinct differences from glycophytic homologues. Plant Cell, Tissue Organ Cult 106:191–206. https://doi.org/10.1007/s11240-010-9906-2
Klucher KM, Chow H, Reiser L, Fischer RL (1996) The AINTEGUMENTA gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene APETALA2. Plant Cell 8:137–153. https://doi.org/10.1105/tpc.8.2.137
Lata C, Prasad M (2011) Role of DREBs in regulation of abiotic stress responses in plants. J Exp Bot 62:4731–4748. https://doi.org/10.1093/jxb/err210
Lee JY, Baum SF, Alvarez J, Patel A, Chitwood DH, Bowman JL (2005) Activation of CRABS CLAW in the nectaries and carpels of Arabidopsis. Plant Cell 17:25–36. https://doi.org/10.1105/tpc.104.026666
Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10:1391–1406
Moose SP, Sisco PH (1996) Glossy15, an APETALA2-like gene from maize that regulates leaf epidermal cell identity. Genes Dev 10:3018–3027
Nakashima K, Shinwari ZK, Sakuma Y, Seki M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K (2000) Organization and expression of two Arabidopsis DREB2 genes encoding DRE-binding proteins involved in dehydration- and high-salinity-responsive gene expression. Plant Mol Biol 42:657–665
Navarro M, Ayax C, Martinez Y, Laur J, El Kayal W, Marque C, Teulieres C (2011) Two EguCBF1 genes overexpressed in Eucalyptus display a different impact on stress tolerance and plant development. Plant Biotechnol J 9:50–63. https://doi.org/10.1111/j.1467-7652.2010.00530.x
Ohme-Takagi M, Shinshi H (1995) Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. Plant Cell 7:173–182. https://doi.org/10.1105/tpc.7.2.173
Okamuro JK, Caster B, Villarroel R, Van Montagu M, Jofuku KD (1997) The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. Proc Natl Acad Sci USA 94:7076–7081
Perveen S, Al-Musayeib N, Malik A, Tareen RB (2014) Brachysides A and B, new lignan glucosides from Caragana brachyantha. J Chem Res. https://doi.org/10.3184/174751914x13863543426727
Ren XZ et al (2010) ABO3, a WRKY transcription factor, mediates plant responses to abscisic acid and drought tolerance in Arabidopsis. Plant J 63:417–429. https://doi.org/10.1111/j.1365-313x.2010.04248.x
Shi Y, Tian S, Hou L, Huang X, Zhang X, Guo H, Yang S (2012) Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and type-A ARR genes in Arabidopsis. Plant Cell 24:2578–2595. https://doi.org/10.1105/tpc.112.098640
Shinwari ZK, Nakashima K, Miura S, Kasuga M, Seki M, Yamaguchishinozaki K, Shinozaki K (1998) An Arabidopsis gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. Biochem Biophys Res Commun 250:161
Siminovitch D, Cloutier Y (1983) Drought and freezing tolerance and adaptation in plants: some evidence of near equivalences. Cryobiology 20:487–503
Stockinger EJ, Gilmour SJ, Thomashow MF (1997) *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc Natl Acad Sci USA 94:1035–1040
Wang Z, Gao HW, Wu YQ, Han JG (2007) Genetic diversity and population structure of *Caragana korshinskii* revealed by AFLP. Crop Sci 47:1737–1743. https://doi.org/10.2135/cropsci2006.09.0562
Wang X, Wang Z, Dong J, Wang M, Gao H (2009) Cloning of a 9-cis-epoxycarotenoid dioxygenase gene and the responses of *Caragana korshinskii* to a variety of abiotic stresses. Genes Genet Syst 84:397–405
Wang XM, Dong J, Liu Y, Gao HW (2010) A novel dehydration-responsive element-binding protein from *Caragana korshinskii* is involved in the response to multiple abiotic stresses and enhances stress tolerance in transgenic tobacco. Plant Mol Biol Rep 28:664–675. https://doi.org/10.1007/s11105-010-0196-y

Wang XM, Chen XF, Liu Y, Gao HW, Wang Z, Sun GZ (2011) CkDREB gene in *Caragana korshinskii* is involved in the regulation of stress response to multiple abiotic stresses as an AP2/EREBP transcription factor. Mol Biol Rep 38:2801–2811. https://doi.org/10.1007/s11033-010-0425-3

Wilson K, Long D, Swinburne J, Coupland G (1996) A dissociation insertion causes a semidominant mutation that increases expression of TINY, an Arabidopsis gene related to APETALA2. Plant Cell 8:659–671. https://doi.org/10.1105/tpc.8.4.659

Wu YP, Hu XW, Wang YR (2009) Growth, water relations, and stomatal development of *Caragana korshinskii* Kom. and *Zygophyllum xanthoxylum* (Bunge) Maxim. seedlings in response to water deficits. N Z J Agric Res 52:185–193. https://doi.org/10.1080/00288230909510503

Xu ZS, Ni ZY, Liu L, Nie LN, Li LC, Chen M, Ma YZ (2008) Characterization of the TaAIDFa gene encoding a CRT/DRE-binding factor responsive to drought, high-salt, and cold stress in wheat. Mol Genet Genom 280:497–508

Xu ZS et al (2009) Isolation and functional characterization of HvDREB1-a gene encoding a dehydration-responsive element binding protein in *Hordeum vulgare*. J Plant Res 122:121–130. https://doi.org/10.1007/s10265-008-0195-3

Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6:251–264. https://doi.org/10.1105/tpc.6.2.251

Yoo SH, Kim BY, Weon HY, Kwon SW, Go SJ, Stackebrandt E (2007) *Burkholderia soli* sp. nov., isolated from soil cultivated with Korean ginseng. Int J Syst Evol Microbiol 57:122–125. https://doi.org/10.1099/ijs.0.64471-0

Zandalinas SI, Mittler R, Balfagon D, Arbona V, Gomez-Cadenas A (2018) Plant adaptations to the combination of drought and high temperatures. Physiol Plant 162:2–12. https://doi.org/10.1111/ppl.12540

Zhang Y et al (2010) Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc Natl Acad Sci USA 107:18220–18225. https://doi.org/10.1073/pnas.1005225107

Zhou ML, Ma JT, Zhao YM, Wei YH, Tang YX, Wu YM (2012) Improvement of drought and salt tolerance in Arabidopsis and Lotus corniculatus by overexpression of a novel DREB transcription factor from *Populus euphratica*. Gene 506:10–17. https://doi.org/10.1016/j.gene.2012.06.089

Zou JJ, Wei FJ, Wang C, Wu JJ, Ratnasekera D, Liu WX, Wu WH (2010) Arabidopsis calcium-dependent protein kinase CPK10 functions in abscisic acid- and Ca^{2+}-mediated stomatal regulation in response to drought stress. Plant Physiol 154:1232–1243. https://doi.org/10.1104/pp.110.157545