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Overexpression of TaBADH increases the salt tolerance in Arabidopsis

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Abstract: Soil salinization is an important threat to wheat growth and production. Previous transcriptome analysis showed that the expression of the betaine aldehyde dehydrogenase (*BADH*) gene differed significantly between the cultivars with strong and weak salinity tolerance. Herein, the *BADH* gene from the wheat cultivar Dongnongdongmai 1 was cloned and transformed into the wild-type *Arabidopsis* to identify its function in salt tolerance. The root length was detected respectively at 0 mM, 50 mM, 100 mM, 150 mM and 20mM NaCl for 7d. The relative electrolytic leakage (REL), GB content and BADH activity were measured at 150 mM NaCl for 1d and 3 d . The result, the BADH activity and GB content of *TaBADH* -overexpressed transgenic

(*TaBADH*^{OE}) lines were significantly higher than the wild type. Salt stress analysis showed that the root length of *TaBADH*^{OE} lines 4, line 18, and line 19 were 0.44cm, 0.54cm and 0.35cm, respectively, which were significantly longer than 0.24cm of wild type in the media containing 150 mM NaCl for 7d. In addition, the REL of transgenic line 4, line 18 and line 19 respectively were 0.37, 0.33, 0.42, respectively, which significantly lower than 0.63 of wild type in media containing 150 mM NaCl for 3d. These results demonstrate that *TaBADH* significantly increased plant salt tolerance, indicating genetic transformation of *TaBADH* may be an effective and sustainable breeding method for increasing salt tolerance in wheat cultivars.

Key words: Wheat, NaCl stress, glycine betaine, *TaBADH*

Soil salinization is a worldwide problem, affecting plant growth, productivity, and agricultural economy by decreasing osmotic potential in soil solution, generating nutritional imbalance, and reducing photosynthetic efficiency (Ashraf 2009; Food and Agriculture Organization of the United Nations 2016; Daoud 2018). Plants utilize a number of protective mechanisms to maintain normal cellular metabolism under salt stress. Plant's adaptation to salt stress involves detoxification pathways (Rodrigues et al. 2006), signal transduction (Flowers and Colmer 2008), ion homeostasis (Rajalakshmi and Parida 2012; Sreeshan et al. 2014; Khan et al. 2016), reactive oxygen species (ROS) scavengers (Himabindu 2016; Avinash and Tanna 2017), and accumulation of osmoprotectants (Mishra and Tanna 2017). Glycine betaine (GB) is osmoprotectant that accumulates in many species under various stresses (Fan et al. 2012; Wang et al. 2018). It can decrease the osmotic potential of plants, helping the plants to retain water and

protecting the cells from salt stress without interfering with other biochemical processes (Ashraf 2007; Wani et al. 2013; Wang et al. 2014). Exogenous application of GB confers tolerance to various abiotic stresses in plants and increases their yield under salt conditions (Chen 2008; Mäkelä et al. 1996). In vascular plants, GB is synthesized from choline through a two-step reaction: The choline is transformed to betaine aldehyde in the presence of choline monooxygenase, and betaine aldehyde is then oxidized to GB in a reaction catalyzed by betaine aldehyde dehydrogenase (BADH) (Yang et al. 2015). BADH locates in chloroplasts, and it is induced by salt (Sun 2017). To date, *BADH* has been cloned from several plants such as *Carthamus tinctorius* (Wang et al. 2014), *Suaeda corniculata* (Wang et al. 2016), *Sesuvium portulacastrum* (Yang et al. 2015), *Leymus chinensis* (Mitsuya et al. 2015), and *Ammopiptanthus nanus* (Yu et al. 2014). Studies have indicated that overexpression of *BADH* can increase GB content, Ca^{2+} influx, activate antioxidative enzymes, and reduce ROS production and electrical conductivity (Fan et al. 2012; Li et al. 2014; Tang et al. 2014; Yang 2015). Thus, cloning of genes from resistant varieties will help to improve salt tolerance of plants by genetic engineering.

Wheat is one of the most important crops in the world. Its growth is affected by salt stress, high temperature, and drought, all of which cause yield losses in wheat (Zhang et al. 2018). Breeding stress-tolerant wheat varieties will contribute to high and stable yield. Dongnongdongmai 1 (DM1) is a winter wheat variety that can survive the harsh winters of Heilongjiang Province, with an overwintering survival rate exceeding 70% (Liu et al. 2013; Xie et al. 2015). DM 1 is superior not only for its cold tolerance, but also its tolerance to salt and drought. Most of the recent research has been focused on cold tolerance mechanisms in DM1 (Zeng et al. 2011; Liu et al. 2013), and Sun et al. (2017) reported a higher expression of BADH in

DM1 than in JM22 under low temperature. Very few studies have examined salt tolerance in DM1. In the present study, we cloned *TaBADH* from DM1 and identified its function under salt stress. This study provides a candidate gene for genetic improvement of salt tolerance of wheat.

MATERIALS AND METHODS

Plant Materials and Treatment

Dongnongdongmai 1(DM1) was developed by Northeast Agricultural University in Harbin City, Heilongjiang Province, China. Although winter temperature in this region may drop as low as -30°C, the overwintering survival rate of DM1 exceeds 70%, reaching up to 90% in some years (Xie et al. 2015).

The experiment was conducted in Northeast Agricultural University. Seeds of DM1 were planted in plastic basins (30 × 40 cm) with holes in the bottom; 35 seeds were planted per basin. Seedlings were grown in an artificial climate chamber at 24°C/18°C (day/night) with 15 h photoperiod. When the seedlings reached the trefoil stage, the meristematic crowns (2-3 cm shoot part above the roots containing tillering nodes) were harvested. The crowns were flash-frozen in liquid nitrogen and stored at -80°C.

Selected BADH gene

The seeds of salt tolerant variety DM1 were planted in a growth chamber with 150μM m-2.s-1 light intensity, 16h-light(22°C)/8h-dark(22°C) photoperiod, and 60%-70% humidity. The 2cm seedlings were cultured with Hoagland nutrient treated with 200mM NaCl for 0 d, 1d and 3d.

Then seedling roots were harvested with three biological replicates for RNA-sequencing. *BADH* was screened from the differentially expressed genes after RNA-sequencing.

Total RNA Isolation and cDNA Synthesis

All the samples were ground into a fine powder in liquid nitrogen with a pestle and mortar. Total RNA was extracted using a Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA concentration was measured by a Nano Drop ultradifferential photometer (ThermoFisher, USA), and the quality of RNA was detected by 1% agarose gel electrophoresis to ensure that the sequencing samples were qualified (Chen et al., 2018). 1 µg total RNA was used for reverse transcription reaction using a RocketScript RT PreMix (Bioneer, Seoul, Korea).

Cloning and Bioinformatics Analysis of *TaBADH*

Traes_6AL_42AEB5299 coding sequence (CDS) was used as template to design forward and reverse primers (*NcoI*-*TaBADHFW*: 5'-CGAACGATAGCCATGGTCGCCACGGCGAAGATCC-3'; *SpeI*-*TaBADHRV*: 5'-GGTAGGATCCACTAGTAGCCGGAGCTTTGTACCATCCC-3'); the primers produced **amplicon of size 1509 bp**. Restriction sites *NcoI* and *SpeI* were introduced into the forward and reverse primer, respectively. The primers were synthesized by the Liuhe Beijing Huada Gene Company. cDNA produced by reverse transcription was used for PCR reaction with the following protocol: initial denaturation at 95°C for 30 s; 35 cycles each consisting of denaturation at 95°C for 15 s, annealing at 58°C for 15 s, and extension at 72°C for 2 min; and final extension at 72°C for 5 min; the samples were conserved at 4°C for 15 min. After sequencing, the obtained

sequences were translated into amino acid sequences using DNAMAN 8 (Lynnon Corp., Quebec, Canada). Amino acid sequences were aligned across conserved domains by DNAMAN 8. The amino acid sequences of *TaBADH* in wheat and other plants were used for phylogenetic analysis using neighbor-joining method in MEGA5.1 (Tamura et al. 2011).

Transformation and Verification of *TaBADH* Transgenic *Arabidopsis*

The PCR product was then cloned into the pCambia1305.1 vector, which contained a flag tag (Promega, Madison, WI, USA), by using an In-Fusion HD Cloning Kit (Takara Bio USA, Inc., Mountain View, CA, USA) as described by the manufacture's protocol. The sequenced positive overexpression vector was transformed into *Agrobacterium tumefaciens* strain GV3101 by using the electric-shock method. Six-week-old *Arabidopsis* wild type Columbia-0 plants were transformed by the *Agrobacterium* carrying the overexpression vector by using the floral dip method (Bechtold et al., 1993). The seeds harvested from the transgenic plants were surface-sterilized and screened for hygromycin resistance on 0.8% solid Murashige and Skoog (MS) medium containing 50 $\mu\text{g mL}^{-1}$ hygromycin for 12 days. Resistant T1 plants were transferred into soil and tested for presence of target gene by using western blot. The positive T1 plants were self-pollinated to produce T2 seeds. The T2 seeds were screened by hygromycin to eliminate the homozygous recessive plants before any further tests. T3 plants from each family were used for phenotype and physiological analyses.

Prokaryotic Expression, Purification, and Enzyme Activity Analysis of *TaBADH*

The full-length CDS of *TaBADH* (double enzyme digestion sites were *EcoRI* and *SalI*) was amplified from DM1 using Phanta high-fidelity DNA polymerase (Vazyme Biotech Co., Ltd.,

Nanjing, China). The PCR product was cloned into pCold-MBP expression vector by In-fusion HD Cloning Kit (TaKaRa, Tokyo, Japan) and transformed into TOP10 competent cells. The positive clones, which were confirmed by sequencing, were expanded and cultured to extract plasmids and then transformed into protein expression strain *Escherichia coli* Transetta. The *E. coli* cells were cultured in 3 mL liquid Luria-Bertani medium with ampicillin resistance at 37°C in a shaker and added isopropyl β -D-1-thiogalactopyranoside (IPTG) inducer. When the OD reached 0.6–0.8, shaker temperature was adjusted to 16°C and the cells were incubated for additional 7 h. *Escherichia coli* cells cultured in the liquid medium without IPTG and incubated under the same conditions were used as a control. Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) was used to detect induced proteins. As the liquid bacterial culture was expanded to 100 mL, protein expression was induced with IPTG. The culture was centrifuged at 4000 rpm for 10 min to collect the bacteria, and the column buffer (200 mM NaCl, 20 mM Tris-HCl, 1 mM PMSF, 1 mM DTT, pH 7.4) was used to resuspend the bacteria. An ultrasonic processor was used for 30 min to rupture the cells, which were then centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was incubated with amylose resin for 1 h at room temperature and then centrifuged at 2000 rpm for 1 min. The pellet was washed three times with column buffer and the supernatant was discarded. Protein was eluted with 200 μ L elution buffer (column buffer + 10 mM maltose), and the purified protein was used for detection of BADH enzyme activity.

Analysis of Salt Stress Tolerance in *TaBADH* Transgenic *Arabidopsis*

Salt tolerance phenotype of transgenic *Arabidopsis thaliana* during germination stage

TaBADH-positive transgenic plants of the T2 generation with 3:1 phenotypic segregation ratio were selected by screening for hygromycin resistance and used to obtain T3 generation; the

screening procedure was conducted as described by Zhang et al (2017). The T3 generation of the transgenic lines and wild type were planted in 1/2 MS medium containing different concentrations of NaCl (0, 50, 100, 150, and 200 mM) and kept for 3 d at 4°C in the dark. Thereafter, they were transferred to an artificial climate chamber and cultured at 22°C/18°C (day/night), under 16 h light /8 h dark photoperiod, and a light intensity of 2500 lux for 7 d. Root length from five plants of each transgenic line was measured during this period. For every line, we used five seedlings and three repetitions, and the data were analyzed using *t*-test.

Salt tolerance phenotype test of transgenic *Arabidopsis thaliana* during the growth period

The generated transgenic and wild-type plants were grown in 1/2 MS medium in the dark for 3 d. When the plants developed 3–5 leaves, they were transferred to the artificial climate chamber and grown at 22°C/18°C (day/night) with 16 h light /8 h dark photoperiod with light intensity of 2500 lx for 10 d. Every third day, the plants were treated with 150 mM NaCl, and growth was observed after 15 d of treatment.

Measurement of GB Content, BADH Activity, and Relative Electrolytic Leakage

Activity of the BADH enzyme was measured according to previously published procedures (Liu et al. 1997). Crown tissue (0.5 g) was homogenized in ice-cold 50 mmol L⁻¹ potassium phosphate buffer [2 mL; pH 7.0, 1 mmol L⁻¹ ethylenediaminetetraacetic acid, 1 mmol L⁻¹ D-isoascorbic acid, 2% (w/v) polyvinylpyrrolidone, and 0.05% (w/v) Triton™ X-100]. The homogenates were centrifuged at 12 000 × *g* for 15 min at 4°C, and the supernatants were collected and used in the enzyme assays. The BADH activity reaction mixture contained 100 mmol L⁻¹ potassium phosphate buffer (pH 8.0), 5 mmol L⁻¹ dithiothreitol, 0.5 mmol L⁻¹

nicotinamide adenine dinucleotide (NAD^+), and 0.5 mmol L^{-1} betaine aldehyde. Measurements of fluorescence were carried out with a fluorescence spectrometer (U3900; Hitachi, Japan; excitation 340 nm, emission 460 nm). Enzyme activity was expressed as the initial rate of the NAD^+ reduction per milligram total protein.

Glycine betaine content was estimated according to methods described by Moghaieb et al. (2004). Crown samples used for betaine determination were transferred to vials, subjected to methanol extraction, and stored in the dark at 4°C . The methanol extracts were passed through Dowex-1-H- and Dowex-50- H^+ columns (Dow Chemical Company, Midland, MI, USA). Betaine was eluted with $6 \text{ mmol L}^{-1} \text{NH}_4\text{OH}$ and then analyzed by high performance liquid chromatography (Agilent 1260 Infinity, Agilent Technology, Waldbronn, Germany) with an SB-C8 Analytical HPLC column. The standard material of betaine (>95%) was purchased from ChromaDex (Irvine, CA, USA).

Relative electrical conductivity (REC) was detected using a conductivity meter (DDS-11A, Shanghai, China; Li 2000) and calculated according to the formula $\text{REC} = R_{\text{fresh}}/R_{\text{kill}}$, where R_{fresh} is the electrical conductivity of the water in which the crown was immersed for 1 h, and R_{kill} is the electrical conductivity of the water after boiling the crown tissue for 15 min.

RESULTS

Selection of BADH Sequence

Common wheat is an allohexaploid plant (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) with three chromosome sets. Therefore, the targeted genes were located on different chromosomes and annotated BADH based on previous transcriptomes. According to the fragments per kilobase

of exon model per million mapped reads (FPKM) value, the gene named *Traes_6AL_42AEB5299* located on chromosome 6A was considered as a candidate gene for putative function in salt tolerance, therefore selected for cloning, expression analysis, and genetic transformation in *Arabidopsis*.

Bioinformatics Analysis of *TaBADH*

Based on the alignment of the conserved domain of the *TaBADH* amino acid sequence, *TaBADH* belongs to the aldehyde dehydrogenase family and contains a typical BADH structural domain (Specific hit: PLN02467, betaine aldehyde dehydrogenase). The gene also has a typical NAD binding site and enzymatic site (Fig. 2A).

The phylogenetic analysis of amino acid sequences of *TaBADH* in wheat and other plants resolved the sequence of *BADH* of DM1 grouped with those of *Aegilops tauschii*, *Triticum urartu*, *Hordeu chinensis*, *Zea mays*, and *Setaria italica*, but it was distant from *Sorghum bicolor*, *Oryza sativa*, *Suaeda glauca*, *Atriplex micrantha*, and *Spinacia oleracea* (Fig. 2B).

The amino acid sequence of *BADH* from DM1 was highly homologous with those of *Hordeum vulgare* (BAB62846.1; 98%), *Arabidopsis thaliana* (NP565094.1; 75%), *Leymus chinensis* (BAD86758.1; 96%), *Brachypodium distachyon* (XP 003574495.1; 93%), *Oryza sativa Japonica* (XP 015650889.1; 88%), *Zea mays* (NP_001157807.1; 87%), *Zoysia tenuifolia* (BAD34947.1; 86%), *Setaria italica* (XP_004973462.1; 73%), and *Brassica napus* (NP 001303116.1; 86%) (Fig. 2C).

Prokaryotic Expression, Purification, and Enzyme Activity of *TaBADH*

The SDS-PAGE results showed that the protein at the location around 100 kDa was induced, indicating that the recombinant proteins fused with MBP tag were induced by IPTG (MBP: 45.2 kDa; TaBADH: 55.6 kDa) (Fig. 3A). Recombinant protein was concentrated and it was the only protein present in the supernatant. The purified protein was quantified, and its enzyme activity detected (Fig. 3B). The protein content was determined using Bradford method from the protein standard curve. The standard curve equation was: $y = 0.0095x - 0.0048$, $R^2 = 0.9945$. The concentration of recombinant protein was $9.87 \mu\text{g} \cdot \mu\text{L}^{-1}$ ($\text{OD}_{549} = 0.374$) (Fig. 3C).

Enzyme activity of the purified protein was examined using GB aldehyde as a substrate and NAD^+ as coenzyme by recording the OD value every 2 min; the reaction liquid without GB aldehyde was used as a control. The OD_{340} value gradually increased with time until reaching the maximum of 0.21 at 20 min of the reaction, with a rapid increase observed between 12-16 min (Fig. 3D). This demonstrates that the purified protein catalyzed the reaction in *E. coli* and had the activity of BADH.

Detection of Transgenic *Arabidopsis thaliana*

The wild-type *Arabidopsis* Col-0 was genetically transformed by *Agrobacterium*-mediated dissemination of the expression vector. Seeds of the T1 generation were obtained by screening for hygromycin resistance on the 1/2 MS medium containing hygromycin and then transferred to a nutrition pot, where they were grown for about 20 d. Their leaves were sampled and subjected to western blot to detect the pCAMBIA1305.1 vector tagged with a flag at the C terminal. Anti-flag and anti-actin (1:3000 dilution, CWBIO, Beijing, China) were used as the primary antibodies. Goat Anti-Mouse IgG (1:3000 dilution, CWBIO, Beijing, China) was used as the second antibody.

Actin antibody was used as a reference. Seven transgenic lines from 19 lines were confirmed positive by western blot (lines 4, 5, 7, 11, 16, 18, and 19; Fig. 4) and lines 4, 18, 19 exhibited high protein levels and were used to obtain T2 and T3 generations. Finally, plant transgenic lines 4, 18, and 19 (Fig. 4) were selected for assessing phenotypic verification and measure of physiological indexes.

Salt stress Tolerance in TaBADH Transgenic Arabidopsis

The wild type and transgenic lines 4, 18, and 19 were sowed on 1/2 MS medium, which contained different NaCl concentration, and grown for 7 d. Growth and development of both the wild-type and transgenic lines were restrained. Germination of the wild type was especially inhibited in the medium supplemented with 200 mM NaCl; its germination rate did not exceed 8%, whereas germination rate of the transgenic lines was >80% (Fig. 5A).

The root lengths of the wild type, line 4, line 18, and line 19 were respectively 2.28 cm, 2.29 cm, 2.31 cm, and 2.33 cm when grown in culture media with 0 mM NaCl. The root lengths of all plants were reduced under salt stress. Compared with plants exposed to no salt stress, the root length of the wild type, line 4, line 18, and line 19 decreased respectively by: 69.4%, 40.0%, 37.8, and 40.4% in media containing 100 mM NaCl; 89.3%, 80.9%, 76.5%, and 85.1% in media supplemented with 150 mM NaCl; and 95.9%, 89.6%, 88.3%, and 91.5% in media with 200 mM NaCl (Fig. 5A).

Measure of the Physiological Index in Transgenic *Arabidopsis*

After 1 d and 3 d of treatment with 150 mM NaCl, the REC of plants increased 115.9% and 304.2% in the wild type, 74.4% and 184% in transgenic line 4, and 79.5% and 225.6% in

transgenic line 19, respectively. The lowest increase in REC, 52.5% and 147.5% at day 1 and 3, respectively, was measured in transgenic line 18 (Fig. 6A).

Concomitantly, the BADH enzyme activity after 1 d and 3 d of salt stress increased 75% and 101.3% in wild-type plants, 150.6% and 331.6% in transgenic line 4, 170.1% and 354.5% in line 18, and 144.1% and 336.4% in line 19, respectively. Thus, the highest increase in BADH enzyme activity under salt stress was detected in transgenic line 18 (Fig. 6B).

Compared with growth under no salt stress, the GB content of wild-type plants decreased 2.91% and 7.76% after 1 d and 3 d of salt stress treatment, respectively, but it increased 167.7% and 208.4% in transgenic line 4, 149.6% and 195.7% in line 18, and 168.8% and 217.6% in line 19 for the same time period. The greatest increase in GB content under salt stress conditions was observed in transgenic line 18 (Fig. 6C).

DISCUSSION

When exposed to stress, plants synthesize osmotic substances (Hagemann 2011; Chen 2012), which play important roles in cells. They maintain the low osmotic potential inside the cells, thereby preventing water loss and facilitating absorption of moisture from the surrounding environment by plant tissues. The osmotic substances also maintain the cell turgor pressure, thus stabilizing plant growth and shape. Finally, they stabilize the structure and function of intracellular macromolecules and biological membranes.

Glycine betaine has been confirmed to improve plant resistance (Ahmad 2013; Li 2014; Liu 2018). *BADH*, a key gene in betaine synthesis, was cloned from many species and verified to improve plant resistance to abiotic stress. A few studies have reported about *TaBADH* cloning, transformation, and its input to abiotic stress resistance in wheat. In the present study, *TaBADH*

was cloned from the resistant variety DM 1. The cloned *TaBADH* contained the typical *BADH* structural domain with NAD binding site and enzymatic site (Fig. 2A). Although *Arabidopsis* plants naturally cannot complete their life cycle when exposed to 100 mM NaCl (Munns and Tester 2008), but in our study, the seedlings of transgenic *Arabidopsis* plants transformed with *TaBADH* grew better than wild type when treated with 150 mM NaCl at the germination stage. At the germination stage, the root length of transgenic *Arabidopsis* plants was longer than that of the wild type when grown in 50 mM, 100 mM, and 150 mM NaCl medium (Fig. 5A). During the growing period, the growth of wide-type plants was visibly inhibited, and the leaves wilted, whereas the growth of transgenic lines was unaffected by salt stress (Fig. 5B). These results showed that *TaBADH* could dramatically improve salt tolerance of wild-type plants as their salt resistance threshold increased to 100 mM NaCl. Previous studies have reported that the expression of *BADH* increases the *BADH* activity and GB synthesis in transgenic plants (Fan 2012; Liu 2018). Consistent with those studies, the results presented herein confirmed increased GB content and *BADH* activity in transgenic lines under salt stress, while electrical conductivity, which reflects the injury of plants, was significantly lower than that of wild-type plants (Fig. 6).

BADH is involved in the early growth of *Arabidopsis* seedlings and salt stress mitigation, and therefore, the overexpression of ortholog *BADH* (*ALDH10A8* and *ALDH10A9*) in *Arabidopsis* delayed the growth of the seedlings early after germination and altered sugar (glucose and fructose) and amino acid contents in transgenic plants compared with wild-type plants (Missihoun 2015). The *AtALDH10A8* mutant reduced GABA accumulation in *Arabidopsis*, a hormone involved in plant response to various abiotic stresses, and was more sensitive to salinity compared with the wild type (Missihoun 2011; Zarei 2016). In our study, we overexpressed *TaBADH* in

Arabidopsis plants, and the resultant plants showed not only increased glycine betaine levels, but also significantly increased salt tolerance compared with the wild type. However, future studies should investigate whether the overexpression of *TaBADH* in *Arabidopsis* plants affects the native expression of *AtALDH10A8* and *ALDH10A9*.

CONCLUSION

In this study, we cloned *TaBADH* gene from a highly salt-tolerant wheat cultivar and overexpressed the gene in *Arabidopsis*. *TaBADH* was efficiently expressed in *Arabidopsis* plants as the resultant transgenic plants had a greater tolerance to salt stress than did the wild type. Therefore, overexpression of *TaBADH* may be a viable method for breeding of salt-tolerant wheat cultivars.

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Figure legends:

Fig. 1. Expression of betaine aldehyde dehydrogenase (*BADH*) on chromosomes under salt stress conditions. FPKM, fragments per kilobase of exon model per million mapped reads.

Fig. 2. Bioinformatics analysis of *TaBADH*. (A) Conserved domain analysis of *TaBADH*; (B) Phylogenetic tree based on amino acid sequences of betaine aldehyde dehydrogenase (BADH) from different plants. (C) Partial multiple alignment of the deduced amino acid sequence of *TaBADH* and BADH sequences from other plant species. Hv (*Hordeum vulgare*), At (*Arabidopsis thaliana*), Ta (*Triticum aestivum*), Lc (*Leymus chinensis*), Bd (*Brachypodium distachyon*), Os (*Oryza sativa*), Zm (*Zea mays*), Zt (*Zoysia tenuifolia*), Si (*Setaria italica*), Ps (*Pascopyrum smithii*), Fa (*Festuca arundinacea*), Bn (*Brassica napus*). Asterisk, colons, and periods indicate perfect (100%), high (75%), and low (50%) conservation of amino acids, respectively.

Fig. 3. SDS-PAGE of *TaBADH* prokaryotic expression and activity assay of betaine aldehyde dehydrogenase (BADH). (A) and (B): SDS-PAGE of *TaBADH* prokaryotic expression. (A) Line 1, marker; Line 2, before the induction by IPTG; Line 3, after the induction by IPTG. (B) Line 1, supernatant; Line 2, pellet; Line 3, marker; Line 4, elution buffer I; Line 5, elution buffer II. (C) Standard curve of protein quantification; (D) activity assay of BADH. Data present with standard error bars per group ($n=3$)

Fig. 4. Western blot detection of transgenic *Arabidopsis thaliana*. The numbers represent different

transformation plant lines.

Fig. 5. Phenotype analysis between (A) Col-0 and transgenic *Arabidopsis thaliana* lines at the germination stage and. (B) Root length of wild type and transgenic at different NaCl stress (C) Between wild-type and transgenic *Arabidopsis thaliana* lines at the development stage and 150 mM NaCl stress. 1-4 represent wild type, transgenic line 4, line 18, and line 19 of *Arabidopsis*, respectively, not subjected to salt stress for 15 d. 5-8 represent wild type, transgenic line 4, line 18, and line 19 of *Arabidopsis*, respectively, treated with 150 mM NaCl for 15 d.

Fig. 6. Comparison of physiological parameters between Col-0 and transgenic *Arabidopsis thaliana* lines.

Fig. 1. Expression of betaine aldehyde dehydrogenase (BADH) on chromosomes under salt stress conditions. FPKM, fragments per kilobase of exon model per million mapped reads.

785x380mm (96 x 96 DPI)

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Fig. 3. SDS-PAGE of TaBADH prokaryotic expression and activity assay of betaine aldehyde dehydrogenase (BADH). (A) and (B): SDS-PAGE of TaBADH prokaryotic expression. (A) Line 1, marker; Line 2, before the induction by IPTG; Line 3, after the induction by IPTG. (B) Line 1, supernatant; Line 2, pellet; Line 3, marker; Line 4, elution buffer I; Line 5, elution buffer II. (C) Standard curve of protein quantification; (D) activity assay of BADH. Data present with standard error bars per group (n=3)

172x122mm (300 x 300 DPI)

Fig. 4. Western blot detection of transgenic *Arabidopsis thaliana*. The numbers represent different transformation plant lines.

551x241mm (96 x 96 DPI)

Fig. 5. Phenotype analysis between (A) Col-0 and transgenic *Arabidopsis thaliana* lines at the germination stage and. (B) Root length of wild type and transgenic at different NaCl stress (C) Between wild-type and transgenic *Arabidopsis thaliana* lines at the development stage and 150 mM NaCl stress. 1-4 represent wild type, transgenic line 4, line 18, and line 19 of *Arabidopsis*, respectively, not subjected to salt stress for 15 d. 5-8 represent wild type, transgenic line 4, line 18, and line 19 of *Arabidopsis*, respectively, treated with 150 mM NaCl for 15 d.

209x243mm (300 x 300 DPI)

Fig. 6. Comparison of physiological parameters between Col-0 and transgenic *Arabidopsis thaliana* lines.

1000x1000mm (96 x 96 DPI)