RESEARCH ARTICLE

Biochemical and molecular study of glycinebetaine synthesis in *Salsola aucheri* under salt stress

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ABSTRACT
Salinity promotes the generation of reactive oxygen species (ROS) and oxidative damages of different cellular components. Thus, salt stress tolerance requires activation of antioxidative pathways to protect plant cells from damages. In this study, seedlings of *Salsola aucheri* were treated with 100, 200, 300, 400 and 500 mM of NaCl and consequently, the activities of some antioxidant enzymes were evaluated. Enhanced activities of catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) were determined by the increasing salinity up to 300 mM. Furthermore, accumulation of glycinebetaine (GB) as a common response to salt stress was assessed. Accordingly, the concentration of GB enhanced by the increasing concentration of NaCl up to 300 mM and decreased at NaCl concentrations of 400 and 500 mM. Likewise, assessing the activity of betaine aldehyde dehydrogenase (BADH) as a key enzyme in the biosynthesis of GB showed that BADH activity enhanced by the increasing salinity (up to 300 mM NaCl) and decreased at the higher concentrations of NaCl. Cloning and characterization of BADH cDNA from *Salsola aucheri* and the expression pattern of BADH transcript were also examined. An open reading frame of 797 bp encoded a protein which showed high homology to BADH enzymes in other plants. These results suggest that the salinity tolerance of *Salsola aucheri* might be closely associated with the increasing function of the antioxidative system to scavenge reactive oxygen species and with the accumulation of the osmoprotectant glycinebetaine.

Key words: *Salsola aucheri*, glycinebetaine, betaine aldehyde dehydrogenase, salinity

Introduction

Salinity is considered as one of the main abiotic factors limiting the growth and productivity of plants. Different plant species have developed diverse adaptation mechanisms in response to the osmotic and ionic stresses caused by salinity. The most widely distributed strategy of plant response to salt stress is the accumulation of compatible solutes, which involves osmotic adaptation, protects cells and allows growth and development (Mishra & Tanna, 2017).

Compatible solutes or osmoprotectants are usually of low molecular weight and highly soluble in water. The accumulation of these compounds is the fundamental mechanism to protect organisms against environmental stresses such as salinity, water stress and freezing. Compatible solutes are divided into four main groups: (1) sugars (e.g., fructose, sucrose and raffinose); (2) amino acids (e.g., proline and citrulline); (3) onium compounds, (e.g., glycine-betaine and 3-dimethylsulfoniopropionate); and (4) polyols and sugar alcohols (e.g., mannitol, glycerol, and sorbitol). The accumulation of these compounds under salt stress leads to salt tolerance of plant cells without interfering cell metabolism. However, plant species show different degrees of tolerance depending on the levels of accumulation of compatible solutes (Giri, 2011; Kalsoom et al., 2016).

The genes involved in the biosynthesis of different types of compatible solutes have been identified in different species. They play significant roles not only in the integrity of membranes and enzymes but also in the mediation of osmotic adjustment in plants grown under stress conditions (Jing et al., 2015).

N,N,N-trimethylglycine or glycinebetaine (GB) as a well-known compatible solute accumulates in cells to protect organisms against abiotic stresses through osmoregulation.

Glycinebetaine maintains the fluidity of the membranes and protects the cellular compartments of organisms under stress (Mitsuya et al., 2015). The main enzyme involved in the biosynthesis of GB is betaine aldehyde dehydrogenase (BADH), which has been identified in different plant species. (Wang et al., 2014).

The induction of oxidative stress due to the accumulation of reactive oxygen species (ROS) is a well-known effect of different environmental stresses, including salinity. Therefore, plants' capacity for scavenging of produced ROS by the antioxidant system is a fundamental characteristic of their tolerance to salinity. Plants have developed some mechanisms by using enzymatic and non-enzymatic components to prevent ROS damages. Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) are some of the key enzymes involved in the antioxidant responses of plants (Pallavi et al., 2012; Wang et al., 2016).

Salsola aucheri (Moq.) Bunge ex Iljin is a native plant to North Iran, Armenia, Turkmenia and neighboring areas (Akhani et al., 2007). In recent years, the distribution of the plant has broadened in the growing regions by the increasing soil salinity. Therefore, understanding the adaptation strategy of this species shed more light on the survival mechanisms of halophytes under salt stress conditions.

In this study, the effect of salinity on the activity of a number of antioxidant enzymes (SOD, POD, CAT) and BADH was studied. The changes in glycinebetaine (GB) content in treated seedlings were determined by spectrophotometer. In addition, the betain aldehyde dehydrogenase (BADH) gene encoding GB synthesis was amplified using PCR from cDNA of salt-stressed *Salsola aucheri*. The amplified gene was cloned and its nucleotide sequence was determined.

Materials and Methods

Seed germination and plant materials

The seeds of *Salsola aucheri* (Moq.) Bunge ex Iljin were collected from Jolfa, East Azerbaijan Province, Iran. The seeds were sterilized by sodium-hypochlorite (1% v/v) and germinated on two layers of moistened filter paper in Petri dishes. Different concentrations of NaCl including 0 (control), 100, 200, 300, 400 and 500 mM were used as salinity treatments in 3 replicates. Two-day-old seedlings were used for biochemical and molecular analyses.

Determination of total protein content and assessment of enzyme activities

A quantity of 0.1 gr of samples were homogenized in ice-cold phosphate-buffered solution (PBS, 50 mM, pH=7) using mortar and pestle. Homogenates were centrifuged at 10000 g for 10 minutes at 4°C. The supernatants were used immediately for determination of the total soluble protein content (Bradford, 1976), as well as the assessment of the activities of SOD, POD and CAT.

The activity of SOD was assessed spectrophotometrically through determination of nitro-blue-tetrazolium (NBT) photoreduction inhibition by extracts (Winterbourn et al., 1976). Reaction mixture (3 ml) comprised of 2.7 ml sodium phosphate solution (60 mM, pH=7.8), 100 µl NBT (0.05 mM), 100 µl EDTA (6.7 mM) containing KCN (0.02 mM), 50 µl of riboflavin (2 µM) and 50 µl enzyme extract. The mixtures were illuminated at a light intensity of 5000 Lux for 12 minutes and the absorbance of the solutions was recorded at 560 nm by a spectrophotometer (analytikjena, SPEKOL 1500, Germany). The amount of the enzyme causing 50% protection of NBT photoreduction was considered as one unit and SOD activity was expressed as U/mg protein.

The activity of POD was determined by recording the increase in absorbance at 470 nm during polymerization of guaiacol to tetraguaiacol (Chance and Maehly, 1955). The reaction mixture (1 ml) contained 300 µl of guaiacol (4 mM), 350 µl of phosphate buffer (10 mM), 300 µl of H_2O_2 (50 mM) and 50 µl of enzyme extract. The reaction was initiated by adding H_2O_2 to reaction mixture and absorbance recorded for 3 minutes. POD specific activity was calculated using the extinction coefficient of 26.6 $mM^{-1}.cm^{-1}$ for guaiacol. One unit of POD activity was measured as the enzyme amount capable of oxidizing 1 µM guaiacol to tetraguaiacol per minute and enzyme specific activity expressed as U/mg protein.

The activity of CAT was evaluated according to the method described by Obinger et al. (1997). The activity of CAT was measured at 240 nm by following the decomposition of H_2O_2 for 3 minutes. The reaction mixture contained 2.5 ml potassium phosphate buffer (50 mM, pH=7), 1 ml H_2O_2 (10 mM) and 500 µl enzyme extract. CAT specific activity was calculated using the extinction coefficient of 0.04 U/mM.cm for H_2O_2 and one unit of enzyme activity was considered as the amount of enzyme necessary for the reduction of 1 µM H_2O_2 per minute. CAT specific activity expressed as U/mg protein.

Determination of Betaine aldehyde dehydrogenase (BADH) activity

The activity of BADH assayed as described by Daniell et al. (2001) with some modifications. To obtain crude protein extracts, plant materials were homogenized in 250 µl homogenization buffer (pH=8) containing 50 mM HEPES-KOH, 1 mM EDTA, 20 mM sodium metabisulfite, 10 mM sodium borate, 5 mM ascorbic acid, 5 mM dithiothreitol, and 2% (w/v) PVPP. Then, homogenates were centrifuged at 10000 g for 15 minutes at 4°C and the obtained supernatants

were used for determination of BADH activity. The BADH activity assayed by monitoring the absorbance at 340 nm with 0.05 mM betaine aldehyde chloride as substrate. The activity was calculated using the extinction coefficient of 6220 $M^{-1}.cm^{-1}$ for NADH and expressed as the formation of NADH (nmol/min.mg protein).

Estimation of malondialdehyde content (MDA)

Malondialdehyde (MDA) content was measured by a technique conveyed by Boominathan and Doran (2002). An amount of 0.1 gr of samples was homogenized with 0.1% (W/V) trichloroacetic acid (TCA) and centrifuged for 5 minutes at 10000 g. Then, 0.5 ml of supernatants were mixed with 2 ml of 20% TCA containing 0.5% of 2-thiobarbituric acid and heated at 95°C for 30 minutes. The mixtures were immediately transferred to an ice bath for 5 minutes and then centrifuged at 10000 g for 15 minutes. Finally, the absorbance of the supernatants recorded at 532 nm and the concentration of MDA was calculated according to a standard curve prepared using 3,1,1,3-tetraethoxy propane (0-100 nM) and expressed as µg/grFW.

Determination of glycinebetaine (GB) content

GB was determined by the method described by Grieve and Grattan (1983) with minor modifications. Dried and finely powdered plant material (0.1 gr) extracted in distilled water. The extracts were diluted 1:1 with 2 N H_2SO_4. Samples were incubated at 60°C for 10 minutes and then centrifuged at 12000 g for 25 minutes at room temperature. A cold KI-I_2 reagent (50 µl) was added to the test tubes containing, 125 µl of supernatants and mixed gently. The tubes were stored at 0-4°C for 16 hours and then centrifuged at 12000 g for 30 minutes at 4°C. The supernatant was aspirated and peridotite crystals were dissolved in 1.4 ml of 1,2-dichloroethane. Finally, the absorbance of samples was measured at 290 nm after 48 hours and GB content expressed as nmol/grFW.

RT-PCR analysis

Total RNA was extracted from 100 mg of treated seedlings using RNX-Plus (CinnaGen, Iran) solution according to the manufacturer's instructions. In order to obtain cDNA, the total RNA samples (after treating with DNase) were used for reverse-transcription. For the synthesis of cDNA, 3 µg total RNA, 1 µl M-MuLV reverse transcriptase, 0.5 µl RNase inhibitor, 1 µl Oligo$(dT)_{18}$ primer, 2 µl dNTP and 4 µl buffer were mixed and incubated at 42°C for 1 hour, then the reaction was terminated at 70°C for 10 min.

cDNA was PCR-amplified using primers of 5´-ATTGCTCCATCTCTTGCTGCTG-3´ and 5´-ACGCTTGACGCCTCCCCAT-3´ for BADH gene. These primers were designed using sequences of BADHs of the following species presented in NCBI by Gene Runner:

Oryza sativa (GenBank accession no. ABB83473.1), *Salicornia bigelovii* (GenBank accession no. AJF23777.1), *Atriplex canescens* (GenBank accession no. AFG28557.1), *Halocnemum strobilaceum* (GenBank accession no. AFB74193.1), *Atriplex centralasiatica* (GenBank accession no. AAM19157.1), *Haloxylon ammodendron* (GenBank accession no. ACS96437.1), *Suaeda salsa* (GenBank accession no. ABG23669.1), *Atriplex prostrate* (GenBank accession no. AAP13999.1), *Triticum aestivum* (GenBank accession no. AAL05264.1), *Suaeda liaotungensis* (GenBank accession no. AAL33906.1), *Atriplex micrantha* (GenBank accession no. ABM97658.1), *Atriplex hortensis* (GenBank accession no. ABF72123.1), *Brassica napus* (GenBank accession no. AAQ55493.1) and Zea mays (GenBank accession no. AAT70230.1).

These primers produced a singular PCR band of 797 bp from cDNA of *Salsola aucheri*. Amplification conditions were as: an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 40 seconds, 58°C for 40 seconds, 72°C for 60 seconds, then a final extension at 72°C for 10 minutes. For internal control, glyceraldehyde-3-phosphate dehydrogenase (GPDH) gene used with the specific primers: 5´-GTGGTGCCAAGAAGGTTGTTAT-3´ and 5´-CTGGGAATGATGTTGAAGGAAG-3´ (Bao et al. 2009) that produced a 286 bp fragment band. Amplification conditions for the GPDH fragment were an initial denaturation at 94°C for 2 minutes, followed by 25 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 45 seconds, and a final extension at 72°C for 8 minutes. The PCR products were electrophoresed on a 1.5 percent agarose gel containing ethidium bromide.

ImageJ software was used to extract the fluorescent intensity levels of gel photographs and to calculate the relative expression of BADH gene using GPDH gene as a normal housekeeping gene (Ferreira & Rasband, 2012). The relative expression was used in statistical analyses.

The resulting PCR products were sequenced and homology analysis of the deduced protein SaBADH was performed. A phylogenetic analysis by the Neighbor-Joining method was undertaken using ClustalW and the MEGA7 software with a bootstrap replication value of 1000.

Statistical analysis

Analysis of variance was conducted with Tukey-Kramer's multiple comparisons test using SPSS software (Version 16) and a difference was considered significant when $p<0.05$. Microsoft Excel 2010 software was used for the preparation of figures.

Results

MDA content

A slight non-significant increase was observed for MDA content by exposing to salt stress ($p<0.05$). Compared to the control sample, MDA content increased by 2.62, 9.13, 12.44, 13 and 18.18%, in plants treated with 100, 200, 300, 400 and 500 mM of NaCl, respectively (Figure 1).

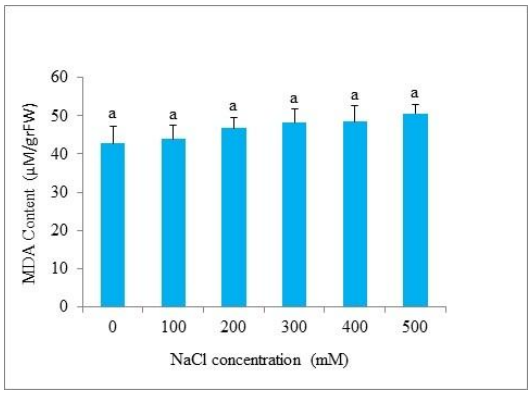
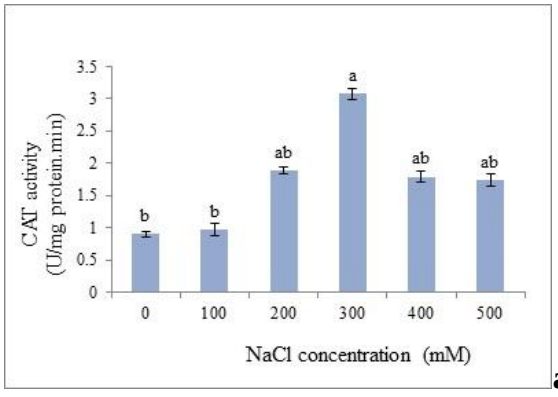


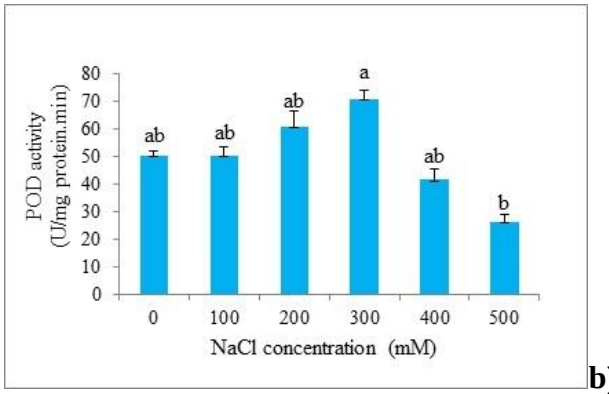
Figure 1. *The effect of different concentrations of NaCl on malondialdehyde (MDA) content (µM/grFW) in Salsola aucheri. The data represent the mean of three replications and error bars indicate SD. The same letters indicate no significant differences ($p<0.05$).*

Activity of antioxidant enzymes

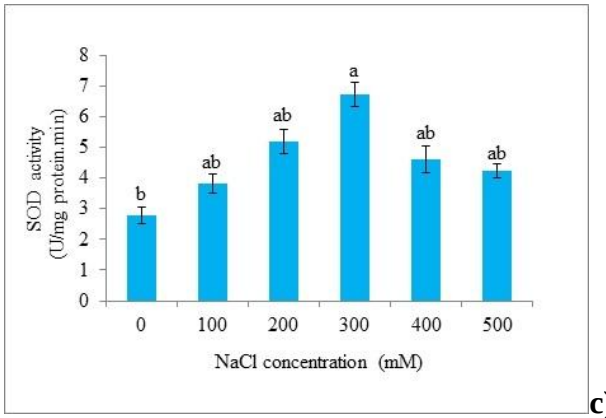
The activity of CAT increased in the seedlings exposed to 300 mM of NaCl in comparison with the control condition, but decreased by 400 and 500 mM of NaCl ($p<0.05$) (Figure 2.a). POD activity in seedlings treated with various concentrations of NaCl was changed in comparison with the control sample and the highest activity was 43.2% by 300 mM of NaCl ($p<0.05$). Intriguingly, in higher salt concentrations than 300 mM, the activity of POD decreased (Figure 2.b). Similarly, SOD activity increased in treatment with 300 mM NaCl by 143.2% compared to the control specimen. By increasing the concentration of NaCl to 400 and 500 mM, the activity of SOD decreased, even though its sum remained by 67.25% and 53.38% higher than the control sample, respectively ($p<0.05$). (Figure 2.c)



a)



b)



c)

Figure 2. *The effect of different concentrations of NaCl on CAT (a), POD (b) and SOD (c) activity (U/mg protein.min) in Salsola aucheri. The data represent the mean of three replications and error bars indicate SD. The same letters indicate no significant differences ($p<0.05$).*

BADH activity

BADH activity significantly enhanced by the increasing NaCl level from 100 to 300 mM compared to the control experiment. However, at the higher concentrations of NaCl (400 and 500 mM), BADH activity decreased significantly ($p<0.05$) (Figure 3).

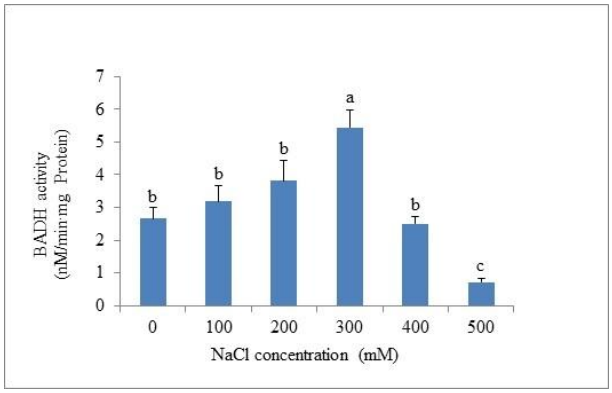


Figure 3. *The effect of different concentrations of NaCl on BADH activity. The data represent the mean of three replications and error bars indicate SD. The same letters above the bars indicate no significant differences ($p<0.05$).*

Glycinebetaine content

In seedlings treated with 300 mM of NaCl, a significant accumulation of GB (550.3 nM/grFW) in comparison to

other treatments has been observed. In comparison, seedlings treated with 500 mM of NaCl showed the lowest GB accumulation (287.4 nM/grFW) (Figure 4).

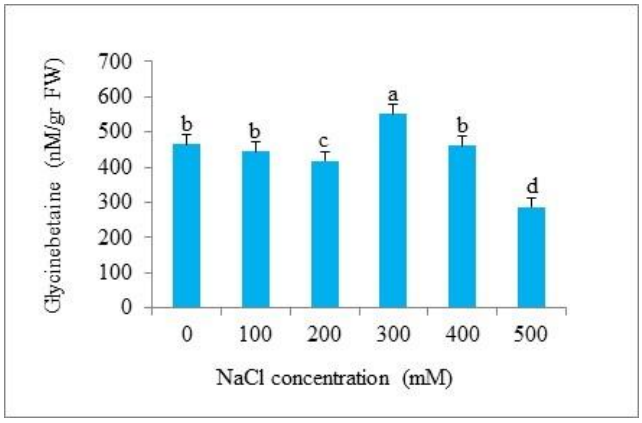


Figure 4. *The effect of different concentrations of NaCl on GB content. The data represent the mean of three replications and error bars indicate SD. The same letters indicate no significant differences (p<0.05).*

Expression of BADH gene

In seedlings, expression of BADH gene increased by 38.88% at 300 mM of NaCl and decreased at higher concentrations compared to the control condition. Comparison of their expressions in treated seedlings based on the band intensity showed that BADH transcripts expression was stronger in 300 mM of NaCl (Figure 5).

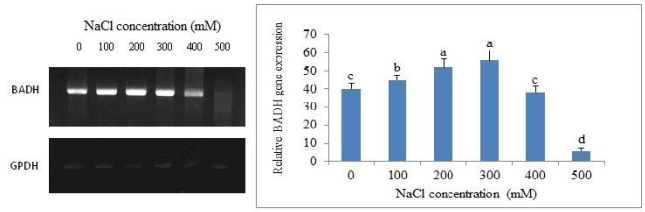


Figure 5. *Expression of BADH gene in seedlings of Salsola aucheri by different concentrations of NaCl. (a) The gel images for BADH and GPDH genes. A single band for each gene was detected. (b) Relative expression of BADH gene detected in gel lane were normalized using GPDH gene intensities. The data represent the mean of three replications and error bars indicate SD. The same letters indicate no significant differences (p<0.05).*

Sequence analyses, alignments and phylogenetic trees

A cDNA fragment of 797 bp was isolated by the RT-PCR method and was sequenced (Figure 6). A nucleotide BLAST search showed that the isolated cDNA fragment from *Salsola aucheri* (recorded in GenBank as accession no.MF399497) shared high sequence homology with many known BADH genes in several plant species. The homologies were 93% with *Haloxylon ammodendron* (GenBank accession no. GQ227730.1), 93% with *Haloxylon persicum* (GenBank accession no. JN547390.1), 91% with *Suaede glauca* (GenBank accession no. KF594413.1), 89% with *Halocnemum strobilaceum* (GenBank accession no. JN969894.1), 89% with *Salicornia bigelovii* (GenBank accession no. KM875506.1), 88% with *Spinacia oleracea* (GenBank accession no. FJ595952.1) and 87% with *Atriplex nummularia* (GenBank accession no. KC785453.1).

An amino acid sequence was proposed for the obtained BADH gene sequence using Generunner software comprises 251 amino acids (Figure 7). Comparison between the sequences of our proposed BADH amino acid and those of BADH protein reported from the well-known plant species presented in NCBI database using BlastP program analysis showed high homology. This homology was by 95% with *Haloxylon ammodendron* (GenBank accession no. ACS96437.1), 94% with *Suaeda glauca* (GenBank accession no. AHB08884.1), 94% with *Haloxylon persicum* (GenBank accession no. AEW31327.1), 90% with *Halocnemum strobilaceum* (GenBank accession no. AFB74193.1).

The CLUSTALW analysis of BADH amino acid sequences proposed in this study and those of BADHs suggested for other plant species showed that our BADH has the closest relationship to BADHs of the genus *Haloxylon* (Amaranthaceae) (Figure 8).

TTTGCTCCCATTCTTGCTGCTGGCTGTACAGCTGTACTCAAGCCATCAGAGTTGGCATCTGTGACTT
GTCTAGAATTCGGTGATGTTTGCAATGAAGTGGGGCTTCCTCCGGGTGTGTTAAATATTTTAACAGG
ATTAGGTCCAGATGCTGGTGCACCATTGGTGTCTCATCCTGATGTTGACAAGGTTGCATTTACTGGA
AGTAGTGTTACTGGCAGCAAGGTTATGGCTTCTGCTGCCCAGTTGGTAAAGCCTGTGACATTAGAA
CTTGGAGGTAAAAGTCCGATAATTGTGTTTGAAGATGTTATTGATCTGGATATAGCTGCTGAATGGA
CCATTTTTGGTGTTTTCTGGACAAATGGTCAAATATGCAGTGCGACGTCTAGGTTGCTTGTGCATGA
AAGTATTGCAGCTGAATTTGTTGATAAGCTTGTAAAATGGACCGAGAAAATAAAGATTTCTGACCC
ATTTGAGGAAGGATGCAGGCTTGGCCCTGTTATTAGCAAGAACCAGTATGACAAAATTATGAAGTA
CATATCCAATGCAAAGAGTGAAGGGGCAACAATTTTGTTTGGGGGTTCCCGTCCTGAGCATCTGAA
GAAAGGGTATTTTATTGAACCAACCATCATAACTGATGTCTCCACTTCAATGCAAATATGGAAAGA
GGAAGTTTTTGGCCCTGTCTTATGTATTAAGACATTTAGTACTGAAGAAGAAGCCATTGAAATGGCA
AATGATACAGAATATGGTTTAGCTGCTGCTGTATTTTCTAAAGACCTTGAGAGGTGTGAAAGGGT

Figure 6. *Nucleic acid sequences of BADH gene obtained from Salsola aucheri seedlings after salinity stress (300 mM of NaCl).*

CTAVLKPSELASVTCLEFGDVCNEVGLPPGVLNILTGLGPDAGAPLVSHPDVDKVAFTGSSVTGS
KVMASAAQLVKPVTLELGGKSPIIVFEDVIDLDIAAEWTIFGVFWTNGQICSATSRLLVHESIAAEF
VDKLVKWTEKIKISDPFEEGCRLGPVISKNQYDKIMKYISNAKSEGATILFGGSRPEHLKKGYFIE
PTIITDVSTSMQIWKEEVFGPVLCIKTFSTEEEAIEMANDTEYGLAAAVFSKD

Figure 7. *The proposed amino acid sequence of BADH gene obtained from Salsola aucheri seedlings (300mM of NaCl).*

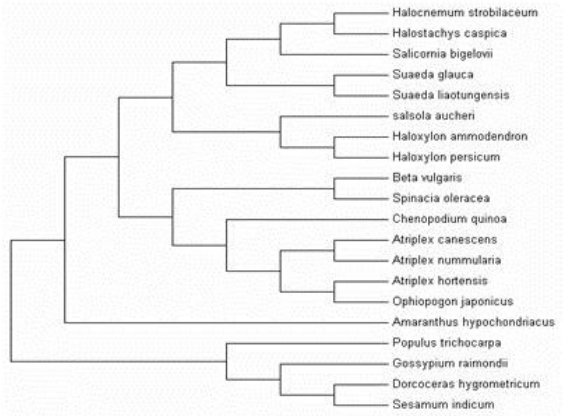


Figure 8. *The phylogenetic tree showing similarity between amino acid sequences of BADH reported in the current study from Salsola aucheri and BADH genes of a number of plant species.*

Discussion

Membrane lipid peroxidation is a useful indicator of the formation and accumulation of ROS in plants exposed to salinity. Accordingly, the content of malondialdehyde was measured as a key parameter in evaluating the degree of oxidative stress damages induced by salinity (Flowers & Colmer, 2015). We found a non-significant change in the content of MDA by increasing NaCl concentration. The level of lipid peroxidation measured by the MDA content did not change significantly due to salinity, suggesting that the halophyte *Salsola aucheri* possesses a very efficient antioxidant defense system to eliminate the production of ROS. Generally, a positive linear relationship between MDA content and salinity was reported for a number of halophytes (Jithesh et al., 2006, Ozgur et al., 2013) such as *Cakile maritima* (Ksouri et al., 2007), *Sesuvium portulacastrum* (Lokhande et al., 2011), *Gypsophila oblanceolata* (Sekmen et al., 2012) and *Sphaerophysa kotschyana* (Yildiztugay et al., 2013). Unchanged lipid peroxidation and membrane permeability seemed to be a characteristic of tolerant plants, which are adapted to salinity (Shalata et al., 2001).

Salinity commonly causes the production of ROS (Ozgur et al., 2013). These ROS are detoxified by using enzymatic and non-enzymatic antioxidant systems. However, a high level of salt stress can make these systems insufficient and cause serious damages that can lead to death (Jithesh et al., 2006). The increase in the activity of SOD is often correlated with a higher tolerance of the plant against environmental stresses (Pallavi et al., 2012). In this study, the activity of SOD, an important indicator for the elimination of ROS, increased significantly after the treatment of plants with NaCl. A similar increase was also observed in the activities of other antioxidant enzymes indicating their role in the detoxification of ROS under salinity stress. The reduction in the activity of antioxidant enzymes at high salt concentrations is probably due to protein degradation as a result of lessening the number of osmolytes such as GB in the cells.

Glycinebetaine (GB) is known to stabilize the structure of macromolecules and the integrity of the membrane. Thus, a change in the quantity of GB in our study revealed an improved cell membrane homeostasis. Therefore, it can be assumed that GB might protect the cell membrane from stress-induced injuries and oxidative stress. These results designated that the enhanced tolerance to salt stress in *Salsola aucheri* as a halophyte is due to the increased accumulation of GB. Based on our findings, increase of the concentration of NaCl, could induce the expression of stress-responsive genes, and cause to activation of ROS scavenging enzymes, and subsequently reduce ROS accumulation in the plant cells, lead to protection of the cell from damage caused by salt stress.

BADH catalyzes the synthesis of glycinebetaine (GB) to protect plants against osmotic stress (Tian et al., 2016). As one of the most important enzymes for the synthesis of GB in plants, BADHs have been intensively studied and have been shown to be associated with stress tolerance in many plant species of a wide taxonomic range especially in members of Chenopodiaceae and Amaranthaceae (Joseph et al, 2013; Kachout et al., 2013; Wang et al., 2015). In another study, *SlBADH* gene from *Suaeda liaotungensis* showed approximately 6.3-fold higher expression under salt stress (400 mM NaCl) compared to the control condition (Zhang et al., 2008). Our results indicated also that the expression of *SaBADH* could be induced by salt treatments.

In this study, we reported a gene sequence encoding BADH isolated from cDNA of *Salsola aucheri* (*SaBADH*). The high homology between our BADH sequence and the sequences reported from other plant species (Brauner et al., 2003; Ye et al., 2005) confirmed that the sequence isolated in the current study is most likely the coding sequence for BADH. In addition, the high match levels (>95 percent) between the BADH amino acid sequence proposed in the current study with reported gene products from several other plant species provided further support for the authenticity of the *SaBADH* cDNA sequence (Ye et al., 2005; Zhang et al., 2008). The phylogenetic analysis confirmed that SaBADH has the closest relationship with BADHs of some plant species of the amaranth family (Figure 8).

Conclusions

The growth of seedlings of *Salsola aucheri* was inhibited at high concentrations of NaCl. Saline stress induces oxidative stress in *Salsola aucheri*. The activities of POD, SOD and CAT as antioxidant enzymes were significantly changed under salinity stress, probably for detoxification of ROS. The results obtained imply that *SaBADH* may play a significant role in the plant to survive under salt stress.

Accordingly, there was a significant correlation between GB content, BADH activity and *SaBADH* expression.

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