

Article

Endophytic Fungi Regulate *HbNHX1* Expression and Ion Balance in *Hordeum bogdanii* under Alkaline Stress

Feng Long ¹ , Meng-Fei Hu ¹, Sheng Chen ¹, Gen-Sheng Bao ², Han Dan ¹ and Shui-Hong Chen ^{1,*}¹ College of Life Science and Technology, Tarim University, Alar 843300, China² Qinghai Academy of Animal and Veterinary Sciences, Xinning 810016, China

* Correspondence: rainbow@taru.edu.cn

Abstract: Plants cope with abiotic stress in several ways, including by collaborating with microorganisms. *Epichloë*, an endophytic fungus, has been shown to improve plant tolerance to extreme external environments. *Hordeum bogdanii* is a known salt-tolerant plant with the potential to improve alkaline lands. *NHX1* plays a key role in the transport of ions in the cell and is overexpressed in plants with increased salt tolerance. However, the expression levels of *HbNHX1* in *Epichloë* endophytic fungal symbionts in *H. bogdanii* have not been elucidated. We used *Hordeum bogdanii* (E+) with the endophytic fungi *Epichloë bromicola* and *H. bogdanii* (E−) without the endophytic fungi and compared the differences in the ion content and *HbNHX1* expression between the shoots and roots of E+ and E− plants under alkaline stress. The absorption capacity of both K⁺ and Na⁺ of *H. bogdanii* with endophytic fungi was higher than that without endophytic fungi. In the absence of alkaline stress, endophytic fungi significantly reduced the Cl[−] content in the host *H. bogdanii*. Alkaline stress reduced SO₄^{2−} content in *H. bogdanii*; however, compared with E−, endophytic fungi increased the content of SO₄^{2−} in E+ plants. With an increase in the alkaline concentration, the expression of *HbNHX1* in the roots of *H. bogdanii* with endophytic fungus exhibited an upward trend, whereas the expression in the shoots exhibited a downward trend first and then an upward trend. Under 100 mmol·L^{−1} mixed alkaline stress, the expression of *HbNHX1* in E+ was significantly higher than that in E−, indicating that endophytic fungi could increase the Na⁺ region in vacuoles. The external environment affects the regulation of endophytic fungi in *H. bogdanii* and that endophytic fungi can play a key role in soil salinization. Therefore, the findings of this study will provide technical support and a theoretical basis for better utilization of endophytic fungi from *H. bogdanii* in saline land improvement.

Keywords: alkali stress; endophytic fungi; *Hordeum bogdanii*; cations; anion; *HbNHX1* expression

Citation: Long, F.; Hu, M.-F.; Chen, S.; Bao, G.-S.; Dan, H.; Chen, S.-H. Endophytic Fungi Regulate *HbNHX1* Expression and Ion Balance in *Hordeum bogdanii* under Alkaline Stress. *J. Fungi* **2023**, *9*, 331. <https://doi.org/10.3390/jof9030331>

Academic Editor: Zonghua Wang

Received: 5 February 2023

Revised: 3 March 2023

Accepted: 6 March 2023

Published: 7 March 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Soil salinization has severely endangered the environment and the production of economic crops in China [1]. High salt levels can lead to ion toxicity, osmotic stress, oxidative damage, and other secondary stresses in plants [2], making it necessary for the plants to maintain the balance between ions in their cells to ensure normal growth and development. A large area of the inland saline-alkaline land in China mainly contains three cations: Na⁺, K⁺, Mg²⁺, and four anions: CO₃^{2−}, HCO₃[−], Cl[−], and SO₄^{2−} [3]. Excess accumulation of Na⁺ and Cl[−] will result in ion toxicity, thus reducing the absorption of essential nutrients such as calcium (Ca), potassium (K), phosphorus (P), and nitrogen (N) [4,5]. As soil salinization involves not only salt stress but also alkaline stress, the same stress factors apply to alkaline stress as they do to salt stress, which also involves high pH stress [6], which leads to a significant amount of metal ions to precipitate, inhibits the absorption of anions, and breaks the ion balance inside and outside cells [7]. Therefore, to slow down the accumulation of cations such as Na⁺ and K⁺ under alkaline stress, plants maintain the balance of anions and cations by accumulating inorganic anions such as Cl[−], NO₃[−], and SO₄^{2−}.

The effects of alkaline stress on the transport of Na^+ and K^+ in plants have been extensively studied. Plants cope with a large amount of Na^+ accumulation under saline-alkaline stress mainly in two ways: *SOS1* gene involved ion efflux and the *NHX1* gene participation in intracellular ion regionalization. *SOS1* is located in the plasma membrane, expressed in the roots of epidermal cells and wood parenchyma cells, and is mainly involved in the efflux of Na^+ from the cytoplasm to the soil and the transport of Na^+ to leaves through transpiration [1,8]. *NHX1* is located in the vacuole membrane and plays an important role in the transport of Na^+ in plants by exchanging Na^+ into vacuoles and H^+ into the cytoplasm. This reduces the excessive accumulation of Na^+ in the cytoplasm and maintains the osmotic potential in cells so that they can absorb water normally [9]. In Arabidopsis and rice, plants overexpressing *NHXs* can retain K^+ in the cytoplasm under saline conditions, thereby improving salt tolerance [10,11]. Pérez-Martín et al. [12] found that high expression of *NHX1* in Arabidopsis leaves under saline and alkaline environment can enhance salt tolerance by promoting Na^+ partition and osmotic regulation. Plants can improve their tolerance to Na^+ by collaborating with microorganisms [13]. For example, in tomatoes and lettuce, inoculation of plant roots with fungal endophytes resulted in an improvement in biomass, photosynthetic capacity, and survival rate under salt stress. At the same time, it enhanced the expression of the *NHX1* gene and promoted Na^+ regionalization [14]. However, many molecular studies have mainly focused on the interaction between root endophytic fungi and plants, whereas there are few studies on the interaction of endophytic fungi in plant stems and leaves.

The endophytic fungus of grass spends part or all its life cycle affecting the growth process of grass and does not cause obvious symptoms in plants [15]. Under saline-alkaline stress, infection with endophytic fungi of Gramineae can improve the tolerance and adaptability of host plants to the external environment [16,17]. Recent research on endophytic fungi of Gramineae mainly focused on the genus *Epichloë* of Clavicipitaceae, Ascomycota [18], which affects the resource allocation and mineral absorption capacity of host plants [19].

H. bogdanii is a salt-tolerant plant with strong ecological adaptability and competitive advantages. It has broad prospects for development as a forage grass and to improve the ecological environments of desert, saline, and alkaline lands [20]. Recently, studies on *H. bogdanii*-endophytic fungi symbionts have mainly focused on the classification and identification of endophytic fungi [21–23] and the effects of endophytic fungi on the growth and development [23,24], physiology, and biochemistry [25,26] of *H. bogdanii* under abiotic stress. However, *HbNHX1* expression and ion balance of *Epichloë* endophytic fungal symbionts in *H. bogdanii* under saline-alkaline stress have not yet been reported.

In this study, we aimed to explore the regulation mechanism of endophytic fungi on the expression of the *HbNHX1* gene of *H. bogdanii* and the balance of anions and cations in plants under alkali concentration stress. To achieve this, we measured *HbNHX1* expression and Na^+ , K^+ , Cl^- , SO_4^{2-} and NO_3^- content in the shoots and roots of E+ and E– *H. bogdanii* plants under alkaline stress, and analyzed the effects of endophytic fungi on *HbNHX1* gene expression and ion balance regulation of the host *H. bogdanii* under alkaline stress.

2. Materials and Methods

2.1. Plant Materials and Culture Conditions

The seeds of Wensu E+ and E– *H. bogdanii* had been previously treated in our laboratory [25]. *Epichloë bogdanii* was used in this study [22]. The seeds were then planted in an experimental field (80°76' E, 41°58' N, H 1514 m) in the experimental station of Tarim University. E+ and E– plump seeds were germinated in water until they grew seedlings and then transplanted into 4 L hydroponic boxes, 19 holes per pot, spaced at 1 seedling per hole for a total of 19. Each basin was connected to an oxygen-charging pump, the air was regularly exchanged every day, and 1/2 Hoagland's solution was replaced every three days. The day/night temperature of the greenhouse was 28 ± 2 °C/ 15 ± 3 °C and the

sunshine duration was approximately 12 h. Once *H. bogdanii* tillered 3–5 plants, aniline blue microscopy [27] was used to determine the presence of endophytic fungi in *H. bogdanii*.

2.2. Stress Treatment

In the mixed alkaline (Na₂CO₃:NaHCO₃ = 1:1) treatment, the concentration gradients were set to 50 mmol·L⁻¹ and 100 mmol·L⁻¹. The experiment had a randomized complete block design with four replicates (hydroponic box) of E+ and E- seedlings. To avoid alkali shock, the alkali concentration was increased by 50 mmol·L⁻¹ every 12 h until all treatments reached the predetermined concentration. A solution without mixed alkali was used as the control for the experiment. The mixed alkali solution with the corresponding concentration was replaced every 7 d, and the sample was taken at 21 d for subsequent ion content and *NHX1* gene expression measurements.

2.3. Determination of Ion Content

After 21 days of alkali stress treatment, the *H. bogdanii* plants were divided into shoots and roots for the determination of ion content. Some samples were placed in an oven at 105 °C for 30 min and then dried to a constant weight at 70 °C. Shoots and roots samples were ground and sieved, weighed to 0.3 g and 0.1 g, respectively, and added to 10 mL of triacid (HNO₃:HClO₄:H₂SO₄ = 8:1:1) for digestion, and diluted to 50 mL. Na⁺ and K⁺ contents were determined using a flame photometer (FP6431) [28]. The other set of samples was placed in a vacuum freeze-drying instrument and freeze-dried for 24 h. Then, the shoots and roots samples were ground, sieved, and weighed to 0.5 g and 0.3 g, respectively. These ground samples were added to 10 mL of deionized water, fully ground, centrifuged at 3000 rpm for 10 min, and the supernatant was collected; this process was repeated three times. The supernatant was combined with deionized water to a volume of 50 mL, filtered with an aqueous phase filter membrane, and the contents of Cl⁻, SO₄²⁻, and NO₃⁻ were determined by ion chromatography (Dionex Integration HPIC high-pressure ion chromatography series) [29]. Each processing set was repeated four times.

2.4. Cloning of *HbNHX1* Gene

Total RNA was extracted using TransZol (Full Gold, Beijing, China) and reverse transcribed into cDNA (TRAN HiFiScript[®] gDNA Removal cDNA Synthesis Kit, Beijing, China). The reference barley Na⁺/H⁺ antiporter *HvNHX1* gene full-length sequence, accession number AB089197.1, designed two pairs of primers (*HbNHX1*): primers and reaction conditions, as shown in Table 1. The *HbNHX1* gene was cloned using Nested PCR via Bio-Rad PCR instrument (Hercules, California, USA, T100). After electrophoretic separation, the target fragment was recovered and constructed into the pMD19-T vector, and inserted into *E. coli* DH5α, cultured in LB liquid medium for 5 h, and then coated on solid LB medium for overnight culture. A single colony was selected and placed in LB liquid medium for amplification and culturing for 6 h. Recombinant bacteria with positive PCR results were sequenced (Xi'an Qingke Biotechnology Co., Ltd., Beijing, China).

Table 1. Gene primer sequence.

Gene	Primer Sequence	Reaction Procedure
<i>HvNHX1</i>	1F: GAAAAGAATAGAGGAGAATCCCGAC 1R: ACCATTACACCAATCCACTAGAAAAG 2F: ATGGCGTTCGAAGTGGTGGC 2R: TCATGCCACGATTACGTTTGGA	The PCR conditions were 95 °C for 3 min, then 30 cycles of 94 °C for 30 s, 56.2 °C for 30 s, 72 °C for 90 s and a final extension at 72 °C for 10 min.
<i>HbNHX1</i>	F: CTGTGTCGATCGCTCTTGCT R: CGGCATTGCTCGATATGGG	The qPCR conditions were 95 °C for 3 min followed by 40 cycles at 95 °C for 10 s and 60 °C for 30 s.
Actin	F: GGCATGGAGTCTTCTGGAATCC R: CCACCACTGAGCACTATGTTTC	

2.5. Expression Analysis of *HbNHX1* Gene

The reagent for fluorescent quantitative PCR was TRAN PerfectStart[®] (Green qPCR SuperMix, Beijing, China), and the real-time fluorescence quantitative PCR instrument (CFX96 Touch) from BIO-RAD Company (Hercules, California, USA) was used. The internal reference actin primer sequence was extracted from Vinje [30]. The qPCR primer design was carried out using the full-length CDS region of the *HbNHX1* gene obtained in the previous step. The primers were synthesized by Xi'an Qingke Biotechnology Co. The relevant information is shown in Table 1. The $2^{-\Delta\Delta CT}$ method was used for calculation, and each treatment was repeated four times.

2.6. Data Analysis

SPSS statistical software (IBMSSSStatistics26) was used for data analysis. The data in the figures are presented as the mean \pm standard error. The effects of different alkali concentrations on the ion content of endophytic fungi in *H. bogdanii* were detected using double-factor ANOVA, and the effects of different alkali concentrations on ion content and *HbNHX1* gene expression in *H. bogdanii* were detected using single-factor ANOVA. An independent sample *t*-test was used to detect the influence of E+ and E− ion content on *HbNHX1* gene expression under the same alkali concentration. Statistical significance was defined at a 95% confidence level. The online software DeepTMHMM was used to predict the transmembrane region of the protein encoded by the *HbNHX1* gene of *H. bogdanii*. Using the Neighbor-Joining method, a phylogenetic analysis of the *NHX1* gene was carried out in MEGAX64 software.

3. Results

3.1. Effect of Alkali Stress on Na^+ , K^+ Content and K^+/Na^+ Ratio in E+, E− *H. bogdanii*

After alkaline treatment, K^+ content in the plants was significantly lower than that in the control group. With an increase in alkaline concentration, the K^+ content decreased continuously, but it was higher in E+ than that in E− (Figure 1A,B), and the content of K^+ at $50 \text{ mmol}\cdot\text{L}^{-1}$ E+ is significantly higher than that of E−. The K^+ content of the E+ shoots was significantly higher than that of E− in the $100 \text{ mmol}\cdot\text{L}^{-1}$ mixed alkaline treatment, but there was no significant difference between E+ and E− in the roots. According to the two-factor ANOVA of variance, endophytic fungi significantly affected K^+ content in the shoots and roots of *H. bogdanii* (Table 2).

Under no stress, Na^+ content was lower in E+ than that in E− and reached a significant level in the roots (Figure 1C,D). It showed that the endophytic fungi could reduce the absorption of Na^+ by the host in the absence of alkali stress. However, it is interesting that the content of Na^+ in the shoots and roots of the plants after alkali treatment shows an upward trend, and the Na^+ content of the E+ plants was significantly higher than that of the E− plants. The possible reason for this phenomenon is that endophytic fungi promote the localization of host plant Na^+ . According to the two-factor ANOVA of variance, endophytic fungi significantly affected Na^+ content in the shoots and roots of *H. bogdanii* (Table 2).

The K^+/Na^+ ratio of E+ was significantly higher than that of E− in the control group. However, after $50 \text{ mmol}\cdot\text{L}^{-1}$ mixed alkali stress, the K^+/Na^+ ratio of E+ was significantly lower than that of E− in the shoots, whereas in the roots, the K^+/Na^+ ratio of E+ was higher than that of E−. In the $100 \text{ mmol}\cdot\text{L}^{-1}$ mixed alkali treatment, the K^+/Na^+ ratio of E+ was significantly higher than that of E− in the shoots, but there was no significant difference in the roots (Figure 1E,F). According to the two-factor ANOVA in Table 2, alkaline treatment and endophytic fungi significantly affected the K^+/Na^+ ratio in the shoots and roots of *H. bogdanii* and there was an interaction between them ($p < 0.05$).

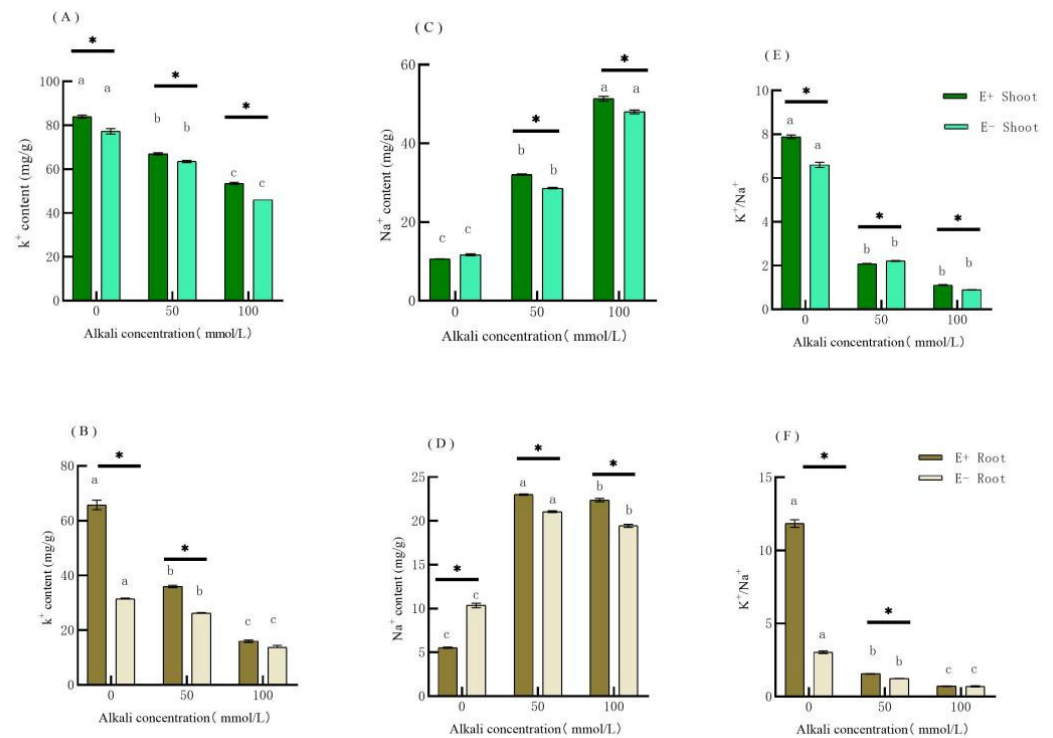


Figure 1. Effects of different alkali concentrations on K^+ (A,B), Na^+ (C,D) content and K^+/Na^+ (E,F) in E+ and E− *H. bogdanii* shoots and roots. Note: Among different groups, when comparing the same E+ or E− plant, different lowercase letters of the column mark indicate a significant difference ($p < 0.05$); in the same group E+ compared with E−, * indicates a significant difference ($p < 0.05$).

Table 2. Two-factor ANOVA of the effect of *Epichloë* endophytic fungi on Na^+ , K^+ content and K^+/Na^+ of *H. bogdanii* shoots and roots under alkali stress.

	deal	df	Na^+		K^+		K^+/Na^+	
			F	p	F	p	F	p
Shoot	E	1	215.727	<0.001	424.706	<0.001	1043.191	<0.001
	S	2	4705.524	<0.001	832.995	<0.001	2069.798	<0.001
	E × S	2	234.500	<0.001	252.975	<0.001	940.424	<0.001
Root	E	1	46.505	<0.001	2.612	<0.001	41.704	<0.001
	S	2	6256.824	<0.001	987.561	0.123	6249.812	<0.001
	E × S	2	28.093	<0.010	57.777	<0.001	101.181	<0.001

S = salt, E = endophytic fungus, E × S is the interaction between the two.

3.2. Effect of Alkali Stress on Cl^- , SO_4^{2-} and NO_3^- Content in E+, E− *H. bogdanii*

In the absence of alkaline stress, the Cl^- content in the shoots and roots of E+ plants was significantly lower than that of E− plants. However, in the 50 $mmol \cdot L^{-1}$ mixed alkaline treatment, the Cl^- content of E− shoots was significantly higher than that of E+ shoots, but the Cl^- content E− of the roots was significantly lower than that of E+ roots. There was no significant difference between the Cl^- content in E+ and E− shoots at 100 $mmol \cdot L^{-1}$ mixed alkaline treatment, but the Cl^- content in E+ roots was significantly lower than that of E− roots (Figure 2A,B). According to the two-factor ANOVA in Table 3, alkaline treatment and endophytic fungi significantly affected the Cl^- content in the shoots and roots of *H. bogdanii*, respectively. Thus, Endophytic fungi interacted with the alkaline stress ($p < 0.05$).

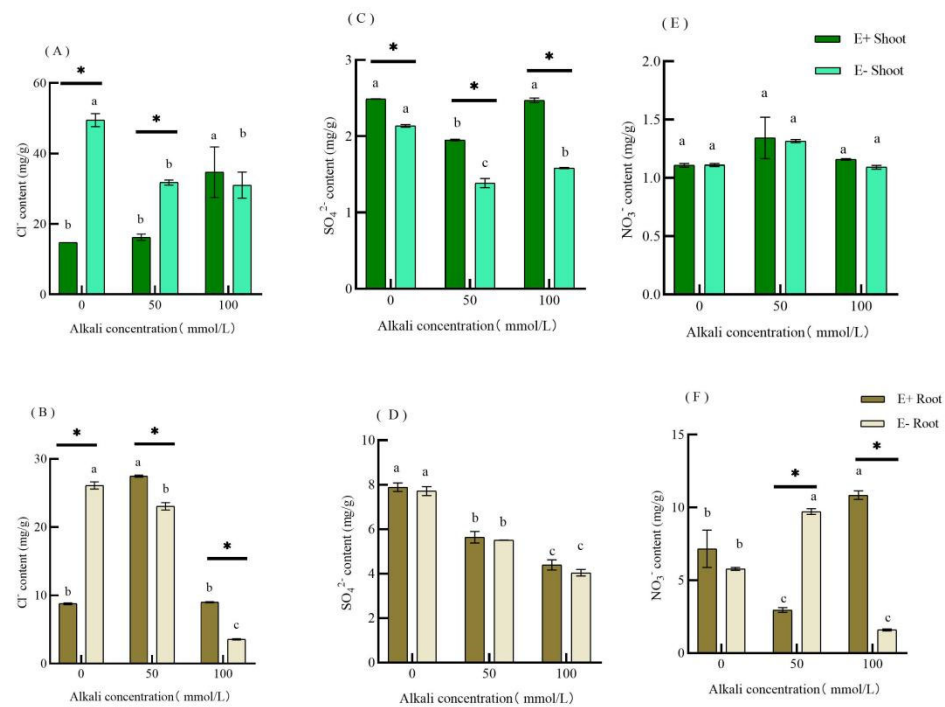


Figure 2. Effects of different alkali concentrations on Cl[−] (A,B), SO₄^{2−} (C,D) and NO₃[−] (E,F) contents in E+ and E− *H. bogdaniii* shoots and roots. Note: Among different groups, when comparing the same E+ or E− plant, different lowercase letters of the column mark indicate a significant difference ($p < 0.05$); in the same group E+ compared with E−, * indicates a significant difference ($p < 0.05$).

Table 3. Two-factor ANOVA of the effect of *Epichloë* endophytic fungi on Cl[−], SO₄^{2−} and NO₃[−] contents in *H. bogdaniii* under alkali stress.

		Cl [−]			SO ₄ ^{2−}		NO ₃ [−]		
		deal	df	F	p	F	p	F	p
Shoot	E		1	536.566	<0.001	0.013	0.910	495.057	<0.001
	S		2	1741.801	<0.001	183.331	<0.001	1115.885	<0.001
	E × S		2	568.100	<0.001	1.190	0.338	1610.349	<0.001
Root	E		1	41.525	<0.001	0.147	0.708	0.908	0.36
	S		2	4.145	0.033	189.965	<0.001	0.506	0.615
	E × S		2	10.504	<0.001	281.279	<0.001	0.236	0.793

S = salt, E = endophytic fungus, E × S is the interaction between the two.

The content of SO₄^{2−} in E+ shoots was significantly higher than that of E− shoots under alkaline-free and alkaline stress conditions (Figure 2C). The content of SO₄^{2−} in the roots of both plants gradually decreased with an increase in the alkaline concentration, and there was no significant difference in the content of SO₄^{2−} in E+ and E− plants (Figure 2D). According to the two-factor variance analysis in Table 3, alkaline stress significantly affected SO₄^{2−} content in the shoots and roots of *H. bogdaniii*; thus, endophytic fungi interacted with alkaline stress in the shoots of *H. bogdaniii* ($p < 0.05$).

Alkaline stress had no significant effect on the NO₃[−] content in the shoots of E+ and E− plants. No significant difference was observed between the E+ and E− groups (Figure 2E). The NO₃[−] content in the roots of E− plants increased and then decreased with an increase in alkaline concentration, whereas in E+ plants, it decreased and then increased accordingly. The NO₃[−] content in E+ roots was significantly lower than that in E− in the 50 mmol·L^{−1} mixed alkaline treatment, but the NO₃[−] content in E+ plants was significantly higher than that in E− in the 100 mmol·L^{−1} mixed alkaline treatment (Figure 2F). According to the two-way analysis of variance in Table 3, alkaline stress and

endophytic fungi significantly affected the NO_3^- content in the roots of *H. bogdanii*, and there was an interaction between them ($p < 0.05$).

3.3. Cloning Results of *HbNHX1* Gene in *H. bogdanii*

Using E- *H. bogdanii* cDNA as the template, nested PCR was conducted to obtain approximately 1700 bp bands (Figure 3). After connecting the T vector with the recovered target band, the total length of the target band was found to be 1708 bp, including 1617 bp in the CDS region, encoding 538 amino acids in Appendix A

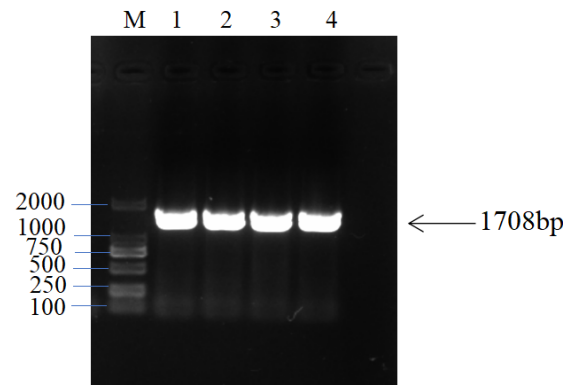


Figure 3. *HbNHX1* Clones. Note: M = Marker (2000 bp); 1, 2, 3, and 4 are *HbNHX1* genes.

3.4. Bioinformatics Analysis of *HbNHX1* Gene in *H. bogdanii*

The physical and chemical properties of the *HbNHX1* protein were analyzed using ProtParam online software. The predicted results revealed that the molecular weight of the *HbNHX1* protein was 59.28 kD, the isoelectric point (PI) was 7.68, and the elemental composition was $\text{C}_{2746}\text{H}_{4286}\text{N}_{668}\text{O}_{739}\text{S}_{25}$. In order to study whether *HbNHX1* protein is a transmembrane protein, we used TMHMM software to analyze its structure. Topological analysis showed that the protein had 13 transmembrane domains and belonged to the transmembrane protein (Figure 4). Through homology comparison, the *HbNHX1* had the highest similarity (96.29%) with the wild barley *NHX1* gene (AB089197.1), belonging to the gramineous barley genus, while the similarity with wheat (MT550001.1) was 94.99%, and the similarity with monocotyledonous model plant rice (KY752545.1) was 86.98% (Figure 5).

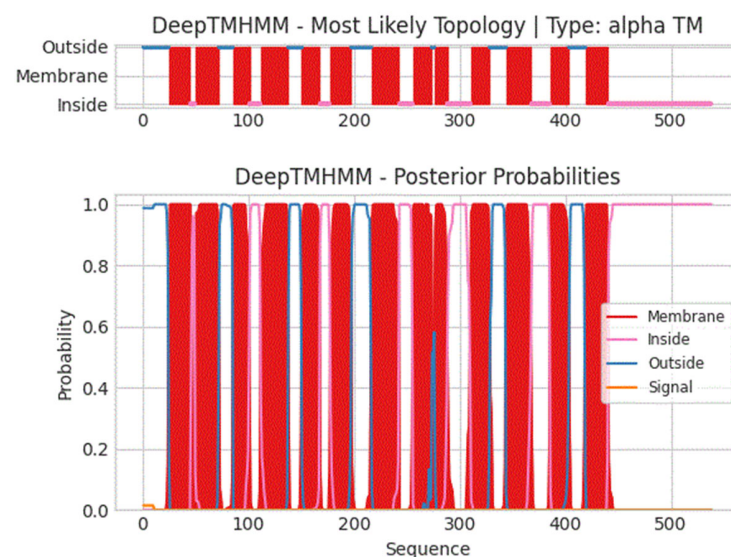


Figure 4. Structural analysis of *HbNHX1* protein. The topological structure of the *HbNHX1* protein was analyzed using TMHMM software (<https://dtu.biolib.com/DeepTMHMM>, accessed on 3 March 2020). The hydrophobic sequence with 13 transmembrane regions is shown in the large rectangle.

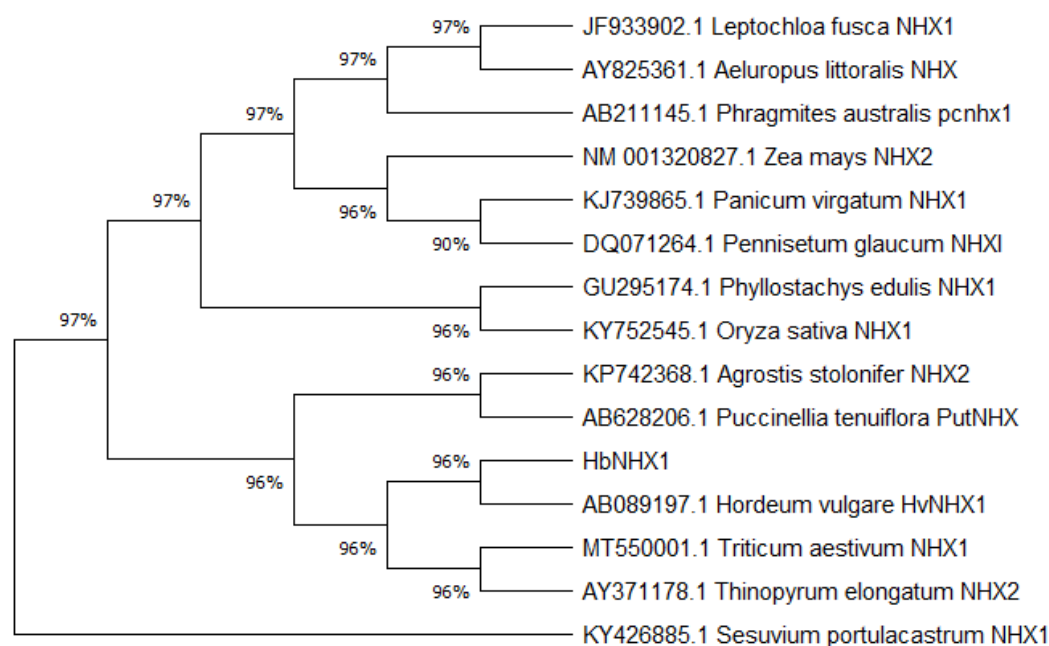


Figure 5. Evolutionary tree analysis of plant *NHX1* gene.

3.5. Effect of Alkali Stress on *HbNHX1* Gene Expression Level in Shoots and Roots of *H. bogdanii*

After alkaline stress, the expression of the *HbNHX1* gene in E+ shoots decreased and then increased with the increase in alkaline concentration compared to the control group. Under different alkaline stress conditions, E− had higher expression of the *HbNHX1* gene under alkaline stress, with the highest level at 50 mmol·L^{−1} mixed alkaline treatment. There was no significant difference in *HbNHX1* expression between E+ and E− shoots in the non-mixed alkaline treatment; however, after inducing alkaline stress, there was a significant difference in the expression of *HbNHX1* between E+ and E− shoots.

In the shoots, compared with the control, the expression of *HbNHX1* gene in E+ decreased and then increased with an increase in alkaline concentration. At the same time, compared with the control, the expression of *HbNHX1* increased in E− plants under different alkaline stress and reached a peak at 50 mmol·L^{−1} mixed alkali treatment. There was no significant difference in the expression of *HbNHX1* between E+ and E− shoots in the 0 mmol·L^{−1} mixed alkaline treatment, but the expression of *HbNHX1* in E+ and E− shoots was significantly different after alkaline stress. The expression of the E+ *HbNHX1* was significantly lower than that of E− at 50 mmol·L^{−1} mixed alkali treatment, while the expression of the E+ *HbNHX1* gene was significantly higher than that of E− at 100 mmol·L^{−1} mixed alkali treatment (Figure 6A). The expression of *HbNHX1* in the roots changed. Alkali treatment significantly increased the expression of *HbNHX1* in the roots of E+ plants, whereas the change in E− plants was not significant. There was no significant difference in the content of *HbNHX1* between E+ and E− in 0 mmol·L^{−1} mixed alkaline treatment roots, but the expression of *HbNHX1* in 50 mmol·L^{−1} and 100 mmol·L^{−1} mixed alkaline treatment E+ plants was significantly higher than that in E− plants (Figure 6B) ($p < 0.05$).

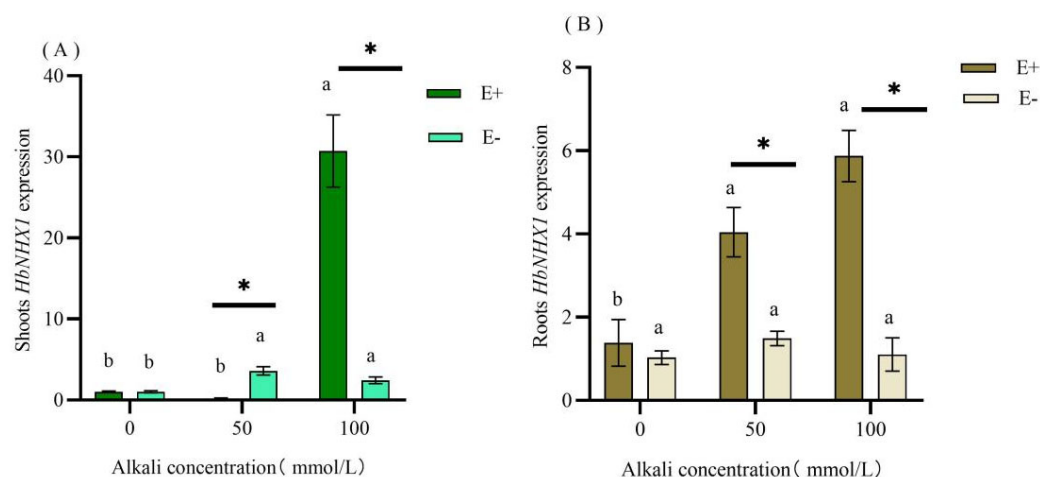


Figure 6. Effect of alkali stress on *HbNHX1* gene expression levels in E+, E− shoots (A) and roots (B) of *H. bogdanii*. Note: Among different groups, when comparing the same E+ or E− plant, different lowercase letters of the column mark indicate a significant difference ($p < 0.05$); in the same group E+ compared with E−, * indicates a significant difference ($p < 0.05$).

4. Discussion

4.1. Effect of Alkali Stress on Na^+ , K^+ Content, and K^+/Na^+ Ratio in *H. bogdanii*

Na^+ and K^+ have similar radii of hydrated ions, so it is difficult to distinguish them, leading to a competitive relationship between the absorption of Na^+ and K^+ by roots [5]. Excessive salt absorption by plants causes ion toxicity. Plants transport Na^+ in cells to vacuoles under salt stress to reduce water loss. In most plants living in saline-alkaline environments, Na^+ accumulates in vacuoles in large quantities and K^+ absorption is inhibited [31]. To cope with the excessive accumulation of Na^+ in the cytoplasm of leaves under salt stress, *Hordeum brevisubulatum* transported it to vacuoles for Na^+ localization to reduce Na^+ toxicity [32]. Many studies have shown that endophytic infection can improve abiotic and biological resistance of host plants [33–35]. However, there have also been reports showing no significant differences between plants with and without endophytic fungi [36]. Our results showed that endophytic fungi and alkaline stress significantly affected the content of Na^+ , K^+ , and K^+/Na^+ in plants. Under alkaline stress, the content of Na^+ in the shoots and roots of E+ and E− *H. bogdanii* exhibited an increasing trend, which is similar to the results published by Song [37]. However, this study found that alkaline stress led to Na^+ content in E+ being significantly higher than that in E−, which may be because endophytic fungi promote the accumulation of Na^+ in vacuoles during alkaline stress, leading to an increase in the content of Na^+ . K^+ is an important mineral nutrient that can protect plants from damage. We found that the K^+ content in the shoots and roots of *H. bogdanii* decreased significantly under alkaline stress, indicating that alkaline stress inhibits the absorption of K^+ by plants. In addition, the K^+ content of E+ was significantly higher than that of E−, indicating that *Epichloë* endophytic fungi can increase the K^+ content in host plants. K^+ content was higher in the shoots than that in the roots under alkaline stress, which is consistent with the results of Chen et al. [38], indicating that plants will accelerate K^+ upward transport when subjected to alkaline stress to alleviate Na^+ toxicity and maintain ionic balance. Alkaline stress increased the plant Na^+ content and continuously decreased the K^+ content, leading to a decrease in the K^+/Na^+ ratio, which was consistent with the trend of K^+/Na^+ ratio decline in *Peucedanum littoralis* and *Hordeum vulgare* [39] after salt stress.

4.2. Effect of Alkali Stress on Cl^- , SO_4^{2-} and NO_3^- Content in *H. bogdanii*

Alkaline stress forces a large amount of Na^+ in plants to increase, leading to an ion imbalance [40,41]. Plants usually accumulate inorganic anions, such as Cl^- [42,43], SO_4^{2-} , and NO_3^- , or synthetic organic anions [44] to regulate ion imbalance. In this study, we

found that when subjected to alkaline stress, Cl^- content was significantly higher than that of the other anions. Additionally, under alkaline stress, the Cl^- content of E+ was lower than that of E−, and the Cl^- content of E− plants decreased continuously but E+ increased, indicating that endophytic fungi can regulate the Cl^- content to regulate the ion balance in plants and reduce the ionic damage in host plants. These results were consistent with studies that reported similar trends in Cl^- content in citrus (*Carrizo citrange*) [45], spring wheat (*Triticum aestivum*), and winter barley (*Hordeum vulgare*) [46] caused by mycorrhiza. We also found that alkaline stress reduced the content of SO_4^{2-} in *H. bogdanii*, which is consistent with the findings of Yang et al. [47]. The SO_4^{2-} content of E+ was higher than that of E− when endophytic fungi infected *H. bogdanii*, indicating that endophytic fungi increased the accumulation of SO_4^{2-} in host plants. In plants, the increase in Cl^- content in the roots is related to a decrease in NO_3^- content [48]. High pH caused by alkali stress may inhibit the absorption of nitrate [49], thus weakening the positive role of NO_3^- in maintaining plant alkaline tolerance [50]. In addition, we found that when subjected to mixed alkali concentrations of $50 \text{ mmol}\cdot\text{L}^{-1}$ and $100 \text{ mmol}\cdot\text{L}^{-1}$, the Cl^- content in the roots of E+ increased then decreased, and the content of NO_3^- decreased and then increased, which also confirms that the increase in Cl^- content is related to the decrease in NO_3^- content. After alkaline stress, the content of SO_4^{2-} and NO_3^- in the roots decreased. Alkaline stress significantly affected the content of NO_3^- in E+ and E− roots. At $50 \text{ mmol}\cdot\text{L}^{-1}$, NO_3^- content in E+ was significantly lower than that in E−, whereas, at $100 \text{ mmol}\cdot\text{L}^{-1}$, NO_3^- content in E+ was significantly higher than that in E−, indicating that the presence of endophytic fungi can regulate the change in NO_3^- content in host plants to maintain the ionic balance after alkaline stress.

4.3. Effect of Alkali Stress on HbNHX1 Expression in *H. bogdanii* Roots and Shoots

Plants maintain ionic stability by controlling the expression of ion transporters. For example, many plants can help reduce Na^+ toxicity and affect the expression of Na^+ -related genes by symbiosis with fungi [51]. *NHX1* is a key gene involved in plant alkaline tolerance. Overexpression of *NHX1* has been shown to improve salt tolerance in many plants. The existence of beneficial fungus mycorrhizae affected the gene expression of host plants of Pakchoi (*Brassica campestris* ssp. *Chinensis*) under salt stress. Moreover, the expression level of *NHX1* was higher in root tissues in inoculated plants under both saline and non-saline stress [52]. In this study, it was found that the expression of *HbNHX1* was highest in the stems and leaves of E+ *H. bogdanii* treated with $100 \text{ mmol}\cdot\text{L}^{-1}$ alkaline, while the expression of *HbNHX1* was lower in the stems and leaves treated with $50 \text{ mmol}\cdot\text{L}^{-1}$, indicating that under high salt, the endophytic fungi promoted the host *H. bogdanii* to regionalize more Na^+ in the vacuoles, promoting the upregulation of *HbNHX1*. Diao [53] found that *Suaeda salsa* inoculated with *Arbuscular mycorrhizal* downregulated the expression of *SsNHX1* in roots under $100 \text{ mmol}\cdot\text{L}^{-1}$ NaCl. However, this study found that under alkaline stress, *HbNHX1* expression increased in the roots of *H. bogdanii* with endophytic fungi, and the expression of *HbNHX1* in E+ was significantly higher than that in E− under alkaline stress. The results demonstrated that endophytic fungi increased the expression of *HbNHX1* in the roots of *H. bogdanii*, and that different fungi had different regulatory effects on plants.

5. Conclusions

The results showed that *Epichloë* endophytic fungi promoted the upregulation of *HbNHX1* in the host *H. bogdanii* in an alkaline environment and participated in the regulation of anion and cation transport in the host plant. This study found that in an alkaline environment, *Epichloë* endophytic fungi reduced Na^+ toxicity in *H. bogdanii* by increasing *HbNHX1* gene expression, thereby regionalizing Na^+ in the vacuole membrane and contributing to the increase in K^+ and related anions to regulate the effect of alkaline stress. This conclusion provides basic data for the innovation of germplasm resources with endophytic fungi from *H. bogdanii*, provides a scientific basis and strong guarantee for the breeding of

new varieties and the healthy and stable development of grassland animal husbandry, and improves the stress tolerance of new varieties in alkaline environments.

Author Contributions: Conceptualization, F.L. and S.-H.C.; methodology, S.-H.C.; and G.-S.B.; software, F.L. and M.-F.H.; validation, F.L. and M.-F.H.; formal analysis, S.C.; investigation, F.L.; resources, S.-H.C.; data curation, H.D.; writing—original draft preparation, F.L.; writing—review and editing, F.L. and S.-H.C.; visualization, M.-F.H.; supervision, S.-H.C.; project administration, S.-H.C.; funding acquisition, S.-H.C. and G.-S.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the National Nature Science Foundation of Project (32260356; 31660689; 32060398; U21A20239) and the project is supported by the President’s Fund of Tarim University (TDZKZD202201).

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Acknowledgments: We are grateful to the National Natural Science Foundation of China and Tarim University for their financial support for this research and Cheng Liang Liang for helpful suggestions in writing the analyses.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

HbNHX1 gene sequence and coding amino acid sequence

Translation of >HbNHX1

ATGGCGTTCTCGAAGTGGTGGCGGCGCAGCTGGAGCGGCTGAGCGGCGCGCTGGGCAC(1-1617)

Total amino acid number: 538, MW=59196

Max ORF starts at AA pos 1(may be DNA pos 1) for 538 AA(1614 bases), MW=59196

1 ATGGCGTTCTCGAAGTGGTGGCGGCGCAGCTGGAGCGGCTGAGCGGCGCGCTGGGCACCTCG
 1 M A F E V V A A Q L E R L S G A L G T S
 61 GACCACGCCTCCGTGGTCTCCATCAACCTCTTCGTGCGGCTGCTCTGCGCCTGCATCATC
 21 D H A S V V S I N L F V A L L C A C I I
 121 CTCGGCCACCTCCTCGAGGAGAACCGCTGGCTCAACGAGTCCATCACCGCCCTCATCATC
 41 L G H L L E E N R W L N E S I T A L I I
 181 GGGCTGTGCACCGCGTGGTGATCCTGATGACCACCAAGGGAAGAGCTCGCACGTGCTC
 61 G L C T G V V I L M T T K G K S S H V L
 241 GTCTTCAGCGAGGACCTCTTCTTCATTTACCTCCTGCCCCCATCATCTTCAACGCCGGT
 81 V F S E D L F F I Y L L P P I I F N A G
 301 TTCCAGGTGAAGAAGAAGCAGTTCTTCCGGAATTTTCATGACAATCACATTATTCGGCGCT
 101 F Q V K K K Q F F R N F M T I T L F G A
 361 GTCGGGACGATGATTTCTTCTTCAATATCTCTCGCTGCCATCGCAATATTCAGCAGG
 121 V G T M I S F F T I S L A A I A I F S R
 421 ATGAACATGGGACACTGGATGTATCAGATTTTCTTGCAATTGGAGCCATCTTTTCCGCG
 141 M N I G T L D V S D F L A I G A I F S A
 481 ACAGATTCAGTCTGCACTTTGCGAGTTCTGAATCAGGATGAGACGCCCTTTTGTACAGT
 161 T D S V C T L Q V L N Q D E T P F L Y S
 541 CTAGTGTTCGGGGAAGGTGTTGTGAACGACGCCACATCAGTCGTGCTTTTCAACGCGCTC
 181 L V F G E G V V N D A T S V V L F N A L
 601 CAGAACTTCGATCCTAACCAAATCGATGCAATCGTCATTCTGAAGTTTTTGGGAACTTC
 201 Q N F D P N Q I D A I V I L K F L G N F
 661 TGCTACTTATTCGTGTCAAGCACCTTCTTGGAGTGTACTGGATTGCTCAGTGCATTC
 221 C Y L F V S S T F L G V F T G L L S A F
 721 GTCATCAAGAAGTTATACATAGGAAGGCATTCTACTGACCGTGAGGTTGCACTTATGATG
 241 V I K K L Y I G R H S T D R E V A L M M
 781 CTCATGGCCTACCTCTCATATATGCTAGCTGAGCTGCTGGATTTGAGTGGCATCCTCACT
 261 L M A Y L S Y M L A E L L D L S G I L T

841 GTATTTTTCTGTGGTATTGTGATGTCGCATTATACATGGCATAATGTCACAGAGAGCTCA
 281 V F F C G I V M S H Y T W H N V T E S S
 901 AGGGTTACAACAAAGCATGCGTTTGCAACCTTGTCTTCATCGCCGAGACTTTTCTCTTC
 301 R V T T K H A F A T L S F I A E T F L F
 961 CTTTATGTTGGGATGGATGCACTAGATATTGAGAAGTGGAATTTGCTAGTGACAGCCCT
 321 L Y V G M D A L D I E K W K F A S D S P
 1021 GGCAAATCCATCGGAATAAGCTCGATTTTGCTAGGATTGGTTCTGGTTGGGAGAGCTGCT
 341 G K S I G I S S I L L G L V L V G R A A
 1081 TTTGTCTCCCGCTTTCGTTCTTATCCAACCTGACAAAGAAGACGGAGCTCGAAAAATA
 361 F V F P L S F L S N L T K K T E L E K I
 1141 AGCTGGAGGCAGCAAGTCGTAATATGGTGGGCTGGGCTGATGAGAGGAGCTGTGTGATC
 381 S W R Q Q V V I W W A G L M R G A V S I
 1201 GCTCTTGCTTACAATAAGTTTACAAGATCTGGTCACACACAGCTACACGGCAACGCGATA
 401 A L A Y N K F T R S G H T Q L H G N A I
 1261 ATGATCACCAGCACCATCACTGTCGTTCTGTTTAGCACTATGCTGTTTGGCATTTTGACA
 421 M I T S T I T V V L F S T M L F G I L T
 1321 AAGCCTCTGATCCGGTTCTGCTGCCATATCGAGCAATGCCGACCCCTCGGAGCCCTCG
 441 K P L I R F L L P I S S N A D P S E P S
 1381 TCACCGAAGTCCCTGCACTCTCCTCCTCACAAGCATGCTAGGCTCGGACATGGAGGCG
 461 S P K S L H S P L L T S M L G S D M E A
 1441 CCTCTCCCATCGTCAGGCCCTCCAGCCTCCGGATGCTCATCACCAGCCGACCCACACC
 481 P L P I V R P S S L R M L I T K P T H T
 1501 ATCCACTACTACTGGCGCAAGTTTGACGACGCGCTGATGCGCCGATGTTGCGTGGGCGC
 501 I H Y Y W R K F D D A L M R P M F G G R
 1561 GGGTTCGTGCCCTTCTCCCCGGATCACCCACCGATCCAAACGTAATCGTGGCATGA
 521 G F V P F S P G S P T D P N V I V A *

References

- Zhu, J.K. Abiotic stress signaling and responses in plants. *Cell* **2016**, *167*, 313–324. [[PubMed](#)]
- Zhu, J.K. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol.* **2002**, *53*, 247–273. [[CrossRef](#)] [[PubMed](#)]
- Zhao, K.F.; Compiled, F.H. *Halophytes and Their Adaptive Physiology to Saline Habitats*; Science Press: Beijing, China, 2005; p. 297. ISBN 7-03-015662-5.
- Zhu, J.K. Plant salt tolerance. *Trends Plant Sci.* **2001**, *6*, 66–71. [[PubMed](#)]
- Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **2008**, *59*, 651–681. [[CrossRef](#)] [[PubMed](#)]
- Yan, H.; Zhao, W.; Sheng, Y.M.; Shi, D.C.; Zhou, D. Effects of alkali-stress on *Aneurolepidium Chinense* and *Helianthus annuus*. *Ying Yong Sheng Tai Xue Bao* **2005**, *16*, 1497–1501.
- Yang, C.; Chong, J.; Li, C.; Kim, C.; Shi, D.; Wang, D. Osmotic adjustment and ion balance traits of an alkali resistant halophyte *Kochia sieversiana* during adaptation to salt and alkali conditions. *Plant Soil* **2007**, *294*, 263–276. [[CrossRef](#)]
- Shi, H.; Quintero, F.J.; Pardo, J.M.; Zhu, J. The putative plasma membrane Na⁺/H⁺ antiporter SOS1 controls long-distance Na⁺ transport in plants. *Plant Cell* **2002**, *14*, 465–477. [[CrossRef](#)]
- Kronzucker, H.J.; Britto, D.T. Sodium transport in plants: A critical review. *New Phytol.* **2011**, *189*, 54–81. [[CrossRef](#)]
- Fukuda, A.; Nakamura, A.; Hara, N.; Toki, S.; Tanaka, Y. Molecular and functional analyses of rice NHX-type Na⁺/H⁺ antiporter genes. *Planta* **2011**, *233*, 175–188. [[CrossRef](#)]
- Barragan, V.; Leidi, E.O.; Andres, Z.; Rubio, L.; De Luca, A.; Fernandez, J.A.; Cubero, B.; Pardo, J.M. Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in *Arabidopsis*. *Plant Cell* **2012**, *24*, 1127–1142.
- Pérez-Martín, L.; Busoms, S.; Almira, M.J.; Azagury, N.; Terés, J.; Tolrà, R.; Poschenrieder, C.; Barcelo, J. Evolution of salt tolerance in *Arabidopsis thaliana* on siliceous soils does not confer tolerance to saline calcareous soils. *Plant Soil* **2022**, *476*, 455–475. [[CrossRef](#)]
- Rho, H.; Hsieh, M.; Kandel, S.L.; Cantillo, J.; Doty, S.L.; Kim, S. Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. *Microb. Ecol.* **2018**, *75*, 407–418. [[CrossRef](#)]
- Molina-Montenegro, M.A.; Acuña-Rodríguez, I.S.; Torres-Díaz, C.; Gundel, P.E.; Dreyer, I. Antarctic root endophytes improve physiological performance and yield in crops under salt stress by enhanced energy production and Na⁺ sequestration. *Sci. Rep.* **2020**, *10*, 5819. [[CrossRef](#)]
- Schardl, C.L.; Leuchtman, A.; Spiering, M.J. Symbioses of grasses with seedborne fungal endophytes. *Annu. Rev. Plant Biol.* **2004**, *55*, 315–340. [[CrossRef](#)]

16. Ren, A.Z.; Gao, Y.B.; Zhang, J.; Zhang, J. Effect of endophyte infection on salt resistance of ryegrass. *Acta Ecologica Sinica*. **2006**, *26*, 1750–1757.
17. Shi, C.; Huang, W.; Wang, C.L. Effects of endophytic fungi on salt tolerance of *Elymus dahuricus*. *J. Xinjiang Agric. Univ.* **2016**, *4*, 277–280.
18. Leuchtman, A.; Bacon, C.W.; Schardl, C.L.; White Jr, J.F.; Tadych, M. Nomenclatural realignment of Neotyphodium species with genus *Epichloë*. *Mycologia* **2014**, *106*, 202–215. [[CrossRef](#)]
19. Malinowski, D.P.; Belesky, D.P. Adaptations of endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance. *Crop Sci.* **2000**, *40*, 923–940. [[CrossRef](#)]
20. Ma, R.C.; Zhang, H.S.; Mu, L.T.; Song, S.J. Regional test of *Hordeum Bogdanii*. *J. Xinjiang Agric. Univ.* **1998**, *21*, 35–38.
21. Chen, T.X. Physiological Mechanism of *Epichloë* Endophyte Infection to Enhance Salt Tolerance of Wild Barley. Ph.D. Thesis, Lanzhou University, Lanzhou, China, 2019.
22. Zhang, E.H.; Yu, X.H.; Da, Y.Q.; Zhao, Y.P.; Wan, G.A.; Xu, Z.X. Classification, identification and biological and physiological characteristics of an endophytic fungus in *Hordeum Bogdanii*. *Q. J. Anim. Husb. Vet. Med.* **2021**, *51*, 1–7.
23. Han, D.; Chen, S.H. Effects of diesel oil contaminated soil on reproductive growth of *Hordeum Bogdanii*. *J. Tarim Univ.* **2021**, *33*, 106–112.
24. Wang, K.; Lin, L.D.; Long, F.; Chen, S.H. Effect of endophytic fungi on fiber and crude fat content of *Hordeum bogdanii* during seed setting under alkali stress. *Heilongjiang Anim. Husb. Vet.* **2022**, 99–104.
25. Wang, K.; Yang, B.Y.; Chen, S.H.; Xi, L.Q. Effects of endophytic fungi on photosynthetic performance and physiological characteristics of *Hordeum bogdanii* under alkali stress. *Acta Agrestia Sin.* **2022**, *30*, 362–369.
26. Long, F.; Wang, K.; Zhu, Y.B.; Chen, S.H. Effects of *Epichloë* endophytic on the growth and physiology of *Hordeum bogdanii* under salt stress. *Heilongjiang Anim. Husb. Vet.* **2022**, 13–17.
27. Li, C.J.; Nan, Z.B.; Liu, Y.; Paul, V.H.; Peter, D. Methodology of Endophyte Detection of Drunken Horse Grass (*Achnatherum inebrians*). In *Hangzhou Joint Annual Meeting of Chinese Society of Plant Diseases and Fungi*; Editorial Department of Chinese Edible Fungi: Beijing, China, 2008; pp. 21–24.
28. Matsushita, N.; Matoh, T.M. Characterization of Na⁺ exclusion mechanisms of salt-tolerant reed plants in comparison with salt-sensitive rice plants. *Physiol. Plant.* **1991**, *83*, 170–176. [[CrossRef](#)]
29. Ma, Y.; Guo, L.Q.; Zhang, S.F.; Wang, X.P.; Shi, D.C. Solute accumulation and distribution traits of an alkali resistant forage plant *kochia sieversiana* and physiological contribution of organic acid under salt and alkali stresses. *Acta Prataculturae Sin.* **2013**, *22*, 193–200.
30. Vinje, M.A.; Willis, D.K.; Duke, S.H.; Henson, C.A. Differential RNA expression of Bmy1 during barley seed development and the association with β -amylase accumulation, activity, and total protein. *Plant Physiol. Biochem.* **2011**, *49*, 39–45. [[CrossRef](#)]
31. Fatehi, F.; Hosseinzadeh, A.; Alizadeh, H.; Brimavandi, T.; Struik, P.C. The proteome response of salt-resistant and salt-sensitive barley genotypes to long-term salinity stress. *Mol. Biol. Rep.* **2012**, *39*, 6387–6397. [[CrossRef](#)]
32. Li, R.F.; Wang, X.Q.; Wang, H.Z. Adaptive mechanisms of salt tolerance in *Hordeum brevisubulatum*. *Sci. Agric. Sin.* **2006**, *39*, 2459–2466.
33. Lee, K.; Missaoui, A.; Mahmud, K.; Presley, H.; Lonnee, M. Interaction between grasses and *Epichloë* endophytes and its significance to biotic and abiotic stress tolerance and the rhizosphere. *Microorganisms* **2021**, *9*, 2186. [[CrossRef](#)]
34. Byregowda, R.; Prasad, S.R.; Oelmüller, R.; Nataraja, K.N.; Prasanna Kumar, M.K. Is endophytic colonization of host plants a method of alleviating drought stress? Conceptualizing the hidden world of endophytes. *Int. J. Mol. Sci.* **2022**, *23*, 9194. [[CrossRef](#)]
35. Ma, M.; Christensen, M.J.; Nan, Z. Effects of the endophyte *Epichloë festucae* var. *lolii* of perennial ryegrass (*Lolium perenne*) on indicators of oxidative stress from pathogenic fungi during seed germination and seedling growth. *Eur. J. Plant Pathol.* **2015**, *141*, 571–583. [[CrossRef](#)]
36. Siegel, M.R.; Johnson, M.C.; Varney, D.R.; Nesmith, W.C.; Buckner, R.C.; Bush, L.P.; Burrus, P.B.; Jones, T.A.; Boling, J.A. A fungal endophyte in tall fescue: Incidence and dissemination. *Phytopathology* **1984**, *74*, 932–937. [[CrossRef](#)]
37. Song, M.L. Mechanisms of Salt Tolerance Improved by *Epichloë* Endophyte in Wild Barley. Ph.D. Thesis, Lanzhou University, Lanzhou, China, 2015.
38. Chen, T.X.; Richard, J.; Chen, S.; Lv, H.; Zhou, J.; Li, C. Infection by the fungal endophyte *Epichloë bromicola* enhances the tolerance of wild barley (*Hordeum brevisubulatum*) to salt and alkali stresses. *Plant Soil* **2018**, *428*, 353–370. [[CrossRef](#)]
39. Liu, L.; Nakamura, Y.; Taliman, N.A.; Sabagh, A.E.; Moghaieb, R.E.; Saneoka, H. Differences in the growth and physiological responses of the leaves of *Peucedanum japonicum* and *Hordeum vulgare* exposed to salinity. *Agriculture* **2020**, *10*, 317. [[CrossRef](#)]
40. Blumwald, E. Sodium transport and salt tolerance in plants. *Curr. Opin. Cell Biol.* **2000**, *12*, 431–434. [[CrossRef](#)]
41. Parida, A.K.; Das, A.B. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.* **2005**, *60*, 324–349. [[CrossRef](#)]
42. Ghoulam, C.; Foursy, A.; Fares, K. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ. Exp. Bot.* **2002**, *47*, 39–50. [[CrossRef](#)]
43. Santa-Cruz, A.; Martinez-Rodriguez, M.M.; Perez-Alfocea, F.; Romero-Aranda, R.; Bolarin, M.C. The rootstock effect on the tomato salinity response depends on the shoot genotype. *Plant Sci.* **2002**, *162*, 825–831. [[CrossRef](#)]
44. Sagi, M.; Dovrat, A.; Kipnis, T.; Lips, H. Ionic balance, biomass production, and organic nitrogen as affected by salinity and nitrogen source in annual ryegrass. *J. Plant Nutr.* **1997**, *20*, 1291–1316. [[CrossRef](#)]

45. Graham, J.H.; Syvertsen, J.P. Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. *New Phytol.* **1984**, *97*, 277–284. [[CrossRef](#)]
46. Buwalda, J.G.; Stribley, D.P.; Tinker, P.B. Increased uptake of bromide and chloride by plants infected with vesicular-arbuscular mycorrhizas. *New Phytol.* **1983**, *93*, 217–225. [[CrossRef](#)]
47. Yang, J.Y.; Zheng, W.; Tian, Y.; Wu, Y.; Zhou, D.W. Effects of various mixed salt-alkaline stresses on growth, photosynthesis, and photosynthetic pigment concentrations of *Medicago ruthenica* seedlings. *Photosynthetica* **2011**, *49*, 275–284. [[CrossRef](#)]
48. Teakle, N.L.; Tyerman, S.D. Mechanisms of Cl-transport contributing to salt tolerance. *Plant, Cell Environ.* **2010**, *33*, 566–589. [[CrossRef](#)]
49. Yang, C.; Shi, D.; Wang, D. Comparative effects of salt and alkali stresses on growth, osmotic adjustment and ionic balance of an alkali-resistant halophyte *Suaeda glauca* (Bge.). *Plant Growth Regul.* **2008**, *56*, 179–190. [[CrossRef](#)]
50. Ding, X.; Tian, C.; Zhang, S.; Song, J.; Zhang, F.; Mi, G.; Feng, G. Effects of NO₃⁻-N on the growth and salinity tolerance of *Tamarix laxa* Willd. *Plant Soil* **2010**, *331*, 57–67. [[CrossRef](#)]
51. Santander, C.; Aroca, R.; Ruiz-Lozano, J.M.; Olave, J.; Cartes, P.; Borie, F.; Cornejo, P. Arbuscular mycorrhiza effects on plant performance under osmotic stress. *Mycorrhiza* **2017**, *27*, 639–657. [[CrossRef](#)]
52. Khalid, M.; Hassani, D.; Liao, J.; Xiong, X.; Bilal, M.; Huang, D. An endosymbiont *Piriformospora indica* reduces adverse effects of salinity by regulating cation transporter genes, phytohormones, and antioxidants in *Brassica campestris* ssp. *Chinensis*. *Environ. Exp. Bot.* **2018**, *153*, 89–99. [[CrossRef](#)]
53. Diao, F.W. Effects of Arbuscular Mycorrhizal Fungi on Salt Tolerance of *Suaeda Salsa* in Molecular Mechanism. Ph.D. Thesis, Inner Mongolia University, Hohhot, China, 2021.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.