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OPEN Effects of brackish water irrigation with different exogenous salt concentrations on the growth and rhizosphere salinity of Lycium barbarum

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To investigate the effects of different typical exogenous salt concentrations on total soil salinity and the growth of Lycium barbarum under brackish water irrigation, and to determine the salinity threshold of irrigated brackish water that is conducive to the normal growth of Lycium barbarum while mitigating soil salinity accumulation. Four typical exogenous salts (NaCl, CaCl₂, NaHCO₃, Na₂SO₄) were selected and set at four concentrations (0.1, 0.5, 2.0, 4.0 q L⁻¹) to conduct a field crossover experiments in the downstream region of the Hetao Irrigation District. The results showed that in the same fertility period, the growth rates of new branches, ground diameter, and crown width first increased and then decreased with rising concentrations of NaCl, CaCl₂, and Na₂SO₄, but showed an inverse relationship with NaHCO₃ concentrations. Furthermore, increasing salt concentrations linearly reduced the yield of dry fruits from Lycium barbarum and led to a notable accumulation of total soil salts. Utilizing an experimental research approach, a comprehensive analysis of involving multiple growth indices, stable yield, and soil salinity control of Lycium barbarum revealed that optimal growth occurs at salt concentrations of 0.1-0.5 q L⁻¹ for different water quality areas within the irrigation area; using the method of path analysis identified the total soil salt and crown width as the primary direct and indirect factors influencing the yield of Lycium barbarum. The results of this study provide scientific basis and significant theoretical support for the safe and rational utilization of brackish water and cultivation of Lycium barbarum in typical regions with varying saline water qualities of Hetao irrigation area.

Keywords Brackish water, Typical salt, Lycium barbarum, Path analysis

Hetao Irrigation District, as Asia's largest one-system irrigation district, serves as a crucial production base for commodity grains and oils for both the country and Inner Mongolia Autonomous Region¹. The irrigated area of the district encompasses 5.7×10^5 hm²², with the primary source of irrigation water being the Yellow River diversion. In recent years, the implementation of national water conservation directives has resulted in a decline in the average annual volume of water diverted from the Yellow River for irrigation, decreasing from approximately 5×10^9 m³ year by year to the 4×10^9 m³ currently, which fails to meet the agricultural irrigation needs of the region^{3,4}. The lower reaches of the river, in particular, do not receive adequate irrigation at critical times, necessitating the identification of alternative water sources. The Hetao irrigation area is rich in underground brackish water (2-5 g L⁻¹), presenting significant development and utilization potential⁵. The scientific and reasonable exploitation of brackish water resources is not only conductive to crop growth, but also effectively mitigates the imbalance between water supply and demand^{6,7}. However, inappropriate salinity associated with brackish water irrigation can adversely affect both crops and the soil. For instance, when irrigated with water having a mineralization greater than 4 g L⁻¹, the high concentration of salt will inhibits the root activity of cotton, resulting in a decline in plant height, stem thickness, and leaf area content8. Wheat seed germination faces a critical salinity threshold of 3 g L⁻¹, as salt concentration increases, the salt infiltrated into the soil, is absorbed

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by the root system and diffuses, leading to reduced of plant height and yield of wheat 9,10 . Similarly, irrigation with brackish water containing 2 g L $^{-1}$ NaCl results in lower water utilization, causing salt accumulation in the soil and water deficit in cucumber, which leads to a 10.35% to 18.34% decrease in yield compared to freshwater irrigation 11 . Therefore, achieving the safe use of brackish water is of paramount importance for enhancing water conservation and irrigation efficiency in the region.

Lycium barbarum is notable for its has biological characteristics of drought resistance, salt tolerance, and significant economic value. It is extensively cultivated in regions like Ningxia and Inner Mongolia. The cultivating area in the Hetao irrigation area reaching as high as 1.33×10^4 hm²¹². As a homologous crop of medicine and food, Ningxia Lycium barbarum was included in the Pharmacopoeia of the People's Republic of China in 2020¹³. Nonetheless, the growth response of Lycium barbarum to various types and concentrations of brackish water salinity varies. Guo Huan et al. 14 found that growth is adversely affected when salinity exceeds the salt tolerance threshold of 2.425 g L^{-1} , with a notable reduction in yield observed at 3 g L⁻¹¹⁵. Salt stress hinders crop development across various stage, with significant growth inhibition occurring at concentrations greater than 200 mM¹⁶. Therefore, establishing a suitable typical salt concentration limit for brackish water irrigation that supports the growth of Lycium barbarum is crucial. Hetao Irrigation District is a typical salinized agricultural irrigation district⁸, the salt content of the soil-forming parent material is high, experiences exacerbated soil degradation under brackish water irrigation¹⁷. Liu Zhenyuan et al. ¹⁰observed an increase in soil salinity with rising salt concentrations, and infiltration following irrigation with NaCl and CaCl₂ at concentrations of 3 g L⁻¹ and 5 g L⁻¹ enhanced soil salt accumulation¹⁸. In summary, different concentrations of brackish water irrigation differently impact soil salinity, physicochemical properties, and crop growth. Due to its inherent salt resistance, Lycium barbarum is ideally suited for cultivation in brackish water-irrigated areas, representing an optimal cash crop to alleviate the scarcity of freshwater resources and the pressures of agricultural production in the Hetao irrigation area. The prevalent brackish water types in the Hetao irrigation area include HCO₃-Na, SO₄-Na, and Cl-Na¹⁹. Among these, the cations are predominantly Na⁺ and Ca²⁺, and Ca²⁺ is the dominant ion in the groundwater of the irrigation area; anions are dominated by Cl⁻, followed by SO₄²⁻ and HCO₃^{-20,21}. Current research primarily focuses on the impact of individual typical salts²²⁻²⁴ and mixed salts^{25,26} on the fruit quality¹⁴, growth structure²², and photosynthetic activity²⁷ of Lycium barbarum, as well as on seed germination and seedling growth. Studies on soil salinity generally concentrate on brackish water irrigation methods²⁸, control of irrigation water quantity, and salinity²⁹. However, comprehensive investigations into the effects of different salinity types on rhizosphere salinity distribution, growth conditions, and yield of Lycium barbarum during the high yielding growth period are less frequently reported, indicating a need for further research. Therefore, this study conducted a field cross test of 4 typical salts and 4 concentration levels for moderately salinized farmland in the Hetao irrigation area to investigate the effects of different typical exogenous salt concentrations on total soil salinity and the growth of Lycium barbarum under brackish water irrigation, and to determine the salinity threshold of irrigated brackish water that is suitable for the normal growth of Lycium barbarum and for alleviating of soil salinity accumulation. The results of the study can provide an important scientific basis for the safe and rational utilization of brackish water and the cultivation of *Lycium barbarum* in areas with varying saline water qualities of Hetao irrigation area.

Materials and methods Overview of the study area

The experiment was conducted at the Hongwei Experimental Station, located in the downstream of the Hetao Irrigation District ($108^{\circ}45'-109^{\circ}36'E$, $40^{\circ}30'-40^{\circ}40'N$) at an altitude of 1040 m. This area is characterized by scarce freshwater resources and higher availability of brackish water, with a mineralization degree of 3.84 g L⁻¹ used for irrigation (Table 1). The climate is classified as a medium-temperate zone with semi-arid continental characteristics. The area experiences an annual average precipitation of 181.95 mm and evaporation of 2383 mm, with an average annual temperature of 7.9° C (Fig. 1). The frost-free period lasts for 146 days. The soil in this region is moderately saline with total salt content ranging from 0.48 to 0.84% (Table 2).

Experimental design

The experiment employed the "Ningqi No. 1" variety of *Lycium barbarum*, now 12 years old, and utilized a randomized block design for a two-factor crossover test involving four typical salts (NaCl, CaCl₂, NaHCO₃ and Na₂SO₄) at four concentration gradients. Within the experimental area, 37 *Lycium barbarum* plants were planted per row, with a plant spacing of 1 m and a row spacing of 3 m. To ensure experimental integrity, 2 plants were positioned at the beginning and end of each plot, and every other row was planted to establish a buffer zone. The remaining 5 plants in each row were successively planted to form an experimental unit measuring 3.0 m × 13 m. The plots were isolated by a 1.5-m-high, seepage-proof plastic sheet. The study included 17 treatment groups, each replicated 3 times, making a total of 51 plots, each covering an area of 39 m². *Lycium barbarum* was irrigated according to the local conventional volume, with 4 irrigation events during the growth period, each delivering 100 mm of water via border irrigation. Table 3 provides detailed information. For this experiment, the range of ion concentrations in the brackish water of the Hetao irrigation area downstream was used as a reference ^{19,30}, with ion concentrations of Na⁺, Ca²⁺, Cl⁻, SO₄²⁻, and HCO₃⁻ ranging from 13.8–100.4, 0.9–13.3, 11.5–108.8,

Norm	CO ₃ ²⁻	HCO ₃ -	Cl-	SO ₄ ²⁻	Ca ²⁺	Mg ²⁺	K++ Na+	Total salt(g L ⁻¹)	
Content	0	0.33	2.03	0.60	0.25	0.21	0.42	3.84	

Table 1. Irrigation of brackish water quality before the test.

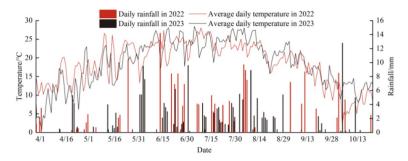


Fig. 1. Daily rainfall and temperature during the fertility period of *Lycium barbarum*.

	Clay	Silt	Sand	Capacity	acity Soil type	
Soil depth m	0-0.002 mm (%)	0.002-0.05 mm (%)	0.05-2 mm (%)	g cm ⁻ 3		
0-0.1	9.57	73.41	17.53	1.36	粉壤土	
0.1-0.2	11.02	71.19	17.79	1.33	粉壤土	
0.2-0.3	3.19	84.79	12.02	1.37	粉土	
0.3-0.4	2.98	87.24	9.78	1.46	粉土	
0.4-0.6	2.76	86.63	10.62	1.44	粉土	
0.6-0.8	4.22	86.99	8.80	1.42	粉土	
0.8-1	2.40	79.47	18.14	1.39	粉壤土	

Table 2. Soil texture before the test.

Processing code	Typical Salt	Salt concentration(g L-1)
NC1		0.1
NC2	NaCl	0.5
NC3	NaCi	2.0
NC4]	4.0
NS1		0.1
NS2	No SO	0.5
NS3	Na ₂ SO ₄	2.0
NS4		4.0
CC1		0.1
CC2	CaCl ₂	0.5
CC3	CaCl ₂	2.0
CC4		4.0
NHC1		0.1
NHC2	NaHCO ₃	0.5
NHC3	NaticO ₃	2.0
NHC4		4.0
CK	None	

Table 3. Experimental design. The types of salts added to the brackish water in the table "NC", "NS", "CC" and "NHC" represent NaCl, Na_2SO_4 , $CaCl_2$ and $NaHCO_3$. 1, 2, 3 and 4 correspond to the concentrations of 0.1, 0.5, 2.0, and 4.0 g L^{-1} , respectively.

1.7-23.6, and 4.0-32.5 mmol L^{-1} , respectively. Based on the water quality of the experimental site (with the ion concentrations of Na⁺, Ca²⁺, Cl⁻, SO₄²⁻ and HCO₃⁻ being 18.6, 8.8, 63.0, 6.3 and 5.5 mmol L^{-1} , respectively), salt concentrations were adjusted for different treatments. NaCl, CaCl₂, NaHCO₃, and Na₂SO₄ were added to the local brackish water (3.84 g L^{-1}) at rates of 0.1, 0.5, 2.0, and 4.0 g L^{-1} . The resulting ion concentration for each treatment fell within the upper limit of the salt ion concentration range found in the experimental and Hetao irrigation areas, providing a reference for the study's significance.

Measurement indices and methods

In this experiment, physiological indices were assessed during the spring shoot growth period (A1), early flowering stage (A2), fruit expansion stage (A3), and prime fruiting period (A4). (Table 4) For each treatment, three plants exhibiting uniform growth were selected for observation. New growth branches were tagged and left unpruned, while other non-essential branches were pruned approximately every 10 days throughout the growth period.

- (1) New branch growth: One new branch was selected from the east, south, west, north, and middle of each plant for measurement using a tape measure. Measurements were taken every 3 days from April 20 to early July, and thereafter every 10 days until leaf fall.
- (2) Crown width: measured east—west and north—south, was recorded with a tape measure prior to budding and subsequently in the middle of each month until the end of fruit harvesting.
- (3) Ground diameter: The ground diameter was measured using a vernier caliper before budding. Subsequent measurements were taken once in the middle of each month, continuing until the end of the fruit harvest.
- (4) Yield: 4 trees with similar growth potential were selected for each treatment for yield assessment. Harvested *Lycium barbarum* fruits were arranged by number on a fruit stack and uniformly dried in a heat pump dryer using a coal fire. The drying room was maintained at humidity of 25% and a temperature of 60–70°C. The fruits were weighed every 2 h, with the drying process lasting about 8 h or until the weight of the *Lycium barbarum* remained unchanged. The dry mass of *Lycium barbarum* from each district was then weighed to determine the yield of dried fruit per unit area.
- (5) Soil total salt content: Soil samples were taken before and after each irrigation during the crop's growth period using a soil auger. Samples were collected from 0 to 0.4 m at every 0.1 m and from 0.4 to 1 m at every 0.2 m, resulting in a total of 7 layers. The content of eight ions (Na⁺, K⁺, Mg²⁺, Ca²⁺, Cl⁻, SO₄²⁻, and HCO₃) in the soil was analyzed according to *LY/T 1251–1999*, *Analysis of Water-soluble salinity in Forest soil*. Soil samples were air-dried, ground and sieved, and a clear liquid was extracted at a soil-to-water ratio of 1:5. The salt ions in the soil solution were analyzed using conventional methods: Cl⁻ was measured through standard silver nitrate titration; water-soluble Na⁺ was assessed using NaOH melt-flame spectrophotometry; K⁺ was extracted via ultrasonic extraction, followed by solid phase extraction and filtrate volume determination; ion chromatography was employed for Ca²⁺ and Mg²⁺, which were determined using EDTA complexometric titration; HCO₃⁻ was quantified through dual indicator-neutralization titration; and SO₄²⁻ was analyzed using indirect titration with EDTA. The sum of these eight ions represented the total salt content of the soil.
- (6) The local brackish water (3.84 g L⁻¹): Prior to sampling, the 500 mL sampling bottle was thoroughly rinsed with distilled water multiple times and dried for future use. The bottle was then rinsed with groundwater samples three to four times to eliminate potential contaminants before subpacking the samples. Following this, filled the bottle with water samples, sealed with a sealing film, taken 3 parallel samples, label them, and send them to Bayannur Institute of Water Conservancy Science for determination within 24 h. Strictly follow the standard determination method proposed by the Ministry of Health of the People's Republic of China (GB/T 5750–2006)³¹.

Data analysis

Each treatment was consisted of three replicates, and the data were presented as mean. Data were processed, plotted, and analyzed using Excel 2020 and Origin 2021 software, while SPSS software (version SPSS 26.0) was utilized for correlation analysis and variance analysis, the least significant difference (LSD) method was used for significance test, and the significance level was 0.05.

Path analysis, based on regression and correlation analysis, established interrelationships between multiple independent variables and the dependent variable, thereby revealing the direct and indirect effects of independent variables on the dependent variable and quantifying their influence.

Results and analysis

Response of physiological traits of *Lycium barbarum* to exogenous salt concentration *Growth rate of new branches*

The growth rate of new branches of *Lycium barbarum* showed consistent trends across all four typical salts $(Na_2SO_4, NaCl, CaCl_2, and NaHCO_3)$ added to brackish water (Fig. 2). For instance, using Na_2SO_4 (Fig. 2a) for detailed analysis, it was found that within a certain salt concentration, significant differences (P < 0.05) were observed in the growth rate of new branches across different fertility periods. The growth rate decreased over time and was 10.86%-15.67%, 26.1%-33.73%, and 61.33%-71.63% lower in the A2, A3, and A4 periods, respectively, compared to the A1 period. The growth rate in A1 increased and then decreased with the rising

spring shoot growth period	Early flowering stage	Fruit expansion stage	Prime fruiting period		
0503-0515	0610-0620	0625-0710	0715-0815		

Table 4. Phenological period of *Lycium barbarum* in the experimental area.

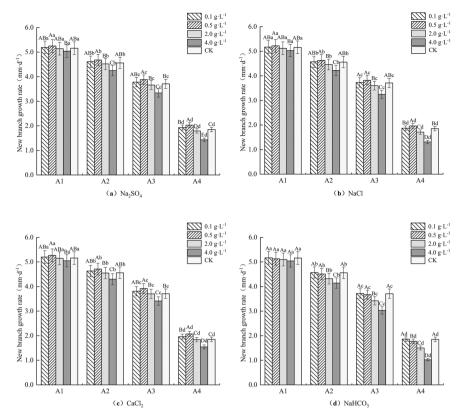


Fig. 2. Effects of different salt concentrations on the growth rate of new branches of *Lycium barbarum*. Different uppercase letters indicate significant differences at the 5% level between different concentrations in the same growth period, and different lowercase letters denote significant differences between different growth periods at the same concentration level, P < 0.05. This notation applies throughout the paper.

salt concentration, peaking at 5.25 mm d $^{-1}$ with a 0.5 g L $^{-1}$ concentration (NS2). This rate was 1.16%, 2.14%, 4.17%, and 1.74% higher than NS1, NS3, NS4, and CK treatments, respectively, with a significant difference noted with NS4 (P < 0.05). Similarly, for NaCl and CaCl $_2$ during A1, the maximum growth rate was observed at a concentration of 0.5 g L $^{-1}$, being 5.22 mm d $^{-1}$ and 5.27 mm d $^{-1}$, respectively. However, NaHCO $_3$ showed an inverse relationship with salt concentration in A1, peaking at 0.1 g L $^{-1}$ (NHC1), which rates higher by 0.19%, 0.78%, 1.37%, and 2.58% compared to CK, NHC2, NHC3, and NHC4, respectively. The remaining changes were consistent with the trend observed for Na $_2$ SO $_4$.

Growth rate of ground diameter

The growth rates of ground diameter under the influence of four typical salts (Na₂SO₄, NaCl, CaCl₂, and NaHCO₃) in brackish water showed similar trends (Fig. 3). Taking NaCl as an example (Fig. 3b), a significant difference was observed in the growth rates across different fertility periods, demonstrating a single-peak distribution. Specifically, there was a 12.28% decrease in the A2 period compared to the A1 period, a 70% decrease in the A4 period compared to the A3 period, and a 44.31% increase in the A3 period compared to the A2 period (P < 0.05). The peak growth rate occurred in the A3 period, averaging 0.498 mm d⁻¹, which initially increased and then decreased with rising salt concentrations. At concentrations of 2.0 and 4.0 g L⁻¹ (NC3 and NC4), the growth rate significantly decreased by 3.52% and 7.63%, respectively, compared to CK (P < 0.5). In contrast, at lower concentrations of 0.1 and 0.5 g L⁻¹ (NC1 and NC2), the differences from CK was not statistically significant (P > 0.5). When the salt concentration of A3 stage was 0.5 g/L and 0.1 g L⁻¹, the growth rate of ground diameter varied with different salt types. At a 0.5 g L⁻¹ concentration during the A3 period, the growth rates for Na₂SO₄, NaCl, and CaCl₂ achieved their maxima (0.519, 0.513, and 0.524 mm d⁻¹, respectively), which were 1.57%, 0.39%, and 2.54% higher than CK, respectively. Meanwhile, NaHCO₃ reached its maximum of 0.512 mm d⁻¹ at a concentration of 0.1 g L⁻¹ (NHC1), showing an increase of 0.20% over CK.

Growth rate of Crown width

The crown width was quantified as the mean of the maximum diameters measured in the east–west and north–south directions of a single $Lycium\ barbarum\ plant$. The growth rates of crown width under different concentrations of the four typical salts (Na₂SO₄, NaCl, CaCl₂, and NaHCO₃) followed a consistent pattern throughout the reproductive period (e.g., Fig. 4). For example, using CaCl₂ (Fig. 4c), influenced by the salt concentration , the crown growth rate varies across different fertility periods (1.28–4.09 mm d⁻¹). Under the same concentration, the rate gradually decreased with the progression of the fertility period. The highest average rate of 4.00 mm d⁻¹

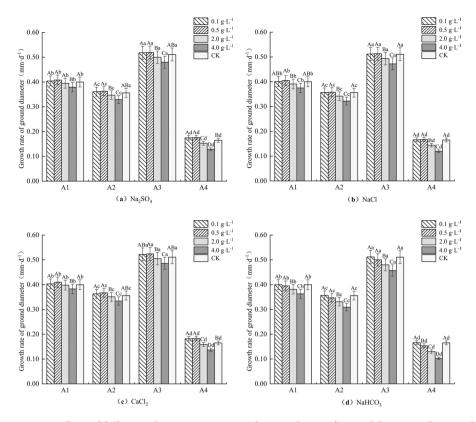


Fig. 3. Effects of different salt concentrations on the growth rate of ground diameter of Lycium barbarum.

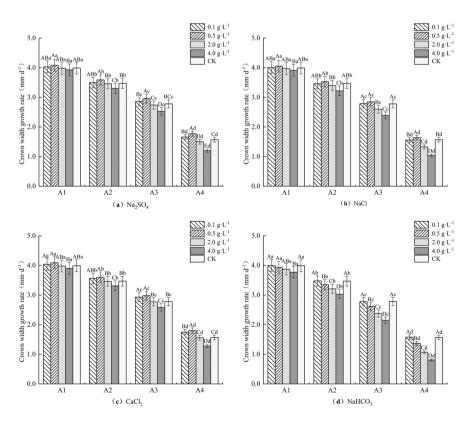


Fig. 4. Effects of different salt concentrations on the growth rate of Lycium barbarum crown width.

was observed in the A1 period, which exhibited an initial increase followed by a decrease as $CaCl_2$ concentration increased. The maximum rate of 4.09 mm d⁻¹ was observed at a concentration of 0.5 g L⁻¹ (CC2 treatment), which was NaHCO₃ concentration in the A1 stage was significantly different compared to other salts. The crown growth rate was inversely related to the NaHCO₃ concentration and peaked at 4.00 mm d⁻¹ at a concentration of 0.1 g L⁻¹ in the A1 period, which was 0.38%, 1.78%, 3.49% and 6.10% higher than that of the CK, NHC2, NHC3, and NHC4 treatments, respectively.

Effects of different typical exogenous salt concentrations on dry fruit yield of Lycium barbarum

As can be seen in Fig. 5, the dry fruit yield of *Lycium barbarum* decreased with increasing concentrations of NaCl, Na₂SO₄, CaCl₂, and NaHCO₃. The effect of different salt concentrations on dry fruit yield was statistically significant (P < 0.5). At a concentration of 0.1 g L⁻¹ (CC1, NS1, NC1, and NHC1 treatments), the yields were 4698.41, 3878.44, 3086.42, and 2710.48 kg hm⁻², respectively, representing the highest yields recorded. Notably, the CC1 treatment increased dry fruit yield by 11.90% compared to CK, conversely, the yields in NS1, NC1, and NHC1 treatments decreased by 7.63%, 26.49%, and 35.45%, respectively, demonstrating the superior effect of Ca²⁺ over Na⁺ in enhancing yield³².

Effects of different typical exogenous salt concentrations on total salt in farmland soil under Lycium barbarum cultivation

Soil total salt content is a critical indicator of soil salinization. Figure 6 illustrates the distribution of total salt content in the 0-1 m soil layer under different typical salt concentrations throughout the entire fertility period of Lycium barbarum under brackish water irrigation. The trend of total soil salinity was consistent across different salt concentrations treatments (NaCl, Na₂SO₄, CaCl₂, and NaHCO₃) irrigation *Lycium barbarum*. Analyzing NaHCO₃ in detail, At the same concentration level during the whole growth period, the total salt content from the 0 to 1 m soil layer followed an order across different fertility periods: A3 > A4 > A2 > A1. During the A1 period, under different concentrations of NaHCO₃, the salt content ranged from 4.382 to 7.348 g kg⁻¹. The accumulation of salt increased with the concentration, and the soil total salt content for the NHC1 treatment (0.1 g L⁻¹) had the lowest value, averaging 5.206 g kg⁻¹. The following treatments-NHC2, NHC3, and NHC4-showed increments of 20.87%, 31.17%, 49.24%, and 56.34% higher than CK, respectively. The soil salt content in the NHC1 treatment during the A1 period showed fluctuations with increasing soil depth. The total soil (0-0.1 m) showed superficial accumulation, being 4.83%, 15.32%, and 31.54% higher than at depths of 0.2, 0.3, and 0.4 m, respectively. The 0.3-0.4 m soil layer presented a low peak, with the smallest salt content recorded at 5.254 g kg⁻¹ in the NHC1 treatment. The 0.4–0.6 m layer, more sensitive to salt changes, showed a slight increase, averaging 6.567 g kg⁻¹, which was 6.65% higher than the 0.3-0.4 m layer. Finally, the 0.6-1 m exhibited a decreasing trend, with salt content ranging from 4.382 to 5.222 g kg⁻¹.

Path analysis of growth indexes, soil total salt, and yield of Lycium barbarum

To further elucidate the direct and indirect effects of various indicators on the yield of *Lycium barbarum*, this study quantified the influence of each growth indicator and soil salinity on the yield and explored their interactions. Based on the results of field experiments, path analysis was conducted growth indicator, soil salt and yield of *Lycium barbarum* for the entire fertility period. The results, with a coefficient of determination R² greater than 0.9, indicate a good fit, accurately reflecting the relationships among growth indices, total soil salinity, and the yield of *Lycium barbarum*. The relationships are illustrated in Fig. 7 and summarized in Table 5.

The analysis revealed that under the treatment of these 4 typical exogenous salts soil total salts exerted a negative impact on the yield of *Lycium barbarum*, acting as limiting factors, while indices such as crown width, new branches, and diameter positively influenced yield to varying extents.

As shown in Fig. 7, neutral salts(NaCl, Na₂SO₄, CaCl₂) , as exemplified in Fig. a, the direct impacts of the indices on yield, in descending order of magnitude(in absolute value), were total soil salt (0.820), crown width (0.144), new branches (0.086), and ground diameter (0.019). Under the alkaline salt NaHCO₃ treatment, the direct effects were 0.790 for total soil salt, 0.195 for crown width, 0.041 for new branches, and 0.022 for ground

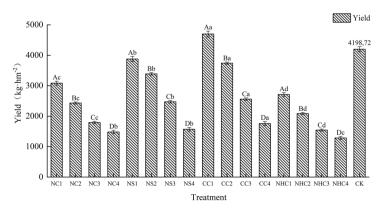


Fig. 5. Effects of different salt concentrations on the yield of dried *Lycium barbarum* fruit.

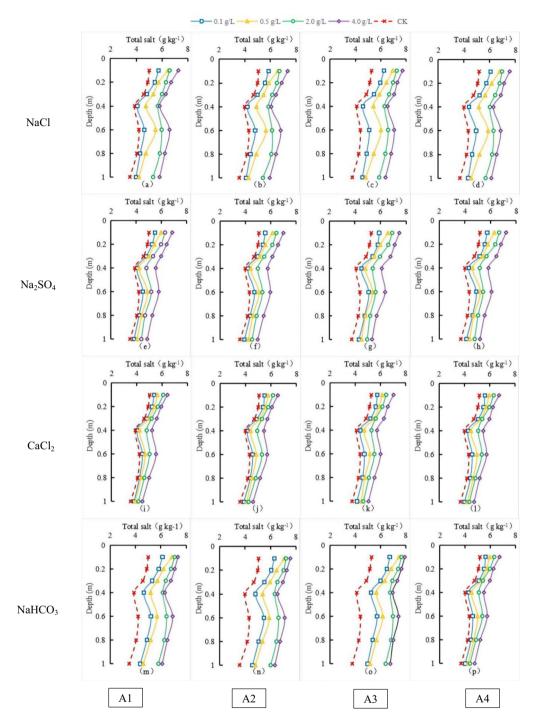


Fig. 6. Effects of different salt concentrations on soil total salt content.

diameter, indicating that total soil salinity consistently had the most substantial direct effect on under both neutral and alkaline conditions. It can be seen that the direct determining effect of total soil salt on *Lycium barbarum* yield was greater in both neutral and alkaline salts, and the other indexes were *Lycium barbarum* crown width, new branches and ground diameter in that order. The interactions among the growth indices showed indirect effects on yield. For instance, under NaCl treatment, the correlation coefficients between new branches and crown width, new branches and ground diameter, and crown width and ground diameter were 0.778, 0.104, and 0.179, respectively. The negative correlations between soil total salt and ground diameter, crown width, and new branches were -0.328, -0.767, and -0.321, respectively. Similarly, under NaHCO $_3$ treatment, the correlation coefficients among growth indices were 0.599, 0.350, and 0.563, respectively, while the correlations between total salt and growth indices were -0.420, -0.756, and -0.402, respectively, it can be seen that the indirect effects

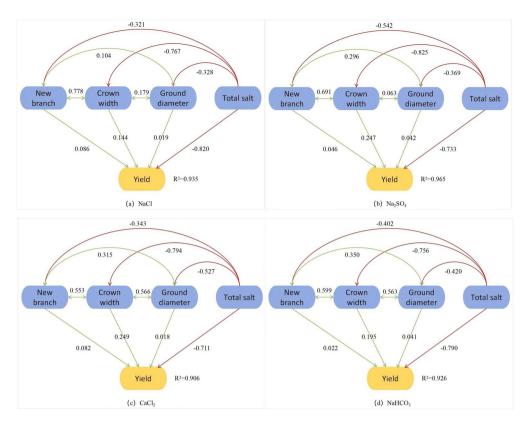


Fig. 7. The results of path analysis for yield of Lycium barbarum yield and associated indices.

Typical Salt		Independent variable	Simple correlation coefficient	Direct passage coefficient	Indirect pas	Indirect passage coefficient				
	Norm				X1	X2	X3	X4	Total	
NaCl		New branch(X1)	0.463	0.086		0.002	0.112	0.263	0.377	
	Yield	Ground diameter(X2)	0.323	0.019	0.009		0.026	0.269	0.304	
	Tield	Crown width(X3)	0.843	0.144	0.067	0.003		0.629	0.699	
		Total salt(X4)	- 0.965	- 0.820	- 0.028	- 0.006	- 0.111		- 0.145	
Na ₂ SO ₄		New branch	0.626	0.046		0.012	0.171	0.397	0.580	
	37: .1.1	Ground diameter	0.343	0.042	0.014		0.016	0.271	0.301	
	Yield	Crown width	0.887	0.247	0.032	0.003		0.605	0.640	
		Total salt	- 0.978	- 0.733	- 0.025	- 0.016	- 0.204		- 0.245	
CaCl ₂		New branch	0.470	0.082		0.006	0.138	0.244	0.388	
	37: .1.1	Ground diameter	0.560	0.018	0.026		0.141	0.375	0.542	
	Yield	Crown width	0.869	0.249	0.045	0.010		0.565	0.620	
		Total salt	- 0.947	- 0.711	- 0.028	- 0.010	- 0.198		- 0.236	
NaHCO ₃		New branch	0.471	0.022		0.014	0.117	0.318	0.449	
	Yield	Ground diameter	0.491	0.041	0.008		0.110	0.332	0.450	
	i ieid	Crown width	0.828	0.195	0.013	0.023		0.597	0.633	
		Total salt	- 0.963	- 0.790	- 0.009	- 0.017	- 0.147		- 0.173	

Table 5. Path analysis between growth indices, soil total salt, and yield of *Lycium barbarum*.

of growth indexes on yield were all mainly affected through the interactions between new branches and crown width, the indirect effect of total soil salt on yield was mainly through its interaction with crown width.

According to Table 3, the simple correlation coefficients of each index with yield, ranked by the magnitude of their absolute values, were X4, X3, X1, and X2. In terms of direct influence, the ranking was the same. However, from the perspective of indirect influence, the order was X3, X1, X2, X4. These results indicate that the direct impacts of soil total salinity and crown width on yield are substantial, with the direct effect of soil total salt being greater than the sum of its indirect effects. This suggests soil salinity predominantly affects yield directly, while the influences of other indicators are mainly manifested through indirect effects and are relatively lesser.

In summary, there is a mutual influence among all indices, with none acting in isolation. Each index affects the yield of *Lycium barbarum* through a combination of direct and indirect actions. To optimize the yield under brackish water irrigation, controlling the total soil salt content is imperative.

Discussion

Salt concentration is one of the main abiotic stress factors affecting plant growth and development, while the external morphology and growth status of plants are the most intuitive expression of the damage degree of saline-alkali stress^{33,34}. Under high concentrations of saline and alkaline stress, plants experience osmotic stress and ionic toxicity, resulting in blocked metabolic functions and reduced intracellular enzyme activity, thereby affecting normal development^{35,36}. However, in accordance with principle of "survival of the fittest," plants can sustain their growth under adverse conditions by regulating inter-root osmotic pressure, changing the structure of cell membrane lipids, and adjusting growth patterns³⁷. This study observed that under brackish water irrigation, the growth parameters of Lycium barbarum, including the number of new branches, ground diameter, and crown width, peaked when the combined concentration of exogenous NaCl, Na₂SO₄, and CaCl₂ (neutral salts) was 0.5 g L⁻¹. Conversely, the optimal growth of *Lycium barbarum* occurred at a concentration of 0.1 g L⁻¹ of NaHCO₃ (basic salt). It is evident that, compared to the other three neutral salts, alkaline salts inflict the most severe damage on various growth indices of Lycium barbarum, likely attributable to the elevated pH induced by alkaline salts³⁸. This finding aligns with the notion that alkaline salt stress exerts a more pronounced inhibitory effect on plants compared to neutral salt stress, as proposed by Guo J et al.³⁹in their study of crop responses to saline and alkaline stresses. Furthermore, our study revealed an inverse relationship between the growth indices of Lycium barbarum and the concentration of the four salts (NaCl, Na₂SO₄, CaCl₂, and NaHCO₃) when the exogenous salt concentration exceeded 0.5 g L⁻¹. This phenomenon occurs because, as salt stress intensifies, a significant influx of Na+ ions hinders the absorption of other essential mineral elements, leading to ion imbalance and inducing ion toxicity. Concurrently, the increase in salt concentration will lead to the weakening of Ca²⁺ absorption by plants, which will cause the lack of Ca²⁺ in plants in the long run, thus inhibiting the growth and development of plants. Furthermore, the high concentration of Ca²⁺ itself is also a stress, and the calcium signal caused by adversity and the excessive reactive oxygen species produced by it are toxic to plants^{40–43}. However, divergent opinions exist regarding the salt tolerance threshold range of Lycium barbarum. Yang Wanpeng et al.²³ conducted potting salt control tests with 6 concentration gradients and found that the tolerated saline stress threshold value during the growth period of *Lycium barbarum* ranged from 50 to 150 mmol L⁻¹, with various growth indicators decreasing with increasing salt concentration. Meanwhile, Hu J et al. 44 through Lycium barbarum cultivation experiments with different NaCl concentrations in Minqin County, Gansu Province, concluded that a concentration of 50 mM NaCl is optimal for Lycium barbarum growth. Although the overall trend of brackish water salt concentration effects on Lycium barbarum stress is consistent, discrepancies in effect magnitude arise due to differences in testing locations and methods. Crop yield is influenced by different brackish water salt concentrations^{45,46}. Li Jinguang et al.⁴⁷ found that corn seed yield decreased when the salinity of brackish water exceeded 2 g L⁻¹, as demonstrated by manually adjusting irrigation water salinity. Similarly, Ma Jiaying et al. 48 observed tomato growth was significantly inhibited by brackish water irrigation at 6 g L⁻¹, resulting in reduced energy yield compared to irrigation at 2 g L⁻¹. In this study, it was observed that among the four exogenous typical salts, at a concentration of 0.1 g L⁻¹, Na⁺ had a greater negative impact on dried *Lycium barbarum* yield than Ca²⁺, whereas Ca²⁺ significantly promoted yield compared to Na⁺³². Additionally, the results showed that the yields of NaCl, Na₂SO₄, and NaHĆO₃ were lower than CK at 0.1 g L⁻¹, whereas the yields of CaCl₂ exceeded that of CK.

In numerous studies, scholars have investigated different levels of mineralization in irrigation water to assess soil salinity. Overall, these studies have consistently shown that soil salt accumulation increases with higher levels of mineralization 49,50. In this study, we found a positive correlation between total soil salinity from 0 to 1 m depth and the salt concentration from different typical salt sources in brackish water throughout the fertility period. Notably, the lowest total soil salinity was recorded when the exogenous salt concentration was 0.1 g L-1. Additionally, salinity levels fluctuated with soil depth, with surface soil exhibiting higher salinity levels. This phenomenon may be attributed to increased temperatures spring, leading to strong soil moisture evaporation, thereby exacerbating surface salt accumulation⁴⁹. Furthermore, high concentrations of salt in brackish water may leach into the soil, elevating soil salt content¹⁵. Leaching of rhizosphere soil during the spring irrigation in the beginning of May resulted in declining salt in the 0 to 0.4 m soil layer, reaching a low peak in the 0.3 to 0.4 m soil layer. However, salinity increased in the 0.4-0.6 m layer, possibly due to salt leaching from deeper layers during fall watering in the previous year, which subsequently froze⁵¹. As soil depth increased, external factors gradually reduced, resulting in a minimal change in the total salt content in the 0.6-1 m soil layer, with an overall decreasing trend. It can be seen that the accumulation of salts in the soil and the leaching effect occur simultaneously in the process of brackish water irrigation. When the accumulation of soil salts is too high, it has a negative effect on the growth of Lycium barbarum, which in turn causes physiological water shortage of Lycium barbarum plants and affects its final yield. At this time, leaching plays a dominant role, and the low concentration (< 0.5 g L⁻¹) of brackish water can be used for irrigation, which does not inhibit the growth of *Lycium barbarum* and even favours the growth of Lycium barbarum, which is probably due to the fact that a moderate amount of brackish water, which will bring the mineral elements to the root system to promote its growth⁴¹. When the brackish water concentration is higher than 0.5 g L⁻¹, the rate of salt accumulation brought in by brackish water infiltration is higher than the rate of drenching, and at this time, in order to ensure that the soil salt accumulation is within the normal range, it is necessary to appropriately increase the measures such as freshwater irrigation, to drench the salt out of the root zone⁵².

In summary, although brackish water irrigation can mitigate water resource supply-demand imbalances in river-loop irrigation areas, irrational salt concentrations in brackish water irrigation can induce salt stress in

Lycium barbarum, leading to physiological water shortages and decreased yields. Considering different brackish water salt concentration treatments, the suitable concentration range for optimal *Lycium barbarum* yield and its constituent factors is 0.1-0.5 g $\rm L^{-1}$.

Conclusions

- (1) The growth rate of new branches, ground diameter, and crown width of *Lycium barbarum* showed a trend of increasing and then decreasing with increasing exogenous salt concentrations of NaCl, Na₂SO₄ and CaCl₂, with the optimal concentration being 0.5 g L⁻¹. Conversely, growth decreased with increasing NaHCO₃ concentration, with the optimal concentration being 0.1 g L⁻¹.
- (2) Throughout the whole reproductive period, when the concentration of the exogenous typical salts in brackish water (NaCl, Na₂SO₄, CaCl₂ and NaHCO₃) was 0.1 g L⁻¹, rhizosphere soil salt accumulation was lower, resulting in a higher dry fruit yield of *Lycium barbarum*.
- (3) Path analysis results showed that under the four exogenous typical salt treatments, soil total salt content was the primary direct factor influencing *Lycium barbarum* yield, while crown width of *Lycium barbarum* was the main indirect factor affecting yield.
- (4) Considering *Lycium barbarum* growth, stable yield, and soil salt control, the suitable concentration range under neutral salt (NaCl, Na₂SO₄ and CaCl₂) treatments was 0.1–0.5 g L⁻¹, whereas under alkaline salt (NaHCO₃), the NHC1 (0.1 g L⁻¹) treatment was the best choice.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

- 1. Zou, Y. F., Cai, H. J., Zhang, T. B., Wang, Y. F. & Xu, J. T. Water use characteristics and profit analysis of spring maize production with different irrigation methods in Hetao irrigation district. *Trans. Chin. Soc. Agric. Mach.* 51, 237–248 (2020).
- 2. Guo, K. & Liu, X. J. Reclamation effect of freezing saline water irrigation on heavy saline-alkali soil in the Hetao irrigation district of north China. CATENA 204, 105420 (2021).
- 3. Shi, H. B. *et al.* Soil water and salt movement and soil salinization control in Hetao irrigation district: Current state and future prospect. *J. Irrigation Drain.* **39**, 1–17 (2020).
- 4. Xue, J. & Ren, L. Conjunctive use of saline and non-saline water in an irrigation district of the yellow river Basin. *Irrigation Drain.* **66**, 147–162 (2017).
- Wei, C. C. et al. Effects of irrigation water salinity on soil properties, N₂O emission and yield of spring maize under mulched drip irrigation. Water. 11, 1548 (2019).
- Guo, Y. et al. Field irrigation using magnetized brackish water affects the growth and water consumption of Haloxylon ammodendron seedlings in an arid area. Front. Plant Sci. 13, 929021–929021 (2022).
- 7. Wei, C. C. et al. Effects of irrigation water salinity and irrigation water amount on greenhouse gas emissions and spring maize growth. *Transactions of the Chinese Society for Agricultural Machinery*. **52**, 251–260+236 (2021).
- 8. Hua, Y. H. & Liu, H. B. Effects of brackish water irrigation with different salinity levels on cotton growth and development. *Shaanxi Water Resour.* **266**, 70–72 (2023).
- 9. He, J. S., Feng, W. D., Liu, W. Q., Bai, H. B. & Zhang, W. J. Effects of NaCl stress on seed germination rate of wheat varieties and their salt tolerance evaluation. *J. Cold-Arid Agric. Sci.* 2, 229–233 (2023).
- Liu, Z. Y. et al. Effects of saline water irrigation with different concentrations on soil water and salt distribution and growth of winter wheat. J. Soil Water Conserv. 38, 378–386 (2024).
- 11. Shen, J. L. Study on the coordinated regulation mechanism of soil-irrigation system in facility cucumber under brackish water irrigation. Master's thesis, Ningxia University. (2022).
- 12. Liu, Y. Study on the mechanism of *Lycium Barbarum's* salt tolerance and microbial-fertilizer salt regulation based on osmotic-photosynthetic feedback under brackish water in Hetao irrigation Area. Ph.D. thesis, Inner Mongolia Agricultural University. (2023).
- 13. Li, Y. P. et al. Correlations between infrared spectrum and phenotypic traits of different Lycium barbarum L.. Germplasms. Food Res. Dev. 44, 178–182 (2023).
- 14. Guo, H., Duan, G. Z., Fan, G. H., Su, C. F. & Wang, Z. L. Effects of irrigation with different concentrations of brackish water on the fruits quality of *Lycium barbarum*. *Northern Horticulture*. **46**, 92–97 (2022).
- 15. Sun, D. Q. Effects of brackish water irrigation on soil water and salt and water consumption characteristics of *Lycium barbarum* and research on planting technology. Master's thesis, Huhhot: Inner Mongolia Agricultural University. (2021).
- Xu, C. J. et al. SiMYB19 from foxtail millet (Setaria italica) confers transgenic rice tolerance to high salt stress in the field. Int. J. Mol. Sci. 23, 756–756 (2022).
- 17. Rajesh, K. S. et al. Sustaining yield of winter wheat under alternate irrigation using saline water at different growth stages: A case study in the north China plain. Sustainability. 11, 4564–4564 (2019).
- 18. Li, H., Lin, Q. & Xu, S. H. Effect of infiltration of saline water/brackish water on soil permeability and transport of salt cations in the soil. *Acta Pedologica Sinica*. 57, 656–666 (2020).
- 19. Li, B., Shi, H. B., Zhang, J. G. & Li, Z. Hydrochemical characteristics of groundwater before and after water-saving reform in Hetao irrigation district, inner Mongolia. *Trans. Chin. Soc. Agric. Eng.* 30, 99–110 (2014).
- 20. Li, Z. Y., Cao, W. G., Wang, Z. R., Li, J. C. & Ren, Y. Hydrochemical characterization and irrigation suitability analysis of shallow groundwater in Hetao irrigation district inner Mongolia. *Geoscience*. 36, 418–426 (2022).
- 21. Du, D. D., Bai, Y. Y. & Yang, D. L. Chemical spatiotemporal characteristics and environmental driving factors of groundwater in Hetao irrigation area. *Environ. Sci.* 47, 1–18 (2023).
- 22. Mao, G. L. et al. Effects of alkali stress on growth, structure and photosynthetic parameters of Lycium barbarum L.. Agric. Res. Arid Areas. 35, 236–242 (2017).

- 23. Yang, W. P., Ma, R., Yang, Y. Y., Ni, Q. & Ma, Y. J. Effects of NaCl treatment on the growth and physiological indexes of *Lycium ruthenicum*. *Molecular Plant Breeding*. 17, 4437–4447 (2019).
- 24. Liu, Q. C., Feng, J. R., Hao, Y. J., Fan, X. M. & Zhang, Y. D. Effects of NaCl stress on seed germination of six *Lycium ruthenicum* Murr. *Types. Xinjiang Agric. Sci.* **53**, 2040–2046 (2016).
- Pan, P. X., Ni, Q., Ma, R., Yang, Y. Y. & Ma, Y. J. Effects of different salt treatments on seed germination and seedling growth of Lycium ruthenicum Murr. Acta Agrestia Sinica. 29, 342–348 (2021).
- 26. Zhan, Z. N., Ma, Q., Wang, W. J. & Na, W. Effect of mixed saline and alkaline stress on seed germination of black fruited *Lycium barbarum. Jiangsu Agric. Sci.* 46, 119–122 (2018).
- Zhang, T. et al. Responses of growth and photosynthesis of Lycium barbarum L. seedling to salt-stress and Alkali-stress. Acta Bot. Boreal. Occident. Sin. 7, 2474–2482 (2017).
- 28. He, Y. H., Tan, J. L., Wang, X. N. & Dong, H. Effects of different brackish water irrigation methods on spatial variability of soil moisture, salinity and pH in gravel-sand mulched soil. *Water Saving Irrigation.* 48, 1–9 (2023).
- 29. Zhao, Y. et al. Analysis of spatial and temporal variability and coupling relationship of soil water and salt in cultivated and wasteland at branch canal scale in the Hetao irrigation district. Agronomy. 13, 2367–2367 (2023).
- 30. Zeng, H. B. et al. Mechanism of salinization of shallow groundwater in western Hetao irrigation area. Earth Sci. 46, 2267–2277 (2021)
- 31. Ministry of Health of the People's Republic of China, China Standardization Administration. Standard testing methods for drinking water. Standards Press of China (2006).
- 32. Lou, S., Yang, S. Q., Zhang, W. F., Liu, Y. & Lv, X. H. Salt concentration threshold of *Lycium barbarum* under different types of Brackish water irrigation in Hetao irrigation area. *Trans. Chin. Soc. Agric. Mach.* 54, 319–334 (2023).
- 33. Li, B. et al. Salt tolerance evaluation of cucumber germplasm under sodium chloride stress. Plants. 12, 2927-2927 (2023).
- 34. Ren, H. X. et al. Effects of biochar on the photosynthetic and antioxidant characteristics of ryegrass and alfalfa under saline-alkali stress. Trans. Chin Soc. Agric. Eng. 37, 116–123 (2021).
- 35. Jiang, Y. et al. A novel transcriptional regulator HbERF6 regulates the HbCIPK2-coordinated pathway conferring salt tolerance in halophytic Hordeum brevisubulatum. Front. Plant Sci. 13, 927253–927253 (2022).
- 36. Wu, G. Q., Wang, J. L., Feng, R. J., Li, S. J. & Wang, C. M. iTRAQ-based comparative proteomic analysis provides insights into molecular mechanisms of salt tolerance in sugar beet (*Beta vulgaris* L.). *Int. J. Mol. Sci.* 19, 3866 (2018).
- 37. Wang, Q. Z., Liu, Q., Gao, Y. N. & Liu, X. Review on the mechanisms of the response to salinity-alkalinity stress in plants. *Acta Ecol. Sinica.* 37, 5565–5577 (2017).
- 38. Yin, L. J., Wei, M. Y., Wu, G. H. & Ren, A. Epichloë endophytes improved *Leymus chinensis* tolerance to both neutral and alkali salt stresses. *Front Plant Sci.* 13, 968774–968774 (2022).
- Guo, J. X., Lu, X. Y., Tao, Y. F., Guo, H. J. & Min, W. Comparative ionomics and metabolic responses and adaptive strategies of cotton to salt and alkali stress. Front. Plant Sci. 13, 871387 (2022).
- 40. Li, N., Cao, B. L., Chen, Z. J. & Xu, K. Root morphology ion absorption and antioxidative defense system of two Chinese cabbage cultivars (*Brassica rapa* L.) reveal the different adaptation mechanisms to salt and alkali stress. *Protoplasma*. **259**, 1–14 (2021).
- 41. Wu, F. Z., Zhu, X. W., Hu, R. F., Wang, H. X. & Chen, Y. M. Effects of NaCl stress on growth ion uptake, transportation and distribution of two blueberry (*Vaccinium corymbosum*) cultivars seedlings. *Sci. Silvae Sinicae*. 53, 40–49 (2017).
- 42. Ji, X. Y., Tang, J. L., Li, A., Zheng, X. & Zhang, J. P. Effects of NaCl stress on physiological and biochemical characteristics and compartmental allocation of mineral element of *Juglans* major seedlings. *Plant Physiol. J.* 60, 40–49 (2024).
- Sun, D. W., Yang, L., Mao, X. & Liu, G. HFu. Physiological mechanism of CaCl2 application relieving salt-stressed seedlings of Cornus hongkongensis subsp tonkinensis. *Acta Botanica Boreali-Occidentalia Sinica*. 43, 441–449 (2023).
- 44. Hu, J., Hu, X. K., Zhang, H. W. & Yu, Q. S. Moderate NaCl alleviates osmotic stress in *Lycium ruthenicum*. *Plant Growth Regulation*. 41, 1–11 (2021).
- 45. Li, Q., Yang, A. & Zhang, W. H. Comparative studies on tolerance of rice genotypes differing in their tolerance to moderate salt stress. *BMC Plant Biol.* 17, 141 (2017).
- 46. Ma, H., Cai, L. L., Lin, J. C., Zhou, K. Y. & Li, Q. S. Divergence in the regulation of the salt tolerant response between arabidopsis thaliana and its halophytic relative *Eutrema salsugineum* by mRNA alternative polyadenylation. *Front. Plant Sci.* 13, 866054–866054 (2022)
- 47. Li, J. G. *et al.* The effects of irrigation water salinity on maize emergence, yield, grain quality and soil salt dynamics. *Water Saving Irrigation.* 49, 1–10 (2024).
- 48. Ma, J. Y. et al. Effects of brackish water irrigation on soil water and salt distribution and yield and quality of facility tomatoes. Agric. Res. Arid Areas. 40, 104–112 (2022).
- 49. Gao, C. S. et al. Annual soil salt balance and crop performance under brackish water irrigation during the winter wheat season. Chin. J. Eco-Agric. 29, 809–820 (2021).
- 50. Huang, M. Y. et al. Effect on soil properties and maize growth by alternate irrigation with brackish water. *Trans. ASABE.* **62**, 485–493 (2019).
- 51. LV, X. H. Response of *Lycium barbarum*-soil and its supporting measures to irrigated with brackish water and micro-fertilizer. Master's thesis, Inner Mongolia Agricultural University. (2021).
- 52. Li, W. J. et al. Soil water and salt distribution and cotton growth under film drip irrigation with different water salinity in cotton field. Agric. Res. Arid Areas. 40, 95–103 (2022).

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Conceptualization: C.Y., L.S. and C.X. Project management: Y.S. Access to funding: Y.S. Experiment performance: C.Y. and L.S. Data curation: C.Y. Data analysis: C.Y. Writing-original draft: C.Y. Writing-review & editing: C.Y. and Y.S. All the authors discussed the results and contributed to the paper.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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