RESEARCH ARTICLE

The effect of water and salt stress and fertilization on the quality of *Licorice* in genuine medicinal materials

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Daoji herbs are higher quality herbs grown in specific regions because stress can have an inhibitory effect on their growth and needs to be eliminated to increase yield and quality. Therefore, it has become necessary to identify the stress factors for the accumulation of Chinese herbs for the development of Chinese traditional medicine. The effects of different water and salt stress and fertilization conditions on the quality of *Licorice*, one of the Daoji medicinal herbs, were studied by hydroponic test, pot test, and field fertilization test. The results showed that, under severe water stress (30%), the relative humidity in the environment was only 30%. The relative contents of glycyrrhetinic acid as well as glycyrrhizin demonstrated a decrease of 16.84% and 47.96%, respectively, while the absolute content of glycyrrhizin alone showed a highly significant decrease of 50.74% ($P < 0.01$). At the same concentration of Na^+, NaCl had the best seed germination potential. Except for the concentration of 25 mmol/L Na^+, all other Na^+ concentrations showed significant differences in seed germination potential compared to Na_2SO_4 and mixed salt. In Na_2CO_3, except for a concentration of 25 mmol/L Na^+, all other concentrations showed significant differences in germination potential indicators compared to $NaHCO_3$ treatment. There was a significant difference in *Licorice* yield among different fertilization treatments, and the difference in *Licorice* yield compared to the control group in all fertilization treatments reached a very significant level ($P < 0.01$). This study discussed the effects of water and salt stress and fertilization on *Licorice* quality and provided theoretical support to the cultivation of high-yielding and high-quality traditional Chinese medicine.

Keywords: water and salt stress; fertilization; Daoji herbs; *Licorice*; quality.

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Introduction

Genuine medicinal herbs refer to Chinese traditional medicinal herbs that grow in specific regions and have better quality compared to the same type of medicinal herbs grown in other regions. The formation of authentic medicinal herbs is closely related to their origin. The main medicinal components of Chinese traditional medicine are secondary metabolites produced under specific environmental conditions. The special habitat conditions determine the geographical location and ecological environment of authentic Chinese medicine production, which are mostly located in fragile ecological environments such as mountainous, hilly, arid, and semi-arid areas [1, 2]. In recent years, due to the increasing demand for Chinese traditional medicine and farmers' unreasonable excavation, a large number of wild Chinese medicine resources have been destroyed, and many Chinese herbal medicines have to be artificially planted. However, due to the factors of environment and cultivation methods, the

quality of artificially planted Chinese herbal medicines cannot meet the medicinal standards. As a major constraint to plant growth, water and salt stress (WAS) can disrupt herb seed germination, affect seedling growth, and even lead to death due to persistent inhibition [3, 4]. At the same time, soil is the most fundamental factor in the production of Chinese herbs, and fertilization can be used to improve the yield and quality of Chinese herbs. However, if fertilizers are applied inappropriately or in excess of what a particular plant can tolerate, its normal growth may still be hindered, leading to a decline in quality. In addition, today's artificial cultivation of Chinese herbs suffers from the problem that yield is the main measure, starting from the elimination of stress factors, which often leads to insufficient content of their medicinal components for medicinal use [5]. *Licorice* is a perennial herbaceous plant belonging to the leguminous family. The root of *Licorice* is its main medicinal part, which has various medicinal functions and uses. *Licorice* usually grows in warm and moist soil and prefers sunny environments. The methods of planting *Licorice* include seed propagation and rhizome division. Seed reproduction requires sowing in spring, while rhizome branching can occur in autumn. *Licorice* has a long growth cycle and usually takes 3-4 years to harvest. After harvesting, the roots are treated and processed to obtain medicinal *Licorice*. *Licorice* has a wide range of medicinal functions including anti-inflammatory and antibacterial, clearing heat and detoxifying, and balancing prescriptions.

In recent years, numerous studies about the WAS and fertilization effects on plants have been conducted. Beyk-Khormizi, *et al*. used vermicompost as an organic fertilizer to grow fennel under different concentrations of physiological salinity and found that increasing salinity significantly stressed the growth of fennel. Fennel could mitigate or limit the negative effects of increased salinity by synthesizing Vitamin C (VC) [6]. Attia, *et al*. proposed a method for comparing seedling growth under salt treatment to address seedling sensitivity to WAS. Seedling germination and mineral nutrient changes were monitored during the process and mRNA abundance in genes was assessed. The results demonstrated that the proposed method provided an accurate analysis of the sensitivity of seedlings in salt-treated environments [7]. Further, Liu and his team studied the effects of titanium dioxide nanoparticles on the peonies in saline stress environments and found that it could accurately determine the physiological effects of saline slope environments on peonies [8]. Paknejad, *et al*. also proposed an analytical method by using standardized value controls for the effect of salinity stress on cotton yield and confirmed that the method could provide an accurate assessment of the effects on cotton [9]. Soltanbeigi, *et al*. investigated the effects of irrigation regimes with water stress and nutrient sources on the growth and essential oil content of dogwood in a greenhouse. The results showed that the plant produced significant amounts of essential oil components with minimal water. Water stress and various sources of nutrients had crucial impacts on the yield and chemical composition of medicinal and aromatic plant growth [10]. Sharma, *et al*. examined salt-tolerant bacteria in the medicinal plants under WAS and evaluated the growth and medicinal value of metabolites of the host plant by this flora. The results showed that the production of nitrogen fixation mechanism was induced in the presence of salt-tolerant bacteria, which further alleviated WAS and improved the production of alkali in medicinal plants, effectively contributing to agroecology and development [11]. Moreover, Rutkay's team used remote sensing technology to study the effects of different WAS concentrations on rosemary growth and quality. The results showed that chlorophyll, brightness, and color values differed in the leaves of different plants under WAS [12]. The production of rhizobia (LB2) in saline environments was analyzed by Verma, *et al*. The results found that LB2 demonstrated high salt tolerance activity when the salt concentration was 4% [13]. Among those previous studies, many of them devoted to the specific growth of plants and crops under

WAS and analyzing and exploring their optimal growing salt concentrations. However, few studies have explored WAS as a starting point, and most of those studies paid more attention to the growth quality of crops, vegetables, and fruits.

The aim of this study was to improve the quality of Chinese medicinal plants and promote the development of the herbal industry. This study took Chinese herbal medicine *Licorice* as an example and WAS as the main starting point to study the effects of different WAS and fertilization conditions on *Licorice* quality. The results of this study would provide reference for improving the quality of Chinese herbal medicine.

Materials and methods

Plant resources and test locations

Glycyrrhiza uralensis Fisch and its seeds were purchased from Jianshi Traditional Chinese Medicine Planting Co. (Baoji, Shaanxi, China). This study consisted of three trials including hydroponic trial, pot trial, and field trial. The hydroponic trials were conducted by using Climatron in Plant Protection College, Northwest A&F University (Yangling, Shaanxi, China) from October 2020 to April 2021. The pot trials were conducted in Shaanxi Changhe Pharmaceutical Co., Ltd (Yangling, Shaanxi, China), a location in a warm temperate monsoon climate zone with an average annual (AA) temperature of 13.96°C, a frost-free period of about 225 days, and an AA rainfall of 649.73 mm [14, 15]. The *Licorice* field test was performed in the Jane's Traditional Chinese Medicine Planting Professional Cooperative (Baoji, Shaanxi, China) with a warm temperate continental monsoon climate in the western part of Guanzhong Plain. The experimental area was 600 m^2 with the AA temperature of 14.54°C, the frost-free period about 260 days, and the AA rainfall about 673.84 mm. The soil at the test site was brown soil.

The hydroponic trials

The seeds of moderate size, plump particles, and free from pests and diseases were selected for the experiment with the sizes of 2.5-4.5 mm long, 2.5-4.0 mm wide, and 1.5-2.7 mm thick. All selected seeds were soaked in 98% sulfuric acid for 30 minutes before in distilled water at 25°C for 24 hours. Drought stress (DS) was simulated by using 5%, 10%, 15%, 20%, and 30% concentrations of PEG-6000 solution (Shanghai Seagate Biotechnology, Shanghai, China). The neutral salt NaCl, Na_2SO_4 and alkaline salt Na_2CO_3, $NaHCO_3$ were prepared at the Na^+ concentrations of 25, 50, 75, 100, 125, 150, 175, and 200 mmol/L before mixing the four Na^+ solutions in a ratio of 12:8:1:9 for salt alkali stress experiment. After washing with distilled water, two layers of filter paper were laid on a 9 cm diameter Petri dish with 100 treated seeds in each Petri dish. Then, 8 mL of each prepared solution was added to Petri dish before incubating in the Climatron for dark germination at 25°C with the culture solution changed every day. Each experiment was set up as triplicates. The details of stress tests were listed in Table 1.

The pot trials

The local soil was used for moisture stress and four moisture gradients were set up according to Hsiao's moisture stress criteria including heavy stress (30%) (S3), medium stress (45%) (S2), light stress (60%) (S1), and normal moisture control (75%) (CK) [16, 17]. Each moisture gradient test was triplicated with a total of 24 pots. To reduce the loss of water content in the pots, a balanced control of water was achieved by weighing and rehydration. The salt stressed soil test was prepared by using local soil with 5 NaCl salinity gradients of 1.0%, 0.8%, 0.6%, 0.4%, and 0.08% as S4, S3, S2, S1, and CK, respectively. Each concentration was also tripled with a total of 15 pots. All the test pots had eight small holes of 2 mm in diameter on the bottom to drain water.

The field fertilization trials

1-year-old *Licorice* was transplanted into a 600 m^2 experimental area. The scheme design adopted a 3-factor, 2-way saturated d-optimal design, and the blank control was not fertilized.

Table 1. The stress tests.

Drought stress PEG-6000 (%)	Saline-alkali stress Na^+(mmol/L)						
	Gradient	$NaHCO_3$	Na_2CO_3	Na_2SO_4	NaCl	Na^+	Mixed salt
Control (CK)	CK	0	0	0	0	0	0
5	1	25	12.5	12.5	25	25	19.25
10	2	50	25	25	50	50	38.5
15	3	75	37.5	37.5	75	75	57.75
20	4	100	50	50	100	100	77
30	5	150	75	75	150	150	115.5

Table 2. The field fertilization trials.

Treatment	N (kg/hm^2)	K_2O (kg/hm^2)	P_2O_5 (kg/hm^2)
Control (CK)	0	0	0
N3	223.83	0	0
P3	0	0	150.00
K3	0	223.83	0
N1P3K3	78.94	223.83	150.00
N3P1K3	223.83	223.83	52.09
N3P3K1	223.83	78.94	150.00
P2K2	0	125.24	88.33
N2K2	125.24	125.24	0
N2P2	125.24	0	88.33

The fertilizer mainly consisted of urea (N, 46%), Potassium sulfate (K_2O, 51%), and Superphosphate (P_2O_5, 12%) (Table 2).

Measurement of indicators

Soil pH was obtained by using MIK-PH 8.0 potentiometry (Hangzhou Meikong Automation Technology Co., Ltd., Hangzhou, Zhejiang, China). Organic matter content was measured by the potassium dichromate volumetric and alkaline nitrogen content was measured by the alkaline diffusion [18, 19]. Effective phosphorus content was measured by 0.5 mol/L $NaHCO_3$ leaching - molybdenum antimony anti-colorimetric and the fast-acting potassium content was measured by 1 mol/L NH_4OAC leaching - flame photometric method using FP6450 multi-element flame photometer (Shanghai Youke Instrument Co., Ltd., Shanghai, China). For *Licorice* physiological indicators, germination indicators were observed from the moment the seeds were in the Petri dish. Germination was judged on the basis of the length of the seed radicle coinciding with the length of the seed, and their number was recorded daily. In particular, germination was calculated by using equation (1).

$$G = n / N \times 100\% \quad (1)$$

where *G* was the germination rate. *n* was the seeds quantity for testing. *N* was the grains quantity that eventually germinated normally. The germination potential was calculated by using equation (2).

$$M = nX / N \times 100\% \quad (2)$$

where *M* represented the germination potential and *nX* represented seed quantity within 3 days after germination. The germination index was then obtained by equation (3).

$$L = \sum (Gt / Dt) \quad (3)$$

where *Dt* was observation days and *Gt* was the normal number of germination on each day. The

vigor index was calculated as shown in equation (4).

$$B = S \times T \quad (4)$$

where *B* represented the vigor index. *T* was the germination index and *S* was the fresh weight of seedlings in g. The salt damage rate was calculated as shown in equation (5).

$$C = \frac{\delta - \gamma}{\delta} \times 100\% \quad (5)$$

where δ was seeds quantity germinated in blank control. γ represented the seeds quantity germinating under WAS and *C* corresponded to the salt damage rate. The salt solution concentration was the appropriate salt tolerance concentration when the germination rate reached 75% of the control germination rate. The salt tolerant semi-lethal concentration corresponded to 50% germination rate and the salt tolerant limit concentration corresponded to 10% germination rate [20, 21]. In terms of growth indicators, it measured the fresh and dry weight of the aboveground parts and roots of Chinese traditional medicinal plants. After washing the root with clean water, the water on the root surface was absorbed, and the fresh weight was obtained. Following the disinfection and sterilization at 105°C for 20 minutes and dry at 70°C to constant weight, the drying rate (ratio of dry weight to fresh weight of root) was calculated. The plant height, stem diameter, root length, root diameter, and lateral root number of the plant were also measured. By using the five-point sampling method, the roots and rhizomes of plant were collected separately. Within 1 m^2 of each treatment, the roots and rhizomes of the plant were collected completely and brought back to the laboratory for the underground dry weight, main root length, and root diameter measurement and the average value calculation. The content of Glycyrrhizin was taken as the main index of quality evaluation in the determination of the medicinal content of *Licorice*. The relative content was the mass percentage content, which referred to the number of specific medicinal ingredients per unit mass number contained in 100 units of the plant. The relative content of Glycyrrhizin was the ratio of the quality of Glycyrrhizin to the quality of *Licorice* multiplied by 100%. The content of the medicinal ingredients of *Licorice* was determined by High-performance liquid chromatography (HPLC), where absolute content referred to the specific medicinal component content of a single medicinal herb.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). WPS Office 2019 (WPS Software PTE. LTD., Singapore) and SPSS 22.0 (IBM, Armonk, New York, USA) were employed for statistical analysis of this study. *P* value less than 0.05 was designated as significant difference, while *P* value less than 0.01 as very significant difference.

Results and discussion

Effects of DS on the germination of licorice seeds

The results of the effects of drought stress (DS) on the germination of *Licorice* seeds were shown in Table 3. With the gradual increase of DS, all four indicators of *Licorice* seed germination including vigor index, germination potential, germination rate, and germination index showed a significant decreasing trend. The germination rate of *Licorice* seeds was higher than that of the control at different concentrations with highly significant differences among all treatments. At 30% concentration, seed germination could only reach 17.01% of the control, confirming the inhibitory effect of DS on seed germination and the inhibitory effect becoming stronger as the stress increased. The differences among the seed germination potential at 5%, 10%, and 15% concentrations were not statistically significant. In contrast, a more pronounced decrease in seed germination rate occurred at the concentrations equal to or greater than 20%, which indicated

Table 3. Effects of DS on the germination of licorice seeds.

Treatment	Vitality index	Germination potential (%)	Germination rate (%)	Germination index
Control (CK)	1.154±0.063a	71.863±3.68a	76.94±4.10a	127.68±8.15a
5%	0.718±0.031b	44.54±1.47b	71.29±3.68b	91.58±2.39b
10%	0.717±0.003c	42.27±1.18b	68.18±3.49b	95.68±0.71b
15%	0.310±0.005d	44.71±2.10b	58.59±3.67c	43.32±0.79c
20%	0.211±0.005e	7.59±1.38c	51.29±0.47d	30.36±0.77d
30%	0.069±0.002f	0d	12.24±2.06e 0d	10.74±0.35e

Note: In the same column, the letters represented the differences at the $P < 0.05$.

Table 4. Growth and yield of *Licorice* under water stress.

Treatment	Number of lateral roots	Root dry weight (g)	Root diameter (mm)	Root length (cm)
Control (CK)	6.98±1.00b	6.77±0.28b	11.86±0.54a	39.95±4.68b
S1	8.55±0.49ab	7.61±0.47b	11.53±0.36a	46.58±4.72a
S2	10.59±2.11a	9.59±1.46a	11.12±0.30b	47.65±0.62a
S3	11.02±1.20a	6.62±0.23b	10.28±0.34c	44.19±2.04ab

Note: In the same column, the letters represented the differences at the $P < 0.05$.

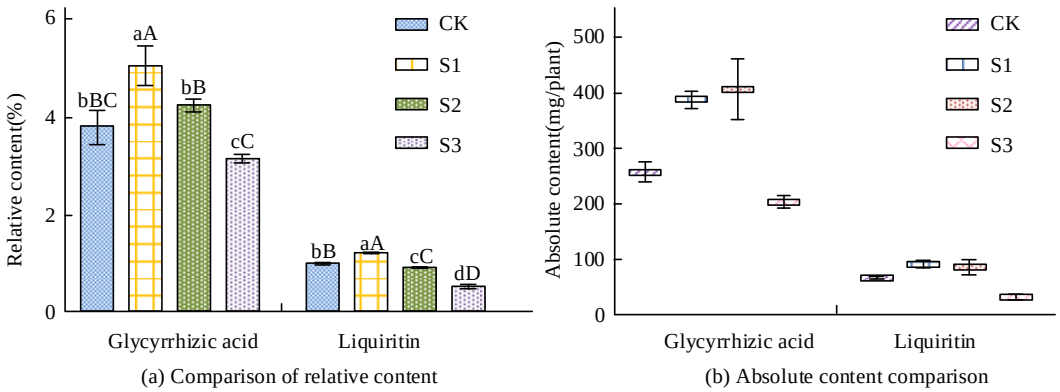

Figure 1. Changes in the contents of glycyrrhetinic acid and glycyrrhizin under water stress.

that *Licorice* seeds had a certain tolerance limit to the type of drought, and, only when this limit was exceeded, the seed germination rate showed a significant decrease.

Effect of water stress on the content of active ingredients, yield, and growth of *Glycyrrhiza glabra*

The changes of growth and yield of *Glycyrrhiza glabra* under moisture stress were shown in Table 4. There was no distinction in all indicators under S1 water stress. The lateral roots, root length, and root dry weight were increased by about 42%, 21%, and 52%, respectively under S2 stress ($P < 0.05$). Under S3 stress, the number of lateral roots increased by about 57% and the root diameter added by about 14% ($P < 0.05$). The results showed that, with the increase of water stress, the water stress of S2 showed a trend of first increasing and then decreasing. The root diameter tended to become progressively smaller, while the number of lateral roots became higher. Figure 1 displayed the changes in glycyrrhetinic acid and glycyrrhetinic acid under water stress. The relative contents of glycyrrhetinic acid and glycyrrhizin increased by 32.89% and 22.45%, respectively. In S1 water stress, it was significantly different to the control

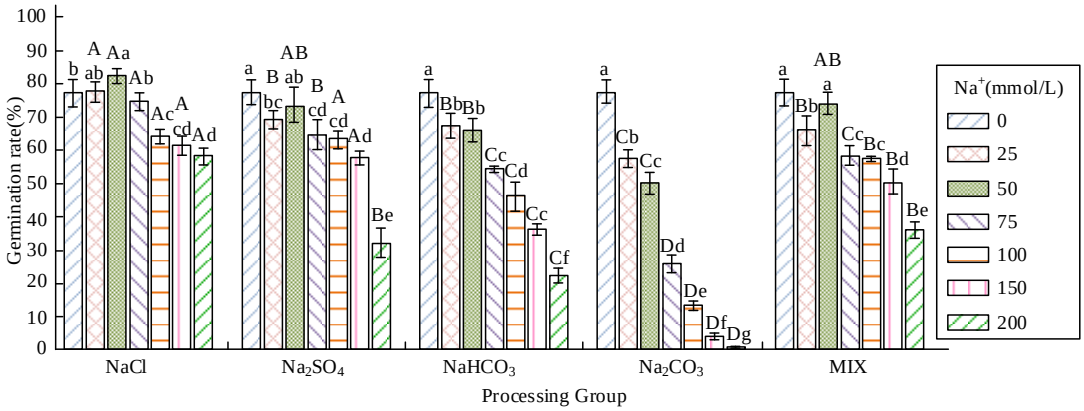

Figure 2. Changes in germination rate of *Licorice* seeds in salt alkali stress.

($P < 0.01$). Meanwhile, the absolute contents of both components were elevated by 50.64% and 37.10%, respectively ($P < 0.01$). Under S2 stress, the difference in relative content was not significant for glycyrrhetinic acid compared to the control, while glycyrrhizin decreased by 8.16% ($P < 0.01$). The absolute contents of glycyrrhetinic acid and glycyrrhizin were elevated by 58.15% and 32.43%, respectively. The differences were very significant for glycyrrhetinic acid ($P < 0.01$) and significant for glycyrrhizin ($P < 0.05$). The relative contents of both chemicals in S3 stress showed a decrease of 16.84% and 47.96%, respectively, while the absolute content of glycyrrhizin showed a highly significant decrease of 50.74% ($P < 0.01$). The result confirmed that the contents of glycyrrhetinic acid and glycyrrhizin were raised first, and then, were fallen during the increasing effect of water stress. When the water holding capacity of the oil field was below the threshold, both chemicals demonstrated a downward trend.

Salt stress on the contents of active ingredients, yield, and growth of *Glycyrrhiza glabra*

The changes in the germination rate of *Licorice* seeds under saline stress were shown in Figure 2. Under the three stress modes of NaCl, Na_2SO_4, and mixed salt, the overall seed germination rate demonstrated a trend of first increasing and then decreasing as the salt concentration gradually increased. Under the two stress modes of $NaHCO_3$ and Na_2CO_3, the seed germination rate always maintained a downward trend. In NaCl stress mode, the seed germination rate tended to be the highest at a Na^+ concentration of 50 mmol/L, which was significantly higher by 4.75%. The germination rate of the control group was significantly higher than that in the experimental groups with the concentration of $Na^+ \geq 100$ mmol/L. The germination rates in the groups of Na_2SO_4 and mixed salt were smaller than the control group in all concentrations. There was no difference in the germination rates when the Na^+ concentration was below 50 mmol/L. The significant differences were observed at the Na^+ concentrations above 75 mmol/L. The germination rates in $NaHCO_3$ and Na_2CO_3 groups were smaller than that of control group and showed significant differences at all concentrations. The result confirmed that low concentration of NaCl could effectively increase the germination rate, while the more concentration of alkaline Na^+ was, the lower the germination rate was. The changes in germination potential and germination index under salinity stress were shown in Figure 3. The germination potential was significantly lower under all treatments compared to the control (Figure 3a). The germination potential at NaCl showed an increasing and then decreasing trend with increased salt concentration, while the remaining four treatments showed mainly a decreasing trend. In the same concentration of Na^+, NaCl corresponded to the best seed germination potential, except for the

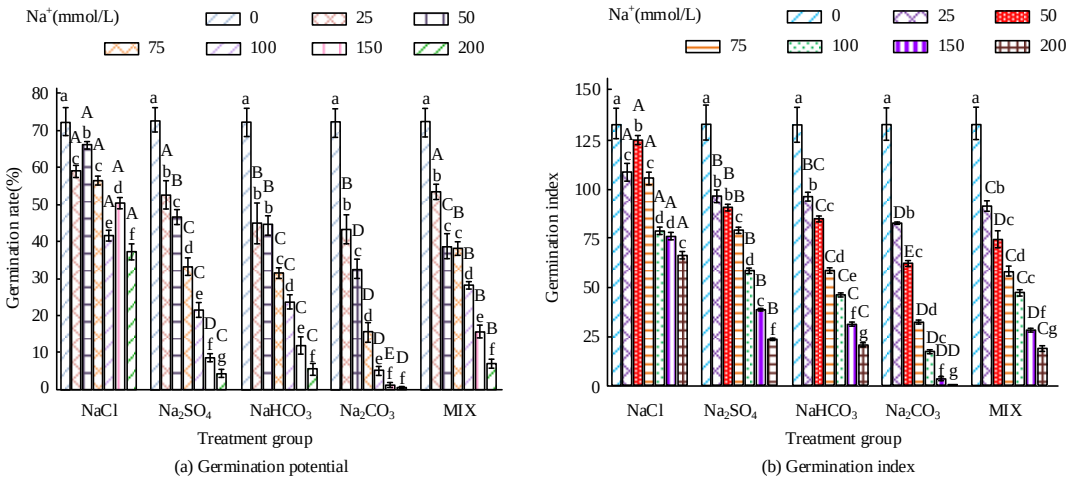

Figure 3. Changes in germination potential and germination index under salt alkali stress.

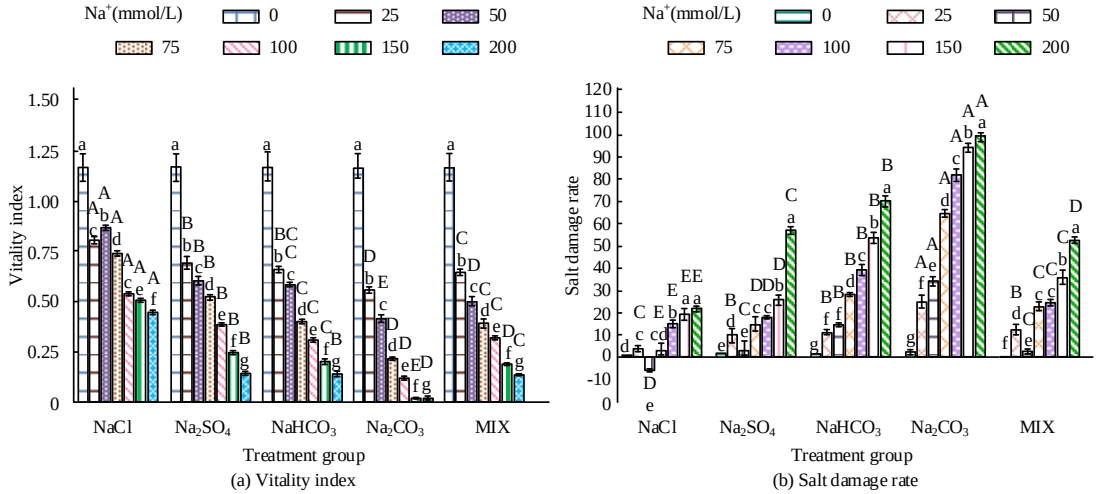

Figure 4. Changes in seed vitality index and salt damage rate under salt alkali stress.

concentration of 25 mmol/L. All other Na^+ concentrations were significantly different from Na_2SO_4 and mixed salt. In Na_2CO_3, all concentrations were significantly different from $NaHCO_3$ treatment except for 25 mmol/L. The results proved that the saline environment acted as an inhibitor of germination potential, and this inhibition was subsequently enhanced when the salt solution concentration continued increasing. The germination index was significantly greater in all five treatment modes (Figure 3b). The germination index for NaCl increased and then decreased during increasing salt concentrations with the highest value occurring at 50 mmol/L. For the other four treatments, the trends were always downward. At the same concentration of Na^+, the germination index of NaCl treatment was significantly higher than the other four treatments, while the germination index of Na_2CO_3 treatment was the opposite one.

Figure 4 demonstrated the changes in seed vigor index and salt damage rate under salinity stress. The vigor index was significantly different in all treatments (Figure 4a). Under NaCl treatment, the activity index first increased and then

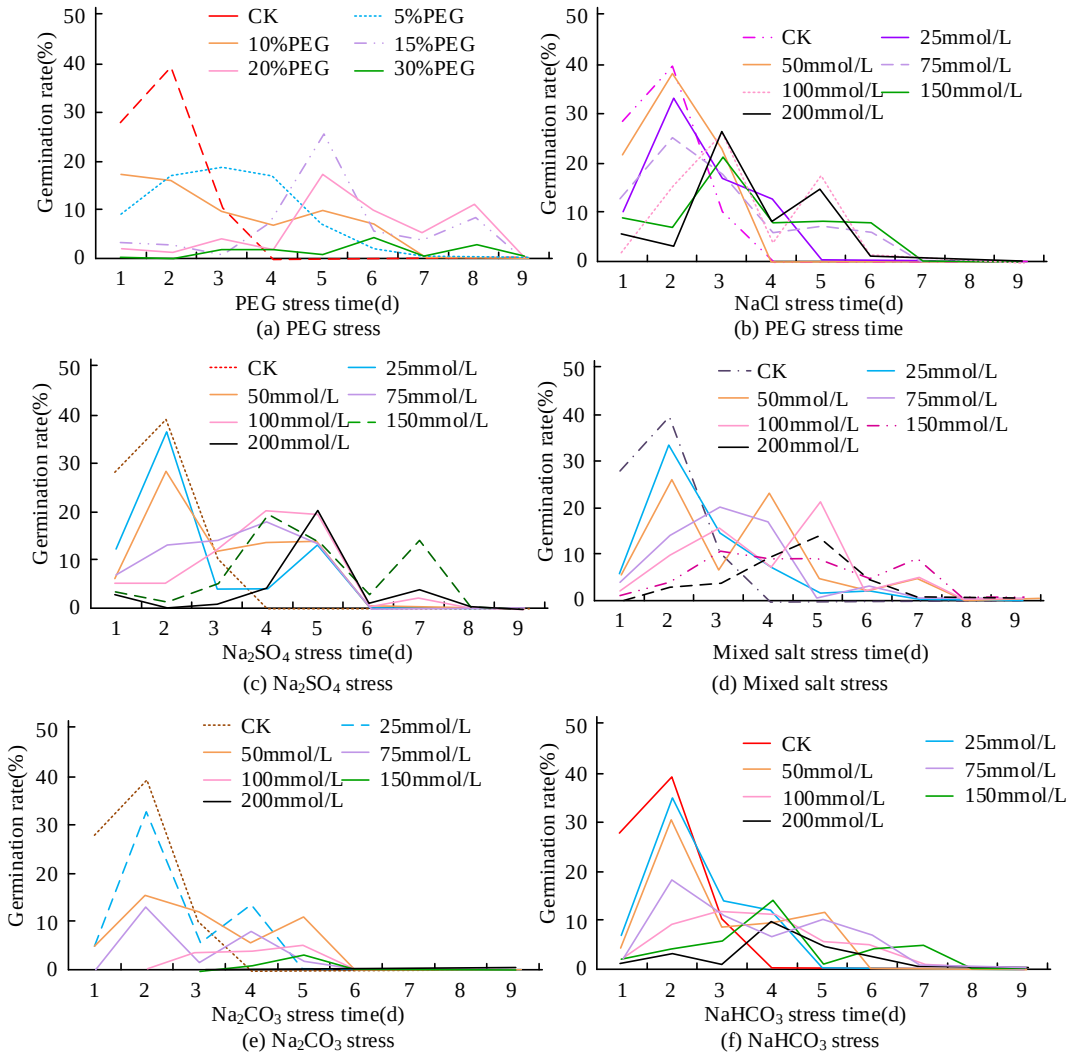

Figure 5. Seed germination process under salt alkali stress.

decreased with the increase of salt concentration, reaching its maximum value at 50 mmol/L. The activity index of the other four treatments showed a downward trend. In the groups of NaCl, Na_2SO_4, and mixed salt, salt damage first became smaller and then increased as the concentration of Na^+ increased with a minimum value occurring at 50 mmol/L in all cases (Figure 4b). There was no distinction between Na_2SO_4 and mixed salt groups. The corresponding salt damage rate for NaCl was -4.77%. The groups of $NaHCO_3$ and Na_2CO_3 both showed an increase in salt damage when Na^+ concentrations were increased. The results showed that the salt damage rate was 100% for Na_2CO_3 counterpart at 200 mmol/L. For the same concentration of Na^+, the salt damage rates were in descending order of NaCl, Na_2SO_4, mixed salt, $NaHCO_3$, Na_2CO_3. The damage caused by Na_2CO_3 was significantly greater than the other treatments, while the damage caused by NaCl was significantly less than the others, which indicated that NaCl had a lower salt damage effect, while Na_2CO_3 corresponded to a significantly greater salt damage effect.

The seed germination process demonstrated a high similarity under different saline stresses (Figure 5). When the saline concentration reached a certain level, there was a certain delay

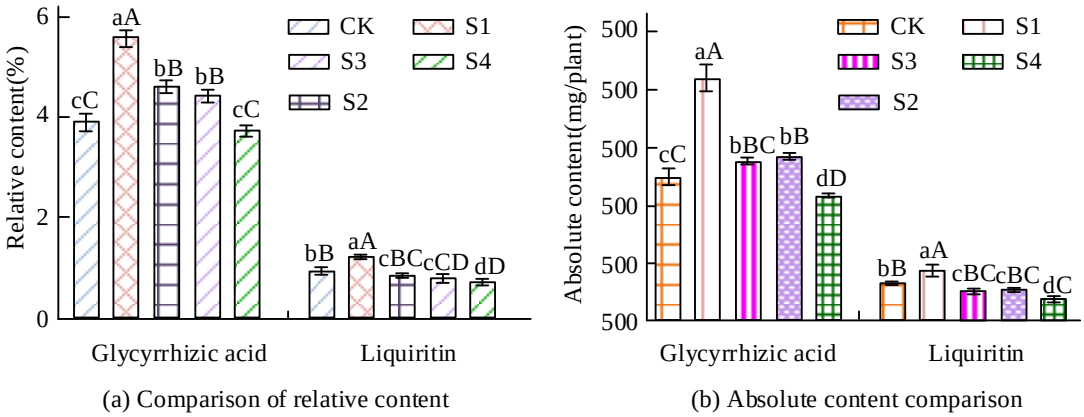

Figure 6. Changes in relative and absolute contents of glycyrrhizin and glycyrrhetinic acid under salt alkali stress.

in both initial germination and peak time. This study found that the deeper the saline stress, the more difficult the germination of seeds. At 200 mmol/L, seeds corresponding to Na_2CO_3 consistently failed to germinate. Germination occurred on day 1 for all modalities except for 200 mmol/L mixed salt, 30% PEG, and 100-150 mmol/L Na_2CO_3. Peak germination occurred on day 3 for the 5% PEG modality and on day 5 and beyond for the 15-30% PEG. The peak germination of 100-200 mmol/L NaCl appeared on day 3, while Na_2SO_4 and $NaHCO_3$ were on day 4, and the mixed salt, Na_2CO_3 were on day 5.

The changes in the relative and absolute contents of glycyrrhizin and glycyrrhetinic acid under salinity stress were shown in Figure 6. The peak relative and absolute contents of glycyrrhetinic acid and glycyrrhizin occurred in S1 in all treatments ($P < 0.01$). The minimum relative and absolute contents of both components occurred in S4 treatment. There was no significant difference in the relative content of glycyrrhetinic acid. However, a 17.20% reduction in the absolute content compared to the control was observed ($P < 0.01$). For glycyrrhizin, the relative content was reduced by 22.34% ($P < 0.01$) and the absolute content by 32.33% ($P < 0.01$). The result demonstrated that the contents of both components increased at first and then decreased under increasing salinity stress. The threshold of increase was in the S1 mode, and when the range of field water holding capacity was within the threshold, the content of both components decreased to different degrees.

Effect of fertilizer application on yield, growth, and component content of *Licorice*

Table 5 showed the changes in root diameter, root length, and folded dryness of *Licorice* caused by different fertilizer application methods. Among all fertilization methods, there was a significant difference in root length between N2K2 and N2P2 ($P < 0.05$) with N2K2 having the largest increase in root length of approximately 24.70%. However, the root diameter showed no significant difference in all fertilization methods. The greatest increase in root diameter was found in the K3 fertilization method with an approximate increase of 10.99%, while the smallest was in N1P3K3 with a decrease of 1.18% ($P < 0.05$). Compared with the control, there was no difference in the percentage of *Licorice* folded dry with the maximum value appearing in the N3P1K3 fertilization method, which increased by approximately 1.58%. The minimum value was the N1P3K3 method, which reduced by 8.37% ($P < 0.05$).

Table 6 showed the changes in the yield, glycyrrhizin, and glycyrrhetinic acid of *Licorice* under different fertilizer application methods. The results showed that there were significant differences in the effects of different fertilization

Table 5. Changes in root diameter, root length, and drying rate of *Licorice* caused by different fertilization methods.

Treatment	Drying rate (%)	Root diameter (mm)	Root length (cm)
N1P3K3	33.79±2.68b	7.48±0.29b	80.38±3.71ab
N3P1K3	37.58±1.47a	7.75±0.59ab	83.48±2.76ab
N3P3K1	37.71±1.83ab	7.62±0.03ab	82.68±6.63ab
N2P2	35.35±0.72ab	7.58±0.45ab	85.11±11.03a
N2K2	35.54±1.38a	8.11±0.08ab	89.19±11.16a
P2K2	35.85±0.33ab	8.24±0.23ab	81.25±1.11ab
N3	37.25±2.74ab	8.08±0.55ab	80.11±4.10ab
P3	34.74±1.09b	8.42±0.21ab	81.08±4.51ab
K3	36.28±1.22ab	8.73±0.28a	81.77±5.72ab
Control (CK)	37.88±1.67ab	7.53±0.31ab	71.42±5.76b

Note: In the same column, the letters represented the differences at the $P < 0.05$.

Table 6. Changes in glycyrrhizic yield, glycyrrhizin, and glycyrrhetinic acid under different fertilization methods.

Treatment	Glycyrrhetinic acid (%)	Glycyrrhizin (%)	Yield (kg/hm)
N1P3K3	3.12±0.06dCD	1.01±0.06deBC	5165.67±160.26dD
N3P1K3	3.18±0.19bcdABC	1.29±0.08aA	5514.15±61.47bB
N3P3K1	3.19±0.15cdBC	1.16±0.09abcdAB	5199.53±164.02dCD
N2P2	3.25±0.11abcABC	1.25±0.06abcdABC	5466.47±12279cdBCD
N2K2	3.48±0.11aA	1.31±0.08abA	5853.22±111.74aA
P2K2	3.07±0.32dCD	1.23±0.09bcdABC	5371.20±46.12bcBC
N3	3.27±0.05abcABC	1.12±0.05cdeABC	5327.03±146.77bcBCD
P3	3.45±0.21abAB	1.09±0.06cdeABC	5537.38±67.31bB
K3	3.43±0.05abAB	1.18±0.08abcAB	6122.53±137.22aA
CK	2.81±0.08eD	0.86±0.05eC	4824.47±142.68eE

Note: In the same column, the letters represented the differences at the $P < 0.05$.

methods on the yield of *Licorice* ($P < 0.01$) with the highest yields observed under the K3 fertilizer application method of 27.03% increasing. The effects of the different fertilizer application methods on the glycyrrhizin and glycyrrhetinic acid content also showed significant differences and were all greater than that of control. N3P1K3, N2K2, and K3 fertilization methods demonstrated very significant differences in glycyrrhizin contents comparing to the control group ($P < 0.01$). Except P2K2 and N1P3K3, the contents of glycyrrhizin in each fertilization mode reached a very significant level ($P < 0.01$).

Conclusion

The determination of stress factors for the medicinal components of Chinese traditional medicinal herbs is an important requirement for the cultivation of herbs. In this study, *Licorice* was chosen as the experimental subject, and different WAS and fertilization methods were tested to investigate the changes in yield and medicinal components of *Licorice*. The results showed that, at a concentration of 30%, seed germination could only reach 17.01% of the control. There was a difference in root length under S1 water stress ($P < 0.05$). Under S2 stress, the lateral roots, root length, and root dry weight increased by about 42%, 21%, and 52%, respectively ($P < 0.05$). In S1 water stress, the relative content of glycyrrhetinic acid and glycyrrhizin increased by 32.89% and 22.45%, respectively ($P < 0.01$). In the same concentration of Na^+, NaCl had the best seed germination potential. Except for the 25 mmol/L Na^+ concentration, all other Na^+ concentrations demonstrated significant

differences compared to Na_2SO_4 and mixed salts. The relative content of glycyrrhetinic acid had no distinction and the absolute content was reduced by 17.20% compared to the control ($P < 0.01$). The yield difference of *Glycyrrhiza uralensis* with different fertilization methods was extremely significant, while other fertilization methods tended to be significant compared to control group ($P < 0.01$). The maximum yield was obtained under the K3 fertilization method with a significant increase of 27.03%. In summary, the suitable external environment for the growth of *Licorice* had been determined. Due to time and conditions limitations, this study did not analyse the effects of light, altitude, and temperature. So further optimization needs to be carried out by integrating various external environmental factors.

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