LRF-691
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RESEARCH ARTICLE Legume Research- An International Journal, Volume Issue : ()

Higher Temperature had Positive Effects on Seed Germination of *Glycyrrhiza glabra* under NaCl Stress

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10.18805/LRF-691

ABSTRACT

Background: Agricultural soils are affected by secondary salinization due to the fragile ecological environment and lack of scientific management. It is of interest to utilize the germplasm resources of salt-tolerant plants and improve their salinity stress resistance. Seed germination of the medicinal halophyte *Glycyrrhiza glabra* is affected by soil salt content and temperature during seeding. This study determined the favourable soil temperature for *G. glabra* seed germination in salinised soil.

Methods: Four sodium chloride concentration gradients (control, hyposaline, medium-saline and hypersaline) and 3 temperatures (low- medium- and high temperature) were used for a total of 12 treatments; 30 seeds were utilised in each treatment and each treatment was repeated 3 times.

Result: *G. glabra* seed germination indexes significantly decreased with increased salt concentration. Interestingly, higher temperature could improve the salt tolerance of seeds. Seed germination percentages of hyposaline, medium-saline and hypersaline treatments were higher at elevated temperature than those at low temperature by 33.53%, 127.1% and 41.7%, respectively and were higher than those at medium temperature by 14.1%, 121.6% and 54.6% respectively. Therefore, *G. glabra* seeds growing in saline habitats should be sown in summer. This study provided a scientific basis for medicinal plant cultivation in salinised soil.

Key words: *G. glabra*, Germination, Licorice, Membership function value, Salinity, Temperature.

INTRODUCTION

Soil salinization is a main global environmental factor influencing seed germination, plant growth, propagation and distribution (Mahmoudi *et al.*, 2020). Incomplete statistics suggests that approximately 1 billion hectares (7% of global land) are affected by salinization (Zhao *et al.*, 2021) and this is predicted to increase. Widespread soil salt accumulation causes poor crop growth and affects global agriculture development (Prakash *et al.*, 2021). Additionally, large salt desert soil areas are not utilised. Therefore, exploiting the germplasm resources of salt-tolerant plants and improving their salt-tolerance will address continual global population growth.

Glycyrrhiza glabra is a perennial herb belonging to the genus *Glycyrrhiza* in the leguminous family used in traditional medicine in many countries. Its dry root and rhizome extracts have many functions including detoxification (Idrees *et al.*, 2021; Muchiri *et al.,* 2022), relieving coughs (Jalali *et al.*, 2021), anti-inflammatory (Inui *et al.*, 2021) and immune regulation (Wang *et al.*, 2013; Srivastava *et al.*, 2019; Simayi *et al.*, 2021). Its stems and leaves are excellent forage for sheep and cattle and are widely used in animal husbandry (Chen *et al.*, 2021). It is mainly distributed in Europe, West Asia, North America and Xinjiang in China (Sarker *et al.*, 2018). Glabridin in its roots and rhizomes has a skin whitening effect known as “skin whitening gold” (Kapkoti *et al.*, 2020). Many local wild populations are endangered following recent international demand for the plant’s underground organs. Therefore, cultivated licorice is becoming the main source of market supply. Unfortunately, licorice cultivation is limited to farmland areas. As *G. glabra* exhibit some tolerance to salinity, it is of great application value to explore the cultivation measures that can improve the salt tolerance of seeds and develop the planting of the licorice on salinized soil.

Seed germination is a crucial plant life cycle stage that is affected by temperature and salinity (Cirka *et al.*, 2021). *G. glabra* exhibits some salinity tolerance. Therefore, it is worthwhile exploring the interaction between temperature and salt stress on licorice seed germination to explore the optimum sowing temperature for improving seedling emergence. Farmers start sowing in early spring to ensure that seedlings have a long growth time in the current year. However, it is unclear whether relatively low temperatures in early spring are favourable for *G. glabra* seed germination. Therefore, we simulated soil temperature conditions in early spring (low temperature), late spring (moderate temperature) and early summer (high temperature) to reveal the influence

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How to cite this article: Dong, X., Zhao, Z., Ma, X., Li, X. and Ma, M. (2022). Higher Temperature had Positive Effects on Seed Germination of *Glycyrrhiza glabra* under NaCl Stress. Legume Research. DOI: 10.18805/LRF-691.

Submitted: 12-04-2022 **Accepted:** 07-07-2022 **Online:** 30-07-2022

of sowing stage on seed germination of the licorice. In addition, the difference in *G. glabra* seed salt tolerance under three temperature conditions were evaluated by the membership function. This provided a scientific basis for the selection of the optimal *G. glabra* sowing time.

MATERIALS AND METHODS

Experiment material

Mature *G. glabra* seeds were collected from Yuli County, Xinjiang, China (41°35′49″N, 86°31′82″E) in September 2020.

Seed germination tests

Healthy, full, uniform seeds were placed in a dry beaker and soaked in 98% sulfuric acid at room temperature for 30 min, then thoroughly washed with distilled water. Seeds were evenly separated into 4 sections, placed into 4 beakers and soaked in NaCl solutions (80-160-and 320 mmol/L to simulate the hyposaline-medium-saline- and hypersaline soil, respectively), or distilled water (control) at room temperature for 8 h. Then, seeds were placed in petri dishes (Φ = 9 cm) covered with 2 layers of sterile filter paper. Thirty seeds were evenly placed in each dish and 10 mL NaCl solution or distilled water was added. Seeds were cultured under 20/15°C (low temperature), 25/20°C (medium temperature) and 30/25°C (high temperature) and each treatment was repeated 3 times. Petri dishes were weighed every day and the lost water was replenished to maintain consistent NaCl concentrations caused by evaporation.

Seeds were germinated when the radicle elongated to ≥2 mm. Germinated seed numbers were recorded every 24 h for 7 consecutive days. Ungerminated seeds after 7 d were thoroughly rinsed with distilled water, placed in new petri dishes with filter paper, then 5 mL of distilled water added and incubated at the same temperature for another 7 d to investigate germination recovery.

Germination percentage (GP), germination index (GI), seedling vigour index (VI), recovery germination percentage (RP) and seed membership function values (MVFs) were calculated using the following formulas:

$$GP\% = n/nT \times 100\%$$

Where

n = Number of germinated seeds.

nT = Total seed number.

$$GI = \sum (Gt/Dt)$$

Where

Gt = Seed number germinated at time “t” days.

Dt = Seed germination days.

$$VI = S \times GI$$

Where

S = Total length of the radicle and shoot (cm).

$$RP\% = \frac{(a-b)}{(c-b)} \times 100\%$$

Where

a = Germinated seed numbers in distilled water.

b = Germinated seed numbers in stress treatments.

c = Total seed number.

Salt tolerance evaluation

G. glabra salt tolerance was evaluated using the MFV at three temperatures using the following equation (Ding *et al.*, 2018):

$$X(\mu) = \frac{X - Xmin}{Xmax - Xmin}$$

Where

X = Plant salt tolerance.

Xmax and Xmin = Maximum and minimum values of the indexes GP, GI, or VI, respectively.

The MFVs of each index in different salts under each temperature treatment were summed and the average was calculated. The subordinate function values of all seed salt resistance indexes at each temperature are added to the average according to the determined seed salt resistance at different temperatures. The MFV reflects the seed stress degree; the larger the MFV, the smaller the influence caused by salt stress and the stronger the plant’s salt tolerance (Choudhary *et al.*, 2021).

Data analysis

SPSS 20.0 software (IBM Corp., USA) was used for data analysis. One-way ANOVA of minimal significant difference (LSD) and Duncan’s multiple comparisons were used to analyse the significance of each parameter among the five NaCl concentrations or three temperatures. The effects of NaCl and temperature interactions on *G. glabra* seed germination were analysed by two-way ANOVA. Origin 2017 (Origin Lab, USA) was used to generate the charts.

RESULTS AND DISCUSSION

Effects of NaCl on *G. glabra* germination

Seed germination is a sensitive plant life cycle period that is crucial for plant growth and population establishment (Mahmoudi *et al.*, 2020). Seed germination percentage and germination speed determines whether *G. glabra* is cultivated in saline soil (Tong *et al.*, 2021). Seed salt tolerance at germination may be assessed by considering the GP, GI and VI values (Gupta *et al.*, 2021). Seed GP, GI and seedling VI decrease as salt concentration increases indicating that high salinity inhibits seed germination which correlates with our findings (Kaya *et al.*, 2021).

G. glabra GIs varied with NaCl concentration under three temperatures indicating that the seed GP-, GI- and VI values significantly decreased with increasing NaCl concentration ($P<0.05$, Fig 1A-I). This indicated that excessive soil salinity negatively affects *G. glabra* seed germination, which consequently had negative effects on its growth and yield. The damage to *G. glabra* may occur through osmotic stress, ion toxicity (Bhatt *et al.*, 2020), or oxidative damage (Cirka *et al.*, 2021).

Effect of temperature on *G. glabra* germination

Sowing at an appropriate temperature is important for seed germination timing and seedling sprouting (Bhatt *et al.*, 2020) by changing hydrolase activity, membrane-binding protein activity and metabolic processes. It is traditionally believed that *G. glabra* can be seeded in spring, summer and autumn. But there are significant differences in soil temperature among different seasons. Therefore, it is worth studying whether sowing in different seasons will affect the seed germination of *G. glabra*.

We simulated soil temperatures in early spring (20/15 °C), late spring (25/20°C) and early summer (30/25°C) to investigate the temperature effect on *G. glabra* salt tolerance during seed germination (Fig 2). *G. glabra* GP, GI and VI significantly increased with elevated temperature. The GP at high temperature in hyposaline, medium-saline and hypersaline salt stress environments increased by 33.5%, 127.1% and 41.7%, GI increased by 18.14%, 86.5% and 78.7% and VI increased by 36.99%, 187.6% and 13.6%, respectively, compared with that at low temperature. Meanwhile, GP increased by 14.8%, 121.6% and 54.5%, at

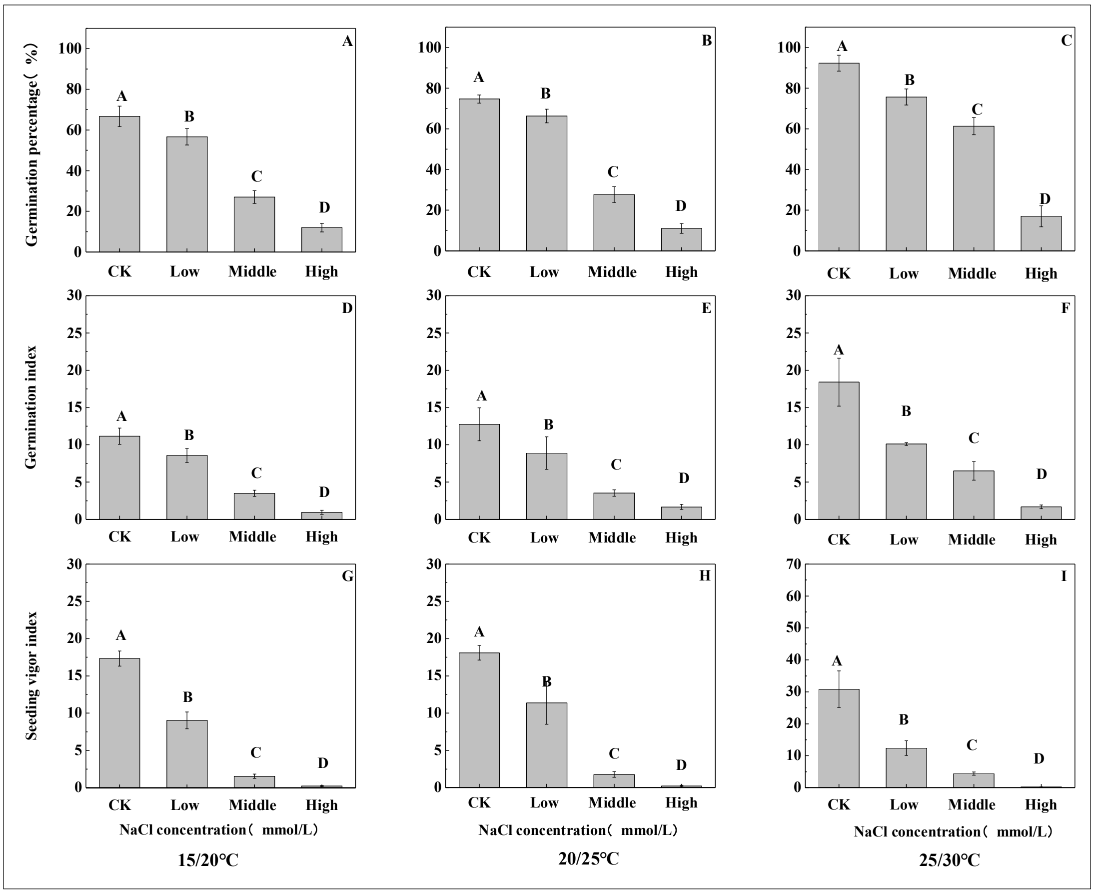

Fig 1: Effects of NaCl on *G. glabra* germination (means ± standard deviation).

Different uppercase letters indicated there is significant difference among different among different NaCl treatments.

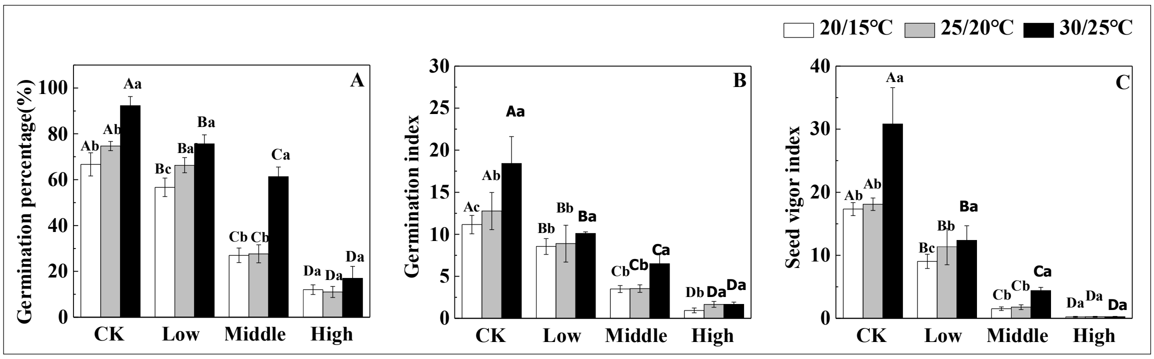

Fig 2: Effects of temperature on seed germination (means ± standard deviation).

Different uppercase letters indicated that there are significant differences between different NaCl treatments at the same temperature and different lowercase letters indicated that there were significant differences between different temperatures at the same NaCl treatment.

high temperature, GI increased by 13.61%, 84.4%and 1.6% and VI ascended by 8.8%, 148.6% and 4.2%, respectively compared with those grown at medium temperature. In conclusion, hypersaline soil inhibited *G. glabra* seed germination; however, higher temperature alleviated salt stress by promoting GP, GI and VI. Summer is the optimal sowing time if *G. glabra* is planted in heavily salinised land.

Effects of NaCl and temperature interaction on seed germination parameters

Temperature and salinity interactions significantly affect seed germination (Hadi *et al*., 2018; AL-Shoaibi, 2020; Piovan *et al*., 2019). Favourable temperature promotes seed germination under salinity stress; however, improper temperature aggravates salt stress on seed germination and even makes seeds lose vigour (Cardona *et al*., 2021). In this study, two-way ANOVA revealed that NaCl concentration significantly affected germination indexes (P< 0.001) (Table 1): increased NaCl concentration decreased germinated seed percentages and seedling vigour and delayed the germination time. Seed germination percentage, emergence speed and seedling vigour significantly increased with elevated temperature (P<0.001). The interaction between temperature and salinity levels significantly affected seed germination: GIs between the high- and medium temperature or between the high- and low temperature increased with elevated NaCl concentration.

G. glabra seeds had higher GP, faster germination speed and maintained higher seeding vigour at high temperature. *Glycine max* L. germination percentage is higher at low temperature than that at high temperature under salt treatments (Cirka *et al.,* 2021). In contrast, raising the temperature to an optimal level improves the salt tolerance of *Astragalus membranaceus* seeds during germination indicating that high temperature might alleviate salt stress (Xu *et al*., 2020) which correlates with our results.

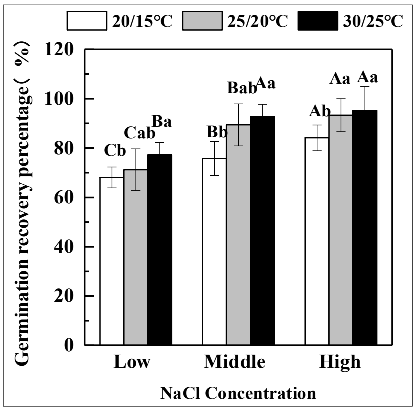

Fig 3: Effects of NaCl concentrations on *G. glabra* germination recovery percentage (mean ± SD).

Recovery test

Plants can develop corresponding growth strategies during seed germination and seedling growth according to the environment. Halophyte seeds maintain vitality in a salinised soil and can germinate after salt dilution by irrigation water or rain (Melendo and Gimenez, 2019). Most ungerminated seeds replaced into distilled water germinated (Fig 3) suggesting that high NaCl concentrations reversibly inhibited licorice seed germination which was alleviated by removing the salt stress. The RP reached 95.24% for seeds under high salinity and temperature conditions while the RP of seeds at low NaCl concentrations and high- and moderate temperature increased by 11.3% and 23.6%, respectively compared with that at low temperature (Fig 3). The RP increased by 25.5% and 31.0% at moderate temperature and high temperature, respectively under moderate NaCl stress compared with that at low temperature. The RP increased by 20.1% and 23.3%, respectively using moderate- and high temperature at high NaCl concentrations compared with that at low temperature. The *G. glabra* RP significantly increased with increasing environmental temperature under mild-, moderate-, or severe stress. This indicated that reversible factors such as osmotic stress and enzyme activity inhibition under NaCl treatment (especially high NaCl concentration) made it difficult for cells to absorb water and caused seeds to enter enforced dormancy to avoid adverse environmental effects. However, seeds germinated when conditions are favourable, especially at higher temperature. Therefore, timely irrigation after seed sowing in a salinised habitat alleviates the impacts of salt stress on *G. glabra* seed germination. This information may be used for planting and seedling protection in saline-alkali land and contributes to *G. glabra* cultivation in saline soil.

Comparison of *G. glabra* seed salt tolerance threshold at different temperatures

The membership function value can reflect the stress degree that the seeds suffered. The larger the MFV, the smaller the influence caused by salt stress and the stronger the salt tolerance of a plant(Choudhary *et al.*, 2021).Higher temperature corelated with higher mean MFV (GP, GI and VI, Table 2) and *G. glabra* salt tolerance was enhanced with increased temperature. Therefore, sowing in summer during

Table 1: Two-way ANOVA within salinity and temperature of germination percentage, germination index and seedling vigor index.

Germination indexes	NaCl			Temperature			NaCl × Temperature		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
GP	3	128.308	<0.001	2	149.68	<0.001	6	8.208	<0.001
GI	3	1035.769	<0.001	2	88.783	<0.001	6	50.957	<0.001
VI	3	411.306	<0.001	2	31.359	<0.001	6	18.506	<0.001

Table 2: Membership function values and the order of salt tolerance of *G. glabra* at germination stage among different temperatures.

Temperatures	MFV			Mean value	Rank
	GP	GI	VI		
20/15°C	0.34	0.26	0.11	0.24	3
25/20°C	0.39	0.29	0.13	0.27	2
30/25°C	0.57	0.42	0.19	0.40	1

high soil temperatures promotes *G. glabra* seed germination in salinised soil.

CONCLUSION

Soil salinization is a serious problem in global agriculture. The key to making good use of salinized soil is to establish the agricultural cultivation management regime which is suitable for salinized soil. Although *G. glabra* exhibit some tolerance to salinity, its seed germination is strongly inhibited by NaCl. Finding the environmental temperature is favorable to improve seeds' salt tolerance, we can choose the corresponding season for seed sowing, which will greatly improve seed germination percentage of the licorice in the salinized soil containing NaCl and the salinized soil may be explored to develop the cultivation of this medicinal plant. It is concluded that higher temperature is conducive to seed germination of *G. glabra* under NaCl stress. It is suggested that the seeds of *G. glabra* should be sown in early summer if the soil is mainly salinized with NaCl and irrigated regularly after sowing to ensure a higher seedling emergence rate.

Conflict of interest: None.

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