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RESEARCH ARTICLE

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Effect of PEG-simulated Drought Stress on Seed Germination of Three Medicinal Liquorice (*Glycyrrhiza*) Species

Mingcai Hou, Miao Ma

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ABSTRACT

Background: *Glycyrrhiza* is an important medicinal plant that has been in shortage in recent years because of high demand and exhaustive harvesting. Investigating the drought tolerance of liquorice species can inform the artificial cultivation of liquorice under different water conditions and help address the shortage.

Methods: Seeds of *G. uralensis*, *G. glabra* and *G. inflata* were treated with different concentrations of polyethylene glycol (PEG)-6000 solutions to simulate different levels of drought stress. The germination process germination rate, germination index, vigour index, stress tolerance index and recovery germination percentage of seeds were measured. Drought tolerance was evaluated using the membership function.

Result: Low concentrations of PEG promoted the seed germination of *G. glabra* and G. *inflata*, whereas high concentrations inhibited the seed germination of all three plants. The degree of inhibition increased with increasing PEG concentration. Under mild PEG stress, the drought tolerance of *G. uralensis* was highest and that of *G. glabra* was the lowest. However, under severe PEG stress, the drought tolerance of *G. glabra* was the highest and that of *G. uralensis* was the lowest. Therefore, seeds of *G. uralensis* should be sown in relatively moist soil and those of *G. glabra* in relatively arid soil.

Key words: Drought, Germination, Liquorice, Membership function value.

INTRODUCTION

Water is an important environmental factor that determines the survival of plants in arid or semi-arid ecosystems it is one of the main factors restricting agricultural production and economic development (Farjam *et al.,* 2015; Elnaggar *et al.*, 2018). Screening drought-tolerant crop varieties and developing water-saving agriculture is an effective strategy to solve water deficiency in arid regions (Li *et al.,* 2016). *Glycyrrhiza glabra* L., *G. uralensis* Fisch. *G. inflata* are perennial herbs of the Leguminosae family.

The roots and rhizomes are used as important traditional medicinal materials (Jiang *et al*., 2020), containing triterpenes and flavonoids as active components. They exhibit various properties such as anti-inflammatory, antitussive expectorant (Ming and Yin, 2013). The stems and leaves are also excellent forage and are widely used in animal husbandry (Wang *et al.,* 2020). In addition, liquorice is an important sand-fixation species that plays an irreplaceable role in the ecological protection and environmental security of desert areas (Chen *et al.,* 2019). In recent years, the demand for liquorice and its products has been increasing sharply, resulting in a shortage of liquorice resources in the market (Chang *et al.,* 2020). However, long-term and exhaustive harvesting has brought many wild populations to the brink of extinction. Therefore, the development of artificially cultivated liquorice to replace wild liquorice is a feasible way to alleviate the shortage.

Seed germination, the initial stage of the plant life cycle, affects the later morphogenesis of the plant and is also the period most susceptible to drought stress (Stefania *et al.,* 2017). Studies have shown that crops with a high tolerance to stress during seed germination can obtain higher yields in stressful environments (Ashraf and Rauf, 2001). Although the seed germination responses of *G. uralensis*, *G. glabra* and *G. inflata* to drought stress have been studied (Wei *et al.,* 2009; Han *et al.,* 2022), the relative degrees of their drought tolerance is still unknown. Therefore, we systematically studied the seed germination responses of these three species to different concentrations of polyethene glycol (PEG) treatment. PEG-6000 is a polymer penetrant with good water and fat solubility (Klarod *et al.,* 2021). A certain concentration of PEG solution can place plant tissues and cells under drought-like stress and effectively simulate the soil drought environment without affecting plant cells, which is the ideal simulated drought stress system widely used in the study of plant drought tolerance (Beyaz and Yildiz, 2020; Lei *et al.,* 2022).

In this study, PEG-6000 was used to simulate drought stress its effects on the germination of three medicinal liquorice species were compared to provide a scientific basis

Ministry of Education Key Laboratory of Xinjiang Phytomedicine Resource Utilization, College of LifeSciences, Shihezi University, Shihezi, Xinjiang 832003, the People's Republic of China

Corresponding Author: Miao Ma, Ministry of Education Key Laboratory of Xinjiang Phytomedicine Resource Utilization, College of LifeSciences, Shihezi University, Shihezi, Xinjiang 832003, The People's Republic of China. Email: mamiaogg@126.com

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for germplasm selection for the cultivation of medicinal liquorices under different water conditions.

MATERIALS AND METHODS

Seed collection and pretreatment

Seeds of *G. uralensis* and *G. inflata* were collected in Yuli County (41°38′ 83″ N, 86°28′ 73″ E) and seeds of *G. glabra* in Bachu County (39°46′41″N, 78°31′11″E) in Xinjiang, China in September 2020. These seeds were used in a germination experiment at the Shihezi University. Seeds with uniform size and full particles were selected and soaked in 98% H_2SO_4 for 30 mins. The acid solution was rinsed off with running water and then the seeds were soaked in 0.3% $KMnO_4$ for 10 min to disinfect the seed surface. Finally, the samples were thoroughly rinsed with distilled water.

Seed germination

Seeds were treated with five concentration levels of PEG-6000 (0% , 5%, 15%, 25% and 30%), with five replicates per treatment. For each treatment, 150 seeds with full grains and uniform size were selected. The seed surfaces were disinfected with 70% alcohol and rinsed with sterile running water three times (2 min each time). Petri dishes (9 cm) with two layers of sterile filter paper were used as germination beds. Each dish was added with 15 mL PEG solution of the appropriate concentration. After the filter paper was fully infiltrated, 30 seeds were evenly placed on each dish. The dishes were covered and placed in an artificial climate incubator (PQX-330A-12H; Ningbo Life Science and Technology Co., Ltd., China) at 25°C, 80% relative humidity, 12 h/d light and 4000 lx light intensity. During germination, water loss due to evaporation was monitored by daily weighing and replenished. The number of seeds that germinated was counted daily. Seeds were considered germinated when the radicle emerged at least 2 mm from the seed coat. Five germinated seeds were randomly selected from each petri dish and the lengths of the seedling radicle and hypocotyl were measured.

After 7 days, seeds that failed to germinate were transferred to distilled water to test their ability to retain viability. The seeds were incubated again under the same conditions as described above. Germinated seeds were counted and removed daily for 7 d.

Measurement Indicators

The formulae used for various germination parameters are as follows:

$$\text{Germination percentage (GP)} = \frac{n}{N} \times 100\%$$

(Jhanji and Dhingra, 2020)

Where

n = Number of germinated seeds in 7 d.

N = Total number of seeds.

Germination index (GI) =

$$(nd_1/1) + (nd_2/2) + (nd_3/3) + \ldots + (nd_i/i)$$

(Xia *et al.*, 2021)

$$\text{Vigour index (VI)} = (GI) \times S$$

(Tong *et al.*, 2021)

Which indicates the germination speed where nd_1, nd_2, …, nd_i,denotes the number of seeds germinated on days 1, 2, …, i S is the average seedling length.

Germination stress tolerance index (GSI) = [PI (stressed seeds) / PI (control seeds)] × 100% = [$(nd_1 \times 1) + (nd_2 \times 0.75) + (nd_3 \times 0.5) + (nd_4 \times 0.25)$ (stressed seeds)] / [$(nd_1 \times 1) + (nd_2 \times 0.75) + (nd_3 \times 0.5) + (nd_4 \times 0.25)$ (control seeds)] (Elnaggar *et al.*, 2018)

which indicates drought tolerance where PI is promptness index.

Recovery germination percentage (RG) =

$$(a - b) / (c - b) \times 100$$

(El-Keblawy *et al.*, 2020)

Where

a = Total number of seeds germinated after being transferred to distilled water.

b = Total number of seeds germinated in PEG solution.

c = Total number of seeds.

The rate of germination was also calculated using the modified Timson index of germination velocity (as described above) to estimate the speed of germination recovery.

Comprehensive evaluation of drought tolerance

Drought tolerance was evaluated using the membership function value (MFV) and the fuzzy comprehensive evaluation method. The MFV for drought tolerance was calculated using the following equation:

$$X_i = \frac{(X - Xmin)}{(Xmax - Xmin)} \times 100\%$$

(Chen *et al.*, 2012)

Where

X_i = Represents the MFV of the index in a specific germplasm.

X = Actual measured value of the index in a specific germplasm.

Xmax and Xmin = Maximum and minimum values observed in all germplasms, respectively.

Statistical analysis

Data were analysed using SPSS version 20.0 (IBM Corp, USA). One-way ANOVA and the least significant difference was used to test for differences in seed germination parameters within the same species under different PEG treatments. Interspecific differences in germination parameters under the same PEG concentration were evaluated using the independent sample *t*-test. Graphing was performed using Origin 2018 (Origin Lab, USA).

RESULTS AND DISCUSSION

Effects of PEG stress on seed germination process

The germination process of all three species was significantly affected by PEG stress ($P<0.05$; Fig 1). In the control, seeds of all three species began to germinate on day 1 and maximum GP was reach on day 3 for *G. uralensis* and *G. glabra* and on day 4 for *G. inflata*. Under mild PEG stress (5%-15%), all three species also began to germinate

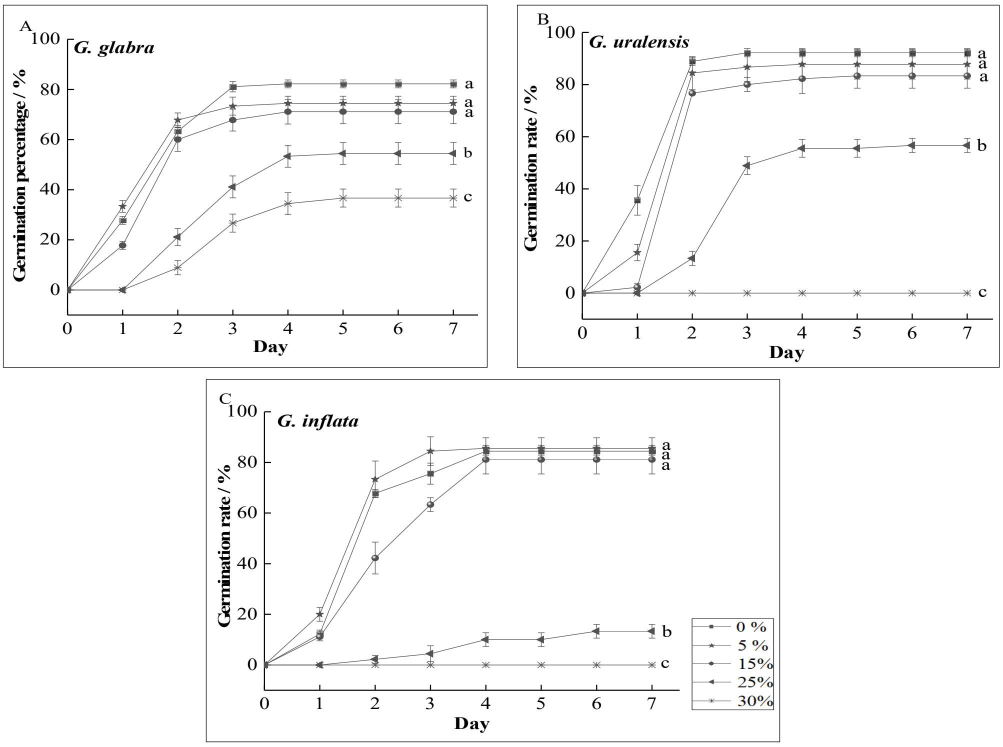

Fig 1: The effects of different concentrations of PEG solution on the germination process of three licorice seeds (Mean ± SE), different lowercase letters in the figure indicate that the same species has significant differences under different concentrations of PEG solution.

on day 1; however, the time to maximum GP for *G. glabra* and *G. uralensis* seeds was delayed by 1 d. Further delays were observed under severe PEG stress (25%-30%). At 25% PEG, the germination onset of *G. uralensis*, *G. glabra G. inflata* seeds was delayed to day 2 and the time to maximum GP was delayed by 2, 3 and 3 days, respectively, compared with the control. At 30% PEG, the seeds of *G. uralensis* and *G. inflata* did not germinate until day 7. These results show that showed that PEG solution could delay the germination initiation time and the time to reach the final germination percentage of the three liquorice seeds, but low concentration (5%) PEG solution could accelerate the seed germination process of *G. glabra* and *G. inflata* improve the final seed germination percentage of *G. inflata* seeds. This may be related to the fact that low concentration PEG solution can be used as a osmotic regulator to regulate the degree and state of water absorption of cells, which can stabilize the water absorption of seeds thus improve the germination rate and tidiness rate of seeds (Jia *et al.,* 2020).

Effects of PEG stress on GP

GP is an important indicator of the germination ability and quality of seeds. PEG stress had a significant effect on the GPs of all three species (P<0.01). As PEG concentration increased, the GPs of all three species showed a downward trend; however, there were interspecific differences in their changing patterns (Fig 2). In the control, the GP of *G. uralensis* seeds was highest. Under mild PEG stress, the GP of *G. uralensis* and *G. inflata* seeds were significantly higher than those of *G. glabra* seeds. Increasing the concentration of PEG from 15% to 25% decreased the GP of *G. inflata* seeds by 71.11%, while increasing the concentration from 25% to 30% decreased the GP of *G. uralensis* seeds by 56.67%. At 30% PEG, the GPs of *G. uralensis* and *G. inflata* seeds were 0, while that of *G. glabra* seeds was 44.60% of that in the control. These results show that under mild PEG stress, *G. uralensis* and *G. inflata* seeds had higher GPs than *G. glabra* seeds; however, under severe PEG stress, the germination of *G. uralensis* and *G. inflata* seeds were severely inhibited, whereas the seeds of *G. glabra* maintained a high GP. The results of this study also showed that high concentration of PEG solution inhibited the germination of three liquorice seeds and the inhibition degree increased with the increase of solution concentration, which might be due to low water potential or high osmotic pressure seriously hindered the absorption of water in the initial stage of seed germination and inhibited the normal germination of seeds (Lamia *et al.*, 2012).

Effects of PEG stress on GI and VI

GI and VI can be used to measure the germination rate and vigour status of certain plant seeds. PEG stress had a significant effect on the GIs and VIs of all three species (P<0.01). As PEG concentration increased, the GI and VI of *G. inflata* seeds first increased then decreased, while the GIs of *G. uralensis* and *G. glabra* seeds gradually decreased

(Fig 3, Fig 4). Under the control and mild PEG treatments, the GI and VI of *G. uralensis* seeds were significantly higher than those of *G. glabra* and *G. inflata* seeds. Increasing the concentration of PEG from 15% to 25% decreased the GI and VI of *G. inflata* seeds by 91.53% and 92.94%, respectively. At 30% PEG, the GIs and VIs of *G. uralensis* and *G. inflata* seeds decreased to 0, whereas those of *G. glabra* seeds were 24.66% and 16.17% of those in the control, respectively.These results show that the seeds of *G. uralensis* had the highest germination speed and seed vigour under mild PEG stress; under severe PEG stress, the germination speeds of *G. uralensis* and *G. inflata* seeds were severely inhibited, while *G. glabra* seeds maintained a high GI and VI. Imbibition is a necessary process of seed germination and the reason for seed imbibition is the hydrophilic substances in the embryo or endosperm, such as protein and starch. And compared to starch, protein is more hydrophilic (Zamora-Briseno and De Jimenez, 2016). The results of our study showed that the seeds of *G. glabra* have a stronger ability to obtain water from a dry or water-deficient environment to make themselves swell and germinate subsequently, while compared with *G. inflata* and *G. uralensis*. However, whether the differences among the three species in their seed germination percentage or germination rate is related to the inter-specific differences in composition of hydrophilic substances in the three seeds is still unknown.

Effects of PEG stress on GSI

GSI can be used to assess the tolerance of plant seeds to drought stress more intuitively. PEG stress had a significant effect on the GSI of all three species (P<0.01). As PEG concentration increased, the GSI of *G. inflata* seeds first increased and then decreased, whereas those of *G.*

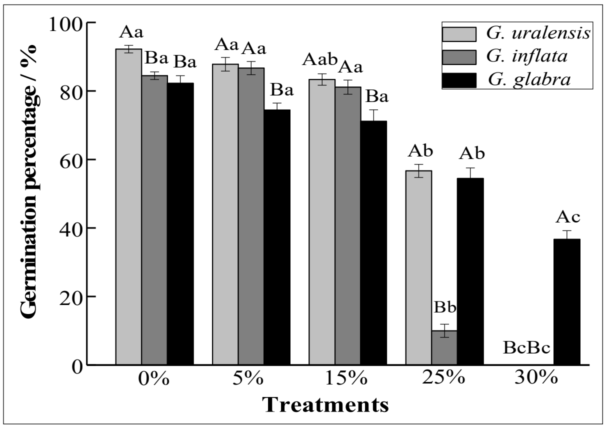

Fig 2: Effects of PEG solution with different concentrations on seed germination percentage of three licorice seeds (Mean ± SE). Different capital letters indicate significant differences among species at the same concentration and different lowercase letters indicate significant intraspecific differences under different PEG solution concentrations.

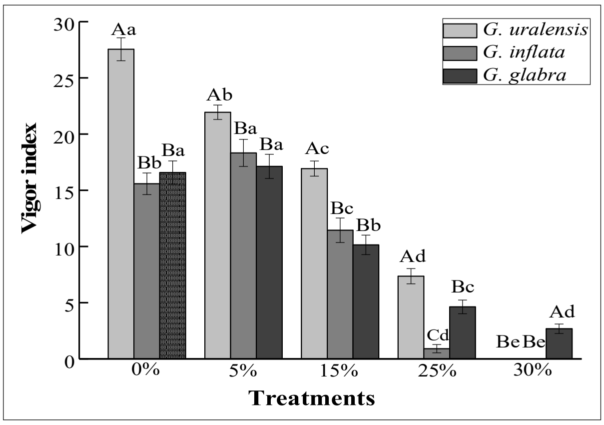

Fig 4: Effects of different concentrations of PEG on the vitality index of three licorice seeds (mean ± SE). Different capital letters indicate significant differences among species at the same concentration and different lowercase letters indicate significant intraspecific differences under different PEG solution concentrations.

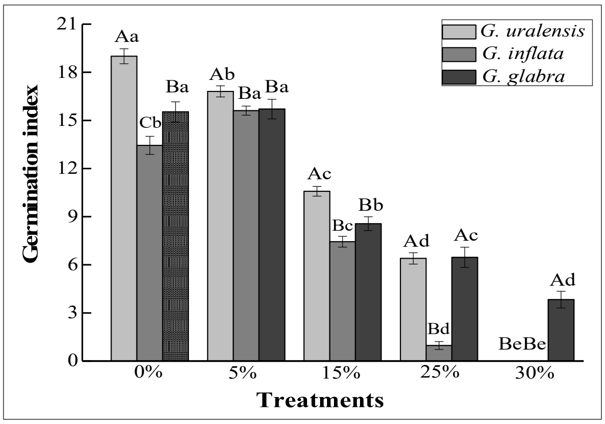

Fig 3: Effect of different concentrations of PEG on the germination index of three licorice seeds (mean ± SE). Different capital letters indicate significant differences among species at the same concentration and different lowercase letters indicate significant intraspecific differences under different PEG solution concentrations.

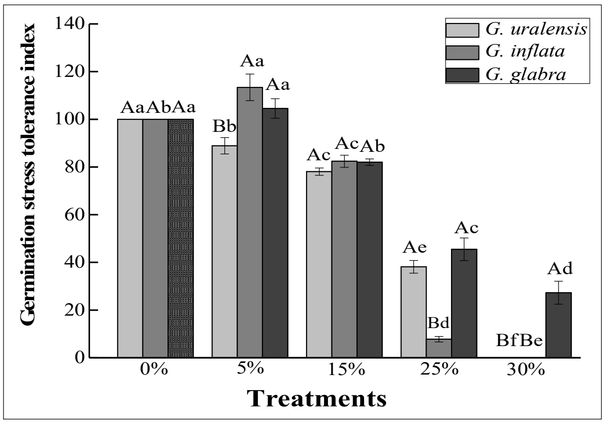

Fig 5: Effect of PEG at different concentrations on the tolerance index of three licorice seeds (mean ± SE). Different capital letters indicate significant differences among species at the same concentration and different lowercase letters indicate significant intraspecific differences under different PEG solution concentrations.

uralensis and *G. glabra* seeds continuously decreased (Fig 5). Under mild PEG stress, the seeds of *G. inflata* had the highest GSI. Increasing the PEG concentration from 15% to 25% decreased the GSI of *G. inflata* seeds by 74.63%. Increasing the concentration from 25% to 30% decreased the GSI of *G. uralensis* seeds by 38.15%. At 30% PEG, the GSIs of *G. uralensis* and *G. inflata* seeds were reduced to 0, whereas that of *G. glabra* seeds remained at 27.26%. These results show that *G. inflata* seeds had the strongest drought tolerance under mild PEG stress and *G. glabra* seeds had the strongest drought tolerance under severe PEG stress. Han *et al*. (2022) pointed out that drought-resistance of *G. inflata* seeds was stronger than those of the other two liquorice, the result was different from ours, which may be due to the genetic differences among the seeds in the two researches, Han's seeds was obtained from Ningxia provience and ours was collected in Xinjiang province. Populations with different genotypes may have different phenitypes while being exposed to drought stress conditions.

Effects of PEG stress on RG

RG reflects the ability of plant seeds to recover to normal germination after being stressed and is an important indicator of plant stress tolerance. PEG stress had a significant effect on the RGs of all three species($P<0.05$). As PEG concentration increased, the RGs of *G. uralensis* and *G. inflata* seeds showed a gradual upward trend, whereas that of *G. glabra* seeds first increased and then decreased (Fig 6). Under mild PEG stress, the RG of *G. glabra* seeds was significantly higher than those of *G. uralensis* and *G. inflata* seeds. Under severe PEG stress, the RG of *G. inflata* seeds was the highest. These results show that *G. glabra* seeds exhibited the strongest germination recovery ability under mild PEG stress and *G. inflata* seeds exhibited the strongest germination recovery ability under severe PEG stress.

Comprehensive evaluation of drought tolerance

The membership function method can eliminate the one-sidedness of individual indicators and reflect the stress resistance of plants more comprehensively and objectively and it has been adopted by many researchers. And MFV ranges between 0 and 1, which simplifies the comparison of resistance performance between species.

Under mild PEG stress, the average MFV of *G. uralensis*, *G. glabra* and *G. inflata* seeds were 0.61, 0.40 and 0.42, respectively (Table 1). The order of drought resistance from strong to weak was: *G. uralensis* > *G. inflata* > *G. glabra*. Under severe PEG stress, the average MFV of *G. uralensis*, *G. glabra* and *G. inflata* seeds were 0.47, 0.76 0.2, respectively (Table 2). The order from strong to weak was: *G. glabra* > *G. uralensis* > *G. inflata*.

Under mild PEG stress, drought tolerance was highest in *G. uralensis* seeds and lowest in *G. glabra* seeds. However, under severe PEG stress, drought tolerance was highest in *G. glabra* seeds and lowest in *G. inflata* seeds.This may explain why *G. uralensis* and *G. inflata* seeds are more sensitive to elevated PEG concentrations than *G. glabra* seeds. Therefore, *G. uralensis* should be preferentially planted in areas with better water conditions or less severe water shortages and *G. glabra* should be preferentially planted in areas with severe drought and water shortage or uncertain water supply.

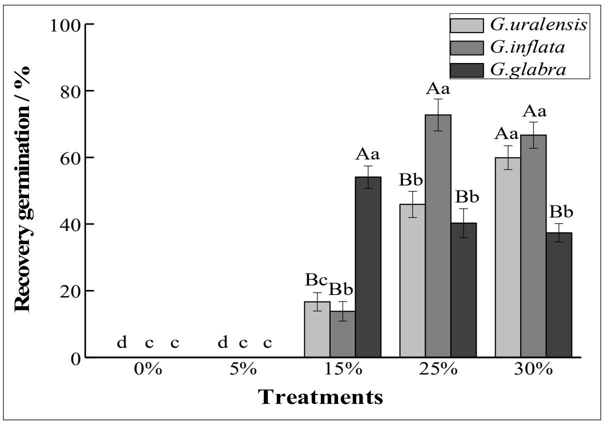

Fig 6: Effect of different concentrations of PEG on recovery germination of three licorice seeds (mean ± SE). Different capital letters indicate significant differences among species at the same concentration and different lowercase letters indicate significant intraspecific differences under different PEG solution concentrations.

Table 1: Comprehensive evaluation of drought resistance of three licorice species seeds under drought stress of low-concentration PEG solution (0% -15%).

Breed	MFV of GP	MFV of GI	MFV of VI	MFV of GSI	MFV of RG	Mean MFV
G.glabra	0	0.22	0	0.76	1	0.396
G.uralensis	1	1	1	0	0.07	0.614
G.inflata	0.87	0	0.22	1	0	0.418

GP-Germination percentage; GI-Germination index; VI-Vigour index; GSI-Germination stress tolerance index; RG-recovery germination.

Table 2: Consive evaluation of drought resistance of three liquorice seeds under high-concentration PEG solution (25% -30%).

Breed	MFV of GP	MFV of GI	MFV of VI	MFV of GSI	MFV of RG	Mean MFV
G.glabra	0.98	1	0.80	1	0	0.76
G.uralensis	0.5	0.49	0.5	0.40	0.47	0.47
G.inflata	0	0	0	0	1	0.2

GP-Germination percentage; GI-Germination index; VI-Vigour index; GSI-Germination stress tolerance index; RG-recovery germination.

CONCLUSION

Drought is one of the key factors that affects seed germination. By measuring seed germination of the three medicinal liquorice species under different degrees of simulated drought conditions, their drought tolerance was studied. This provides a future reference for the selection of drought tolerant varieties. However, the drought resistance of plants during the seedling stage or the flowering stage may not be completely consistent with that in the seed germination period. Therefore, it remains to be further studied whether the liquorice, which shows strong drought tolerance in the seed germination stage, will show strong drought tolerance in other stages of its life history.

Conflict of interest: None.

REFERENCES

Ashraf, M. and Rauf, H. (2001). Inducing salt tolerance in maize (*Zea mays* L.) through seed priming with chloride salts: Growth and ion transport at early growth stages. Acta Phyiologiae Plantarum. 23(4): 407-414.

Beyaz, R. and Yildiz, M. (2020). Influence of Peg-induced Drought Stress on Antioxidant Components of Callus Tissue of Sainfoin (*Onobrychis viciifolia Scop*.) Ecotypes. Legume Research. 44(2): 197-201.

Chang, H.L., Chen, P.Y. and Ma, M. (2020). Feeding preference of Altica deserticola for leaves of *Glycyrrhiza glabra* and *G. uralensis* and its mechanism. Scientific Reports. 10(1): doi: 10.1038/s41598-020-58537-y.

Chen, P.Y., Ma, M. and Shi, L.Y. (2019). Trade-off between salt secretion and gas exchange by stomata in the leaves of *Glycyrrhiza uralensis*. Current Science. 116(7): 1212-1217.

Chen, X., Min, D., Yasir, T.A. and Hu, Y.G. (2012). Evaluation of 14 morphological, yield-related and physiological traits as indicators of drought tolerance in Chinese winter bread wheat revealed by analysis of the membership function value of drought tolerance (MFVD). Field Crops Research. 137: 195-201.

El-Keblawy, A., Elnaggar, A., Tammam, A. and Mosa, K.A. (2020). Seed provenance affects salt tolerance and germination response of the habitat-indifferent *Salsola drummondii* halophyte in the arid Arabian deserts. Flora. 266: doi: 10.1016/j.flora.2020.151592.

Elnaggar, A., El-Keblawy, A., Mosa, K.A. and Solimane, S. (2018). Drought tolerance during germination depends on light and temperature of incubation in *Salsola imbricata*, a desert shrub of Arabian deserts. Flora. 249: 156-163.

Farjam, S.,Kazemi-Arbat, H.,Siosemardeh,A.,Yarnia, M. and Rokhzadi, A. (2015). Effects of salicylic and ascorbic acid applications on growth, yield, water use efficiency and some physiological traits of chickpea (*Cicer arietinum* L.) under reduced irrigation. Legume Research. 38(1): 66-71.

Jhanji, S. and Dhingra, M. (2020). Ameliorative effect of thiourea priming on germination characteristics of mungbean (*Vigna radiata* L.) under water and salinity stress. Legume Research. 43(3): 353-358.

Jia, K., DaCosta, M. and Ebdon, J.S. (2020). Comparative effects of hydro-, hormonal-, osmotic- redox- priming on seed germination of creeping bentgrass under optimal and suboptimal temperatures. Hortscience. 55(9): 1453-1462.

Han, Y.X., Hou, Z.N., Zhang, X.M., Yan, K.J., Liang, Z.S. and He, Q.L. (2022). Important changes in germination, seedling tolerance active components content due to drought stress on three licorice (*Glycyrrhiza*) species. Industrial Crops and Products. 175: doi: 10.1016/J.INDCROP. 2021.114240.

Jiang, M., Zhao, S.J., Yang, S.S., Lin, X., He, X.J., Wei, X., Song, Q., Li, R., Fu. C.M., Zhang, J.M. and Zhang, Z. (2020).An essential herbal medicine-licorice: A review of phytochemicals and its effects in combination preparations. Journal of Ethnopharmacology. 249: doi: 10.1016/j.jep.2019. 112439.

Klarod, K., Dongsansuk, A., Piepho, H.P. and Siri, B. (2021). Seed coating with plant nutrients enhances germination and seedling growth and promotes total dehydrogenase activity during seed germination in tomato (*Lycopersicon esculentum*). Seed Science and Techonlogy. 49(2): 107-124.

Lamia, H., Naoufel, S., Larbi, KM. and Nejib, R.M. (2012). Effect of osmotic stress on Myrtus communis germination. Biologia. 67(1): 132-136.

Lei, C., Bagavathiannan, M., Wang, H.Y., Sharpe, S.M., Meng, W.T. and Yu, J.L. (2022). Osmopriming with polyethylene glycol (PEG) for abiotic stress tolerance in germinating crop seeds: A Review. Agronomy-Basel. 11(11): doi: 10.3390/ agronomy11112194.

Li, C., Wen, X., Wan, X., Liu, Y., Han, J., Liao, Y. and Wu, W. (2016). Towards the highly effective use of precipitation by ridge-furrow with plastic film mulching instead of relying on irrigation resources in a dry semi–humid area. Field Crops Research. 188: 62-73.

Ming, L.J. and Yin, A. (2013). Therapeutic effects of glycyrrhizic acid. Natural Product Communications. 8(3): 415-418.

Qiao, X., Song, W., Ji, S., Wang, Q., Guo, D.A. and Ye, M. (2015). Separation and characterization of phenolic compounds and triterpenoid saponins in licorice (*Glycyrrhiza uralensis*) using mobile phase-dependent reversed-phase × reversed-phase comprehensive two-dimensional liquid chromatography coupled with mass spectrometry. Journal of Chromatography A. 1402: 36-45.

Stefania, T., Daniela, R., Alessandro, T. and Cristina, P. (2017). Effects of drought stress on seed germination of ornamental sunflowers. Acta Physiol Plant. 39(3): doi:10.1007/ s11738-017-2484-8.

Tan, M., Liao, F., Hou, L.T., Wang, J., Wei, L.J., Jian, H.J., Xu, X.F., Li, J.N. and Liu, L.Z. (2017). Genome-wide association analysis of seed germination percentage and germination index in *Brassica napus* L. under salt and drought stresses. Euphytica. 213(2): doi:10.1007/s 10681-016-1832-x.

Tong, R.Q., L, X.Y., Mu, B.F., Wang, J.F., Liu, M.X., Zhou, Y.C., Qi, B.L., Li, Y.A. and Mu, C.S. (2021). Impact of seed maturation and drought on seed germination and early seedling growth in white clover (*Trifolium repens* L.). Legume Research. 44(6): 736-740.

Van, D.B.L. and Zeng, Y.J. (2006). Response of South African indigenous grass species to drought stress induced by polyethylene glycol (PEG) 6000. South African Journal of Botany. 72(2): 284-286.

Wei, S.L., Wang, W.Q., Zhang, Y., Qin, S.Y., Liu, C.L., Zhang, Z.Y. and Di, M. (2009). Geographical variation of *Glycyrrhiza uralensis* seed germination character on water stress. China Journal of Chinese Material Medica. 34(18): 2308-2311.

Xia, F.S., Wang, C.C., Li, Y.Y., Yang, Y.Y., Zheng, C., Fan, H.F. and Zhang, Y.D. (2021). Influence of Priming with Exogenous Selenium on Seed Vigour of Alfalfa (*Medicago sativa* L.). Legume Research. 44(9): 1124-1127.

Zamora-Briseno, J.A. and De Jimenez, E.S. (2016). A LEA 4 protein up-regulated by ABA is involved in drought response in maize roots. Molecular Biology Reports. 43(4): 221-228.