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Drought Resistance under PEG-6000 Stress during Germination of *Onobrychis viciifolia* (Sainfoin) from Different Regions of China

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ABSTRACT

Background: Sainfoin (*Onobrychis viciifolia*) is a nutritious perennial leguminous herb with strong resistance to stress and is a valuable livestock forage and ecologically beneficial crop. It grows in many Chinese provinces, where its stress resistance varies due to different ecological environments. This study aimed to evaluate drought resistance during germination of sainfoin from different regions.

Methods: The registered sainfoin variety *Onobrychis viciifolia* cv. Mengnong was used as a control andaqueous solutions of PEG-6000 (0-20%) were used to simulate drought stress in wild varieties collected from seven regions of China. Seed germination characteristics were determined andthe drought resistance of each variety was comprehensively evaluated using membership function analysis.

Result: As PEG-6000 concentration increased, germination indices initially increased then decreased, showing that mild drought stress was conducive to the germination of sainfoin. Calculation of comprehensive drought resistance coefficients revealed that sainfoin from Shizuishan City exhibited strong drought resistance, those from Hami City and Jiuquan City showed medium resistance, the registered cultivar Mengnong and varieties from Ejin Banner and Yulin City were weakly resistant, while samples from Kunming City were non-resistant.

Key words: Drought resistance, Germination stage, PEG-6000, Sainfoin.

INTRODUCTION

Sainfoin (*Onobrychis viciifolia*), known as the 'queen of forages', is a perennial high-yielding leguminous herb that is rich in protein, crude fat, minerals, amino acids andother nutrients required by livestock (Chen *et al.*, 2024; Macit *et al.*, 2021). It exhibits strong resistance to stress, is rich in condensed tannins andprevents bloating disease (Poudel *et al.*, 2023). It is also active in biological nitrogen fixation, soil improvement andecological and environmental protection and has played an important role in the development of animal husbandry and habitat building in China (Zhu *et al.*, 2025).

Global warming and dramatic environmental changes have increased incidences of drought that adversely impact the growth and development of sainfoin, leading to lower yields and poorer quality (Malisch *et al.*, 2020). Current research in this area aims to improve the drought resistance of sainfoin by breeding water-saving, drought-resistant varieties that use water more efficiently and are high yielding (Yao *et al.*, 2024). The ability of seeds to germinate normally directly affects the emergence speed and quality of sainfoin, determines the number of seedlings and population structure andultimately affects crop yield (Petkova *et al.*, 2024). Evaluation of drought resistance at the seed germination stage is important for identifying suitable sainfoin varieties (lines) and promoting their utilization.

Drought stress has serious impacts on sainfoin seed germination rate and seedling quality (Yao *et al.*, 2024).

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Drought resistance of sainfoin plants from various regions was evaluated under several concentrations of aqueous PEG-6000 that simulated drought conditions, the registered variety *Onobrychis viciifolia* cv. Mengnong being used as a control. The wide range of sainfoin germplasms selected for this study originated from complex ecological environments that encouraged the divergent evolution of different germplasms which ultimately formed different sainfoin ecotypes. Evaluating drought resistance during the germination of sainfoin lays the foundation for the rational selection of forage materials with strong drought resistance. This study will helpful for screening and selecting ideal genotypes of sainfoin that exhibit drought resistance (Beyaz 2019; Jincya *et al.*, 2019).

MATERIALS AND METHODS

This study was conducted in Liaoning Agricultural Vocational and Technical College in December 2024. *Onobrychis viciifolia* cv. Mengnong (MN) provided by Inner Mongolia Agricultural University was used as the control and wild sainfoin plants collected from various regions by Yingkou Shuoguo Technology Co. Ltd. were used as the experimental material. Samples were collected in Ejin Banner in Inner Mongolia (EJN), Jiuquan City in Gansu Province (JQ), Shizuishan City in Ningxia Hui Autonomous Region (SZS), Hami City in Xinjiang Uygur Autonomous Region (HM), Yulin City in Shanxi Province (YL) and Kunming City in Yunnan Province (KM).

Test design and treatment

Aqueous solutions of PEG-6000 (0% CK, 5%, 10%, 15% and 20%) were used to treat the seeds. Plump sainfoin seeds of uniform size were selected, disinfected with sodium hypochlorite (NaClO), then rinsed 7-8 times with sterilized deionized water. PEG-6000 solutions (10 mL) were added to 9 cm-diameter Petri dishes containing two layers of filter paper. For each treatment, 50 randomly selected seeds were placed in Petri dishes and cultured in a constant temperature incubator at 25±1°C (12 h dark/ 12 h light). Each treatment was performed three times. Water (5 mL) was added to all dishes every three days so the PEG-6000 concentration of each treatment remained relatively constant. Germination records were maintained daily until germination was complete after 10 days.

Seed germination survey

In addition to the daily germination records, the germination potential was measured on the third day, while on the tenth day the germination rate was calculated and seedling length was measured (10 randomly selected seedlings in each Petri dish).

Comprehensive drought resistance

The drought resistance coefficient (DC) and comprehensive drought resistance coefficient (CDR) were calculated according to the method of Li *et al.* (2015).

$$DC = \frac{\text{Measured value of treatment group}}{\text{Measured value of control group}}$$

CDR = Arithmetic mean of DCs of each index

CDR can be categorized into four levels of drought resistance: CDR>0.8 is grade 1 (strong resistance), 0.6<CDR≤0.8 is grade 2 (moderate resistance), 0.4<CDR ≤0.6 is grade 3 (weak resistance) and CDR≤0.4 is grade 4 (non-resistant).

Statistical analyses

SPSS 22.0 software was used for one-way analysis of variance (ANOVA).

Germination potential (%) =

$$\frac{\text{Germinated seeds on day 3}}{\text{Total test seeds}} \times 100$$

Germination rate (%) =

$$\frac{\text{Germinated seeds on day 10}}{\text{Total test seeds}} \times 100$$

$$\text{Germination index (GI)} = \Sigma\ (Gt/Dt)$$

Where
Gt = Number of germinated seeds on day.
Dt = Corresponding number of days.

$$\text{Vitality Index (VI)} = GI \times S$$

Where
GI = Germination index.
S = Length of the seedlings.

RESULTS AND DISCUSSION

Effect of PEG-6000 on seed germination

Seed germination rate is a useful measure of seed quality (Nio *et al.*, 2025). Under normal conditions (CK control group), the germination rate of the seven sainfoin varieties exceeded 50%, with the control bred variety MN reaching 78%, which was higher than the wild varieties. As PEG-6000 concentration increased, the germination rate of all varieties tended to increase then decrease, with the rate being highest with 5% PEG-6000 (Fig 1). This indicates that mild drought stress was beneficial to the germination of sainfoin seeds (Beyaz, 2019). However, as drought intensity continued to increase, germination of all seven varieties was significantly inhibited, which is consistent with research findings with alfalfa (Gao *et al.*, 2023). The germination rate of SZS samples at 10% PEG-6000 was significantly higher than CK, suggesting better drought resistance in this region. By contrast, germination rate of KM samples at 20% PEG-6000 was zero, illustrating poor drought resistance. Moreover, root length and seedling growth of two varieties were significantly inhibited at higher drought intensities.

Effect of PEG-6000 on seed germination potential

As PEG-6000 concentration increased to 5%, the germination potential of all the sainfoin varieties increased ($p<0.05$, Fig 2), but decreased at 10% PEG-6000. At 15%, the potentials ranged from 2.7% to 34%, with SZS samples having the highest. At 20% PEG-6000, the germination potential of five of the varieties fell below 10% (SZS and HM samples being the exceptions). Germination potential reflects the speed and uniformity of seed germination (Elaine *et al.*, 2018). Significant differences were evident between the cultivars at each drought stress level as seed germination potential decreased (Shobana devi *et al.*, 2022).

Effect of PEG-6000 on seed germination index

Germination index (GI) refers to the number of seeds germinating per unit time, representing the speed of germination (Lai *et al.*, 2019). GI of all seven sainfoin varieties increased in the presence of 5% PEG-6000 ($p<0.05$, Fig 3). In the CK group, YL and MN samples had the highest GIs, but in the 5% treatment group, YL and SZS

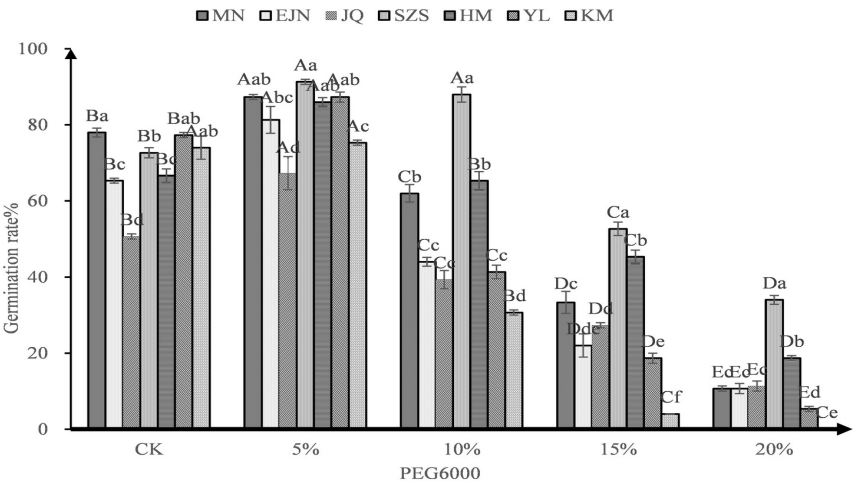
exhibited the highest. Under moderate drought stress (10-20% PEG-6000), the GIs of SZS and HM were higher than the other five varieties ($p<0.05$), demonstrating the relatively fast germination of SZS and HM under these conditions.

Effect of PEG-6000 on seed vigor index

Vigor index (VI) is a comprehensive measure of the germination rate and growth of seeds (Ma *et al.*, 2023). The VI of KM samples decreased gradually as PEG-6000 concentration increased. VI of the other sample groups initially increased, then decreased, with VI at 5% PEG-6000 being higher than under the other treatments ($p<0.05$, Fig 4). VI of SZS under 5% PEG-6000 was higher than the other varieties ($p<0.05$), being 1.93 times that of JQ and 2.5 times that of KM samples. At PEG-6000 concentrations above 15%, the VI of all sainfoin samples was less than one third of the highest value.

Comparison of drought resistance at germination

The germination rate, germination potential, GI and VI of the seven sainfoin seed germplasms were comprehensively evaluated using the membership function method (Table 1) and used to calculate comprehensive drought resistance coefficients (CDR) for each variety. CDR ranged from 0.34 to 0.96 and samples were classified into four levels of



Note: Different uppercase letters indicate that the same sainfoin species differ significantly in different PEG-6000 concentration treatments ($P<0.05$) anddifferent lowercase letters indicate there is a significant difference among different sainfoin species under the same PEG-6000 concentration treatment, the same as below.

MN- *Onobrychis viciifolia* cv. Mengnong; EJN- Sainfoin collected in Ejin Banner in Inner Mongolia; JQ- Sainfoin collected in Jiuquan City in Gansu Province; SZS-Sainfoin collected in Shizuishan City in Ningxia Hui Autonomous Region; HM- Sainfoin collected in Hami City in Xinjiang Uygur Autonomous Region; YL- Sainfoin collected in Yulin City in Shanxi Province; KM- Sainfoin collected in Kunming City in Yunnan Province. The same as below.

Fig 1: The seed germination rate of sainfoin(*Onobrychis viciifolia*) under PEG-6000 treatment.

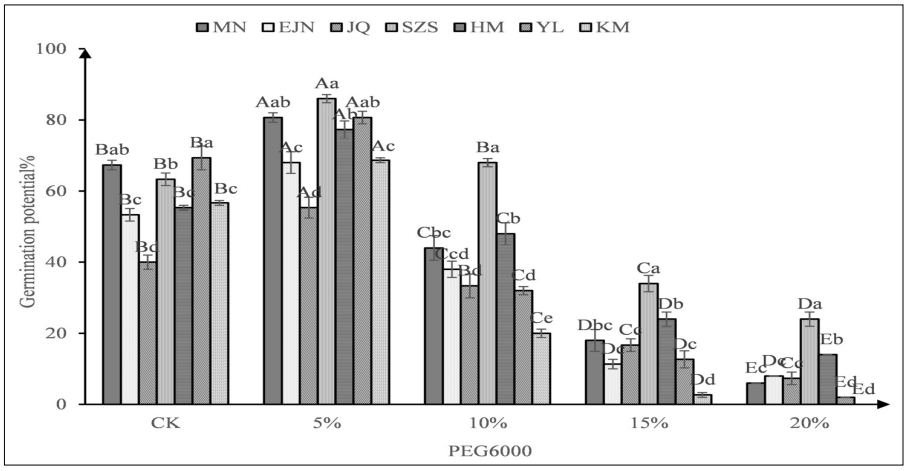


Fig 2: The seed germination potential of sainfoin (*Onobrychis viciifolia*) under PEG-6000 treatment.

drought resistance. Grade 1 (strong resistance) contained only SZS samples, Grade 2 (moderate resistance) included HM and JQ, Grade 3 (weak resistance) included MN, EJN and YL and Grade 4 (non-resistant) contained only KM samples. In general, the wild sainfoin plants collected from SZS, HM and JQ exhibited relatively high drought resistance, making them suitable for use in arid and semi-arid areas.

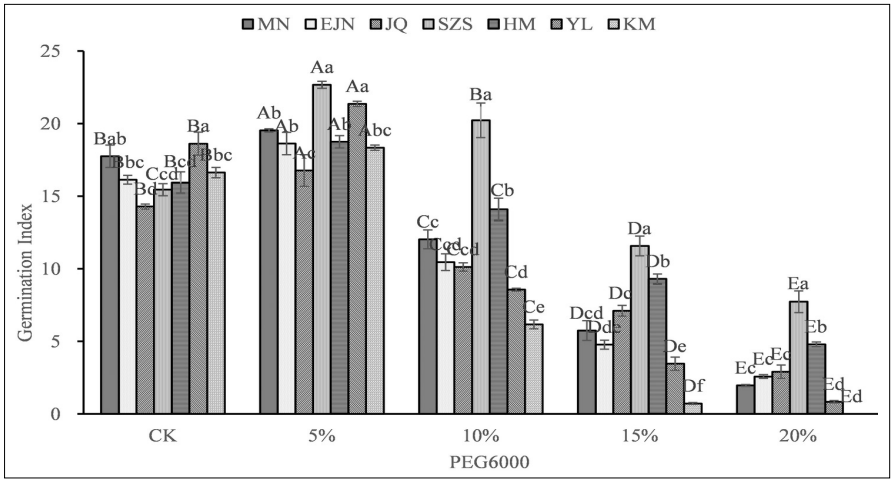


Fig 3: The seed germination index of sainfoin (*Onobrychis viciifolia*) under PEG-6000 treatment.

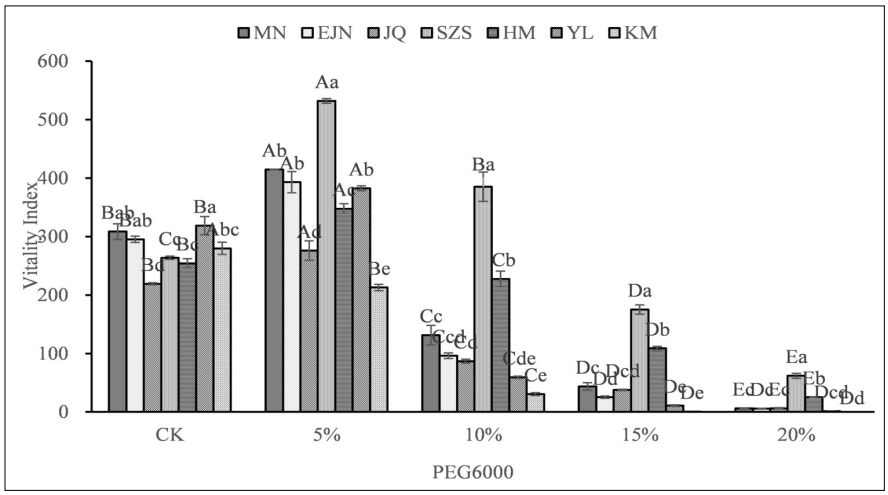


Fig 4: The seed vitality index of sainfoin (*Onobrychis viciifolia*) under PEG-6000 treatment.

Table 1: Comparison of drought resistance at germination of sainfoin (*Onobrychis viciifolia*).

Material	DC				CDR	Drought resistance classification	Rank
	Germination rate	Germination potential	Germination index	Vitality index			
MN	0.62	0.55	0.55	0.48	0.55	Grade 3	4
EJN	0.60	0.59	0.56	0.44	0.55	Grade 3	5
JQ	0.72	0.70	0.65	0.46	0.63	Grade 2	3
SZS	0.92	0.84	1.01	1.09	0.96	Grade 1	1
HM	0.81	0.74	0.74	0.70	0.74	Grade 2	2
YL	0.49	0.46	0.46	0.36	0.44	Grade 3	6
KM	0.37	0.40	0.38	0.22	0.34	Grade 4	7

DC-Drought resistance coefficient; CDR- Comprehensive drought resistance coefficient.

Note: CDR can be categorized into four levels of drought resistance: CDR>0.8 is grade 1 (strong resistance), 0.6<CDR≤0.8 is grade 2 (moderate resistance), 0.4<CDR≤0.6 is grade 3 (weak resistance) and CDR≤0.4 is grade 4 (non-resistant).

CONCLUSION

The screening and selection of desirable genotypes exhibiting drought tolerance is the primary focus of sainfoin breeding programs. This study revealed significant differences between sainfoin cultivars at different drought stress levels, with decreases in germination, germination potential, germination index andvigor index being observed. The cultivar collected from SZS exhibited germination attributes most capable of withstanding drought conditions.

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Disclaimers

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Informed consent

The collection of plant seeds conforms to China's regulatory standards and current laws.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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