



Cold Resistance Evaluation of Alfalfa during the Germination and Seedling Stages and Analysis of its Relationship with Fall Dormancy

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ABSTRACT

Background: Alfalfa (*Medicago sativa* L.), a vital forage crop, demonstrates pronounced variability in growth performance and yield in cold regions, largely determined by its cold tolerance. Fall dormancy ratings, a key indicator of alfalfa's winter dormancy intensity, induce distinct physiological responses and cold tolerance capacities among varieties under low-temperature stress. Therefore, investigating the germination traits and seedling physiological adaptations of alfalfa with varying fall dormancy ratings under cold stress is critical for breeding and cultivating cold-tolerant alfalfa cultivars.

Methods: This experiment was conducted in the Key Laboratory of Grassland Resources, Ministry of Education, Inner Mongolia Agricultural University, from March to September 2024. A total of 28 alfalfa materials with 10 different fall dormancy grades were used. Low-temperature stress experiments were conducted to evaluate critical germination parameters, including germination rate, germination index, vigor index, root length and germination coefficient. Concurrently, seedling physiological responses were investigated under 4°C conditions, focusing on biochemical markers: Malondialdehyde (MDA) content, relative conductivity (REC) and the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), along with proline (Pro) and soluble sugar (SS) accumulation.

Result: Under low-temperature stress, significant reductions were observed in germination parameters, including germination rate, germination index, vigor index, root length and germination coefficient, across alfalfa accessions of different fall dormancy ratings. A strong negative correlation was established between fall dormancy ratings and cold hardiness during germination. Physiological analysis revealed that key biochemical markers-MDA, REC, SOD, POD, CAT, Pro and SS-under 4°C stress conditions. Accessions with lower fall dormancy ratings demonstrated superior cold hardiness, while those with higher fall dormancy ratings showed reduced tolerance. Through comprehensive analysis, REC, SOD, POD and SS were identified as the most critical physiological indicators for evaluating cold hardiness at the seedling stage.

Key words: Alfalfa, Cold hardiness, Correlation, Fall dormancy ratings, Low temperature.

INTRODUCTION

Alfalfa (*Medicago sativa* L.), a perennial leguminous forage crop renowned as the “king of forage grasses”, plays a pivotal role in China's animal husbandry development due to its superior nutritional value, exceptional quality and excellent palatability (Avci *et al.*, 2018; Wang *et al.*, 2016). As a cool-season perennial forage, alfalfa employs a distinct dormancy adaptation mechanism: its meristematic tissues and growth points enter dormancy under low-temperature stress and resume growth under favorable conditions, ensuring winter survival (Luan *et al.*, 2013). Autumn dormancy, characterized by reduced growth responsiveness to seasonal reductions in photoperiod and temperature, serves as a key indicator for assessing growth patterns and cold tolerance in alfalfa cultivars (Tang *et al.*, 2018). In northern regions, low-temperature stress frequently impairs alfalfa's overwintering capacity, posing a major constraint to cultivation (Zandalinas *et al.*, 2021). Cold hardiness during germination and seedling stages critically influences growth dynamics and yield potential. Lower fall dormancy ratings exhibit significantly higher overwintering survival rates and demonstrate enhanced physiological adaptation mechanisms to cold stress

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environments (Ma *et al.*, 2024). However, having evidence reveals a weak genetic correlation between fall dormancy rating traits and overwintering hardiness (Adhikari *et al.*, 2018). While fall dormancy ratings classification is widely used as a phenotypic marker for cold hardiness evaluation and cultivation zoning, existing research has centered on

overwintering capacity, leaving a critical gap in understanding its relationship with cold tolerance during early developmental stages, particularly germination and seedling establishment (Sheaffer *et al.*, 1992).

The germination ability of two alfalfa varieties at 10°C and 20°C was assessed. Under low temperature, the average germination time of seeds was significantly prolonged and seed vigor and seedling growth were affected to a certain extent (Zhang *et al.*, 2024). Fall-dormant cultivars exhibit slower autumn growth rates, prostrate stems and improved cold tolerance compared to non-dormant varieties (Cunningham *et al.*, 1998). Plants subjected to low-temperature stress activate physiological adaptation mechanisms (Aslam *et al.*, 2022). Prolonged exposure to low temperatures can cause an increase in reactive oxygen species within the plant, leading to significant production of MDA from membrane lipid peroxidation (Anwar *et al.*, 2019). The contents of SS, SP, Pro, MDA and CAT in alfalfa roots increased significantly with the decrease of temperature (Guo *et al.*, 2024). Under low temperature stress, the contents of REC, MDA, SS, Pro and APX were significantly increased in *Poa pratensis* seedlings (Li *et al.*, 2023). Under low temperature stress, physiological indices of four plant seedlings were measured, showing significant increases in SS MDA) and Pro contents. This study systematically evaluated cold tolerance mechanisms across 28 alfalfa accessions with well-defined fall dormancy ratings (FDR1-10) through two experimental modules: (1) seed germination under low-temperature stress (4°C/25°C gradient, 12 h photoperiod) and (2) seedling physiological responses to prolonged cold exposure (4°C for 48 h). Integrated analyses (correlation, principal component and regression) explored relationships between fall dormancy ratings and cold tolerance at germination and seedling stages, providing insights for early screening of cold-resistant germplasm and cultivation strategies in cold regions.

MATERIALS AND METHODS

Test material

The experimental materials comprised 28 alfalfa accessions representing fall dormancy ratings (FDR) from 1 to 10, including 6 copies originated from the Branch of Animal Husbandry and Veterinary of Heilongjiang Academy of Agricultural Sciences and 22 copies originated from Beijing Zhengdao Seed Industry Co. It contained FDR1 (n=4), FDR2 (n=4), FDR3 (n=4), FDR4 (n=5), FDR5 (n=4), FDR6 (n=3) and FDR7-10 (n=1 each, experimental lines 07-10).

Test methods

The experiment was conducted in the Key Laboratory of Grassland Resources of Inner Mongolia Agricultural University from March to August 2024.

The low-temperature germination test was carried out in a seed temperature gradient incubator (BGL-GL), set up with 4°C and 25°C temperature treatments. Healthy and

full seeds of 28 alfalfa accessions were selected, the epidermis was polished and then washed with distilled water, 50 seeds for each treatment of each material were put into the treatment chamber of the temperature gradient incubator, each treatment was repeated three times, watering was done regularly every day and the number of germinated seeds was recorded and at the end of the germination test on the 10th day, 10 seedlings were randomly selected for measuring the root length and shoot length and counting the germination rate.

Another 28 alfalfa accessions were taken cleaned seeds in Petri dishes and germinated in a light incubator at 25°C. After the seeds germinated, the seeds with consistent growth were selected and transplanted into pots (height 8 cm, diameter 10 cm), the culture substrate was a 3:1 mixture of peat soil and vermiculite, with 9 plants in each pot, 20 pots per accession and cultivated in a greenhouse at 25°C, watered every 3 days. After 30 days of seedling growth to the three-leaf stage, alfalfa seedlings with good growth and consistent status were selected and transferred to a 4°C low-temperature incubator for 48h of low-temperature treatment, with 25°C as the control and three replications for each treatment and the leaves were collected for the determination of physiological indices.

Measurement items and methods

Measurement of seed germination indicators

Relative germination percentage (RGP), relative germination index (RGI), relative vigor index (RVI), relative root length (RRL), mean germination time (MGT) and germination coefficient (GC) were used as the evaluation indices of germination hardiness to evaluate the germination hardiness of alfalfa at different fall dormancy levels. The calculation formula of each index is as follows:

Germination percentage (GP)(%) =

$$\frac{\text{Number of seeds that eventually germinated normally}}{\text{Number of seeds for testing}} \times 100\%$$

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

Where,

Gt = Number of seedlings on day t.

Dt = Corresponding number of days of seedling emergence.

Vigor index (VI)= GI × Mean shoot length

Relative indicator (%) = $\frac{\text{Stress treatment indicator}}{\text{Control indicator}} \times 100\%$

Mean germination time (MGT) = $\frac{G1T1+G2T2+...+GnTn}{G1+G2+...+Gn}$

Where,

Tn = Number of days to germinate.

Gn = Number of germinated seeds corresponding to the number of days to germinate.

$$\text{Germination coefficient (GC)} = \frac{\text{Germination percentage}}{\text{Average germination time}}$$

Measurement of seedling physiological indices

Malondialdehyde (MDA) via thiobarbituric acid reactive substances (TBARS) assay; relative conductivity (REC) through electrolyte leakage assessment; proline (Pro) employing acid-ninhydrin spectrophotometry; soluble sugar (SS) using anthrone-sulfuric acid assay; superoxide dismutase (SOD) activity measured by nitroblue tetrazolium (NBT) photochemical reduction inhibition; peroxidase (POD) via guaiacol peroxidase assay and catalase (CAT) activity determined by hydrogen peroxide decomposition rate at 240 nm (Yu *et al.*, 2022).

Cold resistance coefficient =

$$\frac{\text{Each index value under low temperature stress}}{\text{Each index value under control condition}}$$

Data processing and analysis

Excel 2021 software was used for data recording and organization, SPSS 27 for statistical analysis of data and Origin 2021 for plotting.

The value of the affiliation function was applied for comprehensive evaluation (Li *et al.*, 2023) and the indicators positively correlated with cold hardiness were calculated using formula (1) and those negatively correlated were calculated using formula (2).

$$F_{ij} = \frac{X_{ij} - X_{imin}}{X_{imax} - X_{imin}} \quad \dots(1)$$

$$F_{ij} = 1 - \frac{X_{ij} - X_{imin}}{X_{imax} - X_{imin}} \quad \dots(2)$$

Where,

F_{ij} = Value of the affiliation function of the j trait of material i.

X_{ij} = Value of the j trait of material i.

X_{imin} = Minimum value of the j trait of material i.

X_{imax} = Maximum value of the j trait of material i.

RESULTS AND DISCUSSION

Response of alfalfa germination to low temperature stress at different fall dormancy ratings

Correlation analysis of cold hardiness indices during the germination period

The correlation analysis of low temperature tolerance indices between fall dormancy ratings and germination stage was carried out (Fig 1). FDR exhibited strong negative correlations with RGI and RVI ($P<0.01$) and significant negative correlated with RGP and GC ($P<0.05$). Strong positive correlations between RGP and RGI, RVI and GC ($P<0.01$), contrasted by a negative correlation with MGT ($P<0.05$) were noted. RGI has shown highly significant positive correlation with RVI and GC ($P<0.01$), while significantly negative correlation with MGT ($P<0.05$). RVI was highly significantly positively correlated with GC

($P<0.01$) and significantly negatively correlated with MGT ($P<0.05$). MGT has recorded highly significant negative correlation with GC ($P<0.01$). The results of correlation analysis showed that most of the individual indicators were highly significantly correlated with each other.

Optimal germination typically requires adequate moisture, sufficient oxygen availability and appropriate temperature regimes (Li *et al.*, 2023). Low temperature greatly reduces the rate of seed uptake and swelling and the germination ability of seeds from different alfalfa accessions varies significantly at low temperatures (Butler *et al.*, 2014). In this study, we used alfalfa seeds with full seeds, good seed embryo and high seed vigor as materials and low temperature was the main factor affecting seed germination. RGP, RGI, RVI and RRL of alfalfa seeds were significantly reduced at 4°C, mainly due to the fact that low temperature reduces enzyme activity in the seeds, so that the enzymatic reactions within the seeds could not be carried out adequately, thus reducing the vigor (Ozkan, 2025). Correlation analysis showed that FDR was significantly negatively correlated with low temperature germination indexes (RGP, RGI, RVI, GC). This suggests that the ability of seeds to germinate at low temperatures and fall dormancy ratings are significantly correlated. Alfalfa accessions with low fall dormancy ratings are not only more susceptible to short sunshine and low temperatures in the fall, but they also halt growth before it enters dormancy. Additionally, they have a strong ability to germinate at low temperatures, as evidenced by higher GP, GI, VI, RL and GC, as well as a shorter germination period. This study revealed that alfalfa seeds with a high fall dormancy ratings had a lower ability to germinate at low temperatures, indicating that these materials were less tolerant and adaptable to low temperatures.

Principal component analysis of cold tolerance indicators at germination stage

Principal component analysis (PCA) reduces multiple highly correlated single indicators to a few independent comprehensive indicators (Cicevan *et al.*, 2016). Two orthogonal principal components were obtained by PCA and the cumulative explained variance was 96.94% (Table 1). PC1 explained 83.732% of the variance ($\lambda=5.024$) and the main loads were RGP, RGI, RVI and GC. PC2 explained 13.208% of the variation ($\lambda=0.792$), which was mainly affected by RRL.

Comprehensive evaluation of cold hardiness during germination

The membership function method was used to analyze the cold resistance of FDR at germination stage. The comprehensive evaluation of cold hardiness of materials in fall dormancy ratings 1~5 matched with fall dormancy ratings to a high degree and there was no obvious pattern in ratings 6~10. The cold hardiness of emergence was negatively correlated with the fall dormancy ratings in general ($R^2=0.659$) (Table 2).

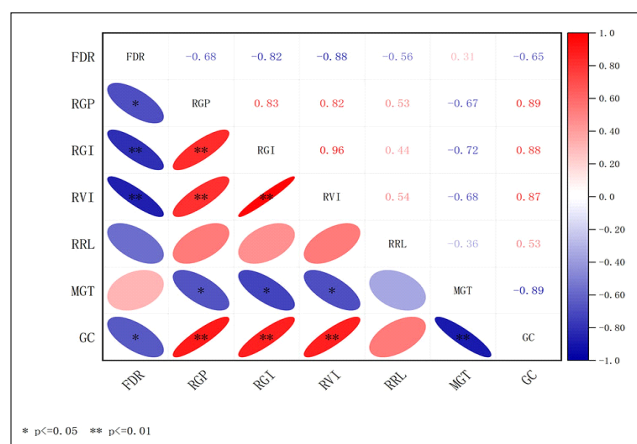
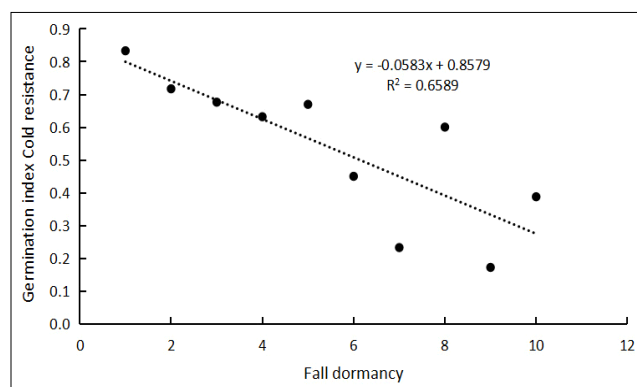
The linear regression method was used to comprehensively evaluate the membership function and

Table 1: Principal component analysis of cold tolerance indices of alfalfa at germination stage.

Principal component	Eigen values%	Proportion%	Cumulative%	Eigen vectors					
				RGP	RGI	RVI	RRL	MGT	GC
PC1	5.024	83.732	83.732	0.436	0.443	0.439	0.226	-0.420	0.440
PC2	0.792	13.208	96.940	-0.186	-0.095	-0.073	0.969	0.096	-0.051

Table 2: Comprehensive evaluation of cold tolerance of different fall dormancy ratings alfalfa at germination stage.

Fall dormancy ratings	Comprehensive evaluation value of membership function	Sort
1	0.833	1
2	0.717	2
3	0.676	3
4	0.632	5
5	0.670	4
6	0.450	7
7	0.233	9
8	0.600	6
9	0.172	10
10	0.388	8

**Fig 1:** Correlation coefficient of cold tolerance indices of alfalfa at germination stage.**Fig 2:** Relationship between different fall dormancy ratings and cold tolerance at germination stage.

fall dormancy level of alfalfa with different fall dormancy levels at the germination stage (Fig 2). It is possible that the relatively more cold-tolerant alfalfa accessions of low fall dormancy ratings showed adaptive responses to low temperatures in seed germination due to the existence of long-term acclimatization to low-temperature environments. In contrast, the seed germination ability of alfalfa with high fall dormancy ratings, which has been cultivated in warm environment for a long time, is a kind of inherent potential performance. Zhao *et al.* (2012) found that there was also no significant correlation between alfalfa germination heat tolerance and fall dormancy ratings, which also side by side confirmed that alfalfa germination response to temperature was not completely consistent with the response to low temperature and short sunshine in fall.

Response of alfalfa seedlings of different fall dormancy ratings to low temperature stress

Cold tolerance coefficients of physiological indicators of seedlings under low temperature stress

Physiological cold tolerance coefficients, calculated as stress-to-control ratios, exhibited distinct ranges across biomarkers: MDA (1.096-1.823), REC (4.295-7.681), SOD (1.290-3.453), POD (1.235-3.358), CAT (1.233-1.755), Pro (1.036-1.877) and SS (1.314-2.462) (Table 3). The stress-to-control ratios of each index were greater than 1, indicating that the contents of the physiological biomarkers measured under low temperature stress increased to different degrees.

Correlation analysis between fall dormancy ratings and cold hardiness coefficients

Multivariate correlation analysis revealed complex interrelationships between cold hardiness biomarkers and fall dormancy ratings (Fig 3). FDR exhibited strong negative correlations with SOD and CAT ($P<0.01$) and significant negative correlation with Pro ($P<0.05$), while showing positive correlation with MDA ($P<0.01$). MDA exhibited strong negative correlations with SOD ($P<0.01$), while significant negative correlation with POD, CAT and Pro ($P<0.05$). SOD exhibited strong significant positive correlation with CAT ($P<0.01$) and significant positive correlation with POD, Pro and SS ($P<0.05$). POD exhibited significant positive correlation with CAT and SS ($P<0.05$). CAT exhibited significant positive correlation with Pro ($P<0.05$). Pro exhibited strong significant positive correlation with SS ($P<0.01$).

Fall dormancy reflects alfalfa's hardiness response to low winter temperatures. Under low temperatures, plants employ various strategies to maintain the metabolic balance of intracellular reactive oxygen species. Low temperatures lead to increased cytoplasmic peroxidation and severe

oxidative damage to the membrane system, resulting in the formation of MDA (Mansoor *et al.*, 2022). Antioxidant enzymes help eliminate reactive oxygen species generated during metabolism, thereby preserving cell membrane stability (Latha

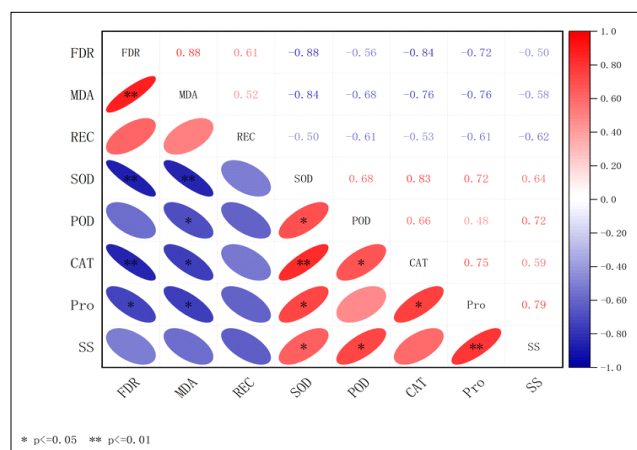


Fig 3: Principal component analysis of cold tolerance indices in alfalfa at seedling stage.

et al., 2024). To counteract low-temperature stress, plants accumulate SP and Pro, which increase intracellular osmotic concentration and reduce damage caused by cold temperatures (Yang *et al.*, 2021). Correlation analyses demonstrated strong negative associations between FDR and cold hardiness indices for SOD and CAT, significantly negatively correlated with the cold hardiness coefficient of Pro, contrasted with positive correlation to MDA; this indicates that the accession with a high index of the fall dormancy ratings has a significant increase in the concentration of MDA and a high degree of peroxidation of the cell membrane under low-temperature stress and the greater the damage suffered by the leaf blade (Hanson *et al.*, 2015). The heightened activity of SOD, POD and CAT enzymes under low-temperature stress demonstrated their protective effects on protoplasts. A negative correlation with the alfalfa's fall dormancy ratings suggested that lower fall dormancy ratings corresponded to stronger cellular antioxidant and repair capabilities under low temperatures. Similarly, the negative correlation between the concentrations of osmoregulators Pro and SS, converted into cold tolerance coefficients and fall dormancy ratings indicated that alfalfa with lower fall dormancy ratings could accumulate

Table 3: Cold tolerance coefficient of physiological indices of different alfalfa accessions at seedling stage.

Material	FDR	MDA	REC	SOD	POD	CAT	Pro	SS
Long mu801	1	1.205	5.885	3.217	2.981	1.755	1.544	2.136
Long mu803	1	1.223	5.301	3.175	2.494	1.451	1.743	2.385
Long mu806	1	1.255	5.202	3.231	2.736	1.448	1.575	2.184
Zhao dong	1	1.096	6.194	2.022	1.988	1.701	1.740	1.943
Ao han	2	1.279	5.741	2.925	2.363	1.480	1.705	1.671
Gong nong1	2	1.174	5.327	2.608	2.896	1.693	1.487	1.590
Bara210SC	2	1.312	6.771	3.453	1.613	1.422	1.410	1.958
WL168HQ	2	1.457	7.101	2.403	2.524	1.366	1.722	1.875
Bara310SC	3	1.400	4.295	1.849	2.400	1.442	1.630	1.561
Bara218TR	3	1.538	5.967	1.814	2.409	1.314	1.430	1.983
WL319HQ	3	1.623	5.201	2.529	2.609	1.351	1.717	1.885
WL298HQ	3	1.463	5.964	2.204	2.702	1.667	1.362	1.997
WL349HQ	4	1.724	4.438	2.000	2.304	1.463	1.877	2.012
New strain2	4	1.766	6.138	1.743	1.582	1.371	1.741	2.462
nice surprise	4	1.442	5.520	1.859	1.495	1.347	1.555	2.022
WL343HQ	4	1.343	4.877	2.127	2.473	1.315	1.877	2.011
New strain1	4	1.823	6.765	2.014	2.197	1.465	1.798	2.238
New strain3	5	1.429	6.186	1.644	2.196	1.343	1.863	2.366
WL366HQ	5	1.461	5.954	2.158	3.358	1.416	1.597	2.196
WL363HQ	5	1.739	4.915	1.415	2.892	1.340	1.240	1.566
Bara520YQ	5	1.502	5.073	2.274	1.884	1.432	1.182	1.825
Fei li	6	1.405	6.227	1.814	2.606	1.345	1.575	2.128
GSM681	6	1.414	5.690	1.815	2.087	1.251	1.398	1.314
WL440HQ	6	1.469	5.794	1.675	2.188	1.412	1.833	2.277
Blue moon	7	1.635	7.681	1.570	1.316	1.246	1.036	1.471
WL525HQ	8	1.689	6.116	1.551	1.235	1.233	1.126	1.584
WL656HQ	9	1.621	6.523	1.916	1.435	1.319	1.422	1.953
Saidi10	10	1.721	7.231	1.290	1.463	1.354	1.342	1.598

Note: MDA: Malondialdehyde; REC: Relative conductivity; SOD: Superoxide dismutase; POD: Peroxidase; CAT: Catalase; Pro: Proline; SS: Soluble sugar.

more osmoregulators to withstand low-temperature stress. REC mainly reflected the integrity of the intracellular membrane system at low temperatures and the correlation with the fall dormancy ratings did not reach a significant level, which might be related to the fact that the stress temperature did not reach below 0°C. The REC of alfalfa at the fall dormancy ratings did not reach a significant level.

Table 4: Principal component analysis of cold tolerance coefficient of physiological indices of alfalfa at seedling stage.

Index	PC1	PC2
Eigenvalues%	5.082	1.010
Proportion%	72.599	14.429
Cumulative%	72.599	87.028
MDA	-0.369	0.484
REC	-0.341	-0.458
SOD	0.377	-0.461
POD	0.389	0.132
CAT	0.396	-0.297
Pro	0.390	0.284
SS	0.382	0.397

Table 5: Comprehensive evaluation of cold tolerance at seedling stage of alfalfa with different fall dormancy ratings.

Fall dormancy ratings	Comprehensive evaluation value of membership function	Predicted value	Sort
1	0.839	0.843	1
2	0.649	0.641	2
3	0.601	0.592	4
4	0.622	0.613	3
5	0.562	0.583	5
6	0.538	0.521	6
7	0.186	0.180	9
8	0.258	0.264	8
9	0.383	0.381	7
10	0.166	0.163	10

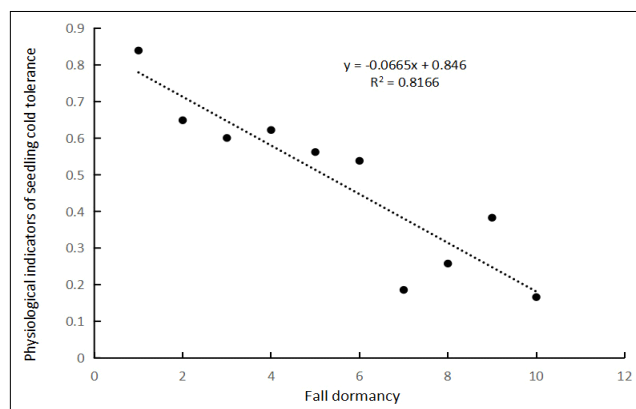


Fig 4: Relationship between different fall dormancy ratings and cold tolerance at seedling stage.

Principal component analysis of cold tolerance coefficients of physiological indicators

PCA was performed on seven seedling-stage cold tolerance biomarkers (Table 4). The eigenvalues (λ) and variance explained revealed: PC1 ($\lambda=5.082$) accounted for 72.599% variance with primary loadings on POD, CAT, Pro. PC2 ($\lambda=1.01$) explained 14.429% variance, dominated by MDA, REC, SOD and POD. The cumulative explained variance reached 87.028%, effectively capturing the multivariate physiological responses.

Comprehensive evaluation of physiological indicators of cold tolerance

For the comprehensive evaluation of cold hardiness of alfalfa seedlings of different fall dormancy ratings (Table 5). The comprehensive evaluation value was used as the dependent variable and seven single indicators with stronger correlations were employed as independent variables for stepwise regression analysis to establish the optimal regression equation $D = -0.017 - 0.061\text{REC} + 0.124\text{SOD} + 0.129\text{POD} + 0.248\text{SS}$, $R^2 = 0.998$, $P < 0.001$, the four independent variables can determine almost all the variances of seedling cold hardiness, the regression equation was used to predict the seedling cold hardiness of different fall dormancy ratings and the predicted values were basically consistent with the order of the comprehensive evaluation value. The regression equation was used to predict the seedling cold hardiness of different fall dormancy ratings. In this study, we found that, alfalfa seedling cold hardiness and fall dormancy ratings showed a strong linear relationship and a negative correlation in general (Fig 4). This clearly indicates that the genetic mechanism regulating fall dormancy ratings not only plays a role in the overwintering process, but also regulates seedling cold hardiness. The lower the fall dormancy ratings of alfalfa, the stronger will be the seedling cold hardiness; and the higher the fall dormancy ratings, the weaker will be the cold hardiness. Tong *et al.* (2020) exposed 16 low-fall-dormancy alfalfa varieties to stress for 24 hours and found a negative correlation between fall dormancy ratings and cold hardiness. Zhu *et al.* (2018) discovered that alfalfa varieties with strong fall dormancy ratings and cold hardiness store more nutrients in their roots, aiding safe overwintering, whereas varieties with weak fall dormancy ratings and poor cold hardiness store fewer nutrients, hindering overwintering. Consequently, in practical applications, fall dormancy ratings can be used to preliminarily assess alfalfa germplasm seedling cold hardiness, helping to select appropriate fall dormancy ratings for planting.

CONCLUSION

This study examined the low-temperature germination of 28 alfalfa accessions with varying fall dormancy ratings and subjected seedlings to low-temperature stress, it was found that the low temperature germination ability of alfalfa accessions with fall dormancy ratings lower than 5 could be reflected by fall dormancy ratings during the

germination period. However, there was no significant correlation between the fall dormancy ratings of accessions with levels 6 or higher and their low-temperature germination ability. A significant negative correlation was observed between the cold hardiness of seedling alfalfa and the fall dormancy ratings. Among physiological indicators, REC, SOD, SS and POD were identified as key factors for assessing cold hardiness in seedlings, providing a basis for predicting fall dormancy ratings and cold hardiness in alfalfa accessions lacking fall dormancy ratings information. The fall dormancy rating offers a theoretical reference for selecting and planting alfalfa varieties in different regions.

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Conflict of interest

All authors declared that there is no conflict of interest.

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