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Root and Crown Characteristics of Alfalfa Varieties in the Establishment Year and Relationship with Overwintering

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ABSTRACT

Background: Alfalfa overwintering ability is a key limiting factor for its stable yield and large-scale popularization in cold regions. Root and crown traits are critical to alfalfa's cold resistance and productivity.

Methods: 25 alfalfa cultivars with different fall dormancy grades planted in the Tumote region of Inner Mongolia were selected as materials; plant height, aboveground biomass, root and crown characteristics in the establishment year and overwintering rate in the next year were measured to explore the relationship between root traits and overwintering rate of alfalfa in the establishment year and select suitable alfalfa varieties for the Tumote region.

Result: Significant differences were observed among alfalfa varieties in aboveground growth, root and crown traits and subsequent overwintering rates. Correlation analysis revealed that crown burial depth and crown diameter were closely associated with overwintering survival. In contrast, taproot diameter and lateral root characteristics primarily influenced aboveground biomass production rather than directly determining winter survival. Based on the subordinate function comprehensive evaluation, WL298, Algonquin and Bara416 attained the highest scores, indicating robust root development and strong overwintering potential. This study clarifies the differentiated cold-resistance functions of crown and root traits; crown traits can serve as useful indicators for evaluating cold resistance. It proposes targeted variety promotion for the Tumote area of Inner Mongolia and provides germplasm resources for breeding cold-resistant, high-yield alfalfa varieties in similar cold northern regions.

Key words: Belowground indicators, Cold resistance, *Medicago sativa*, Overwintering.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a perennial legume forage with the advantages of high protein content and high yield and is widely used in animal husbandry production (Cao *et al.*, 2011). In recent years, with the promotion of China's policies such as "grain for forage" and agricultural planting structure adjustment in the "Sickle Bend Region", the planting area of alfalfa has increased significantly, especially in the northern agro-pastoral ecotone (Xu, 2025). The Inner Mongolia Autonomous Region is an important ecological barrier and animal husbandry production area in China, attaches great importance to the development of the grass industry and has issued a series of supporting policies. However, extreme weather such as frequent frigid, snow-sparse winters and cold wave disasters severely suppresses overwintering survival, causing large yield losses and restricting stable large-scale cultivation (Lu *et al.*, 2026).

Underground morphological traits-including crown and root architectures-function as core cold-resistance organs by storing carbohydrates and protecting regenerative buds and they jointly coordinate aboveground biomass accumulation, making them central to evaluating cultivar winter hardiness and productivity (Marquez-Ortiz *et al.*, 1996; Sun *et al.*, 2001; Wang *et al.*, 2024). Global research has confirmed that crown and root phenotypes affect aboveground biomass (Johnson *et al.*, 1996). Root morphological characteristics reflect root growth status to a certain extent; they can affect biological nitrogen fixation, water use efficiency, regeneration characteristics and cold resistance in alfalfa and play a key role, especially in the multi-year sustainable utilization of alfalfa (Erice *et al.*, 2010; Xu *et al.*, 2021). The crown bears branches and buds and its development status is closely related to production performance and low-temperature tolerance (Marquez-Ortiz *et al.*, 1999; Wang *et al.*, 2016; Xu *et al.*, 2021). In winter, the crown is the tissue closest to the soil surface. Under cold and dry conditions, alfalfa crowns are likely to be damaged by the freezing temperatures (Xu *et al.*, 2022). Therefore, understanding the root and crown development status of alfalfa during the establishment year is crucial for evaluating its cold tolerance and productivity.

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Inner Mongolia is an important animal husbandry production base in China, but local forage shortages have become increasingly prominent and are a key limiting factor for the sustainable and healthy development of animal husbandry (Wang *et al.*, 2022). The local climate is severely cold in winter (Dashtseren *et al.*, 2021) and the root development status and safe overwintering of alfalfa in the establishment year are prerequisites for the successful establishment of alfalfa. Comprehensive evaluations of multiple alfalfa varieties in cold regions have further revealed that root physiological and nutritional components, such as soluble sugars, starch and carbon/nitrogen contents, are major determinants of cold resistance (Guo and Shi, 2024; Li *et al.*, 2024). However, most existing studies have focused either on physiological mechanisms or on variety screening in isolation and the integrated relationships among aboveground growth, root characteristics in the establishment year and subsequent overwintering performance remain inadequately understood-particularly for the Tumote area of Inner Mongolia, where systematic variety comparisons are scarce. To address this gap, the present study selected 25 domestic and international alfalfa varieties for a field experiment in the Tumote area and conducted a preliminary investigation of aboveground growth, root traits in the establishment year and overwintering rate in the following year. The specific objectives were to: (I) Compare root and crown characteristics among different alfalfa varieties; (II) Explore the relationship among overwintering performance, root traits and aboveground growth; and (III) Provide a scientific basis for the introduction and breeding of alfalfa variety resources with excellent characteristics suitable for Inner Mongolia.

MATERIALS AND METHODS

Experimental site

The experimental site is located in the China Chilechuan Modern Agricultural Expo Park of Vocational and Technical College, Inner Mongolia Agricultural University, Tumote Right Banner, Baotou City, Inner Mongolia (40°35′ N, 110°34′ E, altitude 1009 m). It belongs to a temperate continental monsoon climate, with a dry and windy spring, concentrated rainfall in summer, mild and cool autumn and dry, cold and little snow in winter. The annual average temperature is 9°C, the average frost-free period is 150 days, the annual average precipitation is 350 mm and the annual average evaporation is 2055 mm. The soil is sandy loam and the basic soil fertility indices of the plow layer (0-30 cm) are as follows: organic matter content 23.6 g/kg, available nitrogen content 22.3 mg/kg, available phosphorus content 25.8 mg/kg, available potassium content 120.5 mg/kg and pH value 7.5.

Table 1: Tested alfalfa varieties and their sources.

Variety	Fall dormancy rate	Source
Caoyuan No.3	1	Inner Mongolia Agricultural University
Zhongcao No.3	1	Grassland Research Institute, CAAS
WL168	2	Beijing Zhengdao Seed Industry Co., Ltd.
SK3010	2.5	Clover (Beijing) Ecological Technology Co., Ltd.
Algonquin	2-3	Clover (Beijing) Ecological Technology Co., Ltd.
WL298	3	Beijing Zhengdao Seed Industry Co., Ltd.
WL319	3	Beijing Zhengdao Seed Industry Co., Ltd.
WL329	3	Beijing Zhengdao Seed Industry Co., Ltd.
Bara310	3	Barenbrug (Tianjin) International Grass Industry Co., Ltd.
Zhonglan No.2	3	Lanzhou Institute of Animal Husbandry and Veterinary Medicine, CAAS
Algernon	3	Beijing Best Grass Industry Co., Ltd.
Polaris	3	Beijing Best Grass Industry Co., Ltd.
Saskia	3	Beijing Best Grass Industry Co., Ltd.
Juneng No.2	3.2	Clover (Beijing) Ecological Technology Co., Ltd.
Gannong No.3	3-4	Gansu Agricultural University
WL343	4	Beijing Zhengdao Seed Industry Co., Ltd.
WL349	4	Beijing Zhengdao Seed Industry Co., Ltd.
WL354	4	Beijing Zhengdao Seed Industry Co., Ltd.
WL358	4	Beijing Zhengdao Seed Industry Co., Ltd.
MF4020	4	Clover (Beijing) Ecological Technology Co., Ltd.
Bara416	4	Barenbrug (Tianjin) International Grass Industry Co., Ltd.
Bara418	4	Barenbrug (Tianjin) International Grass Industry Co., Ltd.
Bara420	4	Barenbrug (Tianjin) International Grass Industry Co., Ltd.
Bara520	5	Barenbrug (Tianjin) International Grass Industry Co., Ltd.
Juneng 551	5	Clover (Beijing) Ecological Technology Co., Ltd.

Experimental materials

Twenty-five alfalfa varieties at home and abroad were selected. The variety name, fall dormancy class and source of the alfalfa materials are shown in Table 1.

Experimental design

The experiment started on 18 May 2025, adopting a completely randomized block design with a plot area of 6 m^2 (2 m × 3 m), sowing rate of 15 kg/hm^2, row spacing of 30 cm, 50 cm interval between plots and three replications. Routine field management was carried out during the experiment; weeding and pest control were conducted promptly and irrigation was applied once before overwintering.

At the end of September of the same year, aboveground indices were randomly measured in each plot. For each plot, 20 representative plants with uniform growth were randomly selected for plant height measurement and subsequently used for root sampling; these 20 plants were carefully excavated using the trench method (destructive sampling). Alfalfa roots were rinsed with tap water, blotted dry and brought back to the laboratory for root phenotypic trait measurement. For aboveground biomass, a separate 1 m^2 area (non-overlapping with the root-sampled plants) was harvested destructively in each plot. Border rows and 50 cm from each row end of each plot were removed to eliminate edge effects. In early April 2026, the over wintering rate was counted in each plot.

Measurement indicators

Plant height

The vertical distance from the bottom to the top of the plant was measured and the plot mean was calculated.

Aboveground biomass

Fresh grass yield of 1 m^2 was measured in each plot with a stubble height of 5 cm; 500 g was weighed from the mown fresh grass, dried in an oven at 65°C for 24 h, weighed and then dried to constant weight and the hay yield per hectare was converted. Plot mean values were used for statistical analysis.

Root morphological indices were determined using the method described by Marquez-Ortiz *et al.* (1996).

Crown burial depth: The distance from the ground surface to the upper end of the crown.

Crown diameter: The diameter at the swollen part of the crown was measured with a vernier caliper.

Taproot diameter: The diameter of the taproot 1 cm below the crown.

Lateral root position: The distance from the lower end of the crown to the first lateral root.

Lateral root diameter: The diameter of the first lateral root at 1 cm from the taproot.

Lateral root number: Lateral roots with diameter ≥0.5 mm at 1.0 cm from the taproot were counted. All root traits were averaged per plot before further analysis.

Root biomass: Roots of the same 20 plants per plot were weighed fresh, then dried at 65°C to constant weight for dry biomass; plot averages were used in statistical comparisons.

Overwintering rate: One 1 m long sampling segment was randomly selected within each experimental plot.

The numbers of dead and regreened plants were separately investigated during the vegetation recovery stage on April 25th, 2026.

$$\text{Overwintering rate (\%)} = \frac{\text{Number of greening plants}}{(\text{Number of greening plants} + \text{Number of dead plants})} \times 100\%$$

Plot means were calculated from the three replicate segments.

Data analysis

Data processing was performed using Excel 2010 and analysis of variance and correlation analysis were performed using SAS 9.0 software.

The fuzzy mathematics subordinate function method was used to comprehensively evaluate the measured indexes (Han *et al.*, 2006; Yue *et al.*, 2016). For indicators positively correlated with alfalfa cold tolerance during overwintering, the formula is:

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

For indicators negatively correlated with overwintering cold tolerance, the formula is:

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

Note:

F_{ij} = Membership function value of indicator j of alfalfa germplasm i.

X_{ij} = Measured value of indicator j of germplasm i.

X_{imax} and X_{imin} = Maximum and minimum values of indicator j among tested germplasm *i*, respectively.

The average value was obtained by summing up all membership function values of each tested alfalfa variety. A higher average value indicates stronger overwintering cold resistance of alfalfa varieties. Finally, the cold resistance of 25 alfalfa varieties was comprehensively compared to clarify the cold resistance ranking among varieties.

RESULTS AND DISCUSSION

Plant height and aboveground biomass

There were significant differences in plant height and aboveground biomass among the 25 alfalfa varieties before winter. For plant height, Bara416 was the tallest at 66.2 cm, followed by WL168 and WL329 at 61.9 cm and 60.9 cm, respectively, with no significant differences among the three varieties ($P>0.05$). Varieties with plant height lower than 50 cm included WL354, WL319 and Algernon. Among them, Algernon was the shortest at only 47.2 cm, which

was not significantly different from WL319 (P>0.05), but significantly lower than WL354 and other varieties (P<0.05) (Fig 1). For aboveground biomass, Bara416 was the highest at 7789.0 kg/hm^2, followed by WL298 at 7676.7 kg/hm^2; the two varieties showed no significant difference (P>0.05), but were significantly higher than other varieties (P<0.05). Polaris had the lowest biomass at 4118.3 kg/hm^2, which was not significantly different from SK3010, WL354 and Gannong No.3 (P>0.05) but significantly lower than other varieties (P<0.05) (Fig 2).

Root and crown morphological characteristics

There were significant differences in root and crown morphological indices among the 25 alfalfa varieties (Table 2). Specifically, WL343 exhibited the greatest crown depth at 2.50 cm, followed by WL329 at 2.48 cm, with no significant difference between the two (P>0.05). Juneng 551 had the shallowest crown depth, at only 1.35 cm, which was significantly different from WL343 and WL329 (P<0.05). Algonquin had the largest crown diameter at 11.80 mm, followed by MF4020 at 11.03 mm, with no significant difference between them (P>0.05). SK3010 had the smallest crown diameter, at only 6.60 mm, which was significantly different from Algonquin (P<0.05). Similarly, Algonquin had the thickest taproot diameter at 8.67 mm, followed by Zhonglan No. 2 at 8.21 mm, with no significant difference between the two. SK3010 had the smallest taproot diameter, at only 4.27 mm, which was significantly different from Algonquin and Zhonglan No. 2. Zhongcao No. 3 had the most distant lateral root from the crown at 10.23 cm, followed by WL298 at 10.03 cm, with no significant difference between them. WL349 had the closest lateral root to the crown at 3.85 cm, which was significantly different from Zhongcao No. 3 and WL298. WL319 had the largest lateral root diameter at 2.45 mm, followed by WL354 at 2.32 mm, with no significant difference between them. SK3010 had the smallest lateral root diameter, at only 1.17 mm, which was significantly smaller than that of WL319 and WL354. Algonquin had the greatest number of lateral roots at 9.22, followed by Zhonglan No. 2 at 8.13, with no significant difference between them. SK3010 had the fewest lateral roots at only 3.50, which was significantly different from Algonquin and Zhonglan No. 2.

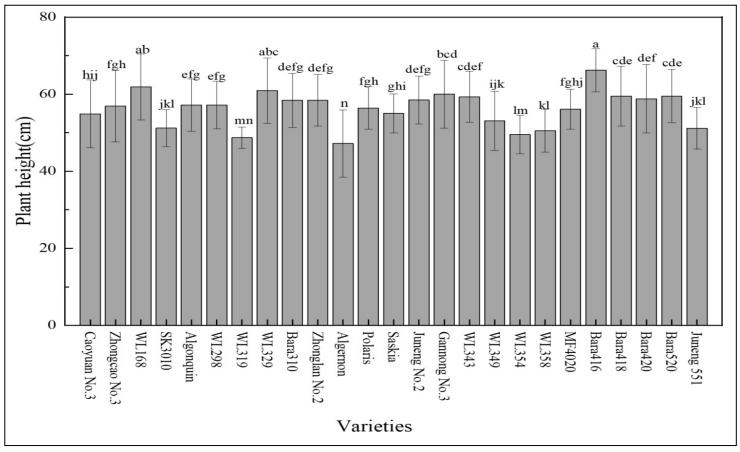


Fig 1: Plant height of different alfalfa varieties.

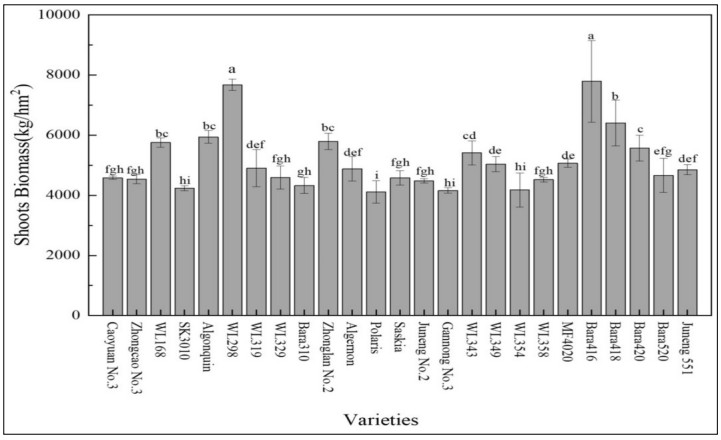


Fig 2: Shoots biomass of different alfalfa varieties.

Table 2: Root and crown morphological characteristics of different alfalfa varieties.

Variety	Crown depth (cm)	Crown diameter (mm)	Taproot diameter (mm)	Lateral root position (cm)	Lateral root diameter (mm)	Lateral root number (n)
Caoyuan No.3	2.00±0.11bcde	8.77±2.14bcdefgh	6.99±1.67abcdefg	8.50±4.60abc	1.82±0.69abcde	6.13±4.29bcdefg
Zhongcao No.3	2.06±0.69abcde	10.13±2.91abcde	7.73±2.06abcd	10.23±7.45a	1.45±0.76cde	6.00±2.14bcdefg
WL168	2.14±0.35abcde	9.70±1.47abcdef	7.01±0.96abcdefg	4.85±3.54bcd	1.75±0.78abcde	7.60±2.22abcd
SK3010	1.73±0.26efgh	6.60±1.20h	4.27±0.75h	5.37±2.57bcd	1.17±0.25e	3.50±1.90g
Algonquin	1.92±0.30cdef	11.80±3.20a	8.67±2.62a	5.78±5.40bcd	1.73±0.61abcde	9.22±4.21a
WL298	2.37±0.55abc	10.11±3.11abcde	6.77±2.01bcdefg	10.03±1.79a	1.64±0.56abcde	7.50±3.41abcde
WL319	1.97±0.40bcde	10.04±1.74abcde	7.35±1.39abcde	5.15±3.70bcd	2.45±0.77a	4.50±0.53efg
WL329	2.48±0.44a	8.44±1.79cdefgh	6.30±1.40cdefg	6.73±2.31abcd	1.26±0.44de	7.50±2.00abcde
Bara310	1.90±0.21cdefg	6.74±1.50h	5.34±1.18gh	5.20±2.44bcd	2.07±1.00abcd	4.50±1.26efg
Zhonglan No.2	1.88±0.46defg	10.07±3.34abcde	8.21±2.56ab	6.31±3.44abcd	1.82±0.42abcde	8.13±4.39ab
Algernon	1.76±0.50efgh	7.74±1.46fgh	6.10±0.84defg	7.13±3.51abcd	1.53±0.55bcde	5.80±2.39bcdefg
Polaris	1.93±0.29bcdef	7.86±2.15defgh	5.79±1.94efgh	4.69±2.43cd	1.47±0.47cde	4.78±2.17defg
Saskia	1.99±0.48bcde	10.14±2.31abcde	7.12±1.61abcdef	5.59±4.89bcd	1.74±0.66abcde	6.22±2.86bcdefg
Juneng No.2	2.11±0.33abcde	9.03±1.83bcdefg	6.71±0.96bcdefg	7.90±3.21abcd	2.16±0.58abc	6.20±2.82bcdefg
Gannong No.3	1.46±0.43gh	7.83±1.38efgh	5.71±1.18efgh	5.94±3.69bcd	1.78±0.65abcde	5.80±2.20bcdef
WL343	2.50±0.67a	6.88±1.57gh	6.54±1.32bcdefg	5.20±2.74bcd	2.02±0.85abcd	5.20±3.22bcdefg
WL349	2.25±0.42abcd	8.35±1.01cdefgh	6.17±0.97defg	3.85±1.73d	1.62±0.55abcde	6.40±1.43abcdefg
WL354	1.42±0.27h	8.46±1.01cdefgh	6.35±0.64cdefg	4.88±2.52bcd	2.32±1.32ab	5.00±2.21cdefg
WL358	2.40±0.52ab	8.53±1.87cdefgh	6.56±1.30bcdefg	5.97±1.91bcd	1.98±0.83abcde	5.40±2.59bcdefg
MF4020	2.00±0.25bcde	11.04±3.61ab	7.99±1.84abc	7.50±4.23abcd	1.99±0.42abcde	8.00±5.26abc
Bara416	2.20±0.42abcde	9.51±2.49bcdef	7.02±2.26abcdefg	8.90±5.17ab	1.48±0.47cde	5.60±2.01bcdefg
Bara418	2.30±0.48abcd	10.16±1.21abcd	6.71±1.19bcdefg	5.70±3.06bcd	1.55±0.33bcde	6.00±2.67bcdefg
Bara420	2.12±0.31abcde	10.55±1.72abc	7.00±1.66abcdefg	7.91±4.38abcd	1.63±0.62abcde	7.30±2.11abcdef
Bara520	1.51±0.51fgh	9.70±2.51abcdef	7.02±1.97abcdefg	6.70±2.89abcd	1.56±0.86bcde	6.00±2.83bcdefg
Juneng 551	1.35±0.41h	7.96±1.05defgh	5.48±0.77fgh	6.80±2.80abcd	1.47±0.61cde	4.40±1.43fg

Note: Different lowercase letters of the same column indicate significant differences (P<0. 05). The same below.

Root biomass

Root biomass differed significantly among the 25 alfalfa varieties (Fig 3). Algonquin had the highest root fresh weight at 14.33 g, followed by WL298 (11.28 g) and Zhonglan No. 2, with no significant difference between the latter two (P>0.05), but both were significantly lower than Algonquin (P<0.05). SK3010 had the lowest root fresh weight, at only 2.88 g, which was significantly lower than that of all other varieties (P<0.05). Similarly, Algonquin had the highest root dry weight at 4.34 g, followed by WL298 at 3.43 g, with a significant difference between them (P<0.05). SK3010 had the lowest root dry weight, at only 0.96 g, which was significantly lower than that of all other varieties (P<0.05).

Overwintering rate

Significant differences in overwintering rate were detected among the 25 alfalfa varieties (Fig 4). WL298 exhibited the highest overwintering rate at 83.7%, followed by Bara416 (82.3%) and WL168 (80.0%), with no significant differences among these three varieties (P>0.05). Polaris showed the lowest overwintering rate at 57.3%, which was not significantly different from that of Zhongcao No. 3, Algernon, Saskia, WL343, WL349, WL354, MF4020, Bara418 and Juneng 551 (P>0.05), but was significantly lower than that of the other varieties (P<0.05).

Correlation analysis

Correlation analysis was conducted among the 11 indicators and the results are presented in Table 3. All the indicators showed correlations of varying degrees. Overwintering rate was extremely significantly positively correlated with plant height and aboveground biomass (P<0.01) and significantly positively correlated with crown burial depth, crown diameter and first lateral root position (P<0.05). Aboveground biomass was extremely significantly positively correlated with root fresh weight and crown diameter (P<0.01) and significantly positively correlated with root dry weight, crown burial depth, taproot diameter and lateral root position (P<0.05). root biomass had significantly positively correlated with crown diameter, taproot diameter and lateral root number (P<0.01).

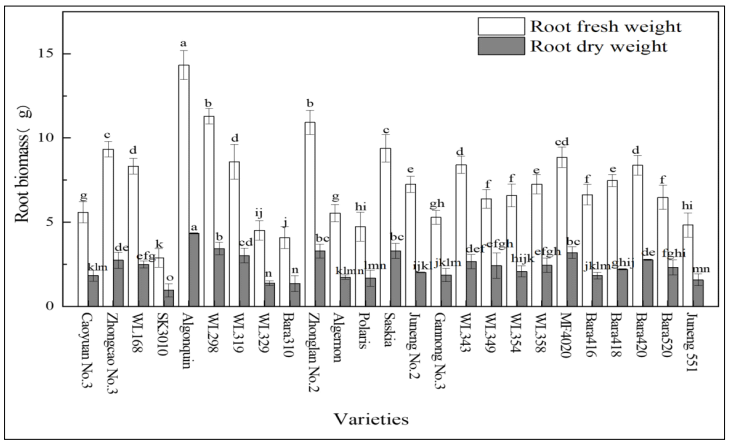


Fig 3: Root biomass of different alfalfa varieties.

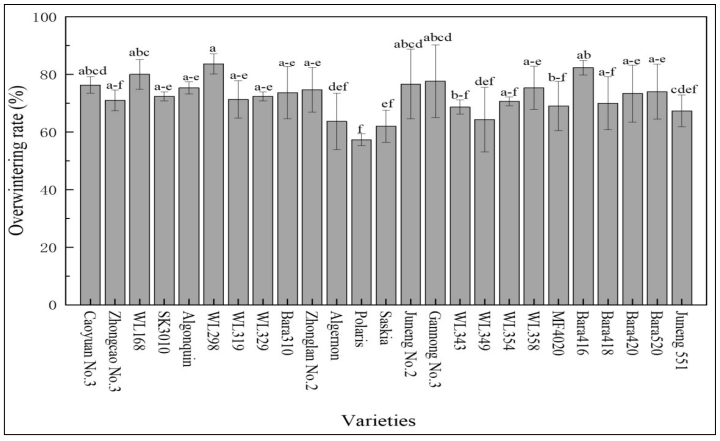


Fig 4: Overwintering rate of different alfalfa varieties.

Table 3: Correlation analysis among various indexes of different alfalfa varieties.

	Overwintering rate	Plant height	Aboveground biomass	Root fresh weight	Root dry weight	Crown burial depth	Crown diameter	Taproot diameter	Lateral root number	Lateral root diameter	Lateral root position
Overwintering rate	1	0.472**	0.509**	0.246	0.122	0.358*	0.340*	0.211	0.299	0.112	0.454*
Plant height		1	0.466**	0.154	0.058	0.346*	0.235	0.251	0.403*	-0.230	0.248
Aboveground biomass			1	0.511**	0.407*	0.432*	0.470**	0.389*	0.427*	-0.153	0.391*
Root fresh weight				1	0.968**	0.270	0.801**	0.855**	0.716**	0.260	0.224
Root dry weight					1	0.225	0.796**	0.837**	0.682**	0.313	0.119
Crown burial depth						1	0.161	0.243	0.313	-0.035	0.152
Crown diameter							1	0.875**	0.734**	0.095	0.352*
Taproot diameter								1	0.749**	0.280	0.312
Lateral root number									1	-0.049	0.255
Lateral root diameter										1	-0.224
Lateral root position											1

Comprehensive evaluation

Based on the 11 indicators as evaluation criteria. The membership function method was adopted to comprehensively assess the cold resistance of different alfalfa cultivars (Table 4). The order of cold resistance of 25 alfalfa varieties was as follows: WL298 (0.74) > Algonquin (0.72) > Bara416 (0.62) = Zhonglan No.2 (0.62) > MF4020 (0.60) > Bara420 (0.58) > WL168 (0.57) > Zhongcao No.3 (0.54) > Juneng No.2 (0.52) > Bara418 (0.51) > WL319 (0.47) = WL343 (0.47) > Saskia (0.46) = Caoyuan No.3 (0.46) > WL358 (0.45) > WL329 (0.43) = Bara520 (0.43) > WL349 (0.36) > Gannong No.3 (0.35) > WL354 (0.32) > Bara310 (0.30) > Algernon (0.28) > Polaris (0.23) = Juneng 551 (0.23) > SK3010 (0.13).

Aboveground growth status of alfalfa in the establishment year

The growth and development of alfalfa during the establishment year not only directly determine the yield obtained in that year, but also affect normal growth and forage yield in subsequent years (Moyer, 1992). Plant height and aboveground biomass in the establishment year are important indicators reflecting the growth and development status of alfalfa. Existing studies have shown that plant height is generally positively correlated with aboveground biomass and both indicators jointly reflect the growth potential and resource-acquisition capacity of alfalfa varieties under current ecological conditions (Li *et al.*, 2021). However, larger above-ground organs are not always beneficial in the establishment year. Especially in regions with severe winters, excessive growth of above-ground organs may consume excessive photosynthates, reduce the allocation of non-structural carbohydrate reserves to roots and consequently weaken the cold resistance and overwintering ability of alfalfa (Beccari and Carmona, 2024; Wang *et al.*, 2023). Wang *et al.* (2008) found that the growth rate of alfalfa varied among cultivars and cutting regrowth cycles. Ji *et al.* (2011) demonstrated that alfalfa cultivars differ in yield potential and environmental sensitivity, resulting in distinct differences in growth rate and biomass accumulation dynamics. Our study revealed significant differences in plant height and aboveground biomass among different alfalfa cultivars in the establishment year. The above-ground growth performance of various alfalfa cultivars was inconsistent with their fall dormancy grades, indicating no inevitable correlation between fall dormancy grade and growth performance in the establishment year. This finding aligns with observations by Cunningham *et al.* (1998), who reported that while fall dormancy is correlated with improved winter survival in alfalfa, the physiological basis for this association is not fully understood and few quantitative trait loci are related to both autumn plant height and winter injury, supporting the observation of no genetic correlation between the two traits. The inconsistency between fall dormancy rating and establishment-year growth may be related to local climate, soil conditions and genetic characteristics of cultivars. Therefore, appropriate

Table 4: Comprehensive evaluation of different alfalfa varieties.

Variety	Overwintering rate	Plant height	Aboveground biomass	Root fresh weight	Root dry weight	Burial depth	Crown diameter	Taproot diameter	Lateral root number	Lateral root diameter	Lateral root position	Mean	Rank
Caoyuan No.3	0.72	0.41	0.13	0.24	0.26	0.57	0.42	0.62	0.46	0.51	0.73	0.46	13
Zhongcao No.3	0.52	0.51	0.11	0.56	0.53	0.62	0.68	0.79	0.44	0.22	1.00	0.54	8
WL168	0.86	0.77	0.45	0.48	0.45	0.69	0.60	0.62	0.72	0.45	0.16	0.57	7
SK3010	0.57	0.21	0.03	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.24	0.13	25
Algonquin	0.68	0.53	0.50	1.00	1.00	0.50	1.00	1.00	1.00	0.44	0.30	0.72	2
WL298	1.00	0.53	0.97	0.73	0.73	0.89	0.68	0.57	0.70	0.37	0.97	0.74	1
WL319	0.53	0.08	0.21	0.50	0.61	0.54	0.66	0.70	0.17	1.00	0.20	0.47	11
WL329	0.57	0.72	0.13	0.14	0.12	0.98	0.36	0.46	0.70	0.07	0.45	0.43	16
Bara310	0.62	0.59	0.06	0.10	0.12	0.48	0.03	0.24	0.17	0.71	0.21	0.30	21
Zhonglan No.2	0.66	0.59	0.46	0.70	0.69	0.46	0.67	0.90	0.81	0.51	0.39	0.62	3
Algernon	0.24	0.00	0.21	0.23	0.23	0.36	0.22	0.42	0.40	0.28	0.51	0.28	22
Polaris	0.00	0.48	0.00	0.16	0.21	0.51	0.24	0.35	0.22	0.24	0.13	0.23	23
Saskia	0.18	0.41	0.13	0.57	0.69	0.56	0.68	0.65	0.48	0.45	0.27	0.46	13
Juneng No.2	0.73	0.59	0.10	0.38	0.31	0.66	0.47	0.55	0.47	0.77	0.64	0.52	9
Gannong No.3	0.77	0.67	0.01	0.21	0.27	0.10	0.24	0.33	0.40	0.48	0.33	0.35	19
WL343	0.43	0.64	0.35	0.48	0.50	1.00	0.05	0.52	0.30	0.67	0.21	0.47	11
WL349	0.27	0.31	0.25	0.31	0.43	0.78	0.34	0.43	0.51	0.35	0.00	0.36	18
WL354	0.51	0.12	0.02	0.32	0.33	0.06	0.36	0.47	0.26	0.90	0.16	0.32	20
WL358	0.68	0.17	0.11	0.38	0.44	0.91	0.37	0.52	0.33	0.64	0.33	0.45	15
MF4020	0.44	0.47	0.26	0.52	0.66	0.57	0.85	0.85	0.79	0.64	0.57	0.60	5
Bara416	0.95	1.00	1.00	0.33	0.26	0.74	0.56	0.63	0.37	0.25	0.79	0.62	3
Bara418	0.48	0.65	0.62	0.40	0.37	0.83	0.69	0.56	0.44	0.30	0.29	0.51	10
Bara420	0.61	0.61	0.40	0.48	0.53	0.67	0.76	0.62	0.66	0.37	0.64	0.58	6
Bara520	0.63	0.65	0.15	0.31	0.40	0.14	0.60	0.63	0.44	0.31	0.45	0.43	16
Juneng 551	0.38	0.21	0.20	0.17	0.18	0.00	0.26	0.28	0.16	0.24	0.46	0.23	23

planting factors (seeding rate, row spacing, sowing depth) and harvesting factors (cutting time, cutting height) are the key determinants of high alfalfa yield (Liu *et al.*, 2015; Malinowski *et al.*, 2007; Rimi *et al.*, 2010).

Root characteristics of alfalfa in the establishment year

Alfalfa roots not only function in nutrient and water absorption, transportation and storage, but also serve as major functional organs closely associated with overwintering and cold resistance. Thick taproots directly affect root volume and root biomass; a larger contact area with soil facilitates the uptake of soil moisture and nutrients by plants. In this study, taproot diameters differed significantly among alfalfa cultivars. Algonquin and Zhonglan No.2 possessed larger taproot diameters, indicating superior taproot development capacity. The emergence positions of lateral roots on taproots reflect the vertical differentiation capacity of cultivars in utilizing soil water, nutrients and trace elements (Wu, 2007). Shi *et al.* (2009) reported that alfalfa lateral roots mainly grow on taproot segments 0 to 20 cm below the soil surface. Wu *et al.* (2019) found that the first lateral roots of alfalfa are mostly distributed 3 to 5 cm away from the crown, with individual cultivars reaching approximately 10 cm. In this experiment, the first lateral roots of tested alfalfa were mainly located 5 to 8 cm from the crown and some extended to 10 cm, which may be attributed to cultivar genetic traits, growth year and soil environmental conditions. Lateral root diameter and quantity directly determine root water and nutrient absorption capacity as well as drought resistance. Larger diameter and greater number of lateral roots lead to larger root volume and surface area, stronger absorption capacity and higher stress resistance and environmental adaptability of cultivars (Wu, 2007). In this study, some cultivars showed distinct environmental adaptability *via* different adaptive strategies. However, correlation analysis revealed that taproot diameter and lateral root number were significantly or extremely significantly positively correlated with aboveground and root biomass, yet had no obvious correlation with overwintering rate. This finding is consistent with recent research by Li *et al.* (2024), during overwintering, soluble proteins in taproots and lateral roots decline significantly, with lateral roots showing a more marked decrease-suggesting higher protein consumption in lateral roots during overwintering, likely because taproots are primarily used for energy storage while lateral roots are more involved in metabolism and growth. This indicates that root phenotypic traits are not core indicators for evaluating alfalfa cold resistance, which is mainly determined by crown characteristics (Malinowski *et al.*, 2007).

Furthermore, non-structural carbohydrates (NSCs) play a critical role in alfalfa cold tolerance. demonstrated that NSCs are important factors influencing the overwintering and regeneration of alfalfa, with soluble sugars and starch contents in the crown being significantly higher than those in deeper roots (Li *et al.*, 2023). The trend of soluble sugar and starch contents was consistent with that of semi-lethal temperature, showing a significant negative correlation between NSC content and low-temperature tolerance. This suggests that root phenotypic traits alone are not core indicators for evaluating alfalfa cold resistance; rather, the physiological and biochemical composition of storage organs-particularly carbohydrate reserves-plays a more decisive role.

Crown characteristics of alfalfa in the establishment year

The root crown is the junction between the stem and root system where buds grow. It is highly sensitive to low temperatures and closely related to the overwintering survival and spring regrowth of alfalfa (Wang *et al.*, 2023). Therefore, crown development in the establishment year directly determines safe overwintering and subsequent yield performance in the following year. Crowns are typically buried 2.5 to 5 cm in the soil, which provides natural protection form air temperatures (Wang *et al.*, 2023). Deeper crown burial thus enhances winter survival by positioning the vulnerable meristematic tissues in a more thermally buffered soil layer. This study showed that crown burial depth and crown diameter differed significantly among alfalfa cultivars, without regular variation along with the increase of fall dormancy grades. Correlation analysis indicated that crown burial depth was closely correlated with overwintering rate and above-ground growth status. Han *et al.* (2008) confirmed that alfalfa cold resistance was associated with crown burial depth; cold-resistant cultivars had deeper buried crowns and stronger overwintering tolerance. Deeper-buried crowns contribute to higher overwintering survival rate in the next year (Wang *et al.*, 2023).

Crown diameter is closely linked to crown bud development and carbohydrate storage capacity. Cunningham *et al.* (1998) demonstrated that parental cultivar differences in fall dormancy and winter survival were associated with changes in crown bud development, elevated total nonstructural carbohydrate and sugar concentrations in buds and sugar and protein accumulation in roots. A larger crown diameter provides greater volume for storage of soluble sugars, starch and vegetative storage proteins, which serve as energy reserves for winter survival and spring regrowth.

Obvious differences in crown traits were observed among tested cultivars, yet no regular correlation was found with fall dormancy grades. This suggested that the phenotypic differences among experimental alfalfa cultivars were mainly determined by their inherent biological characteristics rather than fall dormancy. The relationship between fall dormancy grade and root system traits remains to be further explored (Juan *et al.*, 1994; Sun *et al.*, 2001).

CONCLUSION

Significant differences were observed among the 25 alfalfa varieties in aboveground growth, root and crown traits and subsequent overwintering rates. Correlation analysis revealed that crown burial depth and crown diameter were closely associated with overwintering survival, suggesting these crown traits can serve as useful indicators for evaluating cold resistance. In contrast, taproot diameter and lateral root characteristics primarily influenced aboveground biomass production rather than directly determining winter survival. Based on the subordinate function comprehensive evaluation, WL298, Algonquin and Bara416 attained the highest scores, indicating robust root development and strong overwintering potential. However, due to the limitations of the experimental duration and environmental conditions, although these varieties may be considered as candidate germplasm for future breeding programs, their adaptability and yield stability should be validated under a broader range of environmental conditions over multiple growing seasons.

Author contributions

Methodology, Wang Y.; formal analysis, Wang Y.; investigation, Zhao Y., Li W. Li Z. and Qu G.; resources, Wang Y.; data curation, Wang Y. and Zhao Y.; writing-original draft preparation, Wang Y.; writing-review and editing, Wang Y.; project administration, Wang Y. and Wang F.; funding acquisition, Wang Y. and Wang F. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The data presented in this study are available on request from the corresponding author due to special reasons.

Conflict of interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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