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A Multi-Site Evaluation of Winter Hardiness in Indigenous Alfalfa Cultivars in Northern China

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Abstract: Integration of perennial grass species into the current food production systems, especially in the agropastoral regions worldwide, may produce multiple benefits including, among others, a more stable productivity and a smaller eco-environmental footprint. However, one of the fundamental challenges facing the large-scale adoption of such grass species is their ability to withstand the vagaries of winter in these regions. Here, we present a comprehensive evaluation of the winter hardiness of 50 indigenous Chinese cultivars of alfalfa, a high-quality leguminous perennial grass, in comparison with six introduced U.S. cultivars in a multi-site field experiment in northern China. Our results reveal that indigenous cultivars have stronger winter hardiness than introduced cultivars. Cultivars native in the north performed better than southern cultivars, suggesting that suitability evaluation is an unavoidable step proceeding any regional implementations. Our results also show that the metric we used to assess alfalfa's winter hardiness, the average score index (ASI), produced more consistent results than another more-widely used metric of winter survival rate (WSR). These findings offer a systematic field evidence that supports regional cropping system adjustment and production system betterment to ensure food security under climate change in the region and beyond.

Keywords: alfalfa; average score index; winter survival rate; climate change; adaptation; mitigation

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1. Introduction

Alfalfa (*Medicago sativa* L.) is a perennial high-quality leguminous forage grass, which is widely cultivated around the world. In China, alfalfa cultivation can be dated back to more than 2000 years [1]. By 2016, the alfalfa cultivation area in China has reached 3.84 million hectares, or over 40% of the total area of all high-quality forage grasses in China [2]. The cultivation of alfalfa has played an important role in supporting the healthy development of the modern livestock industry and food security in China. Alfalfa-enabled crop rotations have the potential to transform the low- to medium-yielding lands into high-yielding ones by biological nitrogen fixation [3], demonstrating its role in ensuring national food security [4].

The trend of climate warming has become more evident in recent decades in China. The warming has increased heat resources in most parts of China. As a result, the agro-climatic boundary has been pushed northward, extending the suitability range of alfalfa further into northern China [5,6]. Meanwhile, involving a perennial grass in the current farming or agropastoral systems has been recognized as a valid adaptation measure to climate change [3,7,8]. However, systematic evaluations of the physiological suitability of perennial grasses such as alfalfa are rarely seen in northern China. Cultivation of alfalfa depends on many factors, among which winter hardiness is one of the most important. Winter hardiness is a biological characteristic of forage grasses that affects grassland cultivation and partitioning [9,10]. In alfalfa, winter hardiness is its physiological adaptation capacity of freezing tolerance that allows exposure to subzero temperatures without cellular damages [11], which is regarded as one of its most important characteristics in response to climatic stresses [12]. Although extensive research has been conducted on winter hardiness worldwide, most focused on the physiological indicators of winter hardiness for alfalfa [13–15]. Investigations on morphological indicators are relatively limited [16,17]. To date, a unified winter hardiness grading standard has not been established to measure alfalfa's ability to survive the winter and regenerate in spring [18] or, in other words, its performance to tolerate frost, snow accumulation, freezing, drying and other extreme winter stresses [12,19]. Currently, most studies use winter survival rate (WSR) and autumn dormancy grade [14,18] to characterize alfalfa's winter hardiness, in addition to other physiological indicators, such as malondialdehyde, soluble sugar, soluble protein and catalase [12,14,17,20,21]. One of the longstanding critiques for this approach is that the relationship between autumn dormancy and winter hardiness is not well established [19]. Therefore, a morphology-based grading method, namely, the average score index (ASI), has recently been proposed to quantify the cold resistance of plants [22], which has the potential to measure a plant species' ability to survive winter. The basic idea of ASI is that the plant's ability to undergo cold acclimation in autumn, which is triggered by a decreasing temperature and a shortening photoperiod, is closely related with its ability to survive winter [11,23]. Theoretically, ASI has the advantage to *foresee* the plant's overwintering outcomes in autumn by visually inspecting the changes in plant morphology in situ. However, this potential has rarely been utilized, especially for cultivated species, such as alfalfa. Here, we adopt a combined approach involving both WSR and ASI to evaluate the overwintering performances of 50 indigenous Chinese alfalfa cultivars in a three-site field assessment in northern China to test ASI's applicability and examine the consistency and accuracy of both the ASI and WSR methods.

2. Materials and Methods

2.1. Research Sites

Field experiments were carried out in three research sites, namely Wuyuan, Wuchuan and Tuzuo, in northern China's Inner Mongolia region (Figure 1). Temperate monsoon climate prevails in the region. Average monthly temperature varies between −14.9 °C and 22.2 °C, with a mean temperature of 4.9 °C. The mean annual precipitation ranges from 198 mm to 399 mm during 1957–2010. Further site-specific information is given in Table 1.

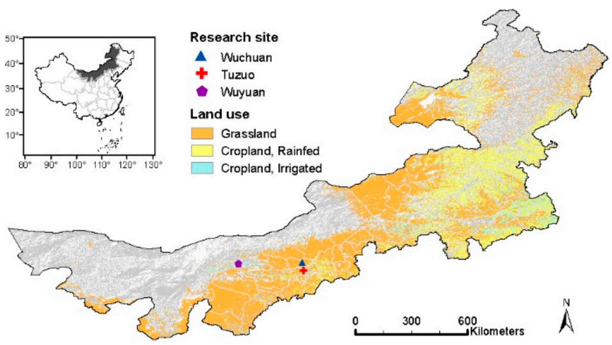

Figure 1. Location of the research sites. Spatial distribution of the grassland and cropland is extracted from ESA CCI 1.3 land cover and upscaled to 1 km grid resolution. The underlying digital terrain is based on NASA's Shuttle Radar Topography Mission, also upscaled to 1 km resolution.

Table 1. Climatic and soil conditions of the experimental sites.

Site	Monthly Temperature (°C)	Annual Precipitation (mm)	Frost-Free Period (d)	Winter Temperature (°C)	Soil
Wuyuan	−14.3~22.2	155~242	130	−12.2	Solonchak
Wuchuan	−14.9~19.7	244~348	150	−12.9	Kastanozem
Tuzuo	−12.1~22.4	302~461	135	−10.0	Kastanozem

2.2. Experimental Design

A total of six standard alfalfa cultivars originally introduced from the U.S. (hereafter referred to as American cultivars) were used in this experiment as control material. Characteristics of these cultivars including their ASI values, fall dormancy ratings and winter survival ratings as reported by McCaslin, Woodward and Undersander [22] are given in Table 2. An additional 50 local cultivars (hereafter referred to as indigenous cultivars) were also included in the experiment (Table S1). The seedlings of these 56 cultivars of alfalfa were grown in a greenhouse at the Wuyuan site in April 2014, using black plastic pots of 20.5 cm in height and 4.0 and 1.5 cm in top and bottom diameter, respectively. Before sowing, 100 g of soil and 70 mL of water were added to each pot. On average, 2–4 seeds were planted in each pot, after being inoculated with the rhizobium bacteria to promote nitrogen fixation by roots. Rhizobium was premixed at a dose of 180 g per 100 g of seeds. Each pot was then topped with a 2 cm layer of vermiculite. Sufficient water was given thereafter. The seedling pots were grouped into samples. Each sample consisted of five replicates, and each replicate included 30 plants. During the seedling stage, the greenhouse temperature was maintained at 24–30 °C with a $\geq$ 16-hr natural light exposure per day. Regular greenhouse management, such as air moisture control, was ensured for normal seedling growth.

Table 2. Standard U.S. alfalfa cultivars used in the experiment.

Cultivar	Serial Number	Fall Dormancy Rating	Winter Survival Rating	Average Score Index
ZG9830	28	1.9	1	1.6
5262	19	3.6	2	2.2
WL325HQ	24	4.3	3	2.9
G-2852	25	5.6	4	3.6
Archer	21	5.1	5	4.0
Cuf101	30	8.8	6	4.8

The seedlings were cut at the height of 5–8 cm in the 8th week and transplanted to the experimental fields in the three study sites. In each site, four replicates were arranged per cultivar. Each replicate consisted of 30 plants. The plants were placed in rows of 60 cm apart with an intra-row spacing of 30 cm. Routine field management was conducted to ensure normal plant growth. The plants were harvested in early autumn, i.e., the second half of August.

The number of plants retained in the first year were counted one week before frost in each experimental site. In the following year, the number of survival plants $\geq$15 cm in height were counted in May. The WSR and ASI indices with a value range of 0–100 and 1–5, respectively, were then derived using Equations (1) and (2), respectively:

$$WSR = \frac{q_t}{q_{t-1}} \cdot 100, \tag{1}$$

$$ASI = \frac{\sum_{i=1}^{5} (q_i \cdot s_i)}{\sum_{i=1}^{5} q_i}, \tag{2}$$

where q_t is the number of survival plants in spring, q_{t-1} is the number of plants counted in autumn of the previous year; q is the number of plants in grade s. In total, five grades were established based on the grading criteria given in Table 3. Examples showing the morphology of a typical plant in each grade are provided in Figure 2.

Table 3. Rating criteria for Alfalfa's wintering performance.

Score	Degree of Injury	Criteria
1	No injury	The plant has uniform, symmetrical appearance, with numerous branches and stems, all shoots are about equal in length.
2	Some injury	The plant is symmetrical, but regrowth is slightly uneven, and bush saturation decreases.
3	Significant injury	The plant is asymmetrical, regrowth varies in length, and it only possess a few branches.
4	Severe injury	The plant has sparse shoots, and regrowth is highly irregular.
5	Dead	The plant is dead.

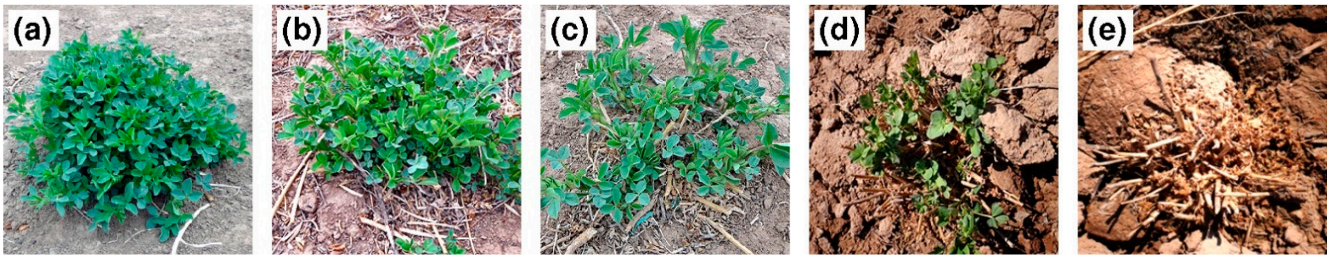

Figure 2. Examples showing alfalfa plant morphology per average scoring index (ASI) grade: (**a**) Grade 1; (**b**) Grade 2; (**c**) Grade 3; (**d**) Grade 4; and (**e**) Grade 5.

2.3. Data Analysis

Microsoft Excel 2013 was used for data processing. SPSS 20.0 was used for statistical analysis. ANOVA, Duncan, and quartile methods were used to test the significance of the statistical results. K-means clustering and Bayesian discriminant methods were used to analyze the consistency between the ASI and WSR measurements.

3. Results

3.1. Validation of the Standard Cultivars

The winter hardiness of the six American alfalfa cultivars measured at each experimental site in terms of ASI is given in Table 4. The obtained measurements show that at all experimental sites, the ASI values of these six cultivars are significantly higher than their reference values reported by McCaslin, Woodward and Undersander [22], indicating that the overwintering abilities of these cultivars are lower in northern China than in the U.S. The correlation analysis between the ASI values obtained here and those reported from the U.S. shows that the ASI values obtained in China are significantly positively correlated with the reported reference ASI values (Table 4). Overall, these results demonstrate that although the wintering ability of these cultivars are somewhat degraded in northern China, their cold resistance performance stays stable and consistent, suggesting that incorporation of these cultivars into the local agropastoral systems in northern China is still considerable.

Table 4. Average score index (ASI) values of the six introduced alfalfa cultivars as measured in northern China and reported as reference by McCaslin, Woodward and Undersander [22].

Cultivar	Measured ASI				Reference ASI
	Wuyuan	Tuzuo	Wuchuan	Average	
ZG9830	3.14	3.39	3.62	3.38	1.6
5262	3.67	4.22	3.48	3.79	2.2
WL325HQ	3.83	3.63	3.96	3.81	2.9
G-2852	4.82	4.69	4.68	4.73	3.6
Archer	4.28	4.79	4.39	4.49	4.0
Cuf101	5.00	4.99	5.00	5.00	4.8
Correlation coefficient	0.930 **,[1]	0.873 *	0.936 **	0.952 **	

[1] Significance levels: * $p < 0.05$; ** $p < 0.01$.

3.2. Evaluation of the Indigenous Cultivars

In order to better compare the cold resistance of different cultivars across experimental sites, multiple analyses involving the quartile range (QR), K-means clustering (KMC) and Bayesian discriminant analysis (BDA) methods were conducted. More specifically, the QR analysis was used to evaluate the cold resistance of cultivars at a single site, while KMC was used to combine the results from multiple sites. Moreover, BDA was employed to judge the validity of the classification results of the KMC method.

3.2.1. Quartile Range and K-Means Clustering Analyses

All 56 alfalfa cultivars from the field experiment were grouped into three classes based on the quartile distribution of their ASI or WSR values, and the obtained results are given in Table 5. The results show that the overall performance of these 56 experimental cultivars in terms of WSR was the lowest at the Tuzuo site, compared to the other two sites. The third and the first quartiles—or in other words, the 75th and the 25th percentiles—of the WSR values were measured at 34.17% and 1.67% at Tuzuo, while the same quartile distributions of the WSR were 70.50% and 23.33%, respectively, at Wuchuan and 78.47% and 41.39%, respectively, at Wuyuan (Table 5). Similar distributions of winter hardiness in terms of ASI were also observed, despite a different value range and an opposite direction. Taking ASI and WSR's quartile distribution results from all three experimental sites together, a batch of best performing cultivars across all three sites were identified as cultivars with the serial numbers of 1, 5, 7, 14, 20, 44 and 46. The winter survival rates of these cultivars were always measured at the fourth quartile in terms of WSR or the first quartile in terms of ASI. More details of the quartile range analysis results, including an individual cultivar's quartile distribution and the summary statistics per class of cultivars can be found in Table 5.

The KMC analysis was next conducted to identify the well, the moderately and the poorly performing cultivars across all three experimental sites. The results are represented in Table 6. The obtained results were found largely in agreement with the QR analysis. For example, cultivars 1, 4, 5, 7, 8, 14, 15, 20, 42, 44, 46 were clustered into the *well* performing class in all three sites and in terms of both ASI and WSR. It is interesting to observe that this cluster of cultivars is a superset of the equivalent class of cultivars identified by QR analysis, meaning that the results obtained using a conventional statistical analyzing method, i.e., quartile distribution, were confirmed by a more computationally sophisticated statistical method, i.e., KMC. Full details of the KMC results, including the site-specific means and standard deviations per class of cultivars in terms of ASI and WSR can be found in Table 6. Overall, similar results were obtained using the QR and the KMC methods. As a summary indicator, the quantity of cultivars in the *well*, *moderately* and *poorly* performing classes were summed at 12, 27 and 7, respectively, in terms of ASI, while in terms of WSR, the cultivars were summed at 13, 28 and 15 in each of the three classes, respectively.

Table 5. Quartile ranges and summary statistics of winter hardiness measurements in 56 alfalfa cultivars.

Metric	Site	IQR [1]	Cultivars	N	Mean	SD [1]	SE [1]	CV [1]
ASI	Tuzuo	≤3.15	1, 4, 5, 7, 8, 14, 15, 16, 20, 42, 44, 46, 47, 48	14	2.33	0.47	0.13	20.09
		3.15–4.72	2, 3, 6, 9, 10, 11, 12, 13, 17, 18, 19, 23, 24, 25, 28, 34, 37, 38, 39, 43, 45, 49, 50, 51, 52, 53, 55, 56	28	3.84	0.45	0.09	11.82
		>4.72	21, 22, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 54	14	4.96	0.06	0.02	1.14
	Wuchuan	≤3.46	1, 4, 5, 6, 7, 8, 12, 13, 14, 17, 20, 42, 44, 46, 48	15	2.87	0.48	0.12	16.75
		3.46–4.67	2, 3, 9, 10, 11, 15, 16, 18, 19, 21, 23, 24, 28, 34, 37, 38, 39, 43, 45, 47, 49, 50, 51, 52, 54, 55, 56	27	3.97	0.32	0.06	8.02
		>4.67	22, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 53	14	4.91	0.12	0.03	2.39
	Wuyuan	≤2.97	1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 14, 16, 20, 44, 46	14	2.57	0.33	0.09	12.94
		2.97–4.51	3, 11, 12, 15, 17, 18, 19, 21, 23, 24, 28, 34, 37, 38, 39, 42, 43, 45, 47, 48, 49, 50, 51, 52, 53, 55, 56	28	3.62	0.40	0.08	11.03
		>4.51	22, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 54	14	4.96	0.07	0.02	1.45
WSR	Tuzuo	>34.17	1, 3, 4, 5, 6, 7, 8, 14, 15, 20, 42, 44, 46, 55	14	55.90	13.60	3.63	24.32
		1.67–34.17	2, 9, 10, 11, 12, 13, 16, 17, 18, 19, 21, 23, 24, 28, 34, 37, 38, 39, 43, 45, 47, 48, 49, 50, 51, 52, 54, 56	28	18.10	8.76	1.66	48.43
		≤1.67	22, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 53	14	0.19	0.31	0.08	164.08
	Wuchuan	>70.50	1, 4, 5, 6, 7, 8, 12, 14, 15, 17, 20, 42, 44, 46	14	86.42	9.41	2.51	10.89
		23.33–70.50	2, 3, 9, 10, 11, 13, 16, 18, 19, 21, 23, 24, 28, 34, 37, 38, 39, 43, 45, 47, 48, 49, 50, 51, 52, 55, 56	27	55.73	10.69	2.06	19.18
		≤23.33	22, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 53, 54	15	7.50	8.71	2.25	116.08
	Wuyuan	>78.47	1, 3, 5, 7, 9, 14, 17, 20, 28, 43, 44, 46, 49, 52	14	82.30	2.82	0.75	3.42
		41.39–78.47	2, 4, 6, 8, 10, 11, 12, 13, 15, 16, 18, 19, 21, 23, 24, 25, 34, 37, 38, 39, 42, 45, 47, 48, 50, 51, 55, 56	28	70.20	8.98	1.70	12.80
		≤41.39	22, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 53, 54	14	10.32	11.48	3.07	111.28

[1] IQR: Interquartile range; SD: Standard deviation; SE: Standard error; CV: Coefficient of variance.

Table 6. Winter hardiness classification of 56 alfalfa cultivars by K-means clustering.

Metric	Class	Cultivar	N	Mean			Standard Deviation		
				Tuzuo	Wuchuan	Wuyuan	Tuzuo	Wuchuan	Wuyuan
ASI	Well	1, 4, 5, 7, 8, 14, 15, 20, 42, 44, 46, 48	12	2.20	2.78	2.73	0.36	0.50	0.54
	Moderate	2, 3, 6, 9, 10, 11, 12, 13, 16, 17, 18, 19, 23, 24, 28, 34, 37, 38, 39, 43, 45, 47, 49, 51, 52, 55, 56	27	3.70	3.83	3.39	0.41	0.34	0.46
	Poor	21, 22, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 50, 53, 54	17	4.90	4.83	4.85	0.15	0.20	0.26
WSR	Well	1, 4, 5, 6, 7, 8, 14, 15, 17, 20, 42, 44, 46	13	56.92	87.52	79.19	13.91	8.81	6.18
	Moderate	2, 3, 9, 10, 11, 12, 13, 16, 18, 19, 21, 23, 24, 28, 34, 37, 38, 39, 43, 45, 47, 48, 49, 50, 51, 52, 55, 56	28	19.55	56.32	72.96	9.13	10.94	8.62
	Poor	22, 25, 26, 27, 29, 30, 31, 32, 33, 35, 36, 40, 41, 53, 54	15	0.31	7.50	12.67	0.56	8.71	14.33

3.2.2. Bayesian Discriminant Analysis

In an effort to provide additional confidence on the classification results, BDA was conducted as a validation of the KMC results. The obtained classification results are shown in Figure 3 in a side-by-side comparison with the KMC results. The comparison showed that the KMC classification was fully in agreement with the BDA classification results based on the ASI measurements obtained from the three experimental sites of this research (Figure 3c,d). When classification was conducted using WSR as the indicator of winter hardiness for all the 56 alfalfa cultivars, one cultivar was found to be misclassified by the KMC method (Figure 3a). Manual examination confirmed that cultivar no. 17—which is named Vernal (Table S1) and should be classified as *moderate*—was indeed misclassified by KMC into the class *well*. An agreement was found between KMC and BDA classification results, confirming the high classification accuracy of the KMC method. This accuracy was 98% for classifications based on WSR measurements (Figure 3a,b) and 100% for classifications based on ASI measurements.

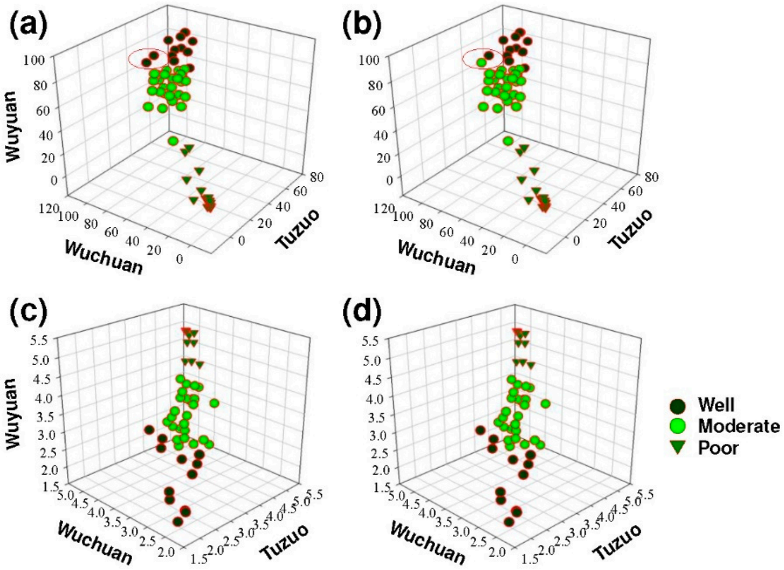

Figure 3. Classification of 56 alfalfa cultivars' winter hardiness performance into three classes using either the K-means clustering (KMC) or the Bayesian discriminant analysis (BDA) method. Winter hardiness is measured in terms of winter survival rate (WSR) or average score index (ASI) based on field experiments in Wuyuan, Wuchuan and Tuzuo sites in northern China. (**a**) Winter hardiness classes in term of WSR based on the KMC method; (**b**) Same as (**a**) but BDA is used as the classification method; (**c**) Winter hardiness classes in terms of ASI based on the KMC method; (**d**) Same as (**c**) but BDA is used as the classification method. The red circle in (**a**) and (**b**) indicates a misclassification involving cultivar no. 17 (unmarked) into the "*Well*" class by KMC.

4. Discussion

Winter hardiness is an inherited plant characteristic as a result of adaptation to the low-temperature environment, which can improve the plant's overwinter survival and regeneration abilities [18,23]. Alfalfa is mainly located at mid- to high latitudes and frigid regions in China [5,24]. Winter hardiness is one of the major characteristics affecting alfalfa growth and productivity [25,26]. The results of this research showed that among the alfalfa cultivars selected for the field experiment, the ASI values of the indigenous cultivars are lower than those of the introduced American cultivars. This was particularly observed in Gongnong-1 (cultivar #1, see Table S1), Caoyuan-3 (#4), Gongnong-5 (#5), Nongmu-801 (#7), Zhaodong (#8) and Zhongcao-3 (#14). Accordingly, the WSR values of the indigenous cultivars are all higher than those of the introduced cultivars, indicating that the wintering performance of these indigenous cultivars are superior to the introduced cultivars, which is consistent with earlier findings of, e.g., Cao, et al. [27]. In production and utilization practice, alfalfa cultivars indigenously found in northern regions are observed to show higher performance in winter hardiness. The cold resistance of alfalfa involves two processes, namely, low-temperature acclimation in autumn and freezing tolerance and adaptation in winter [12,19,23]. During acclimation in autumn, which is induced by a combination of falling temperature and shortening photoperiod, the plant stops the active growing of the stems and leaves, entering physiological dormancy [12]. In alfalfa, the acclimation period leading to complete dormancy begins in late summer or early autumn and is marked by a reduced-rate aboveground biomass accumulation; instead, the photosynthetic products are diverted to the root system [28]. As a result, the roots become stronger and, hence, the plant becomes more cold-tolerant and even freezing-tolerant, as observed in out experiments. Compared to plants that do not undergo low-temperature acclimation, plant species that do undergo low-temperature acclimation gain improvements in winter hardiness. Therefore, low-temperature acclimatization and wintering are two tightly associated characteristics [9,29]. In northern China, the mean temperature during winter is commonly below −10 °C (Table 1) and the lowest temperature in some regions may reach as low as −50 °C. In the end of autumn and early winter, plants can sense the gradual decrease in ambient temperature, and the light cycle induction causes plants to undergo a series of physiological and biochemical changes, thereby increasing the low-temperature resistance during winter [19,30].

There are fewer reports on the use of the ASI in alfalfa [12,19] compared to crop production [24–27,31]. ASI reflects the overall growth status of alfalfa after wintering. Examination of WSR showed that alfalfa cultivars that have the same WSR value differ in branch quantity, plant height, leaf area, biomass, growth, and development speed, etc. ASI is a quantitative indicator that reflects winter hardiness in alfalfa, i.e., the stronger the winter hardiness of alfalfa, the stouter and the greater number of branches. The reference ASIs of those introduced alfalfa cultivars used in this experiment are generally lower than the values obtained from our experimental sites in northern China, indicating that in addition to being related to geographical locations, ASI is also associated with particular climatic conditions, meaning that differences exist in the sensitivity of alfalfa towards temperature, sunlight and precipitation [12,17,20,23]. The most important determinant for alfalfa cultivation in northern China is winter survival, in addition to production conditions [19,23,32]. This paper provides a different perspective from the utilization purpose on introduced cultivars, which are likely planted in relatively more fertile soils with a more favorable management. However, alfalfa cultivars in China are mainly planted in fields of low fertility, such as wasteland under dry conditions, which poses a serious challenge to the survival of alfalfa plants against the vagaries in winter and the nutrient deficiency [5,33]. Usually, indigenous cultivars are more favored in northern China, while introduced cultivars are mostly used in areas to the south. For example, GT13R and Archer II show stronger integrated performance in Kunming [34], Arriba, WL4167 and WL324 in Zhejiang [35], and Shengshi, Fengbao and CW680 in southwest Hunan [36]. In our study, four local cultivars from southern China, namely, Lumu-1 (#43), Liangmu-1 (#41), Yumu-1 (#54) and Huaiyin (#53), were included in the experiments in Inner Mongolia. The results revealed that the ASIs of these four cultivars were measured at 4.98, 3.53, 4.55 and 4.77, respectively, while WSRs were 0.29%, 56.78%, 24.38% and 12.04%, respectively, suggesting that these cultivars are poorly adapted to conditions of the experimental sites and are thus not recommended for cultivation in northern China.

The ASI showed high consistency with WSR in predicting alfalfa's winter hardiness in northern China, indicating that it may be potentially utilized for the evaluation of winter hardiness in alfalfa in wider areas. We further analyzed the prediction accuracy and consistency between ASI and WSR in local versus introduced cultivars. The results of these analyses showed that the overall prediction of ASI and WSR for local cultivars was highly concordant, and the classification result was 100% in agreement between ASI and WSR. Taking together, compared to the introduced cultivars, the indigenous cultivars showed more extensive variability. Overall, winter hardiness can be used as a reliable metric in screening cold-resistant alfalfa cultivars for integration into the regional agroecosystems in the face of climate change [37–41].

The results obtained here have multiple implications to the development of forage industry, enhancement of food security and climate change adaptation and mitigation in China [42]. On the one hand, the successful establishment of alfalfa can provide bulk quantities of high-quality protein forage for animal husbandry, especially for the development of the dairy industry [5,37,40,43–45]. On the other hand, the forage-crop rotation can improve the soil quality, especially in saline-alkaline land, or medium- to low-yielding land areas, which plays a great role in improving soil fertility, consolidating farmland quality, and ensuring food safety and stable production [37,38,43,46–48]. The world is entering an era of low-carbon economy. Alfalfa has multiple functions in line with this trend, such as high carbon fixation capacity [37,49,50], effective facilitation of water infiltration [51,52], and substantially higher water use efficiency [7,15,53]. Moreover, when used in combination with agricultural crops in farming or agropastoral regions, alfalfa has shown benefits in soil function improvement and productivity resilience [7,8,40,49,53]. In a broader spatial context, mosaic landscapes involving alfalfa can provide a range of ecosystem services such as water quality preservation, biodiversity protection, biotic regulation, and stability [3,15,37,43,54–56]. These functions and services are good examples of the win-win options, which are in urgent need in meeting the dual requirements of climate change mitigation and food security enhancement in China and beyond [40,55,57].

5. Conclusions

We conducted field experiments at three Inner Mongolian sites to evaluate the winter hardiness of 50 alfalfa cultivars locally found in China, in comparison with six cultivars introduced from the U.S. We found that, methodologically, ASI is an effective method in evaluating winter hardiness in alfalfa cultivars in northern China. A high degree of agreement between the ASI and the WSR measurements are experimentally confirmed. We also found that satisfactory classification results on alfalfa's overwintering performance can be achieved with the quartile range method, as recursively confirmed by the K-means clustering and the Bayesian discriminant analysis methods. Overall, the indigenous Chinese alfalfa cultivars are more winter hardy than introduced American cultivars. In particular, the cultivars of Gongnong-1 (cultivar #1 in field experiment), Caoyuan-3 (#4), Gongnong-5 (#5), Nongmu-801 (#7), Zhaodong (#8), and Zhongcao-3 (#14) are found highly suitable in northern China, together with a range of local cultivars. These findings provide a solid science base for alfalfa's incorporation into the regional farming or agropastoral systems in simultaneously fighting climate change while strengthening food security.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/atmos12111538/s1, Table S1: List of 50 indigenous Chinese alfalfa cultivars used in the field experiment.

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