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Effects of Sowing Dates and Phosphorus Application on Seed Yield and Yield Components of Alfalfa

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ABSTRACT

Background: Alfalfa (*Medicago sativa* L.) is a globally important cool-season forage grass for semiarid regions and the demand for seeds is increasing with the increase of alfalfa planting area. However, its seed yield is often low or inconsistent.

Methods: A field experiment was conducted to investigate the response of seed yield and yield components of alfalfa to different sowing dates and phosphorus (P) fertilization levels from 2020 to 2022. This research was conducted using a split-plot design in a randomized complete block, with four replications. The main plots were three sowing dates (20 July, 5 and 20 August) and subplots were five different rates (0, 40, 70, 100 and 130 kg P_2O_5 ha^{-1}).

Result: Our results revealed that delaying the sowing dates reduced seed yield, by influencing its yield components, except for the 1000-seed weight, but P fertilization enhanced the seed yield components affected by late sowing and increased seed yield, it is recommended to apply P fertilizer at 77.6~144.8 kg ha^{-1}. Stem density and seeds per pod were the primary factors determining seed yield and they may be useful to consider in developing traits for high-seed-yield alfalfa.

Key words: *Medicago sativa*, Phosphorus, Seed yield, Sowing dates, Yield components.

INTRODUCTION

Alfalfa is the most widely distributed leguminous forage with the largest cultivated area in the world (Shi *et al.*, 2017). Because of its high yield, good quality, many ways of utilization, good palatability and high economic value, alfalfa is the first-class forage for all kinds of livestock, no matter whether green feeding, grazing, or hay (Nian *et al.*, 2021). In recent years, the demand for seeds has increased with the increase of alfalfa planting area year by year. High-quality certified seeds with high levels of genetic and physical purity and vital quality are essential for stands to succeed in all field conditions (Collins, 2003). Thus, forage production will benefit from the specialization of seed production, which aims to produce high-quality seeds consistently and reliably in large quantities (Lannuccia *et al.*, 2002). However, there is little research on alfalfa seed production in China, the production technology is relatively backward and it is difficult to produce and harvest seeds (Zhang *et al.*, 2016).

Seed yield is a complex trait, which is the product of several individual yield components, such as the number of raceme m^{-2}, number of pods raceme^{-1}, number of seeds pod^{-1} and 1000-seed weight (Gholamreza *et al.*, 2010). Seed yield is one of the most complex traits with a generally low heritability, highly affected by agricultural practices as well as environmental factors (Walter and Klliker, 2012; Zhang *et al.*, 2019). Among the yield components, usually, the number of racemes per unit and seed weight undergo the highest and lowest effect of environmental conditions (Askarian *et al.*, 1995; Ozlem and Hakan, 2007). This shows that the seed production level of alfalfa mainly depends on cultivation and management techniques. Thus, the maximum potential of seed production can only be realized if attention is paid to the selection of a favorable environment and the application of appropriate management practices (Lannuccia *et al.*, 2002).

The sowing dates for alfalfa seed production can be sowed in spring or autumn, generally when the ground temperature of 5 cm is above 15°C. However, during the spring from April to May, the temperature is unstable and the climate is very dry. The alfalfa seeds sown in the spring cannot germinate or die soon after germination due to lack of water. Farmers can consider planting and harvesting a short-season cereal crop before sowing alfalfa in the autumn. This approach can not only conserve land and boost economic benefits but also enable alfalfa sowing

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during the rainy seasons of summer and autumn, leading to better seedling emergence. However, alfalfa must undergo a specific growth period before winter to accumulate nutrients and develop roots with sufficient resistance. If the sowing date is not chosen appropriately, it will adversely affect the emergence and growth of alfalfa seeds, making it challenging to overwinter (Wang *et al*., 2008). Therefore, choosing the right autumn sowing dates can improve the overwintering rate of alfalfa plants, promote the greening of alfalfa in the following year and lay a solid foundation for the rapid growth of alfalfa (Wang *et al*., 2015; Zhang *et al*., 2015).

Proper fertilization is one of the key factors that influence the seed yield. Due to its function in root development, establishment, blooming, assimilate partitioning and speeding seed physiological maturation, adequate P nutrition is crucial for high seed output (Wang *et al*., 2017). Loeppky *et al.* (1999) reported that the seed yields of smooth bromegrass (*Bromus inermis* L.), crested wheatgrass (*Agropyron cristatum* L.) and timothy (*Phleum pratense* L.) would be increased at the application of 18 kg P ha^{-1}. Zhu (2005) reported that P fertilizer could improve the growth and development of alfalfa, enhance the ability to disease resistance, promote root development and increase seed yield. Although the application of P fertilizer can increase the seed yield, prolong the life span and enhance the overwintering ability of alfalfa, from an economic point of view, the application of P fertilizer should be determined according to the level of soil P fertilizer. When the content of available P in soil is less than 12.47 mg kg^{-1}, fertilizer should be applied to ensure normal plant growth and increase seed yield, otherwise, there is no need to apply fertilizer (Boschetti *et al*., 2000). Sanderson and Jones (1993) showed that for the alfalfa seed production field, the effect of P fertilizer applied once before sowing or as topdressing every year was similar and the best P_2O_5 application rate was 240 kg ha^{-1} or 80 kg ha^{-1} year^{-1}. When P fertilizer was applied to the alfalfa seed field, the effect of ditch strip application was better than that of surface application, which was mainly due to the large row spacing of the alfalfa seed field and part of the fertilizer could not be absorbed by plants (Malhi *et al*., 2001). In addition, ditching strip application could promote the growth of seedlings, increase the dry matter weight of seedlings by 50% and increase seed yield by 15% in the second year (Malhi *et al*., 2001; Purushotham and Umesh, 2011). Therefore, based on determining the suitable sowing rate and irrigation amount, screening the appropriate fertilization method and fertilizer application rate is the key to obtaining high-quality and high-yield alfalfa seeds.

The central region of Inner Mongolia has a mid-temperate continental monsoon climate, characterized by significant climate variations throughout the four seasons. The summer is short and extremely hot, with autumn usually beginning in mid-August. The temperature fluctuations in autumn are significant. The timing of autumn sowing for alfalfa is crucial. There are few studies on alfalfa seed production technology combined with sowing date and fertilization in Inner Mongolia. The purpose of this study: (I) to clarify the effects of the sowing dates and P fertilizer on seed yield and yield components of alfalfa and then to obtain the best sowing date and P application rate for high seed yield; (II) to determine which are the main seed components of alfalfa for seed production.

MATERIALS AND METHODS

This experiment was carried out for 3 years from 2020, at the Agro-pastoral Experiment Station of Grassland Research Institute of the Chinese Academy of Agricultural Sciences, Hohhot (40°34′N; 111°45′E; 1050 m.a.s.l). The site has a semiarid continental climate and the local environment is marked by drought, cold and wind-blown sand. The mean annual air temperature is 5.6°C and the annual precipitation is about 400 mm, primarily concentrated in June, July and August. Soil samples were taken to a 30 cm depth before planting and were analyzed by a soil nutrient analyzer (Shandong Yuntang Intelligent Technology Co., Ltd., Weifang, China). The soil has the following characteristics: 6.0% organic matter, 1.1 g kg^{-1} total nitrogen, 69.45 mg kg^{-1} available nitrogen, 20.5 mg kg^{-1} available P, 93.0 mg kg^{-1} available K, with a soil pH of 8.5 (Wang *et al*., 2022). The alfalfa variety used in the experiment was Zhongcao NO.3, bred by the Grassland Research Institute of the Chinese Academy of Agricultural Sciences. The treatment design was a factorial combination of sowing dates as main plots and P fertilization as subplots in a split-plot design with four replications. The sowing dates were 20 July, 5 and 20 August (named T1, T2 and T3), respectively. The plot area was 3 × 5 m, the sowing rate was 5.0 kg ha^{-1}, with 60 cm rows and 1~2 cm depth. A 1 m width of Zhongcao NO.3 was sown around the experimental site as border rows. P fertilizer $(NH_4)_2HPO_4$ produced by Henan Jixin Chemical Products Co., Ltd., Zhengzhou, China) was applied at four different rates [40, 70, 100 and 130 kg P_2O_5 ha^{-1} (named P1, P2, P3 and P4)], the control (CK) treatment received no P fertilizer. All P fertilizers were applied in a single application. Irrigation was conducted twice using a sprinkler system in the establishment year and again before flowering in 2021 and 2022. No diseases or pests were observed during the experiment. Weeds were controlled by mowing or hoeing in the year of establishment and in the spring of 2021 and 2022. There were no harvests in the establishment year (Wang *et al*., 2022).

Alfalfa was harvested for seed yield at maturity in early September 2021 and 2022. Seed yield and its components were determined on 8 meters^2 randomly collected from the center of each sub-plot to prevent border effects at seed harvest. The seed yield components considered were: stem density (stems m^{-2}), racemes stem^{-1} (no.), pods raceme^{-1} (no.), seeds pod^{-1} (no.) and 1000-seed weight (g) (Lannuccia *et al*., 2002). The weight (g) of 1000 seeds was calculated by weighing 1000 dried seeds drawn arbitrarily from the seed yield of every treatment using an electronic balance (Al-Kahtani *et al*., 2017). The data were analyzed by the method of analysis of variance as described by (Gomez and Gomez, 1984). A generalized linear mixed model (GLM) was used for treatment comparisons, including sowing dates and P application rates. To determine the correlation between seed yield and seed yield components, the Pearson method and regression equivalence two by two and step by step by MINIT AB were applied.

RESULTS AND DISCUSSION

Main effects of sowing dates and P fertilizer

The sowing dates, P fertilizer and their interaction effects on the seed yield and yield components are shown in Table 1. Both the sowing dates and P fertilizer had significant effects on seed yield and all yield components, except for 1000-seed weight in two years. The interaction between sowing dates and P fertilizer had significant effects on stems m^{-2}, 1000-seed weight and racemes $stem^{-1}$ in 2021. However, their interaction had significant effects only on racemes $stem^{-1}$ and 1000-seed weight in 2022. Therefore, in the subsequent result analysis, we mainly discussed the influence of the main factors such as sowing time and phosphorus fertilizer.

Sowing dates and P fertilizer on seed yield of alfalfa

Both sowing dates and P fertilizer had significant effects on seed yield in two years (Fig 1). Seed yield decreased significantly with the postponement of the sowing dates (Fig 1a). The seed yield of the last two sowing dates decreased by 14.22% and 31.3% compared to the first sowing date in 2021 (P<0.05) and decreased by 10.2% and 26.6% in 2022 (P<0.05). The seed yield increased at first and then decreased with the increase of the P application rate in two years (Fig 1b). When P applied at 100 kg ha^{-1}, the seed yields were the highest, which were 418.7 kg ha^{-1} and 518.3 kg ha^{-1}, respectively, increasing by 23.2% and 28.4% compared to the controls (P<0.05).

There were significant linear regression relationships between seed yield and P application rate on each sowing date in two years (Fig 2). In 2021, the regression equations for each sowing date were: $y=407+1.260x-0.006x^2$ (R^2=0.505, P>0.05), $y=353+0.932x-0.004x^2$ (R^2=0.840, P>0.05) and $y=246+1.936x-0.020x^2$ (R^2=0.931, P<0.05), the suitable amount of P fertilizer were raging form 96.8 kg ha^{-1} to 116.5 kg ha^{-1} for the highest yield. In 2022, the regression equations for each sowing date were: $y=479+1.707x-0.011x^2$ (R^2=0.005, P>0.05), $y=404+1.448x-0.005x^2$ (R^2=0.987, P<0.01) and $y=299+2.512x-0.013x^2$ (R^2=0.416, P>0.05), the suitable amount of P fertilizer were raging form 77.6 kg ha^{-1} to 144.8 kg ha^{-1} for the highest yield.

Sowing dates and P fertilizer on yield components of alfalfa

As the sowing date was delayed, the seed yield components of alfalfa showed the same trend of change

Table 1: Analysis of variance for seed yield, stems m^{-2} (no.), racemes $stem^{-1}$ (no.), pods $raceme^{-1}$ (no.), seeds pod^{-1} (no.) and 1000-seed weight (g) for sowing dates (T) and P treatment (P) in two years.

Source	df+	2021						2022					
		Seed yield	Stem m^{-2}	Racemes $stem^{-1}$	Pods $raceme^{-1}$	Seeds pod^{-1}	1000-seed weight	Seed yield	Stem m^{-2}	Racemes $stem^{-1}$	Pods $raceme^{-1}$	Seeds pod^{-1}	1000-seed weight
T	2	***	***	***	*	***	ns	***	***	**	***	***	ns
P	4	***	***	***	***	***	***	***	***	***	***	***	***
T*P	8	ns	**	*	ns	ns	**	ns	ns	**	ns	ns	*

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

df+ Degrees of freedom.

ns: Not significant at the 0.05 probability level.

within two years (Table 2). The stems m^{-2}, racemes $stem^{-1}$, pods $raceme^{-1}$ and seeds pod^{-1} all significantly decreased with the delay of sowing time (P<0.05), while the 1000-seed weight showed no significant change with the delay of sowing time (P>0.05).

The seed yield components of alfalfa showed a significant trend of change with the increase of P fertilizer in two years (Table 3). In 2021, stems m^{-2}, racemes $stem^{-1}$, pods $raceme^{-1}$ and seeds pod^{-1} all increased first and then decreased with the increase of P application. When P was applied at 100 kg ha^{-1}, the stems m^{-2}, racemes $stem^{-1}$ and pods $raceme^{-1}$ were highest, which were 287.83, 24.15 and 12.48, respectively, increasing by 56.1%, 15.8% and 30.5% compared to the control (P<0.05). When P applied at 70 kg ha^{-1}, the seeds pod^{-1} was the highest, which was 5.51, increasing by 23.3% to the control (P<0.05). However, the 1000-seed weight significantly increased with the increase of P application (P<0.05), up to 2.13 g. In 2022, the stems m^{-2}, racemes $stem^{-1}$ and pods $raceme^{-1}$ all increased first and then decreased with the increase of P application. When P was applied at 100 kg ha^{-1}, the stems m^{-2}, racemes $stem^{-1}$ and pods $raceme^{-1}$ were highest, which were 346.92, 27.53 and 15.03, respectively, increasing by 64.2%, 25.0% and 28.7% compared to the control (P<0.05). However, the seeds pod^{-1} and 1000-seed weight significantly increased with the increase of P application (P<0.05), up to 5.80 seeds pod^{-1} and 2.18 g, respectively.

Relationship between seed yield and yield components

To fully determine the contribution of each yield component to the seed yield of alfalfa, a multiple linear regression analysis was performed. As shown in Table 4, the stems m^{-2} and seeds pod^{-1} had extremely significant or significant impacts on seed yield, while racemes $stem^{-1}$, pods $racemes^{-1}$ and 1000-seed weight did not have significant influences on seed yield in two years (P>0.05). The regression equations for two years are as follows: Y=0.487 *stems m^{-2} +37.172*seeds pod^{-1} and Y=0.715*stems m^{-2} +44.140* seeds pod^{-1}. So, the stems m^{-2} and seeds pod^{-1} made more contributions to the seed yield.

Influence of sowing dates on seed yield and yield components

For seed production of forage legumes especially alfalfa, one of the basic requirements is to have a support vegetative growth. With the delay of the sowing dates, the growth of vegetative organs of alfalfa was affected, in which the root system was the main organ of alfalfa overwintering, the earlier the sowing dates, the thicker the root system, the more nutrients it stores, which is more conducive to alfalfa overwintering (Tian *et al.*, 2020). The higher root reserves can increase the seed yield, by improving the stem density, pods per raceme and seeds per pod (Ahmad *et al.*, 2020). Kumar *et al.* (2017) reported that higher seed yield of alfalfa could be obtained at suitable sowing date and cutting times in India. El-Hifny *et al.* (2019) reported that the sowing

Table 2: Effects of sowing dates on seed yield components of alfalfa.

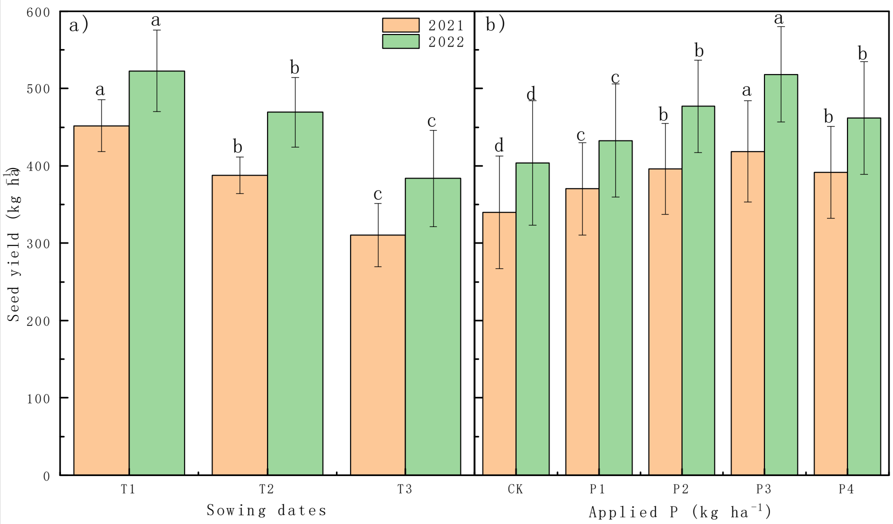
Sowing dates	2021					2022				
	Stems m^{-2}	Racemes $stem^{-1}$	Pods $raceme^{-1}$	Seeds pod^{-1}	1000-seed weight	Stems m^{-2}	Racemes $stem^{-1}$	Pods $raceme^{-1}$	Seeds pod^{-1}	1000-seed weight
T1	290.80±61.07a	24.49±2.61a	11.85±2.11a	5.67±0.61a	2.01±0.17a	335.82±74.47a	25.46±4.20a	14.45±1.49a	5.76±0.43a	2.12±0.08a
T2	220.05±34.39b	21.54±2.24b	11.42±1.91a	5.01±0.49b	2.02±0.14a	268.75±44.90b	25.55±2.41a	13.43±2.32b	5.68±0.43a	2.15±0.08a
T3	202.85±35.85c	20.62±1.16b	10.49±1.00b	4.53±0.49c	2.01±0.18a	245.21±47.06c	23.28±2.16b	12.43±1.26c	5.36±0.22b	2.15±0.11a

Table 3: P fertilizer on seed yield components of alfalfa.

Applied P	2021					2022				
	Stems m^{-2}	Racemes $stem^{-1}$	Pods $raceme^{-1}$	Seeds pod^{-1}	1000-seed weight	Stems m^{-2}	Racemes $stem^{-1}$	Pods $raceme^{-1}$	Seeds pod^{-1}	1000-seed weight
CK	184.33±28.60d	20.85±1.64c	9.56±1.33d	4.47±0.63c	1.76±0.07c	211.25±26.52d	22.03±1.39c	11.68±1.38d	5.22±0.35b	2.05±0.09b
P1	212.42±42.81c	21.82±2.19bc	10.86±1.72c	4.85±0.51b	1.96±0.09b	253.27±50.58c	23.98±2.34b	13.56±1.65bc	5.54±0.28a	2.13±0.06a
P2	260.17±60.91b	22.75±2.37b	11.10±1.32bc	5.51±0.63a	2.13±0.09a	301.87±65.43b	25.00±3.48b	12.72±1.54 c	5.66±0.39a	2.16±0.05a
P3	287.83±65.43a	24.15±3.62a	12.48±1.79a	5.43±0.61b	2.12±0.03a	346.92±58.17a	27.53±3.20a	15.03±1.74a	5.78±0.38a	2.18±0.07a
P4	244.75±28.52b	21.52±2.09bc	12.27±1.33ab	5.08±0.66b	2.10±0.09a	303.00±46.22b	25.26±2.71b	14.20±1.47ab	5.80±0.38a	2.15±0.10a

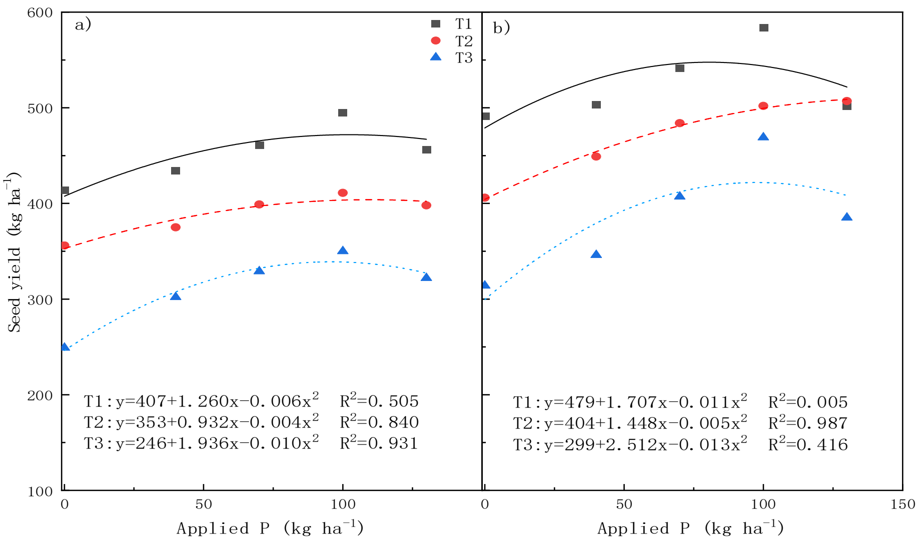
Table 4: The correlation between seed yield and yield components of alfalfa.

Item	2021				2022			
	Coefficient	Standard err	*P* value	VIF	Coefficient	Standard err	*P* value	VIF
Intercept	87.925	75.211	0.248		97.223	183.960	0.599	
Stems m^{-2}	0.487	0.150	0.002	3.434	0.715	0.145	0.000	2.118
Racemes $stem^{-1}$	2.482	2.670	0.357	2.207	1.077	2.616	0.682	1.521
Pods $raceme^{-1}$	5.305	3.311	0.115	1.577	0.145	4.896	0.977	1.902
Seeds pod^{-1}	37.172	10.799	0.001	2.548	44.140	20.161	0.033	1.454
1000-seed weight	-67.479	41.006	0.106	1.570	-60.134	88.448	0.499	1.090



(a): Effects of sowing dates on seed yield of alfalfa; (b): Effects of P fertilizer on seed yield of alfalfa.

Fig 1: Effects of sowing dates and P fertilizer on seed yield of alfalfa.



Each data point represents the average seed yield. Each figure includes data (n=60) from five P fertilizer treatments. T1, T2 and T3 mean different sowing dates (31 July, 10 and 20 August).

Fig 2: Relationships between seed yield and P fertilization rates in two years.

dates, genotypes and their interaction had a significant effect on seed yield and its components, except for 1000-seed weight, which was insignificant. In this experiment, with the delay of the sowing dates, the seed yield and yield components of alfalfa in the following years were significantly reduced, except for the 1000-seed weight. The stems of regrowth alfalfa come from the root crown buds, so the root crown buds are also a direct determinant of the number of stems and a potential factor affecting seed yield. Delaying the sowing of alfalfa will lead to small and thin root crowns before winter, it will reduce the number of stems in the following year and then affect the seed yield.

Influence of P fertilizer on seed yield and yield components

P fertilizer is the key factor affecting seed yield and it has an important effect on the dynamic changes of plant stems, inflorescence number, dry matter accumulation, seed yield and so on (Liu *et al*., 2020; Loeppky *et al*., 1999). Liu *et al.* (2013) reported that under alkaline soil conditions in arid areas when the soil P content reaches a moderate level, a P fertilizer dosage of 150 kg ha^{-2} can promote the increase of alfalfa seed yield. Buglass (1964) reported that there was no significant role for P in increasing forage seed production in southern Saskatchewan. This change reflected the variability of soil P status and the annual (weather) effects were as great as expected. In this experiment, when P fertilizer was applied at 100 kg ha^{-1}, the seed yield increased by 23.2% and 28.4% compared to the control, respectively, in each year. Meanwhile, the stems m^{-2}, racemes $stem^{-1}$ and pods $raceme^{-1}$ were also significantly greater than the controls. Which indicated that P fertilizer can increase the seed yield of alfalfa, but the effect is related to the selected alfalfa varieties, climatic conditions, soil conditions and P fertilizer application (Al-Kahtani *et al*., 2017; Liu *et al*., 2013).

Importance of seed yield components to seed yield

Although all yield components had a direct influence on seed yield, the contribution degree of each component is different. Yu *et al*. (2021) use the grey correlation degree method to find that under the treatment of P application, the correlation between the stem density and seed yield of alfalfa was the highest. El-Hifny *et al*. (2019) employed path-coefficient analysis to ascertain the direct and indirect effects of pods per plant. In our study, we used multiple linear regression analysis and discovered that both stem density and seeds per pod had significant effects on seed yield. This is consistent with the findings of Khrbeet *et al*. (2016), who reported that seed yield was highly positively correlated with seeds per pod, pods per raceme and racemes per stem. There are great genetic variations in seed yield and its components among and within populations of alfalfa (Campbell and He, 1997) and the response of seed yield components to plant genetics and management techniques is also different (El-Hifny *et al*., 2019; Sengul and Suleyman, 2006). But we can use high stems m^{-2} and seeds pod^{-1} as selection targets for breeding high-seed yield alfalfa.

CONCLUSION

In the center of Inner Mongolia, delaying the sowing dates of alfalfa would reduce seed yield, by influencing its yield components, except for the 1000-seed weight, but P fertilization can enhance the seed yield components affected by late sowing and increase seed yield, it is recommended to apply P fertilizer at 77.6~144.8 kg ha^{-1}. Through multiple regression analysis, it was found that stem density and seeds per pod were the primary factors determining seed yield and they may be useful to consider in developing traits for high-seed-yield alfalfa.

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Conflict of interest

The authors declare no conflict of interest.

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