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Effects of Different Cultivation Practices on Alfalfa Seed Yield

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ABSTRACT

Background: Low and unstable seed yield remains a major constraint on alfalfa (*Medicago sativa* L.) seed production in China, increasing production costs and limiting domestic seed supply.

Methods: A four-factor, three-level L9(3^4) orthogonal array was used over two consecutive growing seasons to evaluate cultivar, cutting time, compound fertilizer rate and early-spring mulching time. The established field was divided into three adjacent, equal-area cultivar zones. Within each cultivar zone, the three L9 treatment combinations corresponding to that cultivar were randomized in three blocks, giving three replicate plots per treatment combination and 27 plots in total. The same established plots received the same assigned treatment combinations in 2024 and 2025 and were therefore followed repeatedly across years. The orthogonal array was used to estimate main effects only; interactions were not independently estimable. Within each year, factor-level differences were assessed separately by one-way ANOVA using replicate plot-level observations; the two years were analyzed separately. Seed yield, yield components, agronomic traits and treatment-level economic returns were evaluated in three alfalfa cultivars.

Result: Among the nine tested treatment combinations, Treatment 2 (Zhongmu No. 10, Jingzhe mulching, branching-stage cutting and 450 kg ha^{-1} compound fertilizer) produced the highest observed seed yield in both years, reaching 549.7 kg ha^{-1} in 2024 and 529.6 kg ha^{-1} in 2025. Range analysis showed that fertilizer rate had the largest spread among factor-level means in both years, followed by cutting time and mulching time; this descriptive ranking does not quantify each factor's contribution to total variation. Treatment 2 also gave the highest observed partial-budget return, with RMB 18,405.8 ha^{-1} in 2024 and RMB 17,635.4 ha^{-1} in 2025. The theoretical 2025 combination A3B2C2D2 identified by range analysis was not included in the L9 array and therefore was not field-tested. Overall, Treatment 2 was the highest-yielding and highest-return observed combination across the two years.

Key words: Alfalfa, Cutting time, Fertilizer rate, Mulching, Partial budget, Seed yield.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a perennial legume forage crop valued for its high nutritive value and palatability. Demand for alfalfa in China has continued to rise with the development of the livestock industry, but domestic forage production still falls short of demand. In 2022, China produced approximately 1.8×10^7 t of alfalfa hay, leaving an estimated deficit of 1.2×10^7 t that was partly met through imports from countries such as the United States and Canada (Li, 2024).

Improving seed production is important for China's forage industry, yet domestic alfalfa seed yield and quality remain insufficient for market demand (Su, 1990; Wang *et al.*, 2021). Cultivar selection, fertilization, mulching and cutting can influence seed yield (Huang *et al.*, 2022; Guo *et al.*, 2023; Li *et al.*, 2022). Inner Mongolia provides favorable light and heat resources for alfalfa seed production, while studies have also shown that sowing and cutting management affect alfalfa seed yield and quality (Kumar *et al.*, 2017; Zhao *et al.*, 2020).

Although alfalfa seed-production areas have expanded in Inner Mongolia, Ningxia, Gansu and Xinjiang, commercial seed yield remains unstable and locally adapted integrated management systems are limited (Wang and Han, 2004; Wang *et al.*, 2021). Unlike forage production, seed production depends on flowering, pollination, seed set and filling. Previous studies have mostly examined individual practices, leaving the combined responses to cultivar, cutting time, fertilizer rate and mulching time insufficiently quantified.

Stable seed production requires coordinated water, nutrient, canopy and pollination management (Wang *et al.*, 2013; Wang *et al.*, 2021). Pollination is particularly important for seed set and honey-bee supplementation and other pollinators can affect alfalfa seed yield (Ejaz *et al.*, 2025). Fertilization also influences reproductive growth (Wang *et al.*, 2013; Xiao *et al.*, 2024); phosphorus can increase seed yield under low soil P, whereas excessive fertilizer may reduce input efficiency (Loeppky *et al.*, 1999). Potassium and boron can support physiological

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performance and seed production (Du *et al.*, 2006; Krishna *et al.*, 2013). Irrigation and fertilization can jointly affect seed-yield components (Hui *et al.*, 2025) and a recent study reported effects of irrigation interval and harvest date on alfalfa seed yield (Bahia, 2025).

Cutting alters regrowth, canopy structure and flowering time (Leach, 1968), with reports ranging from increased seed yield after early cutting to reduced thousand-seed weight and yield (Abu-Shakra *et al.*, 1969; Karagic *et al.*, 2007; Wang *et al.*, 2005). Plastic-film mulching can conserve soil moisture and temperature (Meng, 2016); in Ningxia it increased alfalfa seed yield by 64.1% over no mulching (Li *et al.*, 2022), while ridge-furrow mulching improved soil conditions in the semiarid Loess Plateau (Meng *et al.*, 2021). Cultivar performance also varies with environment (Yu, 2008; Zhou, 2007; Mohammed *et al.*, 2023). Therefore, an L9(3^4) orthogonal array was used to compare four factors at three levels with fewer plots than a full 3t factorial. The design estimates main effects; interactions were not independently estimated.

MATERIALS AND METHODS

Trial site overview

The experiment was conducted in the experimental field of the Grassland Research Institute, Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences, located in Tumed Left Banner, Hohhot, Inner Mongolia Autonomous Region (40°06′ N, 111°01′ E; 1,040 m a.s.l.). The site has a temperate continental climate. Northwesterly winds are common in autumn and winter, northerly winds dominate in spring and easterly winds occur more frequently in summer, although wind speed is generally low. In 2024, annual precipitation was 541.3 mm and the mean annual temperature was 4.9°C. In 2025, rainfall was concentrated mainly in July and August; precipitation during these two months reached 404.7 mm, 215.2 mm higher than during the same period in 2024.

The field was equipped for irrigation. Seed-production stands of Zhongmu No. 10, Caoyuan No. 3 and WL319 were established in 2021 by hill seeding at 50-cm row spacing, 30 cm plant spacing and 2-cm sowing depth. During stand establishment and maintenance, irrigation, gap filling, thinning and weeding were carried out as needed. Before the experiment, soil pH was 8.3; organic matter was 15 g kg^{-1} and available nitrogen, available phosphorus and available potassium were 85.26, 13.34 and 151.33 mg kg^{-1}, respectively.

Experimental materials

The tested cultivars were Caoyuan No. 3, Zhongmu No. 10 and WL319. Seed lots were supplied by Inner Mongolia Agricultural University, the Beijing Institute of Animal Science of the Chinese Academy of Agricultural Sciences and Beijing Zhengdao Seed Co., Ltd., respectively. Field observations were made in established alfalfa seed-production stands that were 4-5 yr old during 2024-2025.

Experimental design

A four-factor, three-level L9(3^4) orthogonal design was conducted from April 2024 to September 2025, covering two consecutive growing seasons. After border rows were excluded, the established field was divided along its upper, middle and lower positions into three adjacent areas of equal size, which were occupied by Zhongmu No. 10, Caoyuan No. 3 and WL319, respectively. Within each cultivar area, the three orthogonal treatment combinations assigned to that cultivar in Table 1 were arranged in a randomized complete block design with three blocks. Each block contained one plot of each of the three treatment combinations for that cultivar, giving nine plots per cultivar area and 27 plots in total. Each treatment combination therefore had three replicate plots. Each plot was 20 m^2 (4 m × 5 m) and the plot was the experimental unit. The same 27 established perennial plots received the same treatment combination in both 2024 and 2025, so each plot was observed repeatedly across years; because the treatments were reapplied to perennial stands, possible carry-over effects between years cannot be excluded. Randomization was constrained by the pre-existing cultivar areas rather than by fully interspersing cultivars across the entire field. Accordingly, cultivar comparisons should be interpreted cautiously because cultivar and field position may be partly confounded. The orthogonal array was used to estimate the main effects of four factors; interactions among factors were not independently estimable and were therefore not interpreted. Factor A was cultivar: A1 = Zhongmu No. 10, A2 = Caoyuan No. 3 and A3 = WL319. Factor B was cutting time: B1 = no cutting, B2 = cutting at the branching stage and B3 = cutting at the budding stage. Factor C was NPK compound fertilizer rate: C1 = 300 kg ha^{-1}, C2 = 450 kg ha^{-1} and C3 = 600 kg ha^{-1}. Factor D was early-spring mulching time: D1 = no mulching, D2 = mulching at Jingzhe and D3 = mulching at the Spring Equinox.

For the cutting treatments, B2 plots were cut at the branching stage on 16 May and B3 plots at the budding stage on 4 July, with a 5-cm stubble height. B1 plots were not cut. Fertilizer was applied immediately after cutting in B2 and B3 plots, whereas in the B1 no-cutting treatment it

Table 1: Orthogonal test design.

Treatment	Factor			
	Cultivar	Cutting time	Fertilizer application rate	Mulching time
Trt.1	A1	B1	C1	D1
Trt.2	A1	B2	C2	D2
Trt.3	A1	B3	C3	D3
Trt.4	A2	B1	C2	D3
Trt.5	A2	B2	C3	D1
Trt.6	A2	B3	C1	D2
Trt.7	A3	B1	C3	D2
Trt.8	A3	B2	C1	D3
Trt.9	A3	B3	C2	D1

was applied at the branching stage. The compound fertilizer used was Lanlin 315, with total nutrients ($N + P_2O_5 + K_2O$) of at least 45% and an N:P:K ratio of 11:19:15. Fertilizer was placed in inter-row trenches, then covered with soil and compacted. The mulching treatment used transparent plastic film 0.012 mm thick and 2 m wide, applied by flat-field mulching at the assigned spring date. In both 2024 and 2025, Jingzhe and the Spring Equinox fell on 5 March and 20 March, respectively. Apart from the assigned treatment differences, all plots received the same field management. Irrigation was applied four times during each growing season, at regreening, budding, full flowering and pod setting, with approximately 375 m^3 ha^{-1} at each irrigation. Weed control was conducted approximately once per month. No major pest or disease outbreaks occurred during the experiment. Pollination relied on naturally occurring pollinators; no managed pollinator supplementation was used.

Measurement indices and methods

Plant height (cm): At seed maturity, 10 plants were selected from each plot and measured from the soil surface to the plant top with a measuring tape.

Branch number per plant: At full flowering, 10 representative plants were selected from each plot and the number of primary branches was counted.

Stem diameter (cm): At full flowering, 30 stems were randomly selected from each plot. Stem diameter was measured at the middle of the second aboveground internode using a vernier caliper and converted from millimetres to centimetres before statistical analysis.

Inflorescences per branch: At full flowering, 30 secondary branches were randomly selected from each plot and inflorescences on each branch were counted. These branches were then marked for subsequent observations.

Florets per inflorescence: At full flowering, florets were counted at the same position on the 30 marked secondary branches.

Pods per inflorescence: At the pod-setting stage, pods were counted at the same position on the 30 marked secondary branches.

Seeds per pod: At seed maturity, seeds per pod were counted on the 30 marked secondary branches.

Thousand-seed weight: Mature seeds were harvested, threshed, cleaned and air-dried. Three subsamples of 1,000 seeds were counted and weighed.

Seed yield: At seed maturity, three 1 m^2 quadrats were randomly selected from each plot, avoiding border areas and plants were cut at a stubble height of 3-5 cm. After natural air-drying, seeds were threshed, cleaned and weighed.

Fresh forage yield (kg m^{-2}): Three 1 m × 1 m quadrats were randomly selected from each plot, avoiding border effects. All forage within each quadrat was cut at a stubble height of 3-5 cm, weeds were removed and fresh weight was recorded and expressed per square metre.

Agronomic hay yield (kg m^{-2}): The forage collected for the fresh-forage-yield measurement was oven-dried to constant weight and weighed and dry-matter yield was expressed per square metre. This agronomic hay-yield variable (Table 7-8) was distinguished from the treatment cutting hay yield used in the partial-budget analysis (Table 9-10), which represented hay recovered from the management cutting operation and was expressed as kg ha^{-1}.

Partial-budget economic analysis

The economic analysis was conducted as a treatment-level partial-budget comparison using the following prices and treatment-specific input costs: alfalfa seed, RMB 34/kg; compound fertilizer, RMB 2,820/t; plastic-film mulching, RMB 650 ha^{-1}; cutting, RMB 760 ha^{-1} and hay, RMB 1,000/t. Seed and hay prices were based on local market prices in Tumed Left Banner/Hohhot during 2024-2025 and the same fixed prices were applied consistently to all treatments. Common production costs that were the same across treatments were not included in the comparison. No formal sensitivity analysis was performed; therefore, the economic results should be interpreted as a comparison under the stated fixed local market prices.

Gross treatment return (RMB ha^{-1}) was calculated as:

Seed yield (kg ha^{-1}) × Seed price (RMB/kg) + Treatment cutting hay yield (kg ha^{-1}) ÷ 1000 × Hay price (RMB/t)

Partial-budget return (RMB ha^{-1}) was calculated as gross treatment return - treatment-specific cost. Treatment-specific cost (RMB ha^{-1}) included fertilizer, mulching and cutting costs. For B1 (no-cutting) treatments, cutting hay yield and cutting income were zero.

Statistical analysis

All data were initially organised using Microsoft Office Excel 2019 and statistical analyses were conducted using SPSS 27.0. The plot was treated as the experimental unit. The orthogonal array was used for estimation of factor main effects only. For each year separately, each response variable was analysed by one-way analysis of variance (ANOVA) for cultivar, cutting time, fertilizer rate and mulching time using replicate plot-level observations. In each annual one-way model, the factor under evaluation was treated as a fixed effect and residual variation among replicate plot observations was used as the error term. The randomized blocks were used for field layout and replication but were not entered as an additional term in the one-way ANOVA. Because the 2024 and 2025 datasets were analysed separately, year was not tested as a main effect and year × factor interactions were not estimated. When the ANOVA indicated a significant factor effect, Duncan's multiple range test was used for pairwise comparison of factor-level means and lowercase grouping letters were assigned at $P<0.05$. Pearson correlation coefficients between seed yield and seed-yield components were calculated separately for each year using the 27 plot-level observations

(n = 27 per year). Correlation significance was evaluated at P<0.05, P<0.01 and P<0.001. Graphs were produced using Origin Pro 2021.

RESULTS AND DISCUSSION

Analysis of results at different levels of each treatment

As shown in Table 2, in 2024, range analysis ranked the spread among factor-level means as C (fertilizer rate) > B (cutting time) > D (mulching time) > A (cultivar). The preferred levels were A1, B2, C2 and D2, giving the theoretical combination A1B2C2D2. This combination-Zhongmu No. 10, cutting at the branching stage, 450 kg ha^{-1} fertilizer and Jingzhe mulching-was included as Treatment 2 and recorded the highest observed seed yield in 2024 (549.7 kg ha^{-1}). The R values are descriptive measures of the spread among factor-level means and do not represent percentage contributions to total variance.

As shown in Table 3, the same ordering of R values was obtained in 2025: C > B > D > A. The preferred levels were A3, B2, C2 and D2, giving the theoretical combination A3B2C2D2. Because this combination was not included among the nine L9 treatments, it was not field-tested and no observed yield can be assigned to it. Among the observed treatments, Treatment 2 (A1B2C2D2) again produced the highest seed yield, 529.6 kg ha^{-1}.

Across the two years, the ordering of the descriptive R values was consistent (C > B > D > A). The preferred levels from the range analysis were also consistent for B, C and D: branching-stage cutting, 450 kg ha^{-1} fertilizer and Jingzhe mulching. In contrast, the preferred cultivar differed between years. Because range analysis describes differences among factor-level means, this ordering should not be interpreted as a formal variance-contribution ranking. Treatment 2 had the highest two-year mean seed yield (539.7 kg ha^{-1}), followed by Treatment 4 (517.2 kg ha^{-1}) and Treatment 9 (487.5 kg ha^{-1}), whereas Treatment 3 had the lowest mean yield (415.6 kg ha^{-1}). Thus, among the nine combinations that were actually tested, Treatment 2 was the highest-yielding observed combination across the two years. These observations do not constitute a formal stability analysis.

Seed yield analysis

The factor-level means in Table 4 showed the largest numerical separation among fertilizer levels. Seed yield was numerically highest at 450 kg ha^{-1} in both years. Differences among cultivars, cutting times and mulching times were smaller and are therefore interpreted descriptively together with the range analysis.

The 450 kg ha^{-1} fertilizer level had the highest factor-level mean seed yield in both years. In 2024, its mean did not differ significantly from 300 kg ha^{-1}, although both were higher than 600 kg ha^{-1} according to Duncan's multiple range test following the annual one-way ANOVA. In 2025, 450 kg ha^{-1} was significantly higher than both 300 and 600 kg ha^{-1}. Cutting at the branching stage and mulching at Jingzhe also showed numerical yield advantages, but the corresponding factor levels did not differ significantly within either year.

The numerical difference among fertilizer levels was most evident when 450 kg ha^{-1} was compared with the

Table 2: Descriptive orthogonal range analysis of seed yield in 2024.

Treatment	Factor				Seed yield
	A	B	C	D	(kg ha^{-1})
1	1	1	1	1	512.8
2	1	2	2	2	549.7
3	1	3	3	3	416.4
4	2	1	2	3	511.9
5	2	2	3	1	457.8
6	2	3	1	2	492.6
7	3	1	3	2	452.5
8	3	2	1	3	483.2
9	3	3	2	1	472.4
K1	1478.9	1477.2	1488.6	1443	
K2	1462.3	1490.7	1534	1494.8	
K3	1408.1	1381.4	1326.7	1411.5	
k1	493	492.40	496.20	481	
k2	487.4	496.90	511.33	498.3	
k3	469.4	460.47	442.23	470.5	
R	23.6	36.4	69.1	27.8	
R-value ranking	C>B>D>A				
Preferred levels	A_1	B_2	C_2	D_2	
Range-based candidate	$A_1B_2C_2D_2$				

highest fertilizer rate. In 2024, mean yield at 450 kg ha^{-1} was 69.1 kg ha^{-1} higher than at 600 kg ha^{-1}. In 2025, it was 86.5 kg ha^{-1} higher than at 300 kg ha^{-1} and 45.7 kg ha^{-1} higher than at 600 kg ha^{-1}. Thus, increasing the fertilizer rate from 450 to 600 kg ha^{-1} did not increase the factor-level mean seed yield in either year.

Cutting and mulching showed weaker numerical trends. In both years, branching-stage cutting gave a higher factor-level mean yield than budding-stage cutting and Jingzhe mulching gave the highest mean yield among the mulching levels. However, all three cutting-time levels and all three mulching-time levels shared the same significance group within each year, so these patterns are interpreted as numerical tendencies rather than statistically demonstrated main effects.

Analysis of seed yield components

Table 5 and 6 present the yield components measured in 2024 and 2025. Across both years, the 450 kg ha^{-1} fertilizer level was generally associated with higher values for key components, especially inflorescences per branch, seeds per pod and thousand-seed weight. Cultivar differences were most evident for florets per inflorescence, while cutting time and mulching time produced smaller and less consistent numerical differences among component traits.

Table 3: Descriptive orthogonal range analysis of seed yield in 2025.

Treatment	Factor				Seed yield
	A	B	C	D	(kg ha^{-1})
1	1	1	1	1	416.8
2	1	2	2	2	529.6
3	1	3	3	3	414.7
4	2	1	2	3	522.4
5	2	2	3	1	487.9
6	2	3	1	2	436.1
7	3	1	3	2	514.8
8	3	2	1	3	442.3
9	3	3	2	1	502.6
K1	1361.1	1454	1295.2	1407.3	
K2	1446.4	1459.8	1554.6	1480.5	
K3	1459.7	1353.4	1417.4	1379.4	
k1	453.7	484.67	431.73	469.1	
k2	482.1	486.60	518.20	493.5	
k3	486.6	451.13	472.47	459.8	
R	32.9	35.5	86.5	33.7	
R-value ranking	C>B>D>A				
Preferred levels	A_3	B_2	C_2	D_2	
Range-based candidate	$A_3B_2C_2D_2$				

Table 4: Effects of different factors on seed yield.

Factor	Level	Seed yield (kg ha^{-1})	
		2024	2025
Cultivar	**Zhongmu No. 10**	**492.97±10.6a**	**453.7±12.4a**
	Caoyuan No. 3	487.43±8.9a	482.1±10.8a
	WL319	469.37±11.2a	486.6±11.5a
Cutting time	**No cutting**	**492.40±12.7a**	**484.7±14.6a**
	Cutting at the branching stage	496.90±11.3a	486.6±12.2a
	Cutting at the budding stage	460.47±13.1a	451.1±13.8a
Fertilizer application rate	**300 kg ha^{-1}**	**496.20±8.6a**	**431.7±6.1c**
	450 kg ha^{-1}	511.33±7.4a	518.2±7.8a
	600 kg ha^{-1}	442.23±6.9b	472.5±6.6b
Mulching time	**No mulching**	**481.00±12.1a**	**469.1±12.6a**
	Mulching at Jingzhe	498.27±11.0a	493.5±11.8a
	Mulching at the spring equinox	470.50±12.8a	459.8±13.0a

Values are mean ± SD from replicate plots; different lowercase letters within a factor and year indicate $P<0.05$ (one-way ANOVA followed by Duncan's multiple range test).

The component data show that several reproductive traits were numerically high at 450 kg ha^{-1} rather than being driven by a single component. By contrast, the 600 kg ha^{-1} level did not maintain the same numerical pattern, particularly for seeds per pod and thousand-seed weight, two traits closely related to final seed yield.

In 2024, cultivar differences were significant for florets and pods per inflorescence but not for inflorescences per branch, seeds per pod or thousand-seed weight. Cutting had no significant effect on measured components. At 450 kg ha^{-1}, inflorescences per branch and thousand-seed weight exceeded 300 kg ha^{-1}, while seeds per pod at 450 and 300 kg ha^{-1} exceeded 600 kg ha^{-1}. Mulching effects were not significant.

In 2025, cultivar differences were significant only for florets per inflorescence and cutting or mulching effects were not significant. Fertilizer effects were clearer: 450 kg ha^{-1} gave significantly higher seeds per pod and thousand-seed weight than 300 and 600 kg ha^{-1} and more inflorescences per branch than 300 kg ha^{-1}.

Analysis of agronomic traits

Table 7 and 8 summarize plant height, stem diameter and hay yield. In both years, no-cutting treatments produced taller plants than cutting treatments, whereas stem diameter and hay yield varied only modestly among most factor levels. The 450 kg ha^{-1} fertilizer level and Jingzhe mulching tended to increase hay yield, which partly

Table 5: Effects of different factors on seed-yield components in 2024.

Year	Factor	Level	Inflorescences branch^{-1}	Florets inflorescence^{-1}	Pods inflorescence^{-1}	Seeds pod^{-1}	1000-seed weight (g)
2024	**Cultivar**	**Zhongmu No. 10**	**25.1±1.8a**	**22.4±0.9a**	**10.8±0.4ab**	**7.9±0.3a**	**2.04±0.04a**
		Caoyuan No. 3	20.6±1.7a	18.3±0.8b	11.9±0.5a	7.2±0.4a	1.97±0.05a
		WL319	26.2±2.0a	24.1±1.0a	10.4±0.4b	7.8±0.3a	1.96±0.04a
	Cutting time	No cutting	25.9±1.9a	22.0±1.0a	10.6±0.4a	8.0±0.4a	2.03±0.04a
		Cutting at the branching stage	25.4±1.6a	21.4±1.2a	10.9±0.4a	7.8±0.4a	2.02±0.05a
		Cutting at the budding stage	23.0±1.8a	21.1±1.1a	11.6±0.5a	7.1±0.4a	1.94±0.06a
	Fertilizer application rate	300 kg ha^{-1}	21.1±1.2b	21.3±1.0a	10.7±0.4a	7.4±0.15ab	1.96±0.04b
		450 kg ha^{-1}	28.0±1.1a	22.6±1.1a	11.9±0.6a	8.2±0.18a	2.07±0.03a
		600 kg ha^{-1}	25.4±1.4ab	20.8±1.2a	10.6±0.4a	7.1±0.12c	1.97±0.03ab
	Mulching time	No mulching	22.9±1.7a	20.5±1.1a	10.6±0.5a	7.2±0.4a	1.96±0.04a
		Mulching at Jingzhe	25.7±2.0a	23.1±1.2a	11.0±0.6a	7.9±0.5a	2.04±0.05a
		Mulching at the spring equinox	26.0±1.6a	20.9±1.1a	11.1±0.5a	7.6±0.4a	2.01±0.04a

(mean ± SD from replicate plots; within-factor letters indicate P<0.05, one-way ANOVA followed by Duncan's multiple range test).

Table 6: Effects of different factors on seed-yield components in 2025.

Year	Factor	Level	Inflorescences branch^{-1}	Florets inflorescence^{-1}	Pods inflorescence^{-1}	Seeds pod^{-1}	1000-seed weight (g)
2025	**Cultivar**	**Zhongmu No. 10**	**25.0±1.9a**	**21.8±1.2a**	**11.2±0.4a**	**7.5±0.3a**	**2.05±0.04a**
		Caoyuan No. 3	20.7±1.8a	18.7±0.9b	11.3±0.6a	7.0±0.7a	1.98±0.05a
		WL319	26.1±2.0a	24.0±1.0a	10.8±0.4a	7.5±0.5a	1.99±0.04a
	Cutting time	No cutting	25.1±1.7a	21.7±1.0a	10.9±0.4a	7.4±0.4a	2.05±0.06a
		Cutting at the branching stage	25.0±1.9a	21.0±1.1a	11.1±0.5a	7.6±0.3a	2.00±0.11a
		Cutting at the budding stage	22.0±2.0a	21.7±1.1a	11.5±0.4a	7.0±0.6a	1.98±0.09a
	Fertilizer application rate	300 kg ha^{-1}	20.9±1.2b	21.4±1.1a	11.0±0.3ab	7.2±0.16b	1.94±0.02b
		450 kg ha^{-1}	27.5±1.5a	22.0±1.2a	11.9±0.4a	8.0±0.17a	2.10±0.03a
		600 kg ha^{-1}	24.0±1.4ab	21.0±1.1a	10.5±0.4b	6.9±0.15b	1.98±0.05b
	Mulching time	No mulching	22.3±1.7a	21.4±1.3a	11.0±0.4a	7.3±0.3a	2.02±0.04a
		Mulching at Jingzhe	25.0±2.0a	22.4±1.0a	11.2±0.5a	7.4±0.4a	2.01±0.04a
		Mulching at the spring equinox	24.9±1.8a	20.7±1.1a	11.1±0.4a	7.2±0.3a	1.97±0.05a

(mean ± SD from replicate plots; within-factor letters indicate P<0.05, one-way ANOVA followed by Duncan's multiple range test).

contributed to the economic return of the corresponding treatment combinations.

In 2024, Zhongmu No. 10 was tallest, while stem diameter and hay yield were similar among cultivars. No cutting produced the greatest height. Hay yield was highest at 450 kg ha^{-1} fertilizer and under Jingzhe mulching.

In 2025 (Table 8), Zhongmu No. 10 again had the greatest plant height (103.1 cm). Stem diameter and hay yield were similar among cultivars. No cutting produced taller plants than the two cutting treatments, while stem diameter and hay yield changed little with cutting time. Fertilizer rate and mulching time caused only small differences in plant height, stem diameter and hay yield.

Correlation analysis of seed yield components

The correlation analysis (Fig 1) showed that, in 2024, seed yield was positively correlated with branch number, inflorescences per branch, seeds per pod and thousand-seed weight, but negatively correlated with pods and florets per inflorescence. Branch number had the strongest positive association with seed yield in that year.

In 2025, seed yield was strongly and positively correlated with thousand-seed weight and moderately correlated with branch number, inflorescences per branch and seeds per pod.

Together with the component-trait results, the correlations indicate associations between seed yield and several reproductive traits, including inflorescences per branch, seeds per pod and thousand-seed weight. These correlations indicate associations rather than causal relationships.

Partial-budget analysis of different treatments

In 2024 (Table 9), Treatment 2 had the highest partial-budget return (RMB 18,405.8 ha^{-1}) and Treatment 7 the

Table 7: Effects of various factors on alfalfa agronomic traits in 2024.

Year	Factor	Level	Plant height (cm)	Stem diameter (cm)	Hay yield (kg m^{-2})
2024	Cultivar	Zhongmu No. 10	116.4±2.8a	0.83±0.04a	0.86±0.04a
		Caoyuan No. 3	92.8±2.4b	0.85±0.02a	0.84±0.05a
		WL319	94.1±2.7b	0.79±0.09a	0.80±0.03a
	Cutting time	No cutting	121.2±3.6a	0.82±0.04a	0.82±0.05a
		Cutting at the branching stage	95.5±2.1b	0.84±0.06a	0.83±0.09a
		Cutting at the budding stage	87.2±2.9b	0.80±0.04a	0.81±0.05a
	Fertilizer application rate	300 kg ha^{-1}	105.1±1.6a	0.78±0.02a	0.78±0.03b
		450 kg ha^{-1}	101.3±1.4b	0.86±0.03a	0.87±0.04a
		600 kg ha^{-1}	97.7±1.8b	0.83±0.05a	0.83±0.04ab
	Mulching time	No mulching	106.5±1.8a	0.78±0.02b	0.78±0.02b
		Mulching at Jingzhe	99.8±1.5b	0.87±0.06a	0.88±0.04a
		Mulching at the spring equinox	97.5±1.7b	0.81±0.04ab	0.82±0.02ab

(mean ± SD from replicate plots; within-factor letters indicate P<0.05, one-way ANOVA followed by Duncan's multiple range test).

Table 8: Effects of various factors on alfalfa agronomic traits in 2025.

Year	Factor	Level	Plant height (cm)	Stem diameter (cm)	Hay yield (kg m^{-2})
2025	Cultivar	Zhongmu No. 10	103.1±2.5a	0.81±0.04a	0.81±0.04a
		Caoyuan No. 3	92.7±2.2b	0.84±0.03a	0.84±0.04a
		WL319	92.1±2.4b	0.80±0.04a	0.80±0.02a
	Cutting time	No cutting	108.2±3.1a	0.83±0.03a	0.83±0.05a
		Cutting at the branching stage	93.3±2.0b	0.81±0.03a	0.81±0.03a
		Cutting at the budding stage	87.0±2.3b	0.80±0.06a	0.80±0.04a
	Fertilizer application rate	300 kg ha^{-1}	101.1±5.0a	0.78±0.04a	0.78±0.04a
		450 kg ha^{-1}	94.8±5.4a	0.85±0.05a	0.85±0.03a
		600 kg ha^{-1}	92.8±4.8a	0.82±0.04a	0.82±0.04a
	Mulching time	No mulching	101.3±5.3a	0.81±0.04a	0.81±0.05a
		Mulching at Jingzhe	95.6±4.8a	0.86±0.05a	0.86±0.03a
		Mulching at the spring equinox	91.6±4.9a	0.79±0.04a	0.79±0.02a

(mean ± SD from replicate plots; within-factor letters indicate P<0.05, one-way ANOVA followed by Duncan's multiple range test).

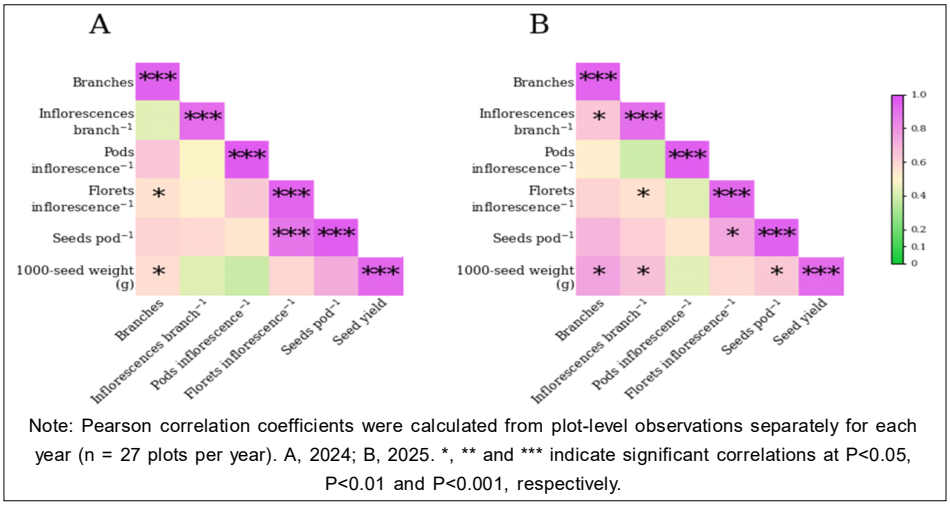
lowest (RMB 13,043.0 ha^{-1}). The highest-return treatments within Zhongmu No. 10, Caoyuan No. 3 and WL319 were Treatments 2, 6 and 9, respectively.

In 2025 (Table 10), Treatment 2 again gave the highest partial-budget return (RMB 17,635.4 ha^{-1}), while Treatment 1 was lowest (RMB 13,325.2 ha^{-1}). The highest-return treatments within the three cultivars were Treatments 2, 5 and 9, respectively.

Across years, Treatment 2 had the highest partial-budget return among the nine observed treatment combinations. This treatment combined Zhongmu No. 10, Jingzhe mulching, branching-stage cutting and 450 kg ha^{-1} fertilizer. Its two-year mean partial-budget return was RMB 18,020.6 ha^{-1}.

Partial-budget ranking differed from seed-yield ranking because hay income and treatment-specific costs were also included.

Among the nine tested treatments, the combination of branching-stage cutting, 450 kg ha^{-1} fertilizer and Jingzhe mulching gave the greatest observed partial-budget return. The highest-return treatment differed among cultivars in



Note: Pearson correlation coefficients were calculated from plot-level observations separately for each year (n = 27 plots per year). A, 2024; B, 2025. *, ** and *** indicate significant correlations at P<0.05, P<0.01 and P<0.001, respectively.

Fig 1: Pearson correlation analysis between seed-yield components and seed yield.

Table 9: Partial-budget analysis of seed production in 2024.

Treatment	Seed yield (kg ha^{-1})	Seed return (RMB ha^{-1})	Cut hay yield (kg ha^{-1})	Hay return (RMB ha^{-1})	Treatment-specific cost (RMB ha^{-1})	Partial-budget return (RMB ha^{-1})
1	512.8	17435.2	0	0	846	16589.2
2	549.7	18689.8	2395	2395	2679	18405.8
3	416.4	14157.6	2644	2644	3102	13699.6
4	511.9	17404.6	0	0	1919	15485.6
5	457.8	15565.2	2381	2381	2452	15494.2
6	492.6	16748.4	2590	2590	2256	17082.4
7	452.5	15385.0	0	0	2342	13043.0
8	483.2	16428.8	2176	2176	2256	16348.8
9	472.4	16061.6	2388	2388	2029	16420.6

Table 10: Partial-budget analysis of seed production in 2025.

Treatment	Seed yield (kg ha^{-1})	Seed return (RMB ha^{-1})	Cut hay yield (kg ha^{-1})	Hay return (RMB ha^{-1})	Treatment-specific cost (RMB ha^{-1})	Partial-budget return (RMB ha^{-1})
1	416.8	14171.2	0	0	846	13325.2
2	529.6	18006.4	2308	2308	2679	17635.4
3	414.7	14099.8	2540	2540	3102	13537.8
4	522.4	17761.6	0	0	1919	15842.6
5	487.9	16588.6	2368	2368	2452	16504.6
6	436.1	14827.4	2495	2495	2256	15066.4
7	514.8	17503.2	0	0	2342	15161.2
8	442.3	15038.2	2120	2120	2256	14902.2
9	502.6	17088.4	2315	2315	2029	17374.4

some cases, but Treatment 2 had the highest observed treatment-level return in both years.

Response to fertilizer application rate

Fertilizer response depends on nutrient rate and ratio, soil fertility, water supply and weather. In this study, 450 kg ha^{-1} compound fertilizer produced the highest factor-level seed yield in both years. It did not differ from 300 kg ha^{-1} in 2024, but both exceeded 600 kg ha^{-1} in 2025 it exceeded both 300 and 600 kg ha^{-1}. Thus, increasing fertilizer from 450 to 600 kg ha^{-1} did not improve yield. Moderate fertilization may support reproductive development, whereas excessive inputs may reduce efficiency or alter assimilate allocation, although these mechanisms were not measured. Similar responses to phosphorus, balanced NPK and coordinated irrigation-fertilization management have been reported (Wang *et al.*, 2013; Jia, 2023; Hui *et al.*, 2025).

Response to cutting time

Cutting affects canopy structure, regrowth and reproductive development (Huang *et al.*, 2022). Branching-stage cutting showed a numerical tendency toward higher seed yield than budding-stage cutting, but most yield components differed little, so this response should not be interpreted as universal. Similar management effects have been reported in ARCC journals (Kumar *et al.*, 2017; Zhao *et al.*, 2020).

Cutting may shift flowering relative to rainfall and alter lodging or canopy conditions, but pollination, lodging and disease were not directly quantified; these explanations therefore remain hypotheses.

Previous work indicates that cutting before the rainy season can shift regrowth and flowering (Zhou *et al.*, 2011), while later cutting may leave insufficient time for recovery and assimilate accumulation (Wang *et al.*, 2005).

Response to mulching time

Mulching conserves soil moisture and temperature and may be beneficial during the dry, windy spring of Inner Mongolia (Li *et al.*, 2022; Meng *et al.*, 2021). Early-spring mulching increased seed yield in Ningxia (Li *et al.*, 2022). In the present study, Jingzhe mulching also showed a numerical yield advantage, but soil temperature and moisture were not measured, so the mechanism remains hypothetical. Irrigation interval and harvest timing can also influence alfalfa seed formation (Bahia, 2025).

Cultivar-related variation in seed yield

Cultivar rankings differed between years: Zhongmu No. 10 ranked highest in 2024, whereas WL319 ranked highest in 2025. Because cultivar differences were smaller than fertilizer differences and the preferred cultivar changed between years, cultivar choice requires validation across environments. Moreover, the three cultivars occupied separate adjacent field areas, so cultivar and field position were partly confounded. The same perennial plots also received the same treatments in both years, allowing possible carry-over effects. Genotype-dependent environmental adaptation has likewise been reported in alfalfa (Mohammed *et al.*, 2023).

CONCLUSION

(1) In the orthogonal range analysis, fertilizer rate showed the largest R value for seed yield in both years, followed by cutting time, mulching time and cultivar. This R ranking is descriptive and reflects the spread among factor-level means; it does not quantify the percentage contribution of each factor to total variance. The preferred levels from range analysis were branching-stage cutting (B2), 450 kg ha^{-1} fertilizer (C2) and Jingzhe mulching (D2), while the preferred cultivar differed between years.

(2) Among the nine treatment combinations actually tested, Treatment 2 (Zhongmu No. 10, branching-stage cutting, 450 kg ha^{-1} fertilizer and Jingzhe mulching) produced the highest observed seed yield and partial-budget return in both 2024 and 2025. The theoretical 2025 combination A3B2C2D2 was not included in the L9 treatments and therefore was not field-validated. Treatment 2 should consequently be regarded as the best observed combination in this experiment rather than as a universally validated optimum.

(3) The annual one-way ANOVAs indicated that the 450 kg ha^{-1} fertilizer level was associated with favorable seed-yield and several yield-component responses, whereas branching-stage cutting and Jingzhe mulching showed smaller numerical tendencies without significant main-effect differences in seed yield. Because the two years were analysed separately and the cultivar areas were spatially constrained, the findings should be interpreted as year-specific evidence from this established field. Further multi-year field validation is recommended before a production-scale management package is adopted.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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