The effect of nitrogen addition on seed yield and yield components of *Leymus chinensis* in Songnen Plain, China

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Abstract

Nitrogen (N) addition strongly influences community composition and ecosystem functioning. However, little is known about their effects on seeds yield attributes of *Leymus chinensis* (Trin.) Tzvel. in Songnen Plain, China. A field experiment was evaluated in Songnen Plain to investigate the effects of N addition on seeds yield and yield components of *L. chinensis*. The experiment consisted of five N addition levels (0, 2.9, 5.9, 9.0, 11.9 g N m² yr⁻¹) was conducted for two consecutive growing seasons. The results showed that, except spikelet number, the other plant characteristic, such as plant height, leaf area, seed setting rate, 1000-grain weight, and seeds yield, were significantly influenced by N addition in the both growing seasons. With increasing N addition, the reproductive seed setting rate, grains number and weight can improve the seed yield in growing season and heading year. Although N addition has the more significantly effect on the seeds yield and yield components (besides spikelet number) in later fall, raised N addition in topdressing did not cause a standing increase trend. Therefore, from our results, the optimal N addition of application 5.90 g m⁻² with a method of topdressing in late fall were recommended to improve the seed yield and yield components of *L. chinensis* in Songnen Plain, China.

Keywords: *Leymus chinensis*, nitrogen addition, yield and yield components, Songnen Plain.

1. Introduction

Leymus chinensis (Poaceae), a member of the family Gramineae, is a perennial rhizome grass (Kuo, 1987). Due to its excellent stress tolerance (Yin *et al.*, 1993; Xiao *et al.*, 1995), grasslands dominated by *L. chinensis* are widely distributed at the eastern end of the Eurasian steppe, from North Korea westward to Mongolia and northern China, and north-westward to Siberia. High palatability and herbage production make the grasslands ideal for grazing and forage production (Li *et al.*, 1983).

Furthermore, because of its high vegetative propagation, *L. chinensis* is used as a soil-binding plant to protect soil from desertification in the arid areas of northern China.

Because of economical and ecological importance, *L. chinensis*, originally a dominant species in the Songnen Plain in China (Yang *et al.*, 2000), has received considerable attention (Wang, 1984). Over the past few years, *L. chinensis* meadows restoration

has attracted the attention of many government officials and researchers. With more attention paid to the environment, people have realized the importance of recovery of the grassland, and have taken many measures in its reclamation. However, it has low heading percentage, low seed setting percentage and low seed yield under natural conditions, which severely limit the further utilizing for it (Yang *et al.*, 1988, 1989).

Up to now, a major problem facing its propagation is its low sexual reproductivity. To better understand the causes of the low fecundity of this grass, several investigations have been conducted (Wang, 1984, 1998; Huang *et al.*, 2002); however, these have focused on the effects of ecological factors. It has been reported that seed production under natural conditions can be <40 % (Wang, 1984). Seeds production by *L. chinensis* may be influenced by climate (Gao *et al.*, 1999; Yang *et al.*, 2000), nutrient uptake (Yang, 1989; Wang, 1998) and vegetative growth (Wang, 1998, Wang and Ripley, 2000), as well as by disturbance by human being and animals (Yang and Zhu, 1989; Wang, 2000). However, the causes of low fecundity are uncertain, largely because many aspects of the reproductive biology of this species remains unknown or incomplete.

Nitrogen element is the nutrient that most frequently limits yield and plays an important role in quality of forage crops. Positive response of nitrogen fertilizers has been reported by Omer (1998), and Gasim (2001). Sharma (1973) observed that addition of nitrogen fertilizer increased plant height. Increase in plant height resulted in an increase in leaf number per plant as reported by Akintoye (1996). Gasim (2001) indicated that the increase in plant height with nitrogen fertilizer is due to the fact that nitrogen promotes plant growth, increases the number of internodes and length of the internodes which results in progressive increase in plant height. Koul (1997) reported similar results. Nitrogen fertilization increased number of leaves per plant and leaf area (Gasim, 2001). Koul (1997) recorded that nitrogen application resulted in greater values of plant height, leaf area, number of leaves and stem diameter of fodder maize, fresh and dry forage

yield were also increased due to addition of nitrogen. Leaf to stem ratio was found also to be increased by nitrogen (Duncan, 1980). These findings are in full agreement with that of Gasim (2001) who reported that the increase in leaf to stem ratio with nitrogen application is probably due to the increase in number of leaves and leaf area under nitrogen treatments, producing more and heavy leaves. However, the characters of morphological variations and mechanism of morphological responses remains unclear. This study was designed to investigate morphological variations of *L. chinensis* under nitrogen addition.

The purpose of the present study was to study the influence of nitrogen addition on the reproductive characteristics of *L. chinensis* widely grown in the Songnen Plain of the northeast China. Our objectives in this study were (i) to compare the impact among nitrogen addition levels on seeds yield and components, and (ii) to determine which nitrogen addition level had higher seeds yield and an effective *L. chinensis* seeds production method.

2. Materials and Methods

2.1. Region and site description

The field experiment was carried out at the Frigid Forage Research Station located at Lanxi county (Figure 1), run by Heilongjiang Academy of Agricultural Sciences (HASS). The station has an altitude of 160 m, longitude of 125°58'E, 46°32' N in Northeast China (Chen *et al.*, 2010). The experimental field is situated in the northwest margin area of the Songnen Plain in 2008. The climate is classified as a typical cold subhumid monsoon. Based on data from 1988 through 2008, the total annual sunshine duration is 2713 hours and the frost-free period is 130 days. The annual mean air temperature is 5.3°C with a maximum temperature of 31.2°C (July) and a minimum temperature of -25.2°C (January). The annual mean accumulated temperature above 10°C is 2,760°C. The annual mean precipitation is 469.7 mm, of which about

75% falls from June to August and the average annual free water evaporation is about 950 mm. The soil is a dark loam (mostly Chernozem, FAO Taxonomy) with high melanic humus. The experimental area had an average soil pH of 8.12, an average soil organic matter content of 6.04%, total N content of 0.34%; the contents of $\text{NO}_3\text{-N}$ was 4.35%, the contents of $\text{NH}_4\text{-N}$ was 6.81% and available P was 22.35 ppm (Olsen method). The experiment was seeded on 1 May 2008 and crops grew for two years until 2010.

Figure 1. The location of Heilongjiang province in China and the location of the study site in Lanxi county

2.2. Experimental design

The population of *L. chinensis* originated from HASS was established in 20 plots (6 m^2) in a randomised complete block design with four replication. Seeds were sown by direct drilling at the rate of 20 kg ha^{-1} with 30 cm between rows in 5 environments of five nitrogen addition levels (0, 2.9, 5.9, 9.0, and $11.9 \text{ g N m}^{-2} \text{ yr}^{-1}$). Nitrogen addition levels are referred to as N_0 , $\text{N}_{2.9}$, $\text{N}_{5.9}$, $\text{N}_{9.0}$ and $\text{N}_{11.9}$, respectively. The nitrogen (as $\text{CO}(\text{NH}_2)_2$) was broadcasted manually in late fall at the beginning of the second growing season. The experiment was carried out for two consecutive years (2009–2010) After seeding,

each plot was constantly fertilised with $21.8 \text{ kg P ha}^{-1}$ and $40.0 \text{ kg N ha}^{-1}$ annually as triple superphosphate (44% P) and ammonium sulphate (21% N) in each fall. Open perimeter area outside the experiment area was reserved to protect the experimental rows from interferential damage. No irrigation was applied during the experimental periods. Plots were hand-weeded during the growing period whenever necessary for proper weed control.

2.3. Sampling and analysis

At final harvest, the samples taken randomly from each plot randomly in september 2009 and 2010 were cut to ground level with manual shears using $0.5 \times 0.5 \text{ m}$ quadrats for determination of the variables. The effects of nitrogen addition on six trait parameters including plant height (cm), leaf area (cm^2), spikelet number (m^2), seed setting rate (%), 1000-grain weight (g), and seeds yield (kg ha^{-1}) were analyzed with one-way ANOVA of General Linear Model (GLM) and a Tukey-Kramer LSD test ($p < 0.05$). Due to a lack of normality, some data were square root transformed as appropriate prior to analysis. Where F-tests were significant ($p < 0.05$), LSD was calculated to compare the means. All statistical analyses were performed using statistical computer software SAS (SAS Institute, Inc.1999).

3. Results

3.1. Plant height

The effect of N addition levels on plant height of *L. chinensis* significantly was observed in both years (Table 1). Average 2-yr varieties for plant height observed in the experiment did not show significant difference in neither year. In 2009 highest plant height obtained by $\text{N}_{5.9}$ treatment compared to other treatments ($p < 0.05$). In 2010 the plant height uniformity had highest height obtained by $\text{N}_{5.9}$ treatment ($p < 0.05$). Plant height from the $\text{N}_{5.9}$ treatment were the tallest (123.15 cm) whereas N_0 (CK) had the shortest (103.15 cm), and those by $\text{N}_{2.9}$, $\text{N}_{9.0}$, $\text{N}_{11.9}$ treatment were intermediate (111.35, 110.50, and 109.90 cm, respectively).

Table 1. Changes in plant height (cm) of *L. chinensis* under N addition in 2009/2010 at Songnen Plain, NE China.

Treatment [¶]	2009	2010	2-year average
	plant height (cm)		
N0(CK)	100.80 ^c	105.50 ^c	103.15
N2.9	112.40 ^b	110.30 ^b	111.35
N5.9	123.80 ^a	122.50 ^a	123.15
N9.0	111.00 ^b	110.00 ^b	110.50
N11.9	110.60 ^b	109.20 ^b	109.90
Average	111.72	111.50	111.61
LSD _{0.05}	10.30	9.50	NS [†]

[¶] N addition levels were 0, 2.9, 5.9, 9.0, and 11.9g N m² yr⁻¹, respectively.

[†] NS indicates no statistically significant difference between group at $p \geq 0.05$. Different letters between rows are statistically different at $p < 0.05$.

Table 2. Changes in leaf area (cm²) of *L. chinensis* under N addition in 2009/2010 at Songnen Plain, NE China.

Treatment [¶]	2009	2010	2-year average
	Leaf area (cm ²)		
N0(CK)	11.29 ^d	11.50 ^b	11.90
N2.9	12.40 ^c	11.30 ^b	11.85
N5.9	14.80 ^a	12.50 ^a	13.65
N9.0	15.00 ^a	13.00 ^a	14.00
N11.9	13.60 ^b	11.20 ^b	12.40
Average	13.42	12.10	12.76
LSD _{0.05}	0.95	0.86	NS [†]

[¶] N addition levels were 0, 2.9, 5.9, 9.0, and 11.9g N m² yr⁻¹, respectively.

[†] NS indicates no statistically significant difference between group at $p \geq 0.05$. Different letters between rows are statistically different at $p < 0.05$.

3.2. Leaf area

The effect of N addition on leaf area of *L. chinensis* significantly was observed in both years (Table. 2). The leaf area obtained in these trial, which ranged from 11.29 cm² to 15.00 cm² in 2009, and which ranged from 11.20

cm² to 13.00 cm² in 2010. In each year similar results was obtained that where the N5.9 and N9.0 treatment had significantly largest leaf area than others ($p < 0.05$). Average 2-yr leaf area of *L. chinensis*, ranged from 11.85 cm² to 14.00 cm² in the experiment, did not show significant difference in both years.

Table 3. Changes in spikelet number (m²) of *L. chinensis* under N addition in 2009/2010 at Songnen Plain, NE China.

Treatment [¶]	2009	2010	2-year average
	Spikelet number (m ²)		
N0(CK)	187.77	196.50	192.14
N2.9	189.40	198.30	193.85
N5.9	180.80	184.50	182.65
N9.0	186.00	190.00	188.00
N11.9	173.60	211.20	192.40
Average	183.51	196.10	192.14
LSD _{0.05}	NS [†]	NS	NS

¶ N addition levels were 0, 2.9, 5.9, 9.0, and 11.9g N m² yr⁻¹, respectively.

† NS indicates no statistically significant difference between group at $p \geq 0.05$. Different letters between rows are statistically different at $p < 0.05$.

3.3. Spikelet number

Data in Table 3 shows that spikelet number of *L. chinensis* was not significantly increased in all treated plants at all sampling occasions during the two seasons. The largest spikelet number of *L. chinensis* was noticed at the N2.9

flowed by N0 (CK) and N9.0 in 2009, whereas the largest spikelet number of *L. chinensis* was noticed at the N11.9 flowed by N2.9 and N0 (CK) in 2010. Average 2-yr spikelet number of *L. chinensis* did not show significant difference in both years.

3.4. Seed setting rate

The effect of different nitrogen addition on seed setting rate of *L. chinensis* during two seasons is presented in Table 4. The revealed values indicated that seed setting rate of *L. chinensis* was significantly affected by applied treatments at all sampling occasions in both seasons (Table 4). In 2009 highest seed setting rate of *L. chinensis* obtained by N5.9 and N9.0 treatment compared to other

treatments ($p < 0.05$). In 2010 year the plant height uniformity had highest seed setting rate obtained by N5.9 treatment ($p < 0.05$). Seed setting rate from the N5.9 treatment were the tallest (20.60%) whereas N0 (CK) had the shortest (17.15%), and those by N2.9, N9.0, N11.9 treatment were intermediate (18.85, 20.00, and 17.90%, respectively). Average 2-yr varieties for seed setting rate of *L. chinensis* observed in the experiment did not show significant difference in neither year.

Table 4. Changes in seed setting rate (%) of *L. chinensis* under N addition in 2009/2010 at Songnen Plain, NE China.

Treatment ¶	2009	2010	2-year average
	Seed setting rate (%)		
N0(CK)	17.80 ^c	16.50 ^d	17.15
N2.9	19.40 ^b	18.30 ^{bc}	18.85
N5.9	20.80 ^a	20.40 ^a	20.60
N9.0	21.00 ^a	19.00 ^{bc}	20.00
N11.9	17.60 ^c	18.20 ^{bc}	17.90
Average	19.32	18.48	18.90
LSD _{0.05}	0.37	0.43	NS [†]

¶ N addition levels were 0, 2.9, 5.9, 9.0, and 11.9 g N m⁻² yr⁻¹, respectively.

† NS indicates no statistically significant difference between group at $p \geq 0.05$. Different letters between rows are statistically different at $p < 0.05$.

3.5. 1000-grain weight

The present study showed that, the 1000-grain weight of *L. chinensis* is significantly affected by different nitrogen addition treatments during the two seasons studied (Table 5). Nitrogen application effected 1000-seed weight as compared to control. In 2009 and 2010, highest 1000-grain weight of *L. chinensis*

obtained by N5.9 treatment compared to other treatments ($p < 0.05$). 1000-grain weight from the N5.9 treatment were the tallest (2.39 g) whereas N0 (CK) had the shortest (2.23g), and those by N2.9, N9.0, N11.9 treatment were intermediate. The experiment did not show significant difference in average 2-yr varieties for 1000-grain weight of *L. chinensis* observed in both seasons.

Table 5. Changes in 1000-grain weight (g) of *L. chinensis* under N addition in 2009/2010 at Songnen Plain, NE China.

Treatment ¶	2009	2010	2-year average
	1000- grain weight (g)		
N0(CK)	2.20 ^c	2.25 ^b	2.23
N2.9	2.25 ^b	2.30 ^b	2.28
N5.9	2.38 ^a	2.40 ^a	2.39
N9.0	2.30 ^b	2.27 ^b	2.29
N11.9	2.27 ^b	2.26 ^b	2.27
Average	2.28	2.30	2.29
LSD _{0.05}	0.07	0.09	NS [†]

¶ N addition levels were 0, 2.9, 5.9, 9.0, and 11.9g N m² yr⁻¹, respectively.

† NS indicates no statistically significant difference between group at $p \geq 0.05$. Different letters between rows are statistically different at $p < 0.05$.

3.6. Seeds yield

During the trial periods, N addition for each year were performed in the first year (2009) and the second year (2010). Positive effect of N addition was recorded in both years (Table 6). In the first year, N5.9 had significantly highest seeds yield of *L. chinensis* than N9.0 and N11.9 (132.20 vs 110.25 and 105.60 kg ha⁻¹, respectively), followed by N2.9 and N0 (CK) (100.50 and 97.20 kg ha⁻¹, respectively) ($p < 0.05$); In the second year, same results also were observed that the effect of N addition on seeds yield of *L. chinensis*

was significantly different among each other ($p < 0.05$). N5.9 also significantly affected seeds yield in 2010 and had highest seeds yield. Average 2-yr seeds yields obtained in these trial, which ranged from 98.60 to 123.25 kg ha⁻¹, where the N9.0 had significantly highest seeds yield. Year for seeds production observed in the experiment did not show significant difference in 2009 and 2010. In general, seeds from the N5.9 treatment showed higher seeds yield compared with seeds from others for 2-year period.

Table 6. Changes in seeds yield (kg ha⁻¹) of *L. chinensis* under N addition in 2009/2010 at Songnen Plain, NE China.

Treatment †	2009	2010	2-year average
	Seeds yield (kg ha ⁻¹)		
N0(CK)	97.20 ^c	100.00 ^c	98.60
N2.9	100.50 ^c	112.30 ^b	106.40
N5.9	132.20 ^a	120.25 ^a	123.25
N9.0	110.25 ^b	114.30 ^b	115.25
N11.9	105.60 ^{bc}	102.20 ^c	103.90
Average	109.15	109.81	109.48
LSD _{0.05}	6.07	5.20	NS [‡]

† N addition levels were 0, 2.9, 5.9, 9.0, and 11.9g N m² yr⁻¹, respectively.

‡ NS indicates no statistically significant difference between group at $p \geq 0.05$. Different letters between rows are statistically different at $p < 0.05$.

4. Discussion

The results obtained in the 2 year investigations indicate that N addition had the dominant effect on the growth and development of seeds of *L. chinensis*. As a result of the differences in plant height, leaf area, spikelet number, seed setting rate, 1000-grain weight, and seeds yield, differences were also recorded depending on N addition in 2009 and 2010.

The increase in plant height with N addition can be attributed to the fact that nitrogen promotes plant growth, increases the number and length of the internodes which results in progressive increase in plant height. Similar results were reported by Koul (1997), Saigusa *et al.* (1999) and Gasim (2001). However, the remarkable increase in plant height attained by N5.9 treatment, in this study can be explained by the optimal N addition which redound to assimilating essential nutrient for the plant. Differences in leaf area per plant were recorded in the 2 year trial period. In the stage of maturation, leaf area was significantly influenced by nitrogen applied in late fall top dressing (5.9 and 9.0

g N m⁻²) though in 2009 and 2010. The increase in leaf area due to application of nitrogen can be explained by the fact that nitrogen enhanced growth and consequently influences leaf expansion and development. These results coincided well with El Noeman *et al.* (1990), Filatov and Afonin (1993) and Sayed (1998). They stated that nitrogen significantly increased leaf area through effect on elongation of leaves. Similar results regarding the increased seed setting rate, 1000-grain weight of *L. chinensis* due to applied nitrogen were obtained by several research workers by Nordestgaard (1986), Havstad *et al.* (2002), and Serin *et al.* (1999). Increasing N addition did not caused a results regarding the increased spikelet number from this study coincided well with some reports by Hampton (1987) Jeremy *et al.* (2002) and Yang *et al.* (2000).

As N addition increase, raised nitrogen rates in topdressing caused decrease of plant height, leaf area, seed setting rate, 1000-grain weight, and seeds yield was significantly different in the maturation stage. Thus, N addition played

an important role in determining both seeds yield and components. In *L. chinensis*, seed setting rate was the most important factor for increasing seed yield. This is in agreement with the reports of Oliva *et al.* (1994a, b) in red and white clovers (*T. pratense* L. and *T. repens* L.), in which the yield components that most affected seed yield were species-specific.

In seeds production, a major goal is to increase seeds yield (Wang *et al.*, 2009). During the two production years, annual mean temperatures in each year were about the long-term average, but there were more rainfall events and total precipitations in 2010. Although there were not large deviations in monthly mean temperatures and accumulated rainfalls during the growing season from year to year, these weather variations were part of the reasons of annual total seeds yield differences of the same varieties, and the year $\times$ N interaction on seeds yields. In this study, the highest seeds yield occurred at N5.9 in both year, which indicated it most suitable for seeds growing development for artificial grassland establishment and eco-environmental restoration in Songnen Plain. Similar results were reported by Wang *et al.* (2000) and Yang *et al.* (2000).

5. Conclusions

Our study provides evidence that: (i) N addition has the more significantly effect on the seeds yield and components (besides spikelet number) in later fall, but raised N addition in topdressing did not cause an standing increase trend; (ii) in Songnen Plain, the optimal N addition is application 5.90 g m⁻² with a method of topdressing in later fall; and (iii) using seeds to reclamation in Songnen Plain will bring a great value for restoration of degraded land.

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