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Full Length Research Paper

Comparative photosynthetic and growth characteristics of *Leymus chinensis* and *Leymus secalinus* in sandy and saline-alkaline soil on the Songnen Plains of China

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***Leymus chinensis* and *Leymus secalinus* are perennial rhizome grasses; photosynthetic physiological characteristics were compared between them in Sandy and Saline-Alkaline Soils on the Songnen Plains of China. The contents of chlorophyll and soluble saccharides were positively correlated with photosynthetic characteristics. The photosynthetic capacity of *L. chinensis* was significantly greater than *L. secalinus*, with great economical and ecological potentials. *L. chinensis* has better adaptaion to the saline-alkali condition than *L. secalinus*.**

Key words: *Leymus chinensis*, *Leymus secalinus*, photosynthesis, water soluble carbohydrate, growth.

INTRODUCTION

Grassland degradation is serious in the Songnen plain (Li and Zheng, 1997), which is already one of the main saline-alkali soil districts in China. The salinized area of the Songnen Grassland forms approximately 60 to 70% of the total grassland. *Leymus chinensis* and *Leymus secalinus* are perennial rhizome grasses distributed on the Songnen grassland (Yang et al., 2003). *L. chinensis* and *L. secalinus* are both economically and ecologically important fodder grasses because they grow rapidly are, rich in vitamins, and have high quality protein, minerals, and carbohydrates (Ma et al., 2007; Yang and Zhang, 2004). Moreover, their physiological characteristics enable them to tolerate water shortage, strong evaporation, poor nutrient, saline-alkaline and dune soil conditions (Zhu, 2004; Zhu et al., 2007; Clayton and Renvoize, 1986). Thus, they play an important role in rehabilitation, reconstruction and dune fixation in arid and desert region of China (Teng et al., 2006; Zhu et al., 2007; Yang et al., 1995).

There have been many studies on these two species, such as physiological characteristics of vegetative organs, especially drought resistance, salt-alkali stress, photosynthetic characteristics, clone growth and the ages structure of them or sexual reproduction and, even plant hybridizable (Wang and Zhao, 2001; Shi and Wang, 2005; Chen et al., 2005; Yang and Zhang, 2004; Huo et al., 2004). Only a few investigations are on the *L. secalinus* photosynthetic processes, and almost no one comprised the photosynthetic and morphological characteristics of *L. chinensis* and *L. secalinus* or provided why two-third of this grassland is dominated by *L. chinensis*, but not *L. secalinus* (Li and Zheng, 1997).

The aim of this study was to analyze and compare the diurnal photosynthesis; the responses of photosynthesis to light and CO_2, the chlorophyll fluorescence parameters and contents of photosynthetic pigments and soluble saccharides of *L. chinensis* and *L. secalinus*. This study could identify which one has more photosynthetic capacity and adapt to survive on the Songnen plains of China; it would provide theoretical foundation to *L. chinensis* and *L. secalinus* growers or specialists for cultivated and artificial grassland construction in restoration of degraded natural grassland.

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Table 1. Chlorophyll fluorescence parameters were calculated according to Genty et al (1989) and Harbinson et al (1989).(In *ETR*, where 0.5 was used as the fraction of excitation energy distributed to PSII, and 0.84 corresponds to the fraction of incident irradiance absorbed by a leaf.)

Chlorophyll fluorescence parameters		Equation
PSII	The maximum quantum efficiency of PSII	F_v/F_m
NPQ	Non-photochemical quenching	$(F_m\text{-}F_m') / F_m$
qP	Photochemical quenching coefficient	$(F_m'\text{-}Fs) / (F_m'\text{-} F_o')$
qN	Non-photochemical quenching coefficient	$1\text{-} (F_m'\text{-} F_o')/ (F_m - F_o)$
Φ_{PSII}	Quantum yield of PSII photochemistry	$1\text{-}F_s / F_m'$
ETR	Apparent photosynthetic electron transports rate	$\Phi_{PSII}\times PFD\times 0.5\times 0.84$

MATERIALS AND METHODS

Study site and soil type

This research was carried out on the Songnen Plains of China (44°34′N, 123°31′E), topographically, situated 141.1 m above sea level, in the Chang Ling Horse Breeding Farm in Jilin Province. The site was located in the southern part of the Songnen Plain, the climate of which is semi-arid with a mean annual rainfall about 400 to 500 mm occurring mainly from June to August, and average annual temperature and accumulated growing degree days in the study area are 4.6 to 6.4°C and 2 545 to 3374°C, respectively (Zhang et al., 1997; Shi and Guo, 2006). The main soil type at the site is sandy with some salinity and alkalinity. In wet period after rain the water penetrates quickly into the sand, whereas during dry periods the soil water is very low. The soil pH of the experiment plot was measured using a HI98129 acidity meter and was measured using EC used DDS-307 conductivity meter (Bao, 2005). The pH is 7.51 and the electrical conductivity is 93.2 μS·cm^{-1}.

Measurement of diurnal photosynthesis

The first full expanded leaves of the two species were measured for photosynthetic characteristics in the grassland. Measurements were carried out with a portable photosynthesis system (LI - 6400), values of diurnal photosynthetic characteristics were taken from 7 am to 17 pm with 1 h intervals on the sunny days in August 2011. The photosynthetically active radiation (PAR) was 1 000±12 μmol·m^{-2}·s^{-1}, CO_2 concentration 350±2 cm^3.m^{-3} leaf temperature 26.0±0.8°C. Gas exchange was measured in full expanded leaves from the same adult plants. Five replications were made for each measurement, 3 replications in each plot.

Measurement of the responses of photosynthesis to light (*A*/*Q* curves) and CO_2 (*A*/*C*i curves)

The responses of photosynthesis to light (*A*/*Q* curves) and CO_2 (*A*/*C*i curves) of leaves were determined between 9:00 to 11:00 from fully expanded first blades, using a portable open flow gas exchange system LI-6400XT (USA) (Wang et al., 2007). The photosynthetic photon flux density levels used for the construction of curves were decreased from 1800 to 0μmol m^{-2} S^{-1}: 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, 50, 0μmol m^{-2} S^{-1} were supplied by Red-Blue light emitting diodes. The air cuvette temperature was maintained at 26. 1(±1.2)℃ and CO_2 concentration was kept at 380 μmol mol^{-1} by 12 g CO_2 cylinder, respectively (Wang and Zhou, 2004; Wu et al, 2007). *A*, *Lcp*, *Lsp* and A_{max} of leaves were calculated according to Prioul and Chartier (1977) and Olsson and Leverenz (1994). The ranges of CO_2 concentration: 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 100, 50 μ mol mol^{-1} volume were given by 12 gram CO_2 cylinder. The air cuvette temperature was maintained at 26. 1(±1.2)℃ and the PPFD was maintained at 1200μmol m^{-2} S^{-1} provided by blue/red light source (Dordas and Sioulas, 2007). This application fits a model curve described by Olsson and Leverenz, (1994) rectangular hyperbola, and V_{cmax}, J_{max}, TPU_{max} and *CE* parameters were determined by Farquhar et al. (1980), as subsequently modified by von Caemmerer and Farquhar (1981), Harley and Sharkey (1991) and Harley et al (1992).

Measurement of chlorophyll fluorescence and photosynthetic pigments

The minimal fluorescence intensity (*F*o), the maximal fluorescence intensity (*F*m), the minimal fluorescence in the light-adapted state (*F*o') and maximal fluorescence in the light-adapted state (*F*m') were determined between 09:00 h and 11:00 h from first fully-expanded leaves using an Li-6400TX. The leaves were held in the dark for about 2 hours before measurement. The intensities of the saturating light settings was 6500 μmol·m^{-2} s^{-1}. The *PSII*, *NPQ*, *qP*, *qN*, Φ_{PSII} and *ETR* were determined according to equation in Table 1.

The contents of carotenoids (Car) and of chlorophylls (Chl) *a* and *b* were extracted using acetone, and spectrophotometeric determination at 440, 645 and 663

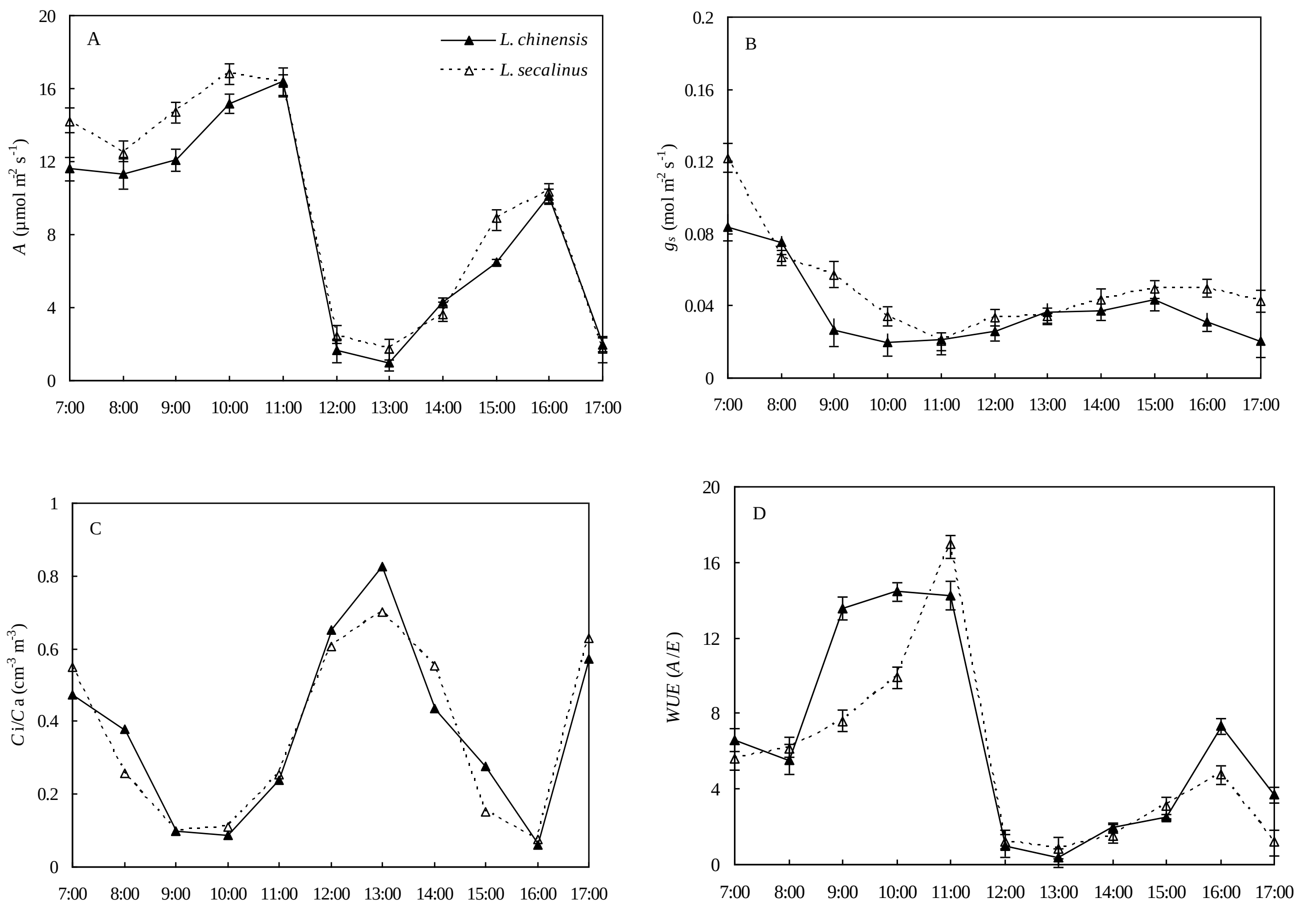

Figure 1. Daily changes in (A) net photosynthetic rate - *A*; (B) stomatal conductance - g_s; (C) ratio of stomatal and sub-stomatal CO_2 concentration - *Ci*/*C*a; and (D) water use efficiency - WUE of *L. chinensis* and *L. secalinus* on the Songnen grassland. Data are the means ± S.E.

nm of each sample was done three times. The calculations used the equations of Arnon (1949). The light response curves of the leaves were determined by the White and Critchley method (1999).

Measurement of biomass and carbohydrates

Morphological parameters of leaves were measured such as the length and width of leaves. Leaf area was measured with a leaf area measurement system (LI-6400, LI-COR, USA). The leaves of samples were collected after measured, the dry weights (DW) determined after drying for 15 min in an oven at 80°C and then in a vacuum dryer at 40°C to constant weight. The total soluble carbohydrate contents of the leaves were determined using a UV-754 spectrophotometer at 620 nm. Fructose and sucrose were determined according to Wrigly (1994) and Li (2003). Each measurement was repeated five times.

Statistical analyses

Statistical analysis included one-way analysis of variance (ANOVA) in SPSS (Version 13.0, SPSS, Chicago, IL, USA). All measurements represent the means and standard errors (SE) of five replicates.

RESULTS

Diurnal photosynthesis

The diurnal patterns of *A* of *L. chinensis* and *L. secalinus* were similar and typical to C_3 plants; belonging to a "W" two-peak type (Figure 1A). The first maximum value appeared at 10:00am in *L. chinensis* and *L. secalinus* at 11:00 am, respectively, and the second maximum value occurred at 16:00 pm in two samplings. There had an obvious middy depressing photosynthesis phenomenon at 13 am. The g_s value trended to decline from 7:00 to 10:00 am, and then values didn't exert significant change (Figure 1B). The daily variation of *Ci*/*C*a could be described as W-type curve, compare with *A* curves just reverse, and the two low hollow appeared 10:00 am and 14:00 pm, the peak occurred 13:00 pm (Figure 1C). The changes of *WUE* of two samples were similar as *A*, it

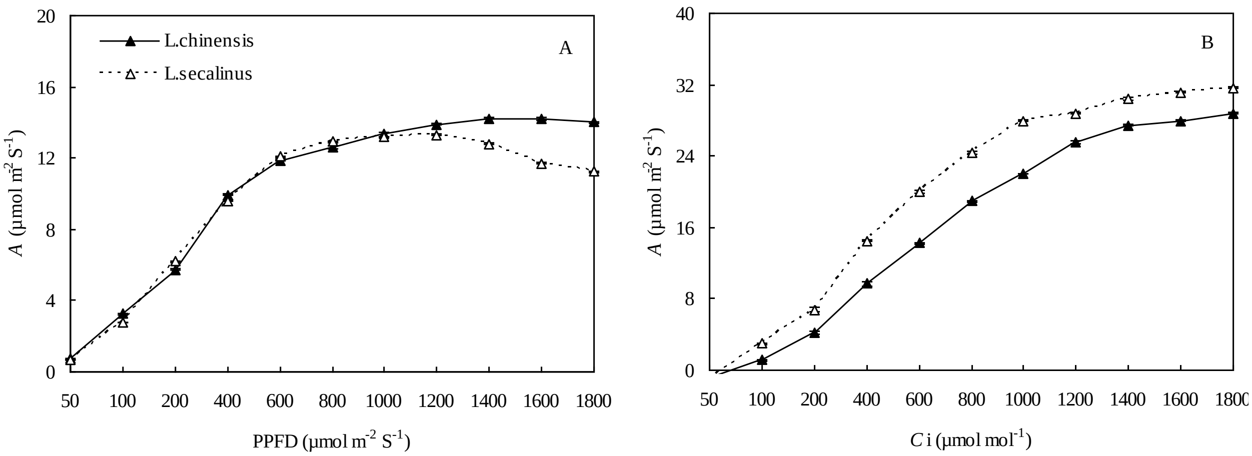

Figure 2. The responses of photosynthesis to light (A) and CO_2 (B) curves of *L.chinensis* and *L.secalinus*. Data are the mean ± SD.

Table 2. The parameters of respond of photosynthesis to light of *L.secalinus* and *L.secalinus*. A_{max} - the maximum net photosynthesis rate, *Lcp* - light compensation point, *Lsp* - light saturation point. Data are the mean ± SD.

	A_{max} (μmolm^{-2}S^{-1})	*Lcp* (μmolm^{-2}S^{-1})	*Lsp* (μmolm^{-2}S^{-1})
L. chinensis	18.3 ± 0.65	29.6 ± 1.12	365 ± 10.91
L. secalinus	13.7 ± 0.93	29.7 ± 1.81	404 ± 9.85

Table 3. The parameters of respond of photosynthesis to CO_2 of *L. secalinus* and *L. secalinus*. A_{sat} - Light-saturated rate of net photosynthesis, CCP -CO_2 compensation point, *Resp*- the parameters leaf respiration. Data are the mean ± SD.

	A_{sat} (μmolm^{-2}S^{-1})	*CCP* (μL L^{-1})	*Resp*
L. chinensis	69.90 ± 3.61	29.23 ± 1.83	22.81 ± 1.15
L. secalinus	40.18 ± 2.18	28.93 ± 1.88	16.42 ± 0.53

show the two-hump curves, the time of peak and low *WUE* values were observed in the same time as *A* daily pattern (Figure 1D).

The responses of photosynthesis to light (*A*/*Q* curves) and CO_2 (*A*/*C*i curves)

Figure 2 shows that increasing light intensity can promote photosynthesis at low light intensity, but when light intensity reaches light saturation point photosynthesis rate does not continue to increase (Figure 2A). The A_{max} of *L. chinensis* was nearly 5 μmol • m^{-2}S^{-1} higher than *L. secalinus*. Only small differences were detected in the light compensation point (*Lcp*) between the two species, but the light saturation point (*Lsp*) of *L. secalinus* was significant lower than *L. chinensis* (Table 2). The patterns of *A* to C_i of *L. chinensis* and *L. secalinus* exhibited a similar trend as *A*/*Q* curves, the A_{sat} of *L. chinensis* and *L.secalinus* was 69.90 and 40.18, respectively (Table 3). The results exhibit both had nearly the same CCP at a rate close to 30μmol·mol^{-1}, the *Resp* of *L. chinensis* was higher than *L. secalinus* (Table 3).

Chlorophyll fluorescence and photosynthetic pigments

The values of PSII, NPQ, qP and Φ_{PSII} of *L. chinensis* were higher than that in *L. secalinus*, but the value of qN of *L. chinensis* was lower than that in *L. secalinus* (Table 4). Table 5 show that the contents of Chla, Chlb and Car of *L. chinensis* were all higher than that in *L. secalinus*, however, the value of Chl a/b of *L. secalinus* was higher than *L. chinensis*.

Biomass and carbohydrates

The Figure 3 showed that the leaf area and L/W ratio of

Table 4. Chlorophyll a fluorescence parameters of leaves of *L. chinensis* and *L.secalinus* leaves on the Songnen grassland. Data are the mean ± SD.

	PSII	NPQ	qP	qN	Φ_{PSII}
L. chinensis	0.83 ± 0.02	0.38 ± 0.01	0.99 ± 0.01	0.25 ± 0.00	0.78 ± 0.02
L. secalinus	0.81 ± 0.04	0.29 ± 0.01	0.95 ± 0.02	0.32 ± 0.01	0.74 ± 0.02

Table 5. The contents of photosynthetic pigments of leaves of *L. chinensis* and *L.secalinus* leaves on the Songnen grassland. Data are the mean ± SD.

Plant species	Chl a (g kg^{-1}FM)	Chl b (g kg^{-1}FM)	Chl a+b (g kg^{-1}FM)	Chl a/b	Car (g kg^{-1}FM)	Total Pigments (g kg^{-1}FM)
L. chinensis	1.68 ± 0.01	0.61 ± 0.01	2.28 ± 0.03	2.79 ± 0.03	0.57 ± 0.02	2.85 ± 0.08
L. secalinus	1.32 ± 0.01	0.41 ± 0.06	1.73 ± 0.02	3.25 ± 0.02	0.47 ± 0.02	2.02 ± 0.05

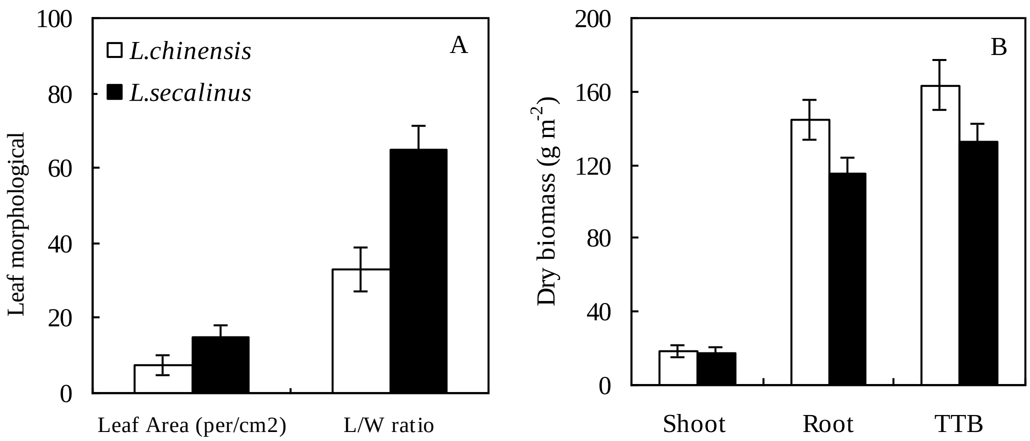

Figure 3. The morphological characters and biomass of leaves of *L. chinensis* and *L. secalinus* on the Songnen grassland. L - length, W - wide, TTB – The total biomass. Data are the mean ± SD.

Table 6. The contents of total carbohydrate (TTC), fructose and sucrose in leaves of *L. chinensis* and *L. secalinus*. The values are the means of five replicates.

	TTC	Fructose	Sucrose
L. chinensis	11.90 ± 1.08	6.81 ± 0.52	3.00 ± 0.15
L. secalinus	8.66 ± 1.12	4.94 ± 0.44	2.66 ± 0.28

L. secalinus were higher than that of *L. chinensis*, while the shoot, root and total dry biomass of *L. secalinus* were lower (Figures 3A and B). TTC, fructose and sucrose in leaves of *L. chinensis* were higher than that of *L. secalinus* (Table 6).

DISCUSSION

Photosynthesis of plants could reflect the physiological functions of plant. Diurnal changes in *A* was important factor determining photosynthetic capacity, the diurnal patterns of photosynthesis of two samplings belong to a two-peak type on the sunny day, the average *A* value of *L. chinensis* was higher about 11.69% than *L. secalinus*. g_s was one of the important indicators of photosynthesis capability, as it closely correlated with the change of environment water potential. The diurnal patterns of g_s did exert significant differences between the two species in the observed process, and the results show that the curve of g_s in *L. chinensis* was more sensitive than *L. secalinus* (Talbott et al., 2003; Warren et al., 2003). The value of *Ci*/*C*a of leaves could reflect the leaf transpiration rate, the results show that though *L. secalinus* had stronger stomatal conductance, *WUE* capability in *L. chinensis* was stronger nearly 30% than *L. secalinus*. The effect of elevated photosynthetic photon flux density and CO_2 concentration and the interaction between them to the photosynthesis has a research focus in this study. Lower A_{max} was in *L. secalinus*, it has been associated with lower photosynthesis capacity, probably due to low chlorophyll content in *L. secalinus*. The result shows that increasing light intensity can promote photosynthesis in the range, but when light intensity reaches light saturation photosynthesis rate did not increased any more. The main reason is the numbers of coloring mater never changed and the activity of stoma has been limited when the light intensity reach saturated, it has been called photoinhibition. The *Lsp* of *L. chinensis* was significantly higher than *L. secalinus*, but the *Lcp* of two plants were similar. The A/C_i curves indicated that *L. chinensis* tops *L. secalinus* in light quantum utilization efficiency, thus boasting higher photosynthetic capacity than the latter. It is the Rubisco enzyme activity in *L. chinensis* was obviously higher than *L. secalinus*, it affects the rate of electron supply, it could express by V_{cmax}, J_{max} and V_{TPU}. The values of *PSII* of them were all higher than 0.80; showing they have high maximum quantum yield (Toivonen and DeEll, 2001) in wild plants. Further analysis indicated, the leaves of *L. chinensis* had higher *PS*Ⅱ activity, light energy transforming efficiency and the transmitting rate of photosynthetic electrons than *L. secalinus*, which could turn the light energy absorbed into chemical energy effectively. Chlorophyll content is not only one of important indicators which direct reflection of the plant's photosynthetic capacity, but also it can use to measure salt-alkaline tolerance of plant and positive correlation ship between them (Wilekens et al., 1994). In the present experiment, it is clearly show that leaf chlorophyll content of *L. chinensis* was higher than *L. secalinus*, so the photosynthetic physiological functions of *L. chinensis* was better than the latter one and it would be used as preference to salt-alkaline tolerance forages specie. The accumulation of dry biomass matter significantly different was most affected by leaf net photosynthesis and the variation of the response of leaf net photosynthesis had significant correlations with leaf morphology such as leaf area, leaf length, leaf width and the ratio of leaf length to breadth.

In conclusion, all results suggested that the photosynthetic capacity, drought-resistance, soluble saccharides content, dry weight of plant and salt-alkaline tolerance of *L. chinensis* were all significantly higher than *L. secalinus*, even they are belong to same family gramineae. So *L. chinensis* has a strong competitive ability in natural habitats. Otherwise, *L. chinensis* could be economically and ecologically better fodder grass. It had been cultivated widely, and it was more suitable survive in the bad environment condition than *L. secalinus*. *L. chinensis* is a very significant research project and assignment for the vegetation – rehabilitation and cultivates the superior variety. And the research of *L. chinensis* and *L. secalinus* have a great significance information parameters and to provide scientific basis for establishing the best management scheme and utilizing grassland rationally.

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