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Responses of chlorophyll fluorescence and nitrogen level of *Leymus chinensis* seedling to changes of soil moisture and temperature

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Abstract: Controlled experiment of *Leymus chinensis* seedlings grown in the environmental growth chambers at 3 soil moisture levels and 3 temperature levels was conducted in order to improve the understanding how leaf photosynthetic parameters will respond to climatic change. The results indicated that soil drought and high temperature decreased the photochemical efficiency of photosystem(F_v/F_m), the overall photochemical quantum yield of PSII(yield), the coefficient of photochemical fluorescence quenching(q_P), but increased the coefficient of non-photochemical fluorescence quenching(q_N). Severe soil drought would decrease F_v/F_m and yield by 3.12% and 37.04% under 26℃ condition, respectively, and 6.60% and 73.33% under 32℃ condition, respectively, suggesting that higher temperature may enhance the negative effects of soil drought. All the soil drought treatments resulted in the decline in leaf nitrogen content. There was no significant effect of temperature on leaf nitrogen level, but higher temperature significantly reduced the root nitrogen content and the ratio of root nitrogen to leaf nitrogen, indicating the different strategies of adaptation to soil drought and temperature. It was also implied that higher temperature would enhance the effect of soil drought on leaf photosynthetic capacity, decrease the adaptability of *Leymus chinensis* to drought.

Keywords: chlorophyll fluorescence; nitrogen level; *Leymus chinensis*; soil moisture; soil temperature

Introduction

The relationship between plant and water has been drawing more and more attention from ecologists and botanists. The temperature changes will affect the biochemical and biophysical processes of plant. Moderately rising temperature could enhance plant photosynthesis and water use availability by increasing leaf water potential (Shaw, 2000), but high temperature may damage chloroplast and biomembranes, reduce photosynthetic electronic transport, and negatively affect chlorophyll fluorescence parameters(Taub, 2000), resulting in a decline in plant leaf photosynthesis(Berry, 1980; Taub, 2000). Water change not only affected plant photosynthesis through affecting temperature change, but also directly did plant growth, productivity and community composition. The grassland productivity and water use efficiency(WUE) were associated with the temporal and spatial distribution of precipitation (O'connor, 2001; Chen, 2002). Drought resulted in the changes of leaf chlorophyll fluorescence parameters (Xiao, 2001), and a decrease in leaf nitrogen content (Sinclair, 2000). The percentage leaf nitrogen may be used to estimate biochemical capacity, e.g. photosynthetic capacity (Peterson, 1999). Global change will rise global temperature, change the distribution of precipitation, intensifying more droughts in arid and semiarid area(Wagner, 1996; Wigleyl, 2001; Morgan, 2001; Qin, 2002). It is important that the responses of plant to the changes of different temperature and soil moisture were investigated in order to relieve the negative impact of global change, and maintain sustainable development of life support system.

The grassland dominated by *Leymus chinensis*, which is a rhizome and native perennial plant with good palatability and high forage value, is widespread in China. The responses of the physiological processes of photosynthesis and gas exchange to environmental changes have been studied extensively(Du, 1995; Wang, 2001), but less emphasis has been placed on understanding how chlorophyll fluorescence parameters of *Leymus chinensis* interact with soil moisture and temperature related to tissue nitrogen level. The purpose of this study was to elucidate the changes in chlorophyll fluorescence parameters and nitrogen levels under three temperature levels and three soil moisture levels so that the theory basis may be provided for understanding the mechanism of adaptation to environmental changes.

1 Materials and methods

Seeds of *Leymus chinensis*(Trin.) Tzvel. obtained from natural grassland in Xilinhot, Inner Mongolia in the fall of 2001 were sterilized by 5% potassium permanganate solution for 8 min, then rinsed, and immersed into water 3 times before they were put into refrigerator below 0℃ for 7 d. They

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were sowed into plastic pots(0.56 L) wrapping with plastic film. Each plastic pot was filled with 0.64 kg dry soil obtained from natural grassland with a density of 6 plants per pot. All experimental pots were put under greenhouse conditions(day/night, 30—33℃/23—28℃) till the seedling had the fifth leaf and then moved into the environmental growth chambers(MLR-350HT, Sanyo, Japan) with a 14 h photoperiod and relative humidity(RH) of about 65%, and the photosynthetic photon flux density(PPFD) of 300 μmol/(m^2·s) on the top of plant canopy. The light was provided by a combination of cool-white fluorescent and incandescent lam PSII as normally supplied with the environmental growth chambers.

Soil water holding treatments were executed after plant tillering stage. The soil water contents were divided into three levels: control(soil moisture accounting for 75%—80% of field moisture capacity), MD (moderate drought, soil moisture for 50%—55% of field moisture capacity) and SD (severe drought, soil moisture for 35%—40% of field moisture capacity). Soil moisture was maintained by weighing method at 5:00 p.m. daily. Temperature treatments were divided into three levels: 20, 26 and 32℃. Each of the treatments had 12 pots as replicates.

The fully youngest expanded leaves were selected to determine the chlorophyll fluorescence dynamic parameters at 22 d after controlling temperature. Three pots were measured in each treatment, and three to four leaves were measured each pot. After 30 min dark adaptation at room temperature (25℃), the parameters were measured by a fluorescence meter(PAM-2000, Walz, German). The parameters were calculated according to the formulae:

PSII photochemical efficiency $F_v/F_m = (F_m - F_0)/F_m$, Yield $= (F'_m - F_S)/F_m'$, photochemical quenching coefficient $q_P = (F'_m - F_S)/(F'_m - F'_0)$, non photochemical quenching coefficient $q_N = 1 - (F'_m - F'_0)/(F_m - F_0)$.

For the biomass measurements, plant samples collected from three pots of each treatment, were separated into the leaves and roots, instantly dried at 80℃ to constant weight, ground in a Wiley Mill to pass a screen with 1 mm openings, and were then subsampled for nitrogen(N) determinations. N content in leaf and root was determined by the standard macro-Kjeldahl procedure(nitrogen analysis system, Büchi, Switzerland). Leaf biomass was expressed as the dry matter per plant, and nitrogen content was expressed on a dry weight basis. All statistical analyses, e.g. GLM-ANOVA, were performed using SPSIIS 10.0(SPSIIS for Windows, Version 10.0).

2 Results

2.1 Responses to different temperatures

Chlorophyll fluorescence may be used as the inner probe of plant to monitor the kinetic changes of PSII activity as the plant was placed from dark to light environment(Lu, 1998; Zhang, 2001). The results showed, in every soil moisture treatment, that the photochemical efficiency of photosystem Ⅱ (F_v/F_m), the overall photochemical quantum yield of PSII(yield) and the coefficient of photochemical fluorescence quenching(q_P) were greater at 26℃ than those at 32℃, except the coefficient of non-photochemical fluorescence quenching(q_N) (Table 1). For all soil moisture treatments, high temperature(32℃) reduced F_v/F_m, yield, and q_P by 7.52%, 42.06% and 20.62% compared to 26℃, respectively, but increased q_N by 5.04%. Temperature did not significantly affect leaf nitrogen content, but rising temperature significantly increased root N content, especially did ratio of root N and leaf N(Table 2). Compared to those at 20℃, root N increased by 10.15% and 17.79% at 26℃ and 32℃, respectively, especially did the ratio of root N and leaf N by 8.71% and 16.42% , respectively(Table 2), implying that higher temperature restricted the transfer of N from root to leaf.

Table 1 Effects of the interaction of temperature and soil moisture on the chlorophyll fluorescence parameter in the leaves of *Leymus chinensis*

Temperature, ℃	Soil moisture	F_v/F_m	Yield	q_P	q_N
	Control	0.765 ± 0.022	0.231 ± 0.070	0.420 ± 0.056	0.750 ± 0.066
26	MD	0.755 ± 0.043	0.196 ± 0.017	0.352 ± 0.011	0.749 ± 0.022
	SD	0.741 ± 0.024*	0.146 ± 0.028*	0.300 ± 0.040	0.741 ± 0.062
	Control	0.724 ± 0.024	0.146 ± 0.023	0.303 ± 0.060	0.715 ± 0.061
32	MD	0.688 ± 0.032*	0.102 ± 0.012*	0.295 ± 0.015	0.789 ± 0.025
	SD	0.679 ± 0.019**	0.084 ± 0.032**	0.253 ± 0.054	0.849 ± 0.051*

Notes: Within each temperature treatment, values followed by * and ** represent significantly difference, $p < 0.05$ and $p < 0.01$, respectively, as compared with that of control. Means ± *SE*, $n = 3$

2.2 Responses to different soil moistures

Within each temperature treatment, soil drought reduced F_v/F_m, yield and q_P, but increase q_N (Table 1). In comparison to control treatment, severe soil drought (SD) reduced F_v/F_m, yield and q_P by 4.65%, 39.07% and 23.53%, respectively, but increased q_N by 8.50%(Table 1). Soil drought significantly reduced leaf N content. Compared to control treatment, moderate soil drought(MD) and SD reduced leaf N by 8.85% and 9.47%, respectively. However, within the same temperature treatment, the status of root N was different from that of leaf N, MD reduced root N, but SD increased. The ratio of root N and leaf N decreased at MD, indicating that higher adaptive response to MD(Table 2).

2.3 Responses to different soil moisture and temperatures

Generally, the results showed that higher temperature enhanced the effect of drought on F_v/F_m and yield. Compared to control treatment, SD reduced F_v/F_m and yield by 3.12% and 37.04% at 26℃, respectively, but 6.60% and 73.33% at 32℃, respectively(Table 1), indicating that higher temperature weakened the adaptability of leaf to soil drought.

Table 2 Effects of the interaction of temperature and soil moisture in nitrogen contents in the leaves and roots of *Leymus chinensis*

Organs	Temperature, ℃	Control	MD	SD
Leaves	20	8.729^{a}	7.614^{b}	6.667^{c}
	26	8.213^{a}	7.82^{ab}	7.13^{b}
	32	8.512^{a}	7.747^{b}	7.193^{c}
Roots	20	4.658^{a}	3.72^{b}	3.502^{b}
	26	4.800^{a}	3.828^{b}	4.369^{b}
	32	5.447^{a*}	4.212^{b*}	5.673^{a*}
N ratio	20	0.534^{a}	0.490^{b}	0.525^{a}
	26	0.584^{a*}	0.490^{b}	0.613^{a*}
	32	0.640^{a**}	0.544^{b*}	0.789^{a**}

Notes: Within each temperature treatment, values followed by the same letter are not significantly different at $p < 0.05$. Within each organ column, values followed by * and * * represent significantly difference, $p < 0.05$ and $p < 0.01$, respectively, as compared with that of 20℃ treatment. Means ± *SE*, $n = 3$

Based on an analysis of the response of root N content to the interaction between soil moisture and temperature, the soil drought enhanced the increase in root nitrogen due to high temperature, indicating the interaction between the high temperature and soil drought was not useful for the N transfer from root to leaf. For all treatments, the correlation analysis among leaf N concentration and fluorescence parameters showed that F_v/F_m was positively and significantly correlated with q_P and yield, respectively, but q_N was negatively correlated with F_v/F_m, yield and q_P, respectively. Leaf N content was positively correlated with F_v/F_m, yield and q_P, respectively, but negatively did with q_N, although the significant difference did not occur, implying that high nitrogen level may help to improve plant capacity of photosynthesis(Table 3).

Table 3 Correlations among fluorescence parameters and nitrogen contents in the leaves of *Leymus chinensis*

	F_v/F_m	Yield	q_P	q_N	N content
F_v/F_m	1.000	0.957^{**}	0.845^{*}	−0.738	0.369
Yield		1.000	0.954^{**}	−0.637	0.508
q_P			1.000	−0.504	0.538
q_N				1.000	−0.605
N content					1.000

Notes: ** and * represent significance at 0.01 and 0.05 level, respectively

2.4 Leaf biomass changes under different soil moisture and temperatures

Leaf biomass was the greatest at 26℃, and the smallest at 32℃ under sufficient soil water condition(Fig. 1). On average over water treatments, it was also the greatest at 26℃, with 71.39 mg/plant, while 48.89 mg/plant and 27.54 mg/plant at 20℃ and 32℃, respectively.

Under moderate temperature condition (26℃, approximating to the 25℃ of plant growth optimal temperature), soil drought significantly reduced leaf biomass. Across temperature treatments, MD and SD reduced leaf biomass by 21.21% and 49.78%, respectively.

Temperature significantly affected the response of leaf biomass to soil drought. Compared to control, MD increased the leaf biomass by 19.94% at 20℃, but reduced by 22.88% and 61.97% at 26℃ and 32℃, respectively. SD reduced leaf biomass by 40.16%, 43.32% and 72.97% at 20℃, 26℃ and 32℃, respectively, indicating that rising temperature would enhance the limitation of soil drought on leaf growth.

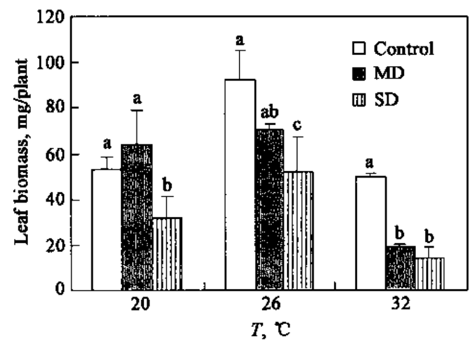

Fig. 1 Effects of the interaction of temperature and soil moisture on leaf biomass of *Leymus chinensis*
Vertical bars indicate ± *SE* of the mean($n = 3$) where these exceed the size of symbol and the figures with the same letters are not different at 0.05 level according to Duncan's multiple test within the same temperature

3 Discussion

The photosynthetic system II(PSII) in plant leaf plays an important role in the processes of photosynthesis; the hotosynthetic capacity depends on its function and integration, which has higher sensitivity to environmental stress e.g. heat stress(Berry, 1980; Zhang, 2001).

The long-term acclimatization of different species to different environmental factors results in different features such as enduring some environmental stress factors e.g. heat stress. The optical temperature and its threshold of plant photosynthesis largely varied with species and environmental conditions, for instance, there was no effect of high temperature on the photosynthesis of some species until 45℃ of high temperature (Berry, 1980). However, the photochemical efficiency of photosystem II (F_v/F_m) of *Cucumis sativus* was significantly reduced as the temperature exceeded 28℃ only for 10 min, it reduced by 50% at 37.9℃(Taub, 2000). The plants of this study grown for around one month under the high constant temperature condition of 32℃, not did only for several hours or days, it appeared therefore a significant effect of high temperature as compared with the accumulated temperature in natural field. This study showed the negative effect on fluorescence parameters increased at 32℃, although high temperature little reduced F_v/F_m, it markedly reduced the overall photochemical quantum yield of PSII (yield), and the coefficient of photochemical fluorescence quenching(q_P), reflecting different responses of different fluorescence parameters to long heat stress, and confirming the negative effects of high temperature on photosynthesis. Soil drought also resulted in same negative consequence. It is suggested that high temperature furthermore would enhance the damage of PSII due to drought. Taub *et al*. (Taub, 2000) reported that elevated CO_2 may retrieve from the negative effect of high temperature. Other studies only focused on the effect of one factor e.g. drought or high temperature(Xiao, 2001).

Most of studies reported the relationship between the leaf nitrogen(N) and photosynthetic capability, which the higher photosynthetic capacity of plant leaf, and suitable fluorescence parameters were associated with higher N level, being significantly correlated among them (Peterson, 1999; Sinclair, 2000). Soil drought caused a decline in N availability and leaf N content, which was consistent with changes of fluorescence parameters. The result confirmed that leaf N level was positively correlated with the fluorescence parameters contributed to photosynthesis, indicating that the N level was associated with photosynthesis. High temperature did not significantly reduce the leaf N content, but significantly reduced activity of leaf PSII. Delgado *et al*. (Delgado, 1994) reported that the nitrogen content averaged leaves of winter wheat decreased, and flag leaf N maintained stable, but flag leaf photosynthesis significantly decreased as temperature rise by 4℃, this was a conclusion confused. It is suggested that the main reason that high temperature reduced photosynthetic capacity may result in the damage of function and integration of PSII, as well as a decline in nitrogen level. The result of Carlen *et al*. (Carlen, 1999) implied the competition of species and adaptability to stress environment did not depend on nitrogen level but did on the availability to organic nitrogen. This study showed that high temperature inhibited the transfer of N from root to leaf, i.e., decreased the nitrogen availability, resulting in a decline in photosynthesis and adaptability to high temperature. On the contrary, soil drought decreased the leaf nitrogen level, suggesting the soil drought would enhance the export transfer of nitrogen from leaf(Sinclair, 2000).

The results from this study have clearly shown that (1) soil drought and high temperature reduced the photochemical efficiency of photosystem Ⅱ (F_v/F_m), the overall photochemical quantum yield of PSII (yield), and the coefficient of photochemical fluorescence quenching(q_P), but increased the coefficient of non-photochemical fluorescence quenching(q_N), indicating that the function and integration of PSII was weaken; (2) all the soil drought treatments resulted in the decline in leaf nitrogen content. There was no significant effect of temperature on leaf nitrogen level, but higher temperature significantly increased the root nitrogen content, especially the ratio of root nitrogen and leaf nitrogen; and (3) the high temperature would enhance the negative effect of soil drought on leaf photosynthetic capacity, suggesting high temperature may decrease the adaptability of *Leymus chinensis* to drought.

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