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Effects of water stress on photosynthesis and nitrogen metabolism in vegetative and reproductive shoots of *Leymus chinensis*

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Abstract

In *Leymus chinensis*, mild water stress (soil moisture 60–65 % of field capacity) had no significant effects on nitrogen metabolism, photosynthesis, and chlorophyll fluorescence. Severe water stress (35–40 %) significantly decreased the activities of nitrate reductase, glutamine synthetase, and glutamate dehydrogenase, net photosynthetic rate, stomatal conductance, transpiration rate, maximal efficiency of photosystem 2 photochemistry (F_v/F_m), actual quantum yield, and photochemical quenching, but increased the endopeptidase activity and malondialdehyde contents. The adverse effects on photosynthesis and N metabolism were markedly greater in reproductive shoots than in vegetative shoots.

Additional key words: chlorophyll fluorescence; endopeptidase; glutamate dehydrogenase; glutamine synthetase; lipid peroxidation; nitrate reductase; soil moisture.

Introduction

Grassland productivity and water use efficiency (WUE) are associated with the temporal and spatial distribution of precipitation (O'Connor *et al.* 2001). A large part of nitrogen (N) is allocated to leaves throughout life of the plant, and a large part of leaf N is invested in photosynthetic apparatus, namely chloroplasts (Makino and Osmond 1991). Photosynthetic capacity is closely associated with the leaf N (Pilbeam *et al.* 2000, Niu *et al.* 2003), and the net photosynthetic rate (P_N) increases with leaf N content (Evans 1983, He *et al*. 2003).

Drought adversely affects the maximal efficiency of photosystem 2 (PS2) photochemistry (Lu and Zhang 1999) and decreases the leaf N content (Sinclair *et al.* 2000). Drought stress and N limitation significantly reduce P_N and ribulose-1,5-bisphoshate carboxylase/oxygenase (RuBPCO) activity, but drought alone does not affect them (Heitholt *et al.* 1991, Cruz *et al.* 2003). However, the decrease in RuBPCO activity and P_N of plants as a response to drought were in agreement with the low foliar N contents (Llorens *et al*. 2003). Plant mitochondria link the cellular processes of carbon (C) and N metabolism through the tricarboxylic acid and photorespiratory cycles. Environmental stresses such as drought lead to damage of specific mitochondrial targets through the direct action of reactive oxygen species and indirect action of lipid peroxidation products (Taylor *et al*. 2004). Oxidative stress is generated as a consequence of drought in plants. Malondialdehyde (MDA) is produced as the decomposition product of polyunsaturated fatty acids of biomembranes. The oxidative stress leads to significant increase in the free MDA pool (Weber *et al.* 2004).

The main link between photosynthesis and supply in organic N-compounds is ensured through glutamine synthetase (GS) present both in cytosolic and chloroplastic compartments (Sibout and Guerrier 1998). Nitrate reductase (NR) is involved in saccharide metabolism (Solomonson and Barber 1990), and its activity is sensitive to water stress. In many plants it is one of the first enzymes affected by declining water status (Hanson and Hitz 1982, Somers *et al.* 1983). Due to its connection with C and N metabolisms, a possible role of glutamate dehydrogenase (GDH) as an adaptive enzyme, whose action depends on the carbon skeleton or glutamate requirements of the cell, has been proposed (Masclaux-

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Daubresse *et al.* 2002, Stitt *et al.* 2002). Water stress affects the activities of GS and GDH (Venekamp 1989, Sibout and Guerrier 1998).

L. chinensis is a native perennial plant with rhizomes, good palatability, and high forage value. However, in recent decades the grassland ecosystem has deteriorated due to current climate change (*e.g.* water deficit) and land-use practices (*e.g.* overgrazing, reclamation) (Zhou *et al.* 2002, Wang and Gao 2003). The plant density and productivity of *L. chinensis* is decreased with decrease in precipitation (Wang and Gao 2003), and the photosynthetic capacity is positively correlated with leaf N content (Niu *et al*. 2003, Xu and Zhou 2004). Wang (2001) reported differences in gas exchange parameters between the vegetative and reproductive shoots of *L. chinensis*, but the responses of photosynthesis and related key enzymes of N metabolism are regulated by abiotic environmental factors (Lam *et al.* 1996, Llorens *et al.* 2003, Larios *et al.* 2004). We used integrated methods to examine the effect of different soil moistures on leaf growth, photosynthesis, N contents, the key enzyme activities of N metabolism, and product of lipid peroxidation of biomembranes in vegetative (VL) and reproductive (RL) shoots of *L. chinensis* plants.

Materials and methods

Plants and treatments: Seeds of *Leymus chinensis* (Trin.) Tzvel. were obtained from field grassland in Xilinhot, Inner Mongolia in the fall of 2001. They were sown into pots (560 cm^3) wrapped with plastic film. Each pot was filled with 0.64 kg dry soil obtained from the same field, and planted with a density of 6 plants per pot. In the chestnut coloured soil, the organic C concentration, total N, and available N were 19.60±0.18, 4.18±0.11, and 89.46±2.37 mg kg^{-1}, respectively. Texture class of the soil was medium. The soil contained 29.0, 31.2, and 39.8 % of clay (<0.005 mm in diameter of particle), silt (0.050–0.005), and sand (>0.05), respectively. Field capacity (FC) (determined in the field 48 h after irrigation) was 25.3 % (m/m), permanent wilting point (PWP) was 6.0 %, and bulk density was 1 210 kg m^{-3}.

Soil water withholding treatments were executed 30 d after sowing (plant initial tillering stage) in the greenhouse: day/night temperature was 25–27/18–20 ºC and maximum photosynthetic photon flux density (PPFD) was 1 000 μmol m^{-2} s^{-1}. The used soil relative water contents (SRWC) were at four levels: control (75–80 % of field capacity), MIW (mild water stress, 60–65 %), MOW (moderate water stress, 50–55 %), and SW (severe water stress, 35–40 %). Soil moisture levels were maintained by manual irrigation by weighing individual pots daily at 05:00. The plants had vegetative and reproductive shoots: the former only grew, but the later could grow and produce ears.

Biomass was measured 46 d after withholding water treatments. Samples of three pots of each treatment were dried at 80 ºC to constant mass and then weighed.

Leaf relative water content and water potential: The detached leaves (about 0.3 g fresh mass) were cut and weighed immediately to obtain fresh mass (FM), and then they were placed overnight in the dark in a beaker (25 cm^3) filled with water. They were reweighed to obtain turgid fresh mass (TM) in the next morning and dry mass (DM) after drying at 80 ºC for 24 h in drying oven. The relative water content (RWC) of the leaves could be calculated as RWC = [(FM – DM)/(TM – DM)] ×100 %. Leaf water potential was measured with a *WP4* dew-point potential meter (*Decagon Device*, Pullman, Washington, USA) after gas exchange measurements.

Leaf gas exchange: Six plants from each treatment were selected from different pots. Gas exchange parameters were measured on an attached youngest and fully expanded leaf at 44–46 d after withholding water, every time in the same order in which the treatments were measured.

P_N and transpiration rate per unit area (E) were measured with a portable photosynthesis system (*LI-6200*, *Li-Cor*, Lincoln, NE, USA) under ambient temperature and irradiance. Readings were terminated after 30 s.

Chlorophyll (Chl) fluorescence: The youngest and fully expanded leaves were selected to determine the Chl fluorescence on 45 d after withholding water treatments. Three pots were measured in each treatment, and three to four leaves were measured in each pot. After 30-min dark adaptation at room temperature (25 ºC), the parameters were determined by a fluorescence meter *PAM-2000* (*Walz*, Effeltrich, Germany). The parameters were calculated according to Genty *et al.* (1989) and Roháček (2002).

N contents: All leaf dried samples were ground in a *Wiley* mill to pass a screen with 1 mm openings, mixed thoroughly, and then sub-sampled for N determinations. N content in plant tissue was determined by the standard macro-Kjeldahl procedure (*N Analysis System*, Büchi, Switzerland).

Soluble protein and free amino acid contents and the activities of key enzymes: The youngest and fully expanded leaves were sampled at 46 d after withholding water at about 09:00, instantly frozen in liquid N for 1 min, and stored at –80 ºC for soluble protein and free amino acid content and key enzyme activity assays. About 0.5 g leaves were homogenized with 10 cm^3 of 50 mM sodium phosphate, pH 7.8, containing 2 mM EDTA and 80 mM L-ascorbic acid. After centrifugation at 15 000×g for 20 min, the supernatants obtained were

used for determining soluble protein and free amino acid contents and the activities of key enzymes (Cruz *et al.* 1970, Alvim *et al.* 2001).

Soluble protein and free amino acid contents in leaves were determined according to Bradford (1976) and Moore (1968), respectively. NR (EC 1.6.6.1), GS (EC 6.3.1.2), GDH (C 1.4.1.2), and EP activities were determined according to Baki *et al.* (2000), Elliot (1953), Loulakakis *et al*. (1994), and Wittenbach (1979), respectively.

MDA estimation: At 46 d after withholding water, the fully expanded leaf material (200 mg) was homogenized in 2 cm^3 of 0.1 % trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 15 000×*g* for 10 min, and 0.5 cm^3 of the supernatant obtained was added to 1.5 cm^3 thiobarbituric acid (TBA) in 20 % TCA. The mixture was incubated at 90 ºC in a shaking water bath for 20 min, and the reaction was stopped by placing the reaction tubes in an ice water bath. Then the samples were centrifuged at 10 000×*g* for 5 min, and the absorbance of the supernatant was read at 532 nm (Hernández and Almansa 2002). The value for non-specific absorption at 600 nm was subtracted. The amount of MDA was calculated from the coefficient of extinction of 155 $mmol^{-1}$ cm^{-1} (Cakmak and Horst 1991).

Statistical analysis: The layout of the experiment was a randomized block design. All statistical GLM-ANOVA analyses were performed using *SPSS 10.0* (*SPSS*, Chicago, IL, USA). Effects of soil moisture were analyzed using a one-way ANOVA and the least significant difference test (LSD) ($p = 0.05$).

Results

Leaf biomass: The difference in leaf biomass was not significant between control and MIW ($p<0.05$), but MOW and SW significantly decreased leaf biomass by 15.6 and 31.7 % in VL and by 34.1 and 45.5 % in RL, respectively, indicating that SW markedly limited leaf growth of *L. chinensis*, especially in RL (Fig. 1*A*).

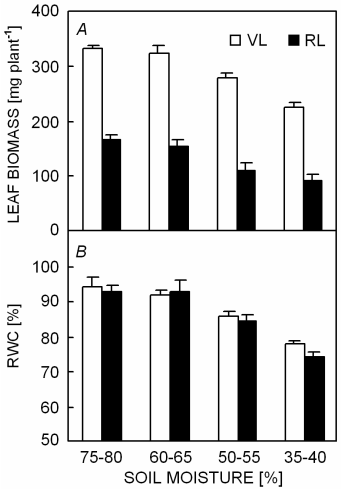

Fig. 1. Leaf biomass (*A*) and relative water content, RWC (*B*) in vegetative (VL: *empty columns*) and reproductive (RL: *full columns*) shoots of *L. chinensis* under four soil moisture regimes [control (75–80 %), MIW (mild water stress, 60–65 %), MOW (moderate water stress, 50–55 %), and SW (severe water stress, 35–40 %)]. Means ± S.E. of three replications.

Leaf relative water content (RWC) and water potential: MIW had no significant effect on RWC, but MOW and SW significantly decreased it by 8.9 and 17.7 % in VL and by 9.2 and 20.0 % in RL, respectively (Fig. 1*B*). The difference was not significant between the two types of shoots at control and MIW, but it was significant at MOW and SW ($p<0.05$). As averaged for all plant leaves, the leaf water potentials of control, MIW, MOW, and SW were –0.1, –0.4, –1.2, and –2.3 MPa, respectively.

Gas exchange and Chl fluorescence: When compared with control, MIW had no significant effect on gas exchange parameters ($p<0.05$), but MOW and SW significantly decreased P_N by 15.5 and 41.5 % in VL and by 28.4 and 53.1 % in RL, stomatal conductance (g_s) by 18.4 and 41.4 % in VL and by 29.3 and 56.6 % in RL, and *E* by 34.3 and 49.7 % in VL and by 40.7 and 53.1 % in RL, respectively (Fig. 2). MOW and SW increased intercellular CO_2 concentration (C_i) by 2.3 and 18.9 % in VL and by 6.6 and 19.6 % in RL, respectively. Hence the effects on gas exchange parameters may be more adversely profound in RL than in VL.

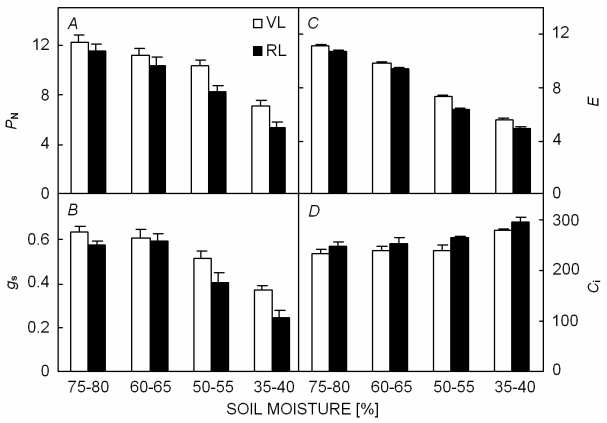

Fig. 2. (*A*) Leaf net photosynthetic rate, P_N [μmol m^{-2} s^{-1}], (*B*) stomatal conductance of CO_2, g_s [mmol m^{-2} s^{-1}], (*C*) transpiration rate, *E* [mmol m^{-2} s^{-1}], and (*D*) intercellular CO_2 concentration, C_i [μmol mol^{-1}] in vegetative (VL: *empty columns*) and reproductive (RL: *full columns*) shoots of *L. chinensis* under four soil moisture regimes (see Fig. 1). Means ± S.E. of nine replications, measured 44–46 d after withholding water.

As compared with control, MIW did not significantly affect leaf Chl fluorescence (p<0.05), but MOW and SW significantly decreased the maximal efficiency of PS2 photochemistry (F_v/F_m) by 7.8 and 14.0 % in VL and by 11.1 and 20.1 % in RL, the actual quantum yield (Φ_p) by 14.9 and 27.6 % in VL and by 17.6 and 33.6 % in RL, respectively (Fig. 3). MOW slightly affected, but SW significant decreased the photochemical quenching (q_P) by 16.1 and 20.5 % in VL and RL, respectively. SW obviously increased the coefficient of non-photochemical quenching (q_N) by 11.5 and 20.2 % in VL and RL, respectively, implicating that severe water stress obviously weakened the activity of PS2, particularly in RL.

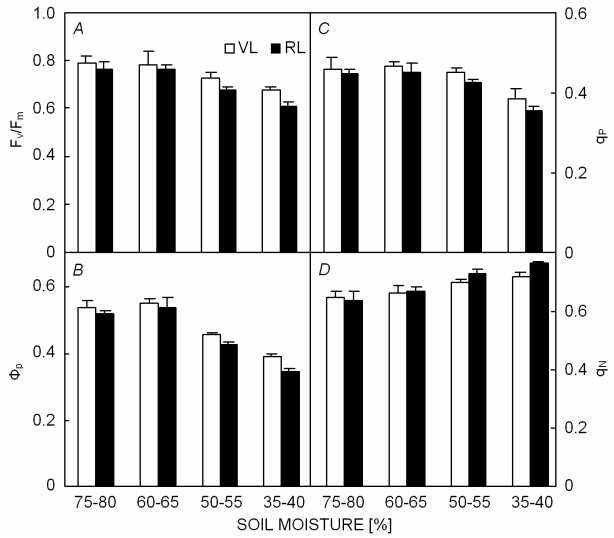

Fig. 3. (*A*) Maximal efficiency of photosystem 2 photochemistry (F_v/F_m), (*B*) actual quantum yield (Φ_p), (*C*) photochemical quenching coefficient (q_P), and (*D*) non-photochemical quenching coefficient (q_N) in vegetative (VL: *empty columns*) and reproductive (RL: *full columns*) shoots of *L. chinensis* under four soil moisture regimes (see Fig. 1). Means ± S.E. of four replications, measured on 45th d after withholding water.

Contents of N, soluble proteins, and free amino acids: MIW had no significant effects on leaf N contents, but MOW and SW markedly reduced leaf N, especially in RL (Fig. 4*A*). The differences in leaf N were not significant between control and MIW, but they were significant between control and MOW or SW. The significant differences between the VL and RL did not occur in the control, but they did at SW (p<0.05). The effects of leaf soluble protein contents were similar to that of leaf N (Fig. 4*B*). The total free amino acid (FAA) accumulation was stimulated by MIW and MOW stresses (Fig. 4*C*), but SW significantly reduced FAA contents by 9.9 and 27.0 % in VL and RL, respectively, when compared with the control ones.

NR, GS, GDH, and EP activities: The differences in NR activity were not significant between control and MIW, but they were significant between control and MOW or SW in both shoot types (p<0.05). MOW and SW significantly decreased NR activities by 7.6 and 28.6 % in VL and by 36.1 and 51.7 % in RL, respectively, indicating that severe soil drought limited the NR activity, particularly in RL (Fig. 5*A*). There were similar responses of GS and GDH activity to soil moisture. MOW and SW all significantly reduced them, and SW has more adverse effect on them in VL than in RL (Fig. 5*B,C*).

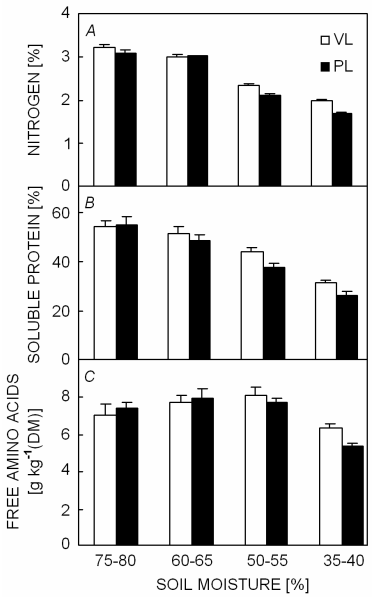

Fig. 4. (*A*) Leaf nitrogen, (*B*) soluble protein, and (*C*) total free amino acid (FAA) contents in vegetative (VL: *empty columns*) and reproductive (RL: *full columns*) shoots of *L. chinensis* under four soil moisture regimes (see Fig. 1). Means ± S.E. of three replications.

We also determined the effects of soil moisture on EP activity (Fig. 5*D*). EP activity was not significantly affected by MIW, but MOW and SW markedly increased it by 11.1 and 40.1 % in VL and by 17.9 and 65.3 % in RL, respectively, suggesting that severe water stress may enhance the protein catabolism, particularly in RL.

MDA contents: As compared with control, MOW and SW significantly increased MDA contents by 10.6 and 22.9 % in VL and by 19.9 and 43.5 % in RL, respectively. The difference in MAD content was not significant between control and MIW (Fig. 6). The difference in MDA content was not significant between VL and RL at control and MIW treatments, but was significant at MOW and SW, implicating that severe water stress accentuated gradual injury to cell membrane of leaves, and RL might be more vulnerable to water stress.

Discussion

Our results confirmed earlier reports (Hanson and Hitz 1982, Somers *et al.* 1983) that NR activity showed a transient decrease as plants were subjected to soil severe water stress. We observed that the other key enzymes, such as GS, GDH, and EP, related with N metabolism were also sensitive to severe soil drought, although they were affected differently in the two different shoot types. There was only a minor effect of MIW on the key enzymes. Heitholt *et al.* (1991) reported that mild soil drought did not affect and even increased the N content in wheat leaves, which was associated with limited irrigation in wheat field which did not significantly weaken photosynthesis, but enhanced WUE (Tavakkoli and Oweis 2004).

During water stress, leaf stomata close as leaves sense water deficit, especially after water potential drops below some threshold (Heitholt *et al.* 1991, García Mata and Lamattina 2001, Pospíšilová 2003), which is associated with a decline in photosynthesis (Kaiser 1987). As the plant was subjected to severe soil drought, function and integration of photosynthetic apparatus were abolished or injured, and this resulted in a decline of photosynthetic capacity (Kaiser 1987). The function of proteins in plant photosynthesis was degraded (Srivalli and Khanna Chopra 1998) and the peroxidation of mesophyll cell was enhanced (Zhang and Kirham 1994). Our results indicated that severe water stress not only led to stomata closure, but increased C_i associated with a reduction in P_N and had an adverse effect on PS2, implying that stomatal and non-stomatal limitations to photosynthesis in plant leaves under severe water stress may occur simultaneously.

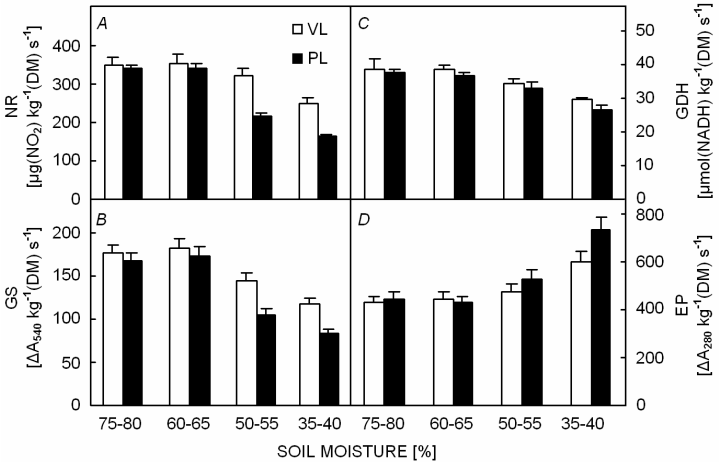

Fig. 5. (*A*) Nitrate reductase (NR), (*B*) glutamine synthetase (GS), (*C*) glutamate dehydrogenase (GDH), and (*D*) endopeptidase (EP) activities in vegetative (VL: *empty columns*) and reproductive (RL: *full columns*) shoots of *L. chinensis* under four soil moisture regimes (see Fig. 1). Means ± S.E. of three replications.

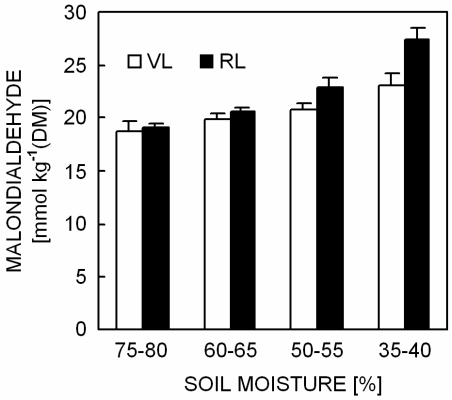

Fig. 6. Malondialdehyde (MDA) contents in vegetative (VL: *empty columns*) and reproductive (RL: *full columns*) shoots of *L. chinensis* under four soil moisture regimes (see Fig. 1). Means ± S.E. of three replications.

The C and N balance is associated with plant growth and leaf senescence (He *et al.* 2003) which determine plant-environment interaction (Raven *et al.* 2004). RuBPCO activity and P_N increase with leaf N content (Evans 1983). The Chl contents are closely correlated with leaf N content in some temperate grasses (Gáborčík 2003). NR is involved in saccharide metabolism (Solomonson and Barber 1990). Drought may have adverse effects on both the NR expression and activity (Baki *et al.* 2000). The protected NR activity in parallel to maintaining a constant protein and N contents may improve plant growth under water stress (Singh and Usha 2003). Glutamate is the main precursor of proline the accumulation of which is large in plants subjected to drought in order to enhance osmoregulation. GS activity was inhibited, but GDH could be stimulated by water stress (Venekamp 1989). The decreased GDH activity leads to a decrease in plant biomass (Lóez-Lefebre *et al.*

2002).

In our study, severe water stress markedly reduced the activities of three enzymes (NR, GDH, and GS) of the N anabolism and enhanced EP activity, especially in reproductive shoots, suggesting the N metabolism may be important in decreasing the tolerance to severe water stress. Furthermore, one of the described damages provoked by water deficit stress is the membrane injury. This is a consequence of an oxidative burst leading to lipid peroxidation and cell death (Bandeoğlu *et al.* 2004). The activity of PS2 is closely associated with lipid peroxidation (Chikov and Bakirova 1999, Bączek-Kwinta and Kościelniak 2003). Our results showed an increase in MDA content with gradual decrease in soil moisture, especially in reproductive shoots, suggesting that severe soil drought may accentuate the injury to photosynthesis due to lipid peroxidation of mesophyll cells.

In *L. chinensis*, a native perennial grass plant, the vegetative shoots largely allocate assimilates to the whole plant, having a larger photosynthetic capacity and WUE (Wang 2001, Wang *et al.* 2001). The reproductive shoots of the grass were more sensitive to water stress than the vegetative ones. Our analysis explained the physiological mechanism in view of gas exchange, Chl fluorescence, N content, activities of key enzymes of N metabolism, and products of lipid peroxidation of biomembranes.

In conclusion, severe water stress adversely affects photosynthesis and N metabolism in *L. chinensis* plants, especially in reproductive shoots. The key enzymes related with N metabolism play a key role in the photosynthetic acclimation of plant to water stress. The reproductive shoots may be more constrained in the regions dominated by *L. chinensis*, where the precipitation will decrease and temperature will elevate in the future.

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