Photosynthetic Recovery of a Perennial Grass *Leymus chinensis* after Different Periods of Soil Drought

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**Abstract**: The effects of rewatering after different periods of soil drought stress on the photosynthetic capacity of *Leymus chinensis* in pots were investigated. The plants were subjected to short-term (10-d), moderate-term (20-day) and long-term (30-d) drought each followed by rewatering. Control plants were well watered during the experimental periods. The long-term water stress without rewatering decreased the chlorophyll content, Chl a/b ratio, carbonic anhydrase (CA) activity, net photosynthetic rate \( A \), and leaf area compared with the control. Rewatering increased the ratio of Chl a/b, CA activity and \( A \), but decreased the leaf area and ion leakage from the cut leaf pieces. The long-term water stress without rewatering reduced the maximal efficiency of PSII \( F_v/F_m \), the actual quantum yield \( \Phi_p \), and photochemical quenching \( q_p \), but these values were increased by rewatering to more than the control level, though non-photochemical quenching \( q_N \) was decreased as compared with the control. This implied that long-term drought aggravated PSII, but rewatering improved it. The net CO\(_2\)-exchange rate showed similar diurnal changes in all treatments, but the rate in the morning was lower in long-term drought (before rewatering) than in the other treatments. These results suggest the photosynthesis of *Leymus chinensis* may be well adapted to episodical soil drought.

**Key words**: Carbonic anhydrase, Chlorophyll fluorescence, Drought adaptation, Gas exchange, *Leymus chinensis* (Trin.) Tzvel., Rewatering.

Water deficit is one of the key environmental factors limiting grassland productivity (Carlen et al., 1999; Bai et al., 2004). Severe water deficit results in the decreases in leaf photosynthetic capacity and the key enzyme activity (Hsiao, 1973; Kaiser, 1987; Inamullah and Isoda, 2005; Xu and Zhou, 2006b), and leads to oxidative damage (García and Lamattina, 2001; Xu and Zhou, 2006a). However, a moderate water deficit improves photosynthetic capacity and regulates carbon allocation due to acclimation to environmental stresses (Hsiao, 1973; Havaux, 1992; Yang et al., 2001; Xu and Zhou, 2005b). Thus, how grassland plants respond to water stress is a critical issue.

Grasslands dominated by *Leymus chinensis*, are widespread from the loess plateau of China (the southeast edge) to Bajkal of Russia (the northeast edge), and from Sanjiang plain of northeastern China (the east edge) to Ulaanbaatar of Mongolia (the west edge). *Leymus chinensis* is a rhizomatous and native perennial plant with good palatability and high forage value. However, the distribution region of *Leymus*-dominated grassland is severely constrained due to climatic change and land-use practices (for example, overgrazing, reclamation, etc.) in the recent decades (Zhou et al., 2002; Bai et al., 2004; Zhou et al., 2006). Many researches indicated that drought and the seasonal precipitation seriously affect the growth and the community composition of grasslands (Distel et al., 1996; O’connor et al., 2001; Kröpfl et al., 2002; Wang and Gao, 2003), and drought is particularly a problem, occurring episodically in many regions (Kang et al., 2002; Shah and Paulsen, 2003). The global climatic change may result in not only the change of total precipitation but also the occurrence of discontinuous drought. How the discontinuous drought induced by the global climatic change will affect plant growth and plant community composition remains unknown, especially for grassland plants that are more sensitive to drought.

In this research, comprehensive experiments with different soil drought and rewatering treatments were conducted in the greenhouse at the Institute of Botany, the Chinese Academy of Sciences in order to elucidate how discontinuous drought affects the photosynthetic capacity of grassland plants. The chlorophyll content, net photosynthetic rate, PSII capacity, a key photosynthetic enzyme activity (carbonic anhydrase), soluble protein and sugar concentrations, as well as ion leakage from the leaf (as a damage indicator) were examined to elucidate the adaptive mechanisms of *Leymus chinensis* plants to soil drought and rewatering. We propose a hypothesis that rewatering promotes recovery the photosynthetic capacity, but not the leaf growth of the plants, from the suppression by soil-
89.46 ± 2.37 mg kg⁻¹ and grown there until the fifth leaf appeared. Each pot was filled with 4.16 kg dry soil obtained from the field and planted with 6 plants per pot. The soil composition is similar to that of the field, and the maximal effi ciency of PSII was shown by \( \frac{F_v}{F_m} \). The relationship between leaf area and dry weight (Kolb and Sperry, 1999).

### Materials and Methods

#### 1. Plant culture and water treatments

Seeds of *Leymus chinensis* (Trin.) Tzvel. were collected from a grassland field in Xilinhot, Inner Mongolia in 2001. They were sterilized with 5% potassium permanganate solution for 5 min, rinsed, and immersed in water for 2 d, and then kept below 0°C in a refrigerator. They were sown in plastic seedling trays (50 cm in length, 30 cm in width and 20 cm in height) on 6 May 2002, and transplanted into plastic pots (5.4 L) wrapped with plastic fi lm for 30 min prior to fl uorescence measurements; and after drying at 80ºC for 24 h in an oven. Leaf relative water content (RWC) of the leaves was expressed as

\[
\text{RWC} = \left(\frac{TW}{FW} - \frac{TW}{FW}\right) \times 100.
\]

#### 2. Leaf area

Leaf fresh samples from whole plants in a pot were dried in an oven at 80°C to a constant weight to measure dry weight. Leaf area was estimated from the relationship between leaf area and dry weight (Kolb and Sperry, 1999).

#### 3. Net CO₂-exchange rate

On the youngest fully-expanded leaves, the net CO₂ exchange rate was measured by an open system (ADC BioScientifi c, Hoddeson, UK) at a CO₂ concentration of 350-360 µmol mol⁻¹, 65-80% relative humidity, and light intensity of 900 µmol m⁻² s⁻¹.

#### 4. Chlorophyll fl uorescence measurements

The youngest expanded leaves were dark-adapted for 30 min prior to fl uorescence measurements; and the chlorophyll fi uorescence was measured at room temperature (25°C) with a portable fl uorometer (PAM-2000, Walz, German). The fl uorescence parameters were calculated according to the formulae (van Kooten and Snel, 1990; Lu and Zhang, 1999):

\[
\Phi_p = \left(\frac{F_m'}{F_0'} - F_0'\right) / \left(F_m' - F_0\right); \quad q_p = \left(F_m' - F_0\right) / \left(F_m' - F_0'\right); \quad \text{and non-photochemical quenching by } q_n = \left(F_m' - F_0'\right) / \left(F_m - F_0\right).
\]

#### 5. Leaf relative water content

The relative water content (RWC) was determined according to the method of Turner (1981), which was modifi ed by Wilkinson et al. (2001). The detached leaves (about 1g fresh mass) were cut, fresh weight (FW) was measured, and then placed in a beaker (25 mL) fi lled with water overnight in the dark. They were reweighed the next morning to obtain the fully turgid weight (TW) and dry weight (DW) was determined after drying at 80°C for 24 h in an oven. Leaf relative water content (RWC) of the leaves was expressed as

\[
\text{RWC} = \left(\frac{FW}{DW} - \frac{TW}{DW}\right) \times 100.
\]

#### 6. Carbonic anhydrase activity assay

The fresh samples of leaves were frozen in liquid N for 1 min and then stored at -80°C for carbonic anhydrase activity assay. About 0.5 g leaves were vigorously ground in a mortar on an ice plate with 40 mmol l⁻¹ Hepes-KOH (pH 8.2), containing 10 mmol...
L dithiothreitol (DTT). After adding additional 1 mL of the buffer mixture, the homogenate was filtered through four-layer gauze cloths. The remainder was diluted in the blending buffer as required (diluted 10 times) and stored at 0°C prior to assay. The rate of CO₂ hydration was measured at 0°C at different pHs. The reaction mixture contained 25 mmol L⁻¹ Hepes-KOH buffer (pH 8.2) in a final 1 mL. The reaction was started by adding a CO₂ solution (distilled water saturated with CO₂ at 0°C). The mixtures were stirred by up and down agitation of the precooled pH electrode probe. Control assays contained boiled extracts. The observed rate of pH was converted into equivalent mol H⁺ generated by titrating the reaction mixture buffer in the range from 8.3 to 7.6 of pH with a standard solution of H₂SO₄ (Dai et al., 2000).

7. Chlorophyll, soluble sugar, and soluble protein concentrations

Chlorophyll (Chl) in leaves was extracted with 10 mL 80% acetone and their compositions were spectrophotometrically calculated according to the equations reported by Lichtenthaler and Wellburn (1983). The Chl concentration was expressed as the percentage of fresh weight (FW) of viable leaf.

Soluble sugar was exhaustively extracted from leaf samples by hot ethanol extraction. Five mL of 70% (v/v) ethanol was mixed by shaking with 100 mg of ground leaf material in a test tube. The sample was then incubated at 80°C for 20 min with shaking, and centrifuged for 5 min at 1,500 g. The freshly prepared anthrone reagent (0.2% anthrone in concentrated H₂SO₄) was pipetted into test tube and chilled in ice water. The extract was thoroughly mixed with the anthrone reagent. The tubes were heated in boiling water for 15 min and then rapidly cooled. The absorbance was read at 630 nm (Yemm and Willis, 1954; Güitman et al., 1991). Total soluble sugar content was calculated as the percentage of dry matter of viable leaf.

About 1 g leaves were homogenized with 10 mL of 50 mmol L⁻¹ sodium phosphate, pH 7.8, containing 2 mmol L⁻¹ EDTA and 80 mmol L⁻¹ L-ascorbic acid. After centrifugation at 15,000 g for 20 min, the supernatants were used to determine soluble protein (Gruz et al., 1970). Soluble protein concentration was measured according to the method of Bradford (1976).

8. Ion leakage from leaves

Ion leakage was determined according to García and Lamattina (2001). Leaves were cut into about 20 mm² pieces (1g fresh mass) and placed in a beaker (25 mL) with 15 mL de-ionized water, and exhausted by rinsing with an air extractor for 10 minutes. After the incubation at 25°C for 2 h, the conductivity of the bathing solution (Xi) was determined with a conductivity meter (HI9033 multi-range, Hanna Instruments, Sigma, Singapore). Then, the samples were heated at 85°C for 2 h, and the conductivity (Xt) was read again in the bathing solution. Electrolyte leakage was expressed as a percentage of the total conductivity after heating at 85°C (Xi/Xt).

9. Statistical analyses

All statistical analyses were performed using SPSS 10.0 (SPSS, Chicago, Illinois, USA). Differences in photosynthetic rate, RWC, Chl content, and CA activity were tested using one-way analysis of variance (ANOVA) (p=0.05 or p=0.01) followed by Duncan’s multiple range test. Interactive effects of treatments and daily times were analyzed using a two-way ANOVA (UNIANOVA) (p=0.05).

Results

1. Relative water content of leaves

On 12 August, the plants in Ltd were still in drought, and their leaf relative water content (RWC) was significantly lower than that in the control (Fig. 2A).
However, RWC in Std and Mtd, which were rewatered, was slightly higher than that in the control. On 22 August, RWC was the highest in Ltd and lowest in the control. Generally, RWC in all treatments on 22 August was lower than that on 12 August, implying that RWC decreased gradually with plant growth.

2. Leaf area
As shown in Fig. 2B, leaf area was largest in Std among the treatments including the control, on both 12 August and 22 August, indicating that rewatering following short-term drought stress stimulated leaf growth but that following long-term drought did not; i.e., leaf growth was not recovered by rewatering.

3. Chlorophyll concentration and Chl a/b ratio
Table 1 shows the effects of different soil drought treatments on chlorophyll (Chl) concentration at two growth stages. These data showed significant differences among different soil drought treatments (p<0.01 for Chl a, a/b, but p<0.05 for Chl b). The concentrations of Chl a, Chl b and Chl a/b ratio in Ltd were 43.4, 28.6 and 21.2%, respectively, lower than those in the control, and 78.6, 29.3 and 37.1% respectively, higher than those in Mtd on 12 August. The concentrations of leaf Chl in both Std and Mtd treatments on 22 August were higher than those in the other treatment (p<0.05). The difference in the Chl concentrations in leaves between Mrd and Ltd and that between the control and Std treatments were not significant. As compared with the control, the concentrations of Chl a and b and a/b ratio in Ltd were 193.0, 54.8 and 97.6%, respectively, higher than those in the control.

4. Chlorophyll fluorescence parameters
Table 2 shows the effects of drought and rewatering on chlorophyll fluorescence. The maximal efficiency of PSII photochemistry (Fv/Fm) represents the most photochemical efficiency or primary efficiency of light energy transitions, the actual quantum yield (Φp) indicates the efficiency of transfer of absorbed photons to the reaction center of PSII, photochemical quenching coefficient (qP) represents the portion of light energy absorbed by PSII, and non-photochemical quenching coefficient (qN) is the portion of absorbed light energy by antenna pigment but not used in electron transport and dissipate as thermal energy. On 12 August, Fv/Fm, Φp, and qP in Ltd were 9.6, 48.4 and 33.3%, respectively lower, and qN in Ltd was 4.5% higher than those in the control, suggesting that long-term soil drought reduces PSII capacity. However, Fv/Fm, Φp, and qP were 4.2, 52.1 and 43.1%, respectively, higher and qN in Mtd was 14.2% lower than those in the control. These data showed significant differences among different soil moisture treatments (p<0.01 for Fv/Fm, Φp, and qP, but p<0.05 for qN). On 22 August, Fv/Fm, Φp, and qP in Ltd were 4.4, 27.0 and 15.9%, respectively, higher, and those in Std were 10.4, 101.2 and 40.8%, respectively, higher than those in the control. However, those in Mtd and qN in Ltd were 9.9 and 8.9%, respectively, lower than those in the control. These findings implied that rewatering, especially Ltd enhances PSII capacity.

5. Ion leakage from leaves
The liberation of ions from the cell to the extracellular space can be used as the index of membrane injury of the plant cell. As shown in Fig. 3A, ion leakage expressed as the percentage of total conductivity (Xi/Xt) in Std and control was 25.5% on the average, but that in Mtd and Ltd was 17.0% on the average in upper leaves, which was significantly lower than in the control and Std. In the lower leaves, similar results were observed but the difference was not significant. These results suggest a higher ability

| Treatment | Measured on 12 August 2002 | Measured on 22 August 2002 |
|-----------|---------------------------|---------------------------|
|           | Chl a (mg g⁻¹ FW) | Chl b (mg g⁻¹ FW) | Chl ratio (a/b) |
| Control   | 0.784±0.014b        | 0.440±0.033b        | 1.797±0.107b    |
| Std       | 0.760±0.015b        | 0.487±0.034ab       | 1.572±0.086b    |
| Mtd       | 1.400±0.113a        | 0.569±0.028a        | 2.464±0.177a    |
| Ltd       | 0.444±0.054c        | 0.314±0.037c        | 1.417±0.074b    |
| Std       | 0.372±0.020c        | 0.290±0.029b        | 1.229±0.058b    |
| Mtd       | 0.517±0.006b        | 0.341±0.001b        | 1.511±0.157b    |
| Ltd       | 1.009±0.081a        | 0.517±0.025a        | 1.951±0.103a    |
| Ltd       | 1.090±0.139a        | 0.449±0.053a        | 2.428±0.045a    |
to adapt to the change in soil water moisture in young leaves than in old leaves.

6. Carbonic anhydrase activity

The carbonic anhydrase (CA) activity in Ltd on 12 August was markedly affected by soil water stress, but that on 22 August was significantly stimulated by rewatering (Fig. 3B). The leaf CA activity on 12 August was highest in Mtd followed by Std, control and Ltd treatments in this order, but that on 22 August was highest in Ltd followed by Mtd, Std and control treatments.

7. Soluble protein and soluble sugar concentrations

On 22 August, Mtd treatment had the highest concentration of leaf soluble protein followed by Ltd, Std and the control (Fig. 4A). Compared with the control, soluble protein concentrations in leaves in Mtd and Ltd were 2.4- and 2.0-fold higher, respectively. The concentration of soluble sugar was also highest in Mtd followed by Ltd, Std and the control in this order (Fig. 4B). The concentration in Mtd and Ltd was 94.9 and 81.5%, respectively, higher than that in the control.

8. Diurnal change of leaf CO₂-exchange rate

Fig. 5A shows the diurnal patterns of net CO₂-exchange rate in leaves of Leymus chinensis measured on 8 August. Daily courses were similar for all treatment; there was a gradual increase from about 7:00 with a peak at about 8:00. Then it decreased and was lowest between 11:00 and 12:00, followed by an increase again with a peak at 14:00. Net photosynthetic rate was reduced to almost zero at 18:00, indicating that the respiratory rate of the leaves might be equal to CO₂-exchange rate without sunlight. The leaf respiration showed little fluctuation at night, and the net photosynthetic rate started to increase again at 4:00.
Fig. 5A also demonstrates the effects of soil drought and rewatering on net CO\(_2\)-exchange rate in leaves. There were significant differences in the CO\(_2\) exchange rate among the treatments. Mtd treatment showed the highest rate before 12:00, particularly between 8:00 and 11:00, but not after 13:00, indicating that rewatering stimulated leaf photosynthesis. Under soil drought conditions, the photosynthetic rate was greatly decreased. However, leaf respiratory rate at night was not significantly different among the treatments.

Fig. 5B presents the diurnal changes of leaf net CO\(_2\)-exchange rate in different soil drought treatments measured on 18 August. The leaf photosynthetic rate increased in the morning in all treatments. The leaf photosynthesis in Ltd treatment showed a higher rate, and the rate in the control treatment the lowest. After midday, the net CO\(_2\)-exchange rate decreased in all treatments although there was a minor peak in the afternoon. The net CO\(_2\)-exchange rate in leaves was close to zero at 7:00 p.m.

Statistical analysis by Univariate Analysis of Variance (UAV) indicated that the net photosynthetic rate significantly varied with the time of day, and the ratio in the upper and lower leaves were significantly different (Table 3). Ltd treatment increased the net CO\(_2\)-exchange rate from 7:00 to 11:00 by 35%
The univariate analysis of variance in the effects of soil moisture and daily time on net photosynthetic rate in upper and lower leaves of *Leymus chinensis*. Measured at 8:00, 9:00, 10:00 and 11:00 AM on 18 Aug 2002.

| Source              | df | Mean square | F     | p     |
|---------------------|----|-------------|-------|-------|
| Upper leaves        |    |             |       |       |
| Time                | 3  | 110.92      | 7.535 | 0.001 |
| Moisture            | 3  | 99.374      | 6.71  | 0.001 |
| Time × Moisture     | 9  | 45.855      | 3.155 | 0.008 |
| Error               | 32 | 14.720      |       |       |
| Lower leaves        |    |             |       |       |
| Time                | 3  | 84.15       | 2.91  | 0.05  |
| Moisture            | 3  | 0.930       | 0.032 | 0.992 |
| Time × Moisture     | 9  | 46.890      | 1.621 | 0.153 |
| Error               | 31 | 15.680      |       |       |

and 4.38% in upper and lower leaves, respectively, as compared with those in the control treatment. There were significant differences among different times, and treatments, and interaction between time and treatment, in the upper leaves (p<0.01), but no significant differences were observed in the lower leaves except at noon. Leaf respiration rates at night, were not greatly influenced by rewatering.

**Discussion**

Two measurement days (Aug 12 and 22) reflected different plant water conditions among different drought treatments. On 12 August, plants in Std and Mtd treatments were under rewatering, while those in Ltd treatment were still under drought stress. On 22 August, all plants were under rewatering, although they differed in duration of rewatering. Thus, Ltd plants on 12 August could be used to assess the effects of long-term drought, while Ltd plants on 22 August to assess the effect of rewatering after the stress. Chl a and Chl b contents and Chl a/b ratios could be used as indices of plant photosynthetic capacity (Johnston et al., 1989). The present results showed that long-term water stress reduced the Chl contents and Chl a/b ratio (Table 1), that represents leaf senescence and a decrease in energy available for the photosystems (Johnston et al., 1989). Carbonic anhydrase (CA) is involved in the first step of CO₂ fixation in leaf mesophyll cells (O’Leary, 1982; Hatch and Burnell, 1990). The present result showed that CA activity decreased when plants were subjected to long-term soil water stress, which is consistent with the result for the mature leaves of rice (*Oryza sativa* L) (Dai et al., 2000). However, Hatch and Burnell (1990) reported that the activity of the enzyme might be just for the conversion of CO₂ to HCO₃⁻ in mesophyll cells, and does not limit photosynthesis. Thus, the role of CA in drought acclimation needs to be assessed in the future.

Under field conditions, plants often suffer from damage including photoinhibition and photooxidation due to soil drought stress (Chaves et al., 2003). Rainfall occurs after various durations of dry periods in natural fields. The drought treatments in this study simulated drought conditions in natural fields, and the results may represent the plant responses to drought stress in natural fields. The chlorophyll fluorescence parameters measured on plant leaves may be used to assess of PSII capacity (van Kooten and Snel, 1990; Taub et al., 2000; Lu et al., 2003). This experiment showed that rewatering increased PSII capacity. Severe soil drought resulted in cell-membrane damage (Benhassaine-Kesri et al., 2002), and a decrease in leaf photosynthetic capacity (García and Lamattina, 2001; Xu and Zhou, 2006b). However, rewatering after long-term drought may repair the damage and stimulate the well photosynthesis. The other studies indicated that elevated CO₂ could protect photosynthesis against high-temperature and drought damage (Hamerlynck et al., 2000; Taub et al., 2000), and water stress or salinity increased the ability to protect plant from high temperatures (Lu and Zhang, 1999; Lu et al., 2003; Qiu and Lu, 2003). However, extreme drought weakens the photosynthetic adaptation to a high temperature (Xu and Zhou, 2005a; Xu and Zhou, 2006a), and the leaf photoprotective mechanism is associated with leaf temperature change, depending on plant species (Inamullah and Isoda, 2005). The adaptive mechanisms may be involved in the stress history of the plant. There is evidence that plants, which have been previously exposed to water stress, show an improved tolerance to water stress through increase in solute levels (Takami et al., 1981; Morgan, 1984). In the present study, the concentrations of soluble protein and sugars in the leaves were higher in the plants previously subjected to soil drought than in those grown in ample soil moisture. The drought treatment followed by rewatering also increased Chl a/b ratio, CA activity and PSII capacity. The osmoregulation under drought treatment may have increased the tolerance to drought.

The present results showed that a decrease in leaf physiological capacity (activity) by long-term drought might be overcome by rewatering. The capacity (activity) can be increased over that in well-watered treatment (control). Investigations on the stimulating effect of drought treatment followed by rewatering in wheat by Liu et al. (2001) indicated that severe water stress increased the leaf area more greater than the moderate water stress. Recently, Siopongco et al. (2005, 2006) reported that the stimulation of rice plant growth by drought followed by rewatering depended on the genotype. In the present study, although
rewatering stimulated Chl content, Chl a/b ratio, and CA activity, the leaf growth of the plants subjected to rewatering following long-term water stress was inferior to that of the control. It is suggested that leaf expansion is more sensitive to water stress than many other physiological processes in *Leymus chinensis*. This point highlights the difference between the responses of plant growth and some physiological processes to soil moisture.

Note that we reported here only the experiment, the physiological responses of the grassland plant at juvenile stage. The responses at adult stage with rhizomes and extensive root system may be largely different. On the other hand, the plant growth in pots is different from that in the field, because of different environmental conditions, such as temperature, light. The limited volume of soil in pots might also be responsible (Kang et al., 2002). However, the data may be comparable among different soil water treatments because all plants were grown in the same size pots and under same environmental conditions. Thus, the present results may provide insight into underlying mechanism of the grassland plant in response to soil drought and rewatering at the juvenile stage. However, more investigation is required in the field at different plant growth stages in the future.

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**References**

Bai, Y., Han, X., Wu, J., Chen, Z. and Li, L. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. Nature 431 : 181-184.

Benhassaine-Kesri, G.B., Aid, F., Demandre, C., Kader, J.C. and Mazliak, P. 2002. Drought stress affects chloroplast lipid metabolism in rape (*Brassica napus*) leaves. Physiol. Plant. 115 : 221-227.

Bradford, M.M. 1976. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 : 248-254.

Carlen, C., Kölliker, R. and Nösberger, J. 1999. Dry matter allocation and nitrogen productivity explain growth responses to photoperiod and temperature in forage grasses. Oecologia 121 : 441-446.

Chaves, M.M., Maroco, J.P. and Pereira, J.S. 2003. Understanding plant responses to drought - from genes to the whole plant. Funct. Plant Biol. 30 : 239-264.

Cruz, L.J., Cagampang, G.B. and Juliano, B.O. 1970. Biochemical factors affecting protein accumulation in the rice grain. Plant Physiol. 46 : 743-747.

Dai, X.B., Zhai, H.Q., Zhang, H.S. and Zhang, R.X. 2000. Effect of soil drought stress on photosynthetic rate and carbonic anhydrase activity of leaf. Acta Phytophysiol. Sin. 26 : 133-136.

Distel, R.A., Peláez, D.V., Bóo, R.M., Mayor, M.D. and Elia, O.R. 1996. Growth of *Prosopis caldenia* seedling in the field as related to grazing history of the site and in a greenhouse as related to different levels of composition from *Stipa tenus*. J. Arid Environ. 32 : 251-257.

Garcia, M.C. and Lamattina, L. 2001. Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiol. 126 : 1196-1204.

Güttman, M.R., Arnozis, P.A. and Barneix, A.J. 1991. Effect of source-sink relations and nitrogen nutrition on senescence and N remobilization in the flag leaf of wheat. Physiol. Plant. 82 : 278-284.

Hamerlynck, E.P., Huxman, T.E., Loik, M.E. and Smith, S.D. 2000. Effects extreme high temperature, drought and elevated CO₂ on photosynthesis of the Mojave Desert evergreen shrub, *Larrea tridentata*. Plant Ecol. 148 : 183-193.

Hatch, M.D. and Burnell, J.N. 1990. Carbonic anhydrase activity in leaves and its role in the first step of C₄ photosynthesis. Plant Physiol. 93 : 825-828.

Havaux, M. 1992. Stress tolerance of photosystem II in vivo. Antagonistic effects of water, heat, and photoinhibition stress. Plant Physiol. 100 : 424-432.

Hsiao, T.C. 1973. Plant responses to water stress. Ann. Rev. Plant Physiol. 24 : 519-570.

Inamullah and Isoda, A. 2005. Adaptive responses of soybean and cotton to water stress II. Changes in CO₂ assimilation rate, chlorophyll fluorescence and photochemical reflectance index in relation to leaf temperature. Plant Prod. Sci. 8 : 131-138.

Johnston, M., Grof, C.P.L. and Brownell, P.E. 1989. Chlorophyll a/b ratios and photosystem activity of mesophyll and bundle sheath fractions from sodium-deficient C₄ plants. Aust. J. Plant Physiol. 16 : 449-457.

Kaiser, W.M. 1987. Effects of water deficit on photosynthetic capacity. Physiol. Plant. 71 : 142-149.

Kang, S.Z., Shi, W.J., Cao, H.X. and Zhang, J.H. 2002. Alternate watering in soil vertical profile improved water use efficiency of *Zea mays*. Field Crops Res. 77 : 31-41.

Kolb, K.J. and Sperry, J.S. 1999. Transport constraints on water use by the great basin shrub, * Artemisia tridentata*. Plant Cell Environ. 22 : 925-935.

Kröpfl, A.I., Cecchiw, G.A., Villasuso, N.M. and Distelz, R.A. 2002. The influence of *Larrea divaricata* on soil moisture and on water status and growth of *Stipa tenus* in southern Argentina. J. Arid Environ. 52 : 29-35.

Lichtenthaler, H.K. and Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. T. 603 : 591-592.

Liu, X.Y., Luo, Y.P. and Shi, Y.C. 2001. The stimulating effects of rewetting in subjecting to water stress on leaf area of winter wheat. Sci. Agr. Sin. 34 : 422-428.

Lu, C. and Zhang, J. 1999. Effects of water stress on photosystem II photochemistry and its thermostability in wheat plants. J. Exp. Bot. 50 : 1199-1206.

Lu, C., Qiu, N., Wang, G.S. and Zhang, J.H. 2003. Salinity...
treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte *Suaeda salsa*. J. Exp. Bot. 54 : 851-860.

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. Ann. Rev. Plant Physiol. 35 : 299-319.

O’connor, T.G., Haines, L.M. and Snyman, H.A. 2001. Influence of precipitation and species composition on phytomass of a semi-arid African grassland. J. Ecol. 89 : 850-860.

O’Leary, M. 1982. Phosphoeno/pyruvate carboxylase : an enzymologist’s view. Ann. Rev. Plant Physiol. 33 : 297-315.

Qiu, N. and Lu, C. 2003. Enhanced tolerance of photosynthesis against high temperature damage in salt-adapted halophyte *Atriplex centralasiatica* plants. Plant Cell Environ. 26 : 1137-1145.

Shah, N.H. and Paulsen, G.M. 2003. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant Soil. 257 : 219-226.

Siopongco, J.D.L.C., Yamauchi, A., Salekdeh, H., Bennett, J. and Wade, L.J. 2005. Root growth and water extraction response of doubled-haploid rice lines to drought and rewatering during the vegetative stage. Plant Prod. Sci. 8 : 497-508.

Siopongco, J.D.L.C., Yamauchi, A., Salekdeh, H., Bennett, J. and Wade, L.J. 2006. Growth and water use response of doubled-haploid rice lines to drought and rewatering during the vegetative stage. Plant Prod. Sci. 9 : 141-151.

Takami, S., Turner, N.C. and Rawson, H.M. 1981. Leaf expansion of four sunflower (*Helianthus annuus* L.) cultivars in relation to water deficits. I. Patterns during plant development. Plant Cell Environ. 4 : 399-407.

Taub, D.R., Seemann, J.R. and Coleman, J.S. 2000. Growth in elevated CO₂ protects photosynthesis against high-temperature damage. Plant Cell Environ. 23 : 649-656.

Turner, N.C. 1981. Techniques and experimental approaches for the measurement of plant water status. Plant Soil 58 : 339-366.

van Kooten, O. and Snel, J.F.H. 1990. The use chlorophyll fluorescence nomenclature in plant stress physiology. Photosynth. Res. 25 : 147-150.

Yang, J.C., Zhang, J.H., Wang, Z.Q., Zhu, Q.S. and Wang, W. 2001. Hormonal changes in the grains of rice subjected to water stress during grain filling. Plant Physiol. 127 : 515-323.

Yemm, E.W. and Willis, A.J. 1954. The estimation of carbohydrates on plant extracts by anthrone. Biochem. J. 57 : 508-514.

Zhou, G.S., Wang, Y.H. and Wang, S.P. 2002. Responses of grassland ecosystems to precipitation and land use along the Northeast China Transect. J. Veg. Sci. 13 : 361-368.

Zhou, Z., Sun, O.J., Huang, J.H., Gao, Y. and Han, X. 2006. Land use affects the relationship between species diversity and productivity at the local scale in a semi-arid steppe ecosystem. Funct. Ecol. 20 : 753-762.