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Water relations, gas exchange, photochemical efficiency, and peroxidative stress of four plant species in the Heihe drainage basin of northern China

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Abstract

Haloxylon ammodendron, *Calligonum mongolicum*, *Elaeagnus angustifolia*, and *Populus hosiensis* had different adaptations to limited water availability, high temperature, and high irradiance. *C. mongolicum* used water more efficiently than did the other species. Because of low transpiration rate (*E*) and low water potential, *H. ammodendron* had low water loss suitable for desert conditions. Water use efficiency (WUE) was high in *E. angustifolia*, but high *E* and larger leaf area made this species more suitable for mesic habitats; consequently, this species is important in tree shelterbelts. *P. hosiensis* had low WUE, *E*, and photosynthesis rates, and therefore, does not prosper in arid areas without irrigation. High irradiances caused photoinhibition of the four plants. The decrease of photochemical efficiency was a possible non-stomata factor for the midday depression of *C. mongolicum*. However, the species exhibited different protective mechanisms against high irradiance under drought stress. *H. ammodendron* and *C. mongolicum* possessed a more effective antioxidant defence system than *E. angustifolia*. These three species showed different means of coping with oxidative stress. Hence an enzymatic balance is maintained in these plants under adverse stress conditions, and the concerted action of both enzymatic and non-enzymatic reactive oxygen species scavenging mechanisms is vital to survive adverse conditions.

Additional key words: active oxygen; antioxidative ability; desert plants; drought stress; mesophytic plants; photochemical efficiency; photosynthesis; stomatal conductance; transpiration rate; water use efficiency.

Introduction

Plants have developed physiological responses, in addition to ecological strategies, to cope with water shortages by either stress avoidance or stress tolerance. These responses allow plants to survive and even to continue growth under adverse conditions. Species in arid areas adapt to their environment through different eco-physiological responses such as regulating productivity through gas exchange and carbon assimilation (Manes *et al.* 1998), reducing leaf water potential (Ψ_l) and having sensitive stomatal control (Deng *et al.* 2002, Flexas and Medrano 2002), and regulating the net photosynthetic rate (P_N) and translation, and photosystem 2 (PS2) efficiency under high light and temperature stress (Jiang and Zhu 2001, Liu *et al.* 2003).

Every year, environmental stresses cause considerable losses in crop quality and productivity. One of the major mechanisms causing plant damage during adverse environmental conditions is the excess production of active oxygen species (AOS), such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH), a general phenomenon that creates oxidative stress (Bowler and Van 1992). In photosynthetic

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Abbreviations: AOS – active oxygen species; APX – ascorbate peroxidase; AsA – ascorbic acid; CAT – catalase; C_i – intercellular CO_2; C_i/C_a – intercellular CO_2 concentration/ambient CO_2; Chl – chlorophyll; E – leaf transpiration rate; F_v/F_m – maximal photochemical efficiency of photosystem; g_s – stomatal conductance; GSH – reduced glutathione; H_2O_2 – hydrogen peroxide; OH – hydroxyl radical; MDH – malondialdehyde; P_N – net photosynthetic rate; POD – peroxidase; PPFD – photosynthetic photon flux density; PS – photosystem; ROS – reactive oxygen species; SOD – superoxide dismutase; T_a – air temperature; T_l – leaf temperature; TCA – trichloroacetic acid; VPD – vapour pressure deficit; WUE – water use efficiency; Ψ_l – water potential.
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organisms, environmental factors such as excessive irradiation or high temperature, particularly in combination with high iradiance and drought, can create oxidative stress; this causes oxidative injury to plant cells by increased production of reactive oxygen species (ROS), including O_2^-, singlet oxygen ($^1O_2$), H_2O_2, and OH (Asada 1999). To defend against oxidants, plants increase contents of their antioxidant defensive enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and other compounds such as glutathione, tocopherols, carotenoids, and ascorbic acid (AsA) (Asada and Takahashi 1987, Halliwell and Gutteridge 1989). In most cases, these defensive mechanisms work in combination to reduce contents of ROS in the cells. Generally, stress-tolerant species have a more effective defensive system against ROS than do stress-susceptible species. Defensive systems can be activated continuously or induced through exposure to oxidative stress (Buchanan *et al.* 2000). In desert areas, conditions are usually adverse and characterised by drought, high temperature, high irradiance, and hot, dry air. However, many desert plants can survive and reproduce by adapting to these conditions (Li *et al.* 2003). It is important to understand both the ecological strategies and the mechanisms of physiological and biochemical plant responses to their natural habitats. There is a large body of literature regarding the various aspects of life of desert plants. However, field studies that consider ecological strategies and physiological and biochemical responses are scarce, especially for desert plants.

The HeXi corridor is one of the most seriously desertified areas of China. Moving sand dunes and blown sand on the Gobi surface continually encroach on the oasis, threatening living conditions. Thus, it is crucial to control desertification and reclaim degraded areas. The goals of this work were to study the ecological strategies and physiological and biochemical adaptations of four local species under limited water availability, high temperature, and high irradiance; examine the responses of water relations, gas exchange, and anti-oxidative systems to adverse field conditions, and determine the nature of interactions among environmental factors as they affect gas exchange. We also examined the characteristics of mesophytic plants growing in the study area in comparison to the desert plants.

Materials and methods

Study site: Studies were conducted at the edge of the Linze Oasis in the HeXi corridor, adjacent to the Badain Juran desert of northern China (38°57'N, 9°51'E). This area has a temperate arid climate; the mean annual precipitation and latent evaporation are 118 and 2 390 mm, respectively, with 65 % of the total precipitation occurring between July and September. The mean annual temperature is 7.7 °C, with a maximum and minimum of 25.1 and –11.1 °C, respectively. The frost-free period is 179 d, and the average total number of sunlight hours reaches 3 045 annually.

Plants: *Haloxylon ammodendron*, *Calligonum mongolicum*, *Elaeagnus angustifolia*, and *Populus hosiensis* were selected for this study. *H. ammodendron* and *C. mongolicum* are widely distributed over the desert regions of China; at our study site, these are the main sand-binding species on sand dunes around the oasis edge. As one of the most important sand-fixing species in northern China, the mesophyte *E. angustifolia* develops in flat sands and grows naturally near the inland river. *P. hosiensis* is a fast-growing species of the shelterbelt. From 1976 to 1979, in order to protect oases and reclaim desert, *H. ammodendron* and *C. mongolicum* were planted on sand dunes, and *E. angustifolia* and *P. hosiensis* were planted on flat sand.

Field measurements and sampling were conducted near the Linze Desert Experimental Station on 20 and 22 July 2001, under sunlit conditions. During measurement and sampling, the maximum photosynthetic photon flux density (PPFD), and air temperature (T_a) during measurements of *H. ammodendron*, *C. mongolicum*, *E. angustifolia*, and *P. hosiensis* were 2 014, 2 046, 1 945, and 1 921 μmol m^{-2} s^{-1} and 39.36, 39.32, 38.53, and 38.99 °C, respectively (Fig. 1*A*,*B*).

Soil water content was determined from soil samples taken from the centre of the root zone (a depth of 180 cm) on the dates of measurement (Table 1). The samples were dried to constant mass at 105 °C and the mass was expressed as a percentage of the water content. The water content of forest belt soils was higher than that of sand dune soils.

Table 1. Soil water content [%] of four plant species at different depths.

Species	Depth [cm] 0–10	10–20	20–40	40–60	60–80	80–100	100–120	120–140	140–160	160–180
Haloxylon ammodendron *Calligonum mongolicum*	1.48	0.41	0.59	0.84	1.57	1.69	1.85	1.67	1.82	2.03
Elaeagnus angustifolia *Populus hosiensis*	1.28	0.55	0.79	1.52	2.07	2.1	3.55	3.5	1.73	2.14

Physiological measurements: Daily courses of stomatal conductance (g_s), leaf transpiration rate (E), P_N, air temperature (T_a), leaf temperature (T_l), photosynthetic photon flux density (PPFD), vapour pressure deficit (VPD), and intercellular CO_2 (C_i) were measured using a portable leaf chamber in an open system (*Li-6400*; *Li-COR*, Lincoln, NE, USA) throughout the day on clear days. Instantaneous water use efficiency (WUE) was estimated using the relationship P_N/E. The diurnal course of gas exchange was monitored at 1-h intervals; five replications were measured for the current mature foliage on each individual (four individuals per species). After gas exchange was measured on attached leaves, the leaves were excised for determination of Ψ_1 using a pressure chamber (*ZLZ-4*, China) at 06:00, 10:00, 14:00, and 18:00 (Beijing time). Mature leaves were excised and soaked in water. After recording fresh masses, plant samples were oven-dried at 80 °C and the dry masses were determined. Water deficit was calculated according to Huang *et al*. (2000).

Chlorophyll (Chl) fluorescence was measured on adequately dark-adapted leaves using a portable plant efficiency analyser (*PEA*, *Hansatech*, King's Lynn, UK). F_0 (minimal fluorescence), F_m (maximal fluorescence), F_v (variable fluorescence), and F_v/F_m were determined in the field on attached leaves.

After measuring eco-physiological characteristics, samples were collected and frozen in liquid nitrogen for biochemical analyses.

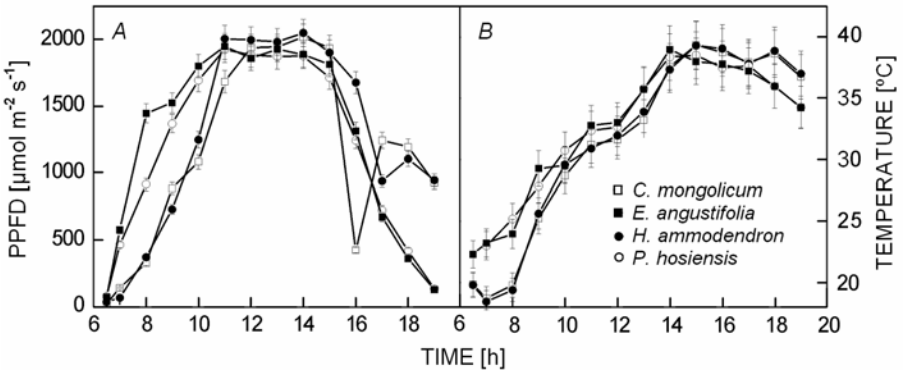

Fig. 1. Diurnal changes in photosynthetic photon flux density (PPFD) and air temperature. Means ± SE of four individuals for each species.

Lipid peroxidation was estimated by measuring the malondialdehyde (MDA) content (Dhindsa and Matowe 1981). Samples (0.1 g) were ground in liquid nitrogen and dissolved in 2 cm^3 of 20 % trichloroacetic acid (TCA) and 0.5 % thiobarbituric acid. The solution was heated for 30 min at 90 °C and then cooled on ice. The homogenate was centrifuged at 10 000×g for 10 min at 4 °C and the supernatant was measured at 532 and 600 nm using MDA as a standard.

H_2O_2 contents in leaves were determined as described by Mukherjee and Choudhuri (1983). Samples (1 g) were ground with cold acetone and then centrifuged at 5 000×g for 10 min. After adding 0.1 cm^3 of 5 % titanium-sulphate and 0.2 cm^3 of ammonia to 1 cm^3 of the supernatant, the samples were further centrifuged at 3 000×g for 10 min. The pellet was dissolved in 2 M H_2SO_4 and measured at 415 nm. A standard curve was prepared based on solutions with different concentrations of H_2O_2.

O_2^- in leaves was estimated as described by Wang and Luo (1990).

Enzyme assays: Samples (1 g) were ground in liquid nitrogen and extracted in 5 cm^3 of 50 mM potassium phosphate buffer (pH 7.0) containing 1 % (m/v) polyvinylpyrrolidone and 10 mM thioglycollic alcohol. The extraction was then centrifuged for 20 min at 15 000×g at 4 °C. The resulting supernatant was used for the various enzyme assays.

The assay for total superoxide dismutase (SOD, EC 1.15.1.1) activity was based on the method described by Pan *et al*. (1995). One unit of SOD activity was defined as the amount of enzyme required to inhibit the reduction of nitroblue tetrazolium (NBT) by 50 %. The reaction mixture (3 cm^3) contained 50 mM potassium phosphate buffer (pH 7.8) with 0.1 mM ethylene diaminotetraacetic acid (EDTA), 2.25 µM NBT, 39 mM methionine, 2 µM riboflavin, and 25 mm^3 of enzyme extract. Reactions were performed at 25 °C under irradiance sufficient to cause an increase for 10 min. The reaction was finished in the dark and was deducted from A_{560}.

For the measurement of CAT activity, 50 mm^3 of supernatant was added to 150 mM potassium phosphate buffer (pH 7.0) and 15 mM H_2O_2. The consumption of H_2O_2 by CAT was detected by measuring the decrease in absorbance at 240 nm for 2–4 min at 25 °C (Zeng *et al.* 1991).

Ascorbate peroxidase (APX) activity was determined as described by Nakano and Asada (1981) by measuring the decrease in A_{290} due to ascorbic acid (AsA) oxidation (E = 2.8 mM^{-1} cm^{-1}). The reaction mixture (3 cm^3)

contained 50 mM potassium phosphate buffer (pH 7.0), 0.3 mM EDTA, 2 mM H_2O_2, 1.5 mM AsA, and 25 mm^3 of enzyme extract.

The total soluble protein content was determined using the Coomassie blue-dye binding assay (Bradford 1976). A standard curve of protein quantity was obtained using bovine serum albumin.

Peroxidase (POD) activity was measured by following the change in absorption at 470 nm due to guaiacol oxidation (Liu and Zhang 1994). The reaction solution (3 cm^3) was composed of 100 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 20 mm^3 H_2O_2, and 100 mm^3 of enzyme extract.

Reduced glutathione (GSH) content was measured as described by Guri (1983). Samples (1 g) were ground in extraction solution of 5 mM EDTA–3 % TCA, and were then centrifuged at 5 000×*g* for 10 min. The supernatant (1 cm^3) was added to 1.2 cm^3 of 0.1 M NaOH, 2.8 cm^3 of 0.2 M potassium phosphate buffer (pH 7.0), and 50 cm^3 of dithiobis-2-nitrobenzoic acid. The absorbance was measured at 412 nm after 30 min.

AsA content was measured as described by Arakawa *et al.* (1981). Samples (1 g) were ground in 5 cm^3 of 5 % TCA, and were then centrifuged at 5 000×*g* for 10 min. The supernatant (1 cm^3) was diluted to 5 cm^3 and 1 cm^3 of DCIP was added to 3 cm^3 of the dilution. The absorbance was measured at 600 nm.

Data analysis: Measurements for each species were compared using analysis of variance (ANOVA) in SPSS. Differences among the species were evaluated using the least significance difference (LSD) method with a significance level of $p<0.05$.

Results

Water relations: The year 2001 was the driest of the last 50 years, especially between January and June (Table 2). Only 7.0 mm of precipitation had fallen in this area since January 2001 and before the experiment. Therefore, the plants had been subjected to serious drought stress.

The four study species showed different degrees of water deficit and had undergone obvious diurnal changes. The water deficit of the mesophytic plants (*P. hosiensis* and *E. angustifolia*) fluctuated widely; at noon the water deficit was higher than that of the desert plants (*C. mongolicum* and *H. ammodendron*; Fig. 2*A*). The Ψ_1 of all four species decreased from 06:00 and reached its lowest at about 14:00. *H. ammodendron* had lower water potential than the other species (Fig. 2*B*). The diurnal changes in *E* of *E. angustifolia* and *C. mongolicum* showed two and one peaks, respectively, whereas those of *H. ammodendron* and *P. hosiensis* fluctuated gently (Fig. 2*C*). The first peak in *E* of *E. angustifolia* appeared at about 09:00 and the second one at about 14:00, while the peak in *C. mongolicum* occurred at about 15:00. Of the four species, *E. angustifolia* had the highest *E*, followed by *C. mongolicum*; *P. hosiensis* had the lowest *E* ($p<0.05$). WUE was highest at about 08:00 h and lowest at about 16:00 h under high heat and low humidity. The daily mean WUEs were in the order: *C. mongolicum* > *E. angustifolia* > *H. ammodendron* > *P. hosiensis* (Fig. 2*D*).

Table 2. Precipitation [mm] in the Linze area.

Month	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
Precipitation in 2001	0.5	0	0	3.8	1.4	1.3	29.1	4.3	39.7	11.4	2.0	1.4	94.9
Mean annual precipitation	1.6	0.8	4.1	4.2	10.6	15.5	30.6	21.7	17.2	4.0	2.0	1.2	113.5

Gas exchange and Chl fluorescence: The diurnal pattern of photosynthesis in *C. mongolicum*, *H. ammodendron,* and *E. angustifolia* showed two peaks, indicating a midday depression around noon, whereas *P. hosiensis* had one peak in photosynthetic rate. The peak in P_N of the mesophytes (*E. angustifolia* and *P. hosiensis*) occurred earlier than that of the xerophytes (*C. mongolicum* and *H. ammodendron*; Fig. 3*A*). Among the four species, *C. mongolicum* had the highest P_N, with a maximum of 58.6 % over that of *P. hosiensis* ($p<0.05$), which had the lowest rate.

Diurnal changes in g_s in the four species indicated that closed stomata opened gradually with increasing irradiance and temperature in the morning (Fig. 3*B*). The g_s reached a maximum at about 09:00 in *C. mongolicum*, *E. angustifolia*, and *P. hosiensis* (08:00 in *H. ammodendron*), and then decreased until sunset, when stomata closed gradually. The g_s in *E. angustifolia* and *C. mongolicum* increased between 12:00 and 14:00 and between 11:00 and 12:00, respectively. Diurnal changes in g_s in *E. angustifolia* had a tendency similar to that of the diurnal pattern of P_N.

Intercellular CO_2 concentration/ambient CO_2 (C_i/C_a) of *C. mongolicum* increased from 12:00 to 13:00, but C_i decreased with the occurrence of the midday depression in photosynthesis (Fig. 3*A*,*C*), suggesting that the midday depression was attributable primarily to a reduced biochemical capacity for photosynthesis, rather than to an increased stomatal limitation of photosynthesis. Thus, non-stomatal factors led probably to photosynthetic depression. In *E. angustifolia*, the decrease in P_N was accompanied by decreases in C_i/C_a and C_i, suggesting

that stomatal closure was responsible for the midday photosynthetic depression.

The photochemical efficiency (F_v/F_m) in leaves reached a low at dawn and sunset, and a minimum around noon, indicating that photoinhibition occurs in these species. Photoinhibition in the mesophytes (*E. angustifolia* and *P. hosiensis*) occurred earlier than in the two desert plants (*C. mongolicum* and *H. ammodendron*; see Fig. 3*D*). The lowest F_v/F_m in *E. angustifolia* and *P. hosiensis* appeared at 10:00 h, whereas those in *C. mongolicum* and *H. ammodendron* occurred at 13:00 and 15:00, respectively.

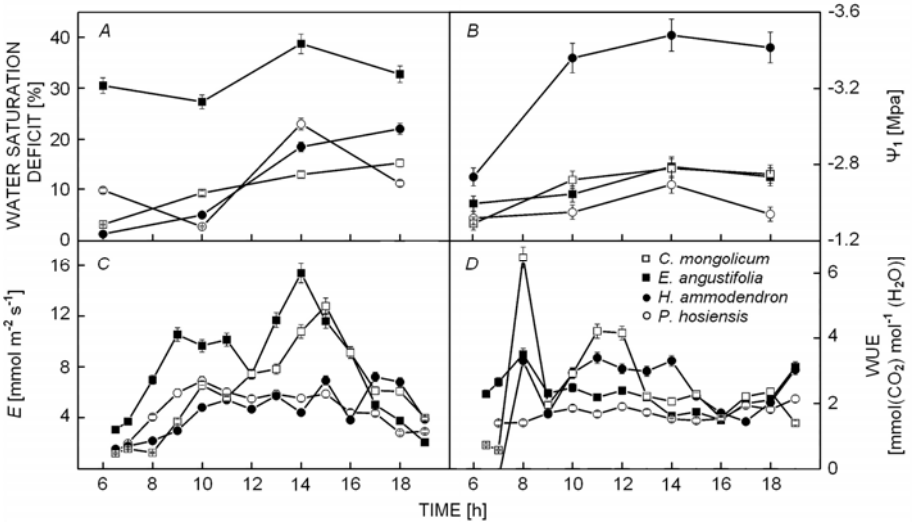

Fig. 2. Diurnal changes in water deficit (*A*), water potential (*B*), transpiration rate (*C*), and water use efficiency (*D*) for four plant species during the day. Means ± SE of four individuals for each species.

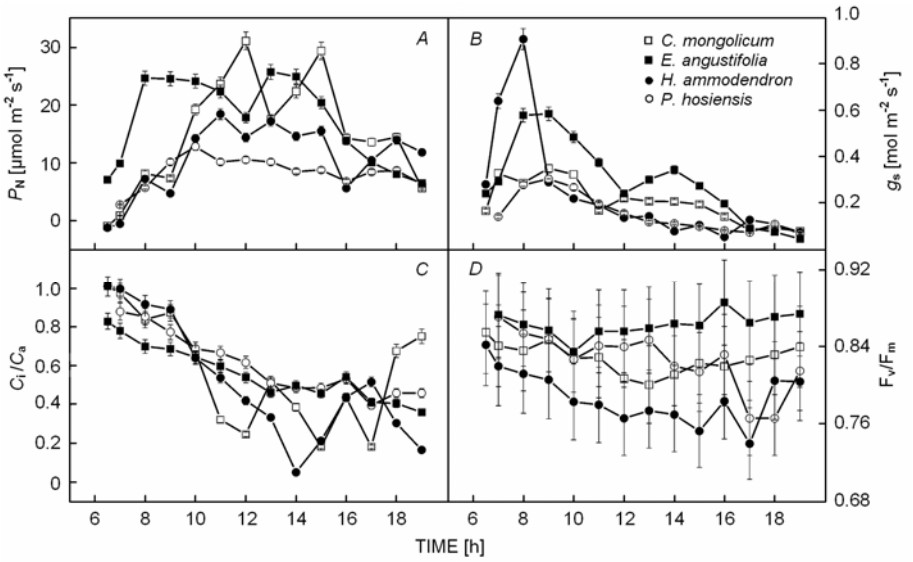

Fig. 3. Diurnal changes in net photosynthetic rate (*A*), stomatal conductance (*B*), intercellular CO_2 concentration/ambient CO_2 concentration, C_i/C_a (*C*), and photochemical efficiency of photosystem 2, F_v/F_m (*D*). Means ± SE of four individuals for each species.

Oxidative stress: From the eco-physiological characteristics of these species, we conclude that *P. hosiensis* has significantly lower WUE, *E*, and P_N than the other species, suggesting that it cannot flourish without irrigation. Therefore, we analysed oxidative stress and activities of AOS-scavenging enzymes in the other three species that are able to produce forests without irrigation.

The changes in MDA content differed among the three species with changes in Ψ_1 and temperature (Fig. 4*A*). MDA content increased with a decrease in Ψ_1 before 10:00, and then decreased continually until 18:00, except in *E. angustifolia*, which began to increase at 14:00. Therefore, the anti-oxidative ability of *E. angustifolia* was weaker than that of *H. ammodendron* or

C. mongolicum. These species have different means of coping with oxidation stress. Hence membranes are damaged to different degrees under adverse conditions; damage to the membranes of *E. angustifolia* was more severe than to those of *H. ammodendron* and *C. mongolicum*.

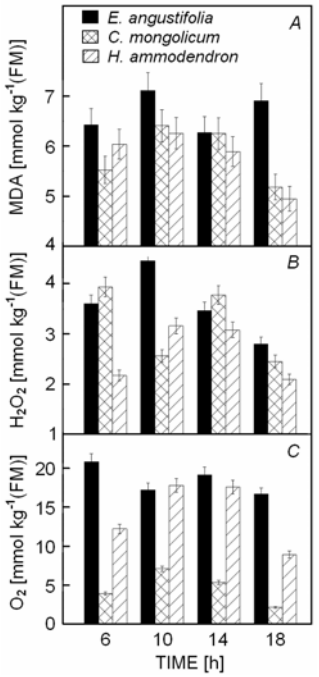

Fig. 4. Diurnal changes in malondialdehyde, MDA (*A*), H_2O_2 (*B*), and O_2^- (*C*) contents in three plant species during the day. Means ± SE of four individuals for each species.

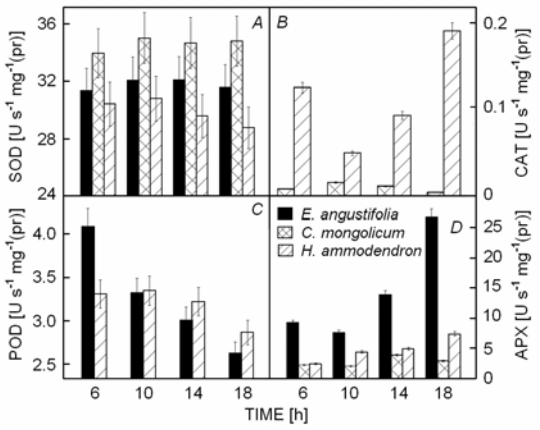

Fig. 5. Diurnal changes in superoxide dismutase, SOD (*A*), catalase, CAT (*B*), peroxidase, POD (*C*), and ascorbate peroxidase, APX (*D*) activities in three plant species during the day. Means ± SE of four individuals for each species.

Changes in H_2O_2 content indicate the status of the ROS-cleansing capacity of plants under oxidative stress. The content of H_2O_2 in *E. angustifolia* and *H. ammodendron* increased with increasing water and temperature stress and reached a maximum at 10:00, and then decreased gradually. The H_2O_2 content of the three species followed different daily patterns. H_2O_2 was highest in *E. angustifolia* and fluctuated greatly in *C. mongolicum*; in all species, however, H_2O_2 reached a minimum at 18:00 (Fig. 4*B*).

All three species accumulated O_2^- to different degrees. *E. angustifolia* had the highest O_2^- content at 1.3 and 4.0 times that of *C. mongolicum* and *H. ammodendron,* respectively (p<0.05; Fig. 4*C*).

Activity of AOS-scavenging enzymes: SOD activity in *C. mongolicum* was much higher than in *H. ammodendron* and *E. angustifolia* (p<0.05), resulting in a lower O_2^- content. SOD activity in the three species did not show any significant change with changes in water potential (Fig. 5*A*), indicating that SOD was not the only O_2^- scavenger. CAT is an enzyme with high capacity but low oxygen affinity that destroys hydrogen peroxide. Its activity in *H. ammodendron* was 11.5 times higher than in *C. mongolicum* (p<0.05; Fig. 5*B*). CAT activity in *E. angustifolia* could not be assayed. In *C. mongolicum*, CAT activity increased with a decrease in water potential and reached a maximum at 10:00, after which it gradually decreased; this resulted from protein de-naturation or impedance of CAT biosynthesis. Thus, CAT was not the only enzyme operating to eliminate H_2O_2. However, CAT activity in *H. ammodendron* decreased with increasing stress to a low at about 10:00; thereafter, CAT rose gradually.

Changes in POD activity in *E. angustifolia* and *H. ammodendron* showed similar tendencies under similar water stress. POD activity decreased with increasing water stress (Fig. 5*C*); POD activity was not measured in *C. mongolicum*. APX activity in *E. angustifolia* was at 5.3 and 3.0 times higher that in *H. ammodendron* and *C. mongolicum,* respectively (p<0.05; Fig. 5*D*). All three species showed obvious diurnal variation in APX activity. At about 10:00, APX activity reached a loss, but the H_2O_2 content continued to rise, indicating that APX is not activated by decreases in water potential. Subsequent increases in APX and reductions in H_2O_2 indicated that APX was the main enzyme to eliminate H_2O_2 in *E. angustifolia*. APX activity in *H. ammodendron* increased with decreases in Ψ_1 to reduce H_2O_2 accumulation, scavenging the AOS, reducing oxygen, and subsequently reducing lipid peroxidation. APX activity changed slightly in *C. mongolicum*.

GSH is an antioxidant that eliminates H_2O_2. Among the three species, diurnal changes in GSH content in *C. mongolicum* were opposite to those of H_2O_2 and fluctuated greatly with changes in water stress (Fig. 6*A*). Because CAT activity in *C. mongolicum* was low and decreased after 10:00, GSH was the major antioxidant used to cleanse H_2O_2. The GSH content in *E. angustifolia* was low at 10:00, increased until 14:00, and then declined until 18:00, but the H_2O_2 content peaked at 10:00 and then decreased continuously. This indicates that GSH eliminated H_2O_2. In *H. ammodendron,* the GSH content followed the same diurnal pattern as H_2O_2, indicating that GSH is not the main antioxidant in this species.

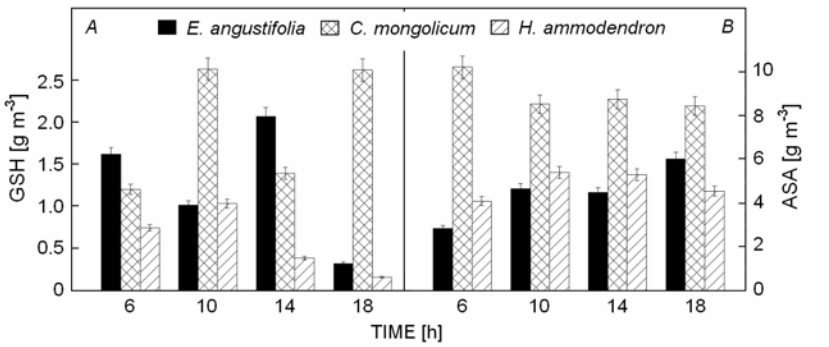

Fig. 6. Diurnal changes in reduced glutathione, GSH (*A*) and ascorbic acid, AsA (*B*) contents in three plant species during the day. Means ± SE of four individuals for each species.

As an antioxidant in plants, AsA not only protects cell damage resulting from the lipid peroxidation, but also rapidly reduces AOS such as O_2^- and OH^-, and regenerates V_E (α-tocopherol), which correlates with the improved stress-reducing antioxidative capability. The AsA content in *C. mongolicum* was higher than in *H. ammodendron* and *E. angustifolia* (p<0.05; Fig. 6*B*), consistent with its lower O_2^- content, indicating that higher contents of AsA were correlated with an improved ability to reduce oxidative stress. The activity of AsA in *E. angustifolia* followed a pattern opposite to the H_2O_2 content.

Discussion

Water deficit is a valid index of the water equilibrium in plants (Salisbury and Ross 1969). All four species showed different degrees of water deficit, indicating that water absorption cannot satisfy the high transpiration demand under hot, dry conditions. The water deficit in *P. hosiensis* and *E. angustifolia* was characterised by wide diurnal fluctuations; it reached a peak at noon (higher than that of other two desert plants), but recovered in the evening. Water potential is an important index of water conditions in plants (Slatyer and Taylor 1960). *H. ammodendron* had lower water potential than the other species, which helped it to absorb water from the soil and enhanced its drought resistance (Fig. 2*B*). As water potential declines, live cells accumulate osmotically active compounds that reduce the osmotic potential, and therefore, help maintain turgor and enable the plants to continue to acquire water from the soil at low water potentials (Kramer 1988, Huang 1991). Water potential decreased to a minimum at noon; this is the physiological response of plants to drought and high temperatures.

Transpiration patterns showed peak in *E. angustifolia* at about 14:00, when the air temperature was highest (39 °C). This is considered a protective mechanism, whereby transpiration cools the leaves. Similarly, in *C. mongolicum,* the only peak was found at 15:00, indicating the same protective mechanism. In *P. hosiensis, E* decreased with increasing temperature and decreasing water potential, suggesting that soil water could not meet the needs of transpiration. Schimper considered that xerophytes generally have low *E* (Liu *et al.* 1987). However, only succulent xerophytes maintain low *E* to reduce water loss, and some less-succulent xerophytes have higher *E* than do mesophytes (Liu *et al.* 1987). *E. angustifolia* and *C. mongolicum* had high *E*, while *P. hosiensis* and *H. ammodendron* had low *E*; therefore, not all succulent xerophytes have low *E* in drought-prone habitats.

WUE is an important index with which to measure a plant ability to maintain water equilibrium. The daily average WUEs in *C. mongolicum*, *H. ammodendron*, *E. angustifolia,* and *P. hosiensis* were 2.51, 2.15, 2.29, and 1.71 mmol(CO_2) mol^{-1}(H_2O), respectively. Despite lower WUE compared to *C. mongolicum, H. ammodendron* is still suitable for desert habitats because of its lower *E* and higher P_N and its ability to endure drought with a very low water potential. *E. angustifolia* had high WUE, but its high *E* and large leaf area make it more suitable for mesic habitats. Thus, it is a suitable species for a forest shelterbelt. In contrast, *P. hosiensis* had low WUE, *E*, and P_N, and thus cannot produce a forest in arid areas without irrigation.

High irradiance and high temperatures often coincide with drought, so photoinhibition may be involved (Lambers 1998). Decreases in photosynthesis are influenced by stomatal and non-stomatal limitations (Wilson *et al.* 2000). With the occurrence of a midday depression in P_N for *C. ammodendron*, C_i/C_a increased and C_i decreased, suggesting that the midday depression was primarily attributable to a reduction in the biochemical capacity rather than to stomatal limitation of photosynthesis. The g_s in *E. angustifolia* and *C. mongolicum* increased between 12:00 and 14:00 and between 11:00 and 12:00, respectively. It may be an adaptive

mechanism in these two species to maintain the water balance by lowering stomatal resistance and enhancing transpiration to absorb water from the soil under high temperatures and drought. In *E. angustifolia*, P_N decreased, accompanied by decreases in C_i/C_a and C_i, indicating that stomatal closure is the main cause of the midday depression in P_N.

Chl fluorescence is a quick and non-intrusive probe for the measurement of photosynthetic function (Genty *et al.* 1989). Chl fluorescence decreases with photoinhibition (Strand and Lundmark 1987). Fig. 3*C* shows that the efficiency of photosystem 2 (PS2), F_v/F_m declined at midday, suggesting that the PS2 centre has excessive energy and that photochemical efficiency decreases and photosynthesis is inhibited. Fig. 3*C* shows that in *C. mongolicum*, photoinhibition takes place during the midday photosynthetic depression when the plant experiences high temperatures and irradiance. Thus, photoinhibition is one of the non-stomatal factors involved in the midday depression of photosynthesis in *C. mongolicum*. Reversible changes in F_v/F_m that decreased from morning to midday and recovered in the evening suggest another mechanism for the depression in photosynthesis. The decrease in F_v/F_m at midday reflects protective mechanisms to avoid photo-damage to the photosynthetic apparatus under excess irradiance. The F_v/F_m of *C. mongolicum* and *E. angustifolia* recovered very quickly, but that of *H. ammodendron* and *P. hosiensis* fluctuated greatly and recovered slowly. However, all species recovered in the evening.

Lipid peroxidation is a complex process caused by either enzymatic or non-enzymatic reactions (Seel *et al.* 1992, Jiang *et al.* 1993). Lipid peroxidation is commonly regarded as an indicator of oxidative stress. Our results showed that there were specific MDAs in three of the species, indicating the occurrence of different degrees of oxidative stress and membrane damage in arid habitats. Membrane damage in *E. angustifolia* was more severe than in *H. ammodendron* or *C. mongolicum*. The MDA content changed daily with water potential in these species. The accumulation of O_2^- and H_2O_2 in *E. angustifolia* was higher than in *H. ammodendron* and *C. mongolicum*, consistent with the accumulation of MDA. These results imply that the anti-oxidative ability of *E. angustifolia* was weaker than that of *H. ammodendron* and *C. mongolicum*.

Plants use their available machinery to combat oxidative stress by scavenging excess ROS through enhancing activity of various antioxidant enzymes and non-enzyme antioxidants such as ASC and GSH (Seel *et al*. 1992, Patsikka *et al*. 2002). SOD activity is positively correlated with plant anti-oxidative ability (Bor *et al.* 2003). SOD activity was significantly higher in *C. mongolicum* than in *H. ammodendron* and *E. angustifolia*. Thus, *C. mongolicum* had a greater ability to regulate oxidative stress. SOD activity in *H. ammodendron* and *E. angustifolia* showed no obvious diurnal changes; however, this does not mean that they experienced little or no oxidative stress because the accumulation of O_2^- was high, especially in *E. angustifolia*. This implies that *H. ammodendron* and *E. angustifolia* do not primarily depend on SOD activity for detoxication of O_2^-, but rather, there is an alternative, non-enzymatic route for conversion of O_2^- using antioxidants such as AsA.

The intracellular content of H_2O_2 is regulated by a wide variety of enzymes, the most important being CAT and peroxidases (POXs). Ascorbate peroxidases (APXs) are the most important H_2O_2 scavengers operating in both the cytosol and chloroplasts (Yordanova *et al.* 2004). Interestingly, CAT activity in *H. ammodendron* was much higher than in the other species. The lowest CAT activity occurred at 10:00, but H_2O_2 showed the opposite trend, which may have occurred if CAT was not activated early enough. However, CAT activity subsequently increased and the accumulation of H_2O_2 decreased. AsA–POD activity showed the same diurnal patterns as that of CAT. Therefore, these components play an important role in scavenging H_2O_2 in *H. ammodendron*. *C. mongolicum* had low CAT and AsA–POD activity; however, the accumulation of H_2O_2 changed greatly, implying that other antioxidants function in *C. mongolicum*. A negative correlation existed between GSH and H_2O_2. Thus, the non-enzyme antioxidant GSH played a major role in cleansing H_2O_2. CAT was not detected in *E. angustifolia*, possibly for three reasons: (*1*) CAT production was not initiated to scavenge the AOS; (*2*) CAT does not occur in *E. angustifolia*; or (*3*) CAT cannot be extracted. AsA–POD activity was higher in *E. angustifolia* than in *H. ammodendron* and *C. mongolicum*, and increased with increasing water stress. Thus, AsA–POD may be activated to compensate for the absence of CAT. Therefore, AsA–POD is the main antioxidant enzyme that cleanses toxic H_2O_2 in *E. angustifolia*, along with other antioxidants. The coexistence of enzymatic and non-enzymatic ROS-scavenging mechanisms in these species shows that these plants maintain the equilibrium of various enzymes and mobilise them as a defence system against oxidative damage induced by water stress.

There are disagreements over the defensive functions of POD to peroxidation. In an investigation of two kinds of rice under *Paraquat* and H_2O_2 stress, Del-Longo *et al.* (1993) showed that drought-resistant plants exhibited higher POD activity than did drought-sensitive plants. Guo *et al.* (1997) demonstrated similar results. However, POD activity in water-tolerant species (*e.g. Artemisia ordosica*) decreases with increasing water stress (Gong *et al.* 2002). Zeng *et al*. (1991) showed that POD was involved in the degradation of Chl under senescence and chilling in the light. Niinomi *et al*. (1987) demonstrated that POD produced polyunsaturated acid peroxide. Our study showed that POD activity in *E. angustifolia* and *H. ammodendron* decreased with increasing water stress, similar to the results of Niinomi *et al.* (1987). With

increasing water stress, POD activity decreased to reduce Chl degradation and produce polyunsaturated acid peroxide.

In conclusion**,** a comparison of the eco-physiological responses of *H. ammodendron*, *C. mongolicum*, *E. angustifolia*, and *P. hosiensis* suggests different strategies of controlling water relations. WUE in *C. ammodendron* was higher than that of the other three species. Despite lower WUE compared to *C. ammodendron*, Ψ_l of *H. ammodendron* was lower than that of the other three species, and aided in water absorption from the soil and resistance to drought. Additionally, *H. ammodendron* had lower *E*, which implies low water loss and resistance to drought. Thus, this species is water-saving, drought-tolerant, and suitable for desert habitats. The eco-physiological responses of *C. ammodendron* and *E. angustifolia* were very similar. However, *E. angustifolia* had a large leaf surface area and plant size, high *E*, and experienced more severe membrane damage than did the other two species, thus exhibiting weaker anti-oxidative ability. Therefore, *E. angustifolia* is more suitable for mesic habitats.

SOD activity and AsA content in *C. mongolicum* were much higher than in *H. ammodendron* and *E. angustifolia*, and resulted in a lower O_2^- content. With regard to the cleansing of toxic H_2O_2, the antioxidant GSH operated chiefly in *C. mongolicum*, whereas CAT played a major role in *H. ammodendron,* with auxiliary antioxidant activity. AsA–POD was the main antioxidant enzyme used to cleanse toxic H_2O_2 in *E. angustifolia,* along with other minor antioxidants. Therefore, an enzyme balance is maintained in plants to resist adverse stresses, and the concerted action of both enzymatic and non-enzymatic ROS-scavenging mechanisms is vital to overcome adverse conditions in these plants.

References

Arakawa, N., Tsutsumi, K., Sanceda, N.G.: A rapid and sensitive method for determination of ascorbic acid. – Agr. biol. Chem. **45**: 1289, 1981.

Asada, K.: The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. – Annu. Rev. Plant Physiol. Plant mol. Biol. **5**0: 601-639, 1999.

Asada, K., Takahashi, M.: Production and scavenging of active oxygen in photosynthesis. – In: Kyle, D.J., Osmond, C.B., Arntzen, C.J. (ed.): Photoinhibition. Pp. 227-287. Elsevier, Amsterdam – New York – Oxford 1987.

Bor, M., Ozdemir, F., Turkan, I.: The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. – Plant Sci. **164**: 77-84, 2003.

Bowler, C., Van, M.: Superoxide dismutase and stress tolerance. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 83-116, 1992.

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

Buchanan, B.B., Gruissem, W., Jones, R.L.: Biochemistry and Molecular Biology of Plants. – American Society of Plant Physiologists, Rockville 2000.

Del Longo, O.T., González, C.A., Pastori, G.M., Trippi, V.S.: Antioxidant defences under hyperoxygenic and hyperosmotic conditions in leaves of two lines of maize with differential sensitivity to drought. – Plant Cell Physiol. **34**: 1023–1028, 1993.

Deng, X., Li, X.M., Zhang, X.M., Ye, W.H.: A study of the gas exchange characteristics of four desert plants. – Acta physiol. sin. **26**: 605-612, 2002.

Dhindsa, R.S., Matowe, W.: Drought tolerance in two mosses: correlated with enzymatic defence against lipid peroxidation. – J. exp. Bot. **32**: 79-91, 1981.

Flexas, J., Medrano, H.: Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. – Ann. Bot. **89**: 183-189, 2002.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. biophys. Acta **900**: 87-92, 1989.

Gong, J.R., Zhang, L.X., Zhao, A.F., Bi, Y.R.: Elementary studies on physiological and biochemical anti-drought features of *Artemisia ordosica*. – J. Desert Res. **22**: 387-392, 2002.

Guo, Z.F., Lu, S.Y., Li, B.S., Li, M.Q.: Responses of rice seedlings in different drought tolerant cultivars to oxidative stress. – Acta bot. sin. **39**: 748-752, 1997.

Guri, A.: Variation in glutathione and ascorbate acid content among selected cultivars of *Phaseolus vulgaris* prior to and after exposure to ozone. – Can. J. Plant Sci. **63**: 733-737, 1983.

Halliwell, B., Gutteridge, J.M.C.: Free Radicals in Biology and Medicine. 2nd Ed. – Oxford Univ. Press, New York 1989.

Huang, Z.C.: Study on Physioecological Characteristics of Desert Plants. – Educational Science Publishing House, Beijing 1991.

Huang, Z.C., Shen, W.S.: The Water Relations and Drought Resistance of Plants in the Arid Area. – Pp. 26-27. China Environmental Science Press, Beijing 2000.

Jiang, G.M., Zhu, G.J.: Different patterns of gas exchange and photochemical efficiency in three desert shrub species under two natural temperatures and irradiances in Mu Us Sandy Area of China. – Photosynthetica **39**: 257-262, 2001.

Jiang, M.Y., Jing, J.H., Wang, S.T.: Generation of hydroxyl radical in plants and its relation to the initiation of lipid peroxidation. – Plant Physiol. Commun. **29**: 30, 1993.

Kramer, P.J.: Water Relations of Plants. – Academic Press, New York 1988.

Lambers, H.: Responses to availability of water. – In: Lambers, H., Stuart Chapin, F., III, Pons, T.L.: Plant Physiological Ecology. Pp. 154-191. Springer-Verlag, New York – Berlin – Heidelberg 1998.

Li, Y.G., Jiang, G.M., Niu, S.L., Liu, M.Z., Peng, Y., Yu, S.L., Gao, L.M.: Gas exchange and water use efficiency of three native tree species in Hunshandak Sandland of China. – Photosynthetica **41**: 227-232, 2003.

Liu, J.Q., Pu, J.C., Liu, X.M.: Comparative studies on water relations and xeromorphic structures of some plant species in the middle part of the desert zone in China. – Acta bot. sin.

30: 85-95, 1987.
Liu, M.Z., Jiang, G.M., Li, Y.G., Gao, L.M., Niu, S.L., Cui, H.X., Ding, L.: Gas exchange, photochemical efficiency, and leaf water potential in three *Salix* species. – Photosynthetica **41**: 393-398, 2003.
Liu, Z.Q., Zhang, S.C.: Anti-activities Physiology. – China Agriculture Press, Beijing 1994.
Manes, F., Vitale, M., Donate, E.: Relations between environmental parameters and ecophysiological status of pubescent oak during summer. – Monti Boschi **49**: 29–33,1998.
Matos, M.C., Matos, A.A., Mantas, A., Cordeiro, V., Vieira da Silva, J.B.: Diurnal and seasonal changes in *Prunus amygdalus* gas exchange. – Photosynthetica **35**: 517-524, 1998.
Mukherjee, S.P., Choudhuri, M.A.: Implications of water stress induced change in the level of endogenous ascorbic acid under hydrogen peroxide in *Vigna* seedlings. – Physiol. Plant. **58**: 166-170, 1983.
Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.
Niinomi, A., Morimoto, M., Shimiza, S.: Lipid peroxidation by the (peroxidase/H_2 O_2/phenolic) system. – Plant Cell Physiol. **28**: 731-734, 1987.
Pan, R.C., Dou, Z.J., Ye, Q.S.: Effect of methyl jasmonate on SOD activity and membrane-lipid peroxidation in peanut seedlings during water stress. – Acta phytophysiol. sin. **21**: 221-228, 1995.
Patsikka, E., Kairavuo, M., Sersen, F., Aro, E.M., Tyystjarvi, E.: Excess copper predisposes photosystem II to photoinhibition *in vivo* by outcompeting iron and causing decrease in leaf chlorophyll. – Plant Physiol. **129**: 1359-1367, 2002.
Salisbury, F.B., Ross, C.: Plant Physiology. – Pp. 73-74. Wadsworth Publishing Company, New York 1969.
Seel, W.E., Hendry, G.A.F., Lee, J.A.: The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. – J. exp. Bot. **4**: 1023-1031, 1992.
Slatyer, R.O., Taylor, S.A.: Terminology in plant and soil water relations. – Nature **187**: 22-24, 1960.
Strand, M., Lundmark, T.: Effects of low night temperature and light on chlorophyll fluorescence of field-grown seedlings of Scots pine. – Tree Physiol. **3**: 211-224, 1987.
Wang, A.G., Luo, G.H.: Quantitative relations between the reaction of hydroxylamine and superoxide anion radicals in plants. – Plant Physiol. Commun. **6**: 55-57, 1990.
Wilson, K.B., Baldocchi, D.D., Hanson, P.J.: Quantifying stomatal and non-stomatal limitations to carbon assimilation resulting from leaf aging and drought in mature deciduous tree species. – Tree Physiol. **20**: 787-797, 2000.
Yordanova, R.Y., Christov, K.N., Popova, L.P.: Antioxidative enzymes in barley plants subjected to soil flooding. – Environ. exp. Bot. **51**: 93-101, 2004.
Zeng, S.-X., Wang, Y.-R., Liu, H.-X.: [Some enzymatic reactions related to chlorophyll degradation in cucumber cotyledons under chilling in the light.] – Acta phytophysiol. sin. **17**: 177-182, 1991. [In Chin.]

Rama Das, V.S.: **Photosynthesis. Regulation Under Varying Light Regimes.** – Science Publishers, Enfield – Plymouth 2004. ISBN 1-57808-343-5. VIII + 175 pp., USD 65.00.

The reviewed hard-bound book contains 121 pages of text. The rest is list of cited papers and good indexes (author and subject). This shows that it is more a textbook or introductory text for researchers interested in control of photosynthetic processes than a monograph. Seven chapters contain all the basic necessary information.

The introductory chapter explains main facts – photosynthetic pigments and photon harvesting, composition and structure of photosystems (antennae, components, thylakoid structure, core complexes, reaction centres). Chapter 2 deals with the occurrence of photoinhibition of both photosystems, its main mechanism, *i.e.* the degradation of D1 protein and its re-synthesis. Next chapter explains the photoprotection mechanisms (thermal dissipation of excitation energy, xanthophyll cycle) and plant response to related stresses such as lipid oxidation stress, production of active oxygen and scavenging it. In chapter 4 leaf movements regulating photon interception (paraheliotropism and diaheliotropism) are shown, as well as sites of perception of signals for these movements and their mechanisms. Chapter 5 deals with acclimation of photosynthesis to irradiation environments. Transgenic and biotechnological aspects are the topics of chapter 6.

Chapter 7 contains brief concluding remarks (over two pages). The author included also some recent findings (*e.g.* lutein epoxide cycle) and explained terms not always clearly used in the literature (*e.g.* why Mehler peroxidase reaction = water-water cycle).

The explanations are pedagogically clear, which shows the teaching experience of the author. The text is accompanied by not too many figures, tables, and schemes that help in understanding the more complicated facts. Cited are almost 600 papers. I wonder why only three of them are those published in Photosynthetica because the journal presents many papers dealing with this interesting topic. I miss also some of the papers which are often cited in articles on photoinhibition.

The editorial work was certainly not very careful. There are many misprints and inaccuracies both in the text and in references. I wonder why the author did not unify nomenclature – for example, he uses alternately both old-fashioned and modern terms (light intensity, light level, irradiance).

Generally, the book will certainly find its readers among students and young researchers. I only regret that its production was not done with more care.

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