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C_4 PHOTOSYNTHETIC CHARACTERISTICS AND ANTIOXIDATIVE PROTECTION OF C_3 DESERT SHRUB *HEDYSARUM SCOPARIUM* IN NORTHWEST CHINA

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Abstract

To understand the adaptive mechanisms, and the strategy and direction of evolution of exploiting plants for living in oasis-desert transitional areas where there was a water gradient, mechanisms of C_4 photosynthesis and antioxidative protection were studied in leaves and assimilating shoots of C_3 desert shrub *Hedysarum scoparium* under water gradient accompanied with high irradiance and high temperature in simulated water gradient trial site that lies in the desert. In this study, C_4 pathway was detected in the leaves and assimilating shoots of *H. scoparium* and the antioxidative enzymatic system was found to be very important in the protection against oxidants, both increasing considerably antioxidative enzymes and C_4 photosynthetic enzymes under increasing water stress in C_3 plant. The results indicated that the C_4 photosynthetic response of assimilating shoots was more positive than that of leaves with water condition exacerbate. It was proposed that the direction of evolution of organs of C_4 photosynthesis and positive interaction between antioxidative enzymes and C_4 photosynthetic key enzymes in C_3 desert species were important factors for plant growth, survival and reproduction in water gradient environment.

Introduction

Hedysarum scoparium (Leguminosae), a desert pioneer plant, is famous for its capacity to resist aridity and fixate dune. It is a small-leaf desert shrub, distributing very widely in the arid regions of China with dry air, excess sunshine, and annual precipitation less than 130 mm (Li *et al*., 1995). *H. scoparium* makes use of leaves and assimilating shoots performing photosynthesis as different photosynthetic organs. Some reports have been shown on the photosynthetic attribution in leaves of *H. scoparium* (Chen *et al*., 1961; Li *et al*., 1995), but less is known about its leaf-axis-like assimilating shoots. Su *et al*., (2003) confirmed *H. scoparium* was C_3 plant by its characteristics of $\delta^{13}C$ value (-26.38 ‰) and gas exchanges (high CO_2 compensation point, 90 μmol· mol^{-1}), but studies on photosynthetic enzymes were absent. In general, C_4 plants originated from tropical arid areas, which possess higher photosynthetic rates, lower photorespiration and better capacity to endure leanness than that of C_3 plants. The desert plants are often exposed to different stressful conditions such as high irradiance, extreme temperature, water deficit and air-dryness. Especially, water deficit is perhaps one principal factor of

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impacts on plants growth and development, and causes physiological and biochemical responses. On the one hand, cellular malfunction caused by active oxygen species and lipid peroxidation was mitigated by antioxidative enzymes such as SOD (EC 1.15.1.1), POD (EC 1.11.1.7), CAT (EC 1.11.1.6). Antioxidant enzymes are known to increase in response to drought (Menconi *et al*., 1995; Zhang & Kirkham, 1996; Sairam & Saxena, 2000; Sairam & Srivastava, 2001), high temperature (Upadhyaya *et al*., 1990; Sairam *et al*., 2000), external calcium under water stressed conditions (Li *et al*., 2003). On the other hand, the reproductive parts of wheat (C_3 plant), chickpea (*Cicer arientinum*) and rapeseed (*Brassica campestris*) contain high activities of C_4 enzymes (Singal *et al*., 1987; Singh, 1993), such as PEPCase and NADP-ME in the pods, which were purified and characterized by Das *et al*., (1986) and Singal & Singh (1986) respectively. Voznesenskaya *et al*., (2001) showed C_4 photosynthesis could function within a plant that its carbon isotope composition was like that of C_4, but lacked Kranz anatomy. Hibberd & Quick (2002) also reported that tobacco and celery (a typical C_3 plant) showed characteristics of C_4 photosynthesis in cells of stems and petioles surrounding the xylem and phloem. Moreover, the enzymatic activities of C_4 pathway in soybean have positive relations to net photosynthetic rate (Li *et al*., 2000). These results implied that some C_3 species might extend their photosynthetic types in different assimilating organs without Kranz anatomy along the increasing environmental stress.

Fryer *et al*., (1998) studied the relationships between CO_2 assimilation, photosynthetic electron transport and antioxidant enzyme activities in field-grown maize and found that the donation of electrons to oxygen by the photosynthetic electron transport chain was elevated by growth at low temperatures (Fryer *et al*., 1998). There was a positive correlation between the photosystem stoichiometries and active oxygen protective mechanisms (Yamazaki & Kamimura, 2001). Further research on the correlation between adaptable changes in photosynthetic pathway and antioxidative protection in different photosynthetic organs of desert plant is essential to understand the adaptive mechanisms and the strategy and direction of evolution of exploiting plants for living in oasis-desert transitional areas since the adaptation of desert vegetation across oasis-desert transitional areas was important for the oasis to maintain its stability. Water availability was the major limiting environmental factor for the growth of desert plants within desert ecosystem (Mittler *et al*., 2001). So, the experiment is conducted in a simulated trial site in the desert area with a water gradient, attempting to investigate the following hypotheses: (1) C_3 dominant desert species *Hedysarum scoparium* extends its photosynthetic pathway to improve its survival and adaptation; (2) The positive interactions between antioxidative enzymes and C_4 photosynthetic key enzymes in C_3 desert species are important factors for the growth, survival and reproduction; (3) Assimilating shoots become an adaptable strategy and direction of evolution with water condition exacerbate in many of these species.

Materials and Methods

Habitat of trial site: The study was carried out near the town of Minqin, northwest of China, which is located in a joint region of Tengger Desert and Badain Jaran Desert (38°38′N, 103°05′E). The trial site lies in the Plant Transpiration Consume Water Observation Station of the Desert Plantation of Gansu Provincial Institute of Desert Control Research. There are many sand dunes in a protected area in nature, correlating to *Nitraria tangutorum* and *Reaumuria soongorica* communities.

The average annual precipitation is 115 mm with the largest precipitation in July, August and September. The average annual evaporation is 2643.9 mm, 23 times of the average annual precipitation. The annual average temperature is 7.8°C with the highest temperature of 38.1°C and the lowest temperature of – 28.8°C. There are 165 days of non-frost days, 139 days of blowing sand and 37 days of sandstorm. The soil is sandy soil with the depth of ground water of 18 m (Zhao *et al*., 2003a).

The growth conditions and plant materials procedures: *Hedysarum scoparium* was growing in the Plant Transpiration Consume Water Observation Station which be consisted of 66 cultivated pools filling with sandy soil, establishing in a natural environment for plant growth. Cultivated pools were 1 m in both length and width with different depth of 1.6 m, 2.6 m and 3.6 m. The cobble and crushed stones were 11 cm in depth acting as a layer for water storage, where there was dune soil as the nutrient soil layer. The water that plants need was supplied by the groundwater eternally through the compensatory apparatus, which could stabilize water storage level. The Plant Transpiration Consume Water Observation Station has 3 depths of ground water (DGW), i.e., 1.4 m, 2.4 m and 3.4 m (Guo & Zhao, 1988). *H. ammodendron* and *H. scoparium* of two years old were transplanted to cultivated pools in 1995 (Zhao *et al*., 2003a). Every water gradient and every plant have 4 repeats. Because the level of water storage of transpiration and the permeation instrument (were made up of cultivated pools and groundwater eternal compensatory apparatus) were stable, soil water contents of every layer of cultivated pools were almost constant, which created a more stable soil water environment for plants growth (Zhao *et al*., 2003b).

The samples were all collected at growing and developing stage in August 2003. The collected leaves and assimilating shoots were frozen in liquid nitrogen immediately after sampling.

Determination of PFD and $T._{Air}$: We measured the photon flux density (PFD) and air temperature ($T._{Air}$) with portable photosynthetic gas analysis systems (CIRAS-1, PP Systems Corporation in Britain) before sampling on sunny and clear days.

Determination of soil water content: Water contents of the soil were determined by the fresh and drying weight of the soil. Soil samples were randomly collected from the ground every 20 cm in depth. This procedure was repeated 3 times.

The relative water content (RWC) of leaves and assimilating shoots were estimated by the fresh mass (FM) and saturated mass (SM). 0.5 g fresh leaf samples first were kept in water for 24 h and then dried in hot air oven till constant dry mass (DM) was achieved according to Barrs & Weatherley (1962).

$$RWC(\%)=((FM-DM)/(SM-DM)) \times 100\%$$

Antioxidative enzymatic assays: Leaf tissue of 0.5 g was frozen in liquid nitrogen and ground to fine powder with a pre-cold mortar and pestle. The powder was homogenized in 1: 5 (w/v) 50 mmol L^{-1} phosphate buffer (pH 7.8) containing 1 % PVP (MW 40,000) and 10 mmol L^{-1} sulfhedryl alcohol. The homogenate was centrifuged at 15,000 g for 20 min and the supernatant obtained was used immediately for assay as enzyme extract. All steps in the preparation of the enzyme extract were carried out at 4°C. Determination of all enzyme activities was conducted through UV-751 ultraviolet spectrophotometer. An aliquot of the extract was used to determine the protein content according to Bradford (1976) using bovine serum albumin as a standard.

SOD (EC 1.15.1.1) activity was measured by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) using the method of Beauchamp & Fridovich (1971) as modified by Dhindsa & Matowe (1981). The reaction mixture (3 mL) contained 50 mmol L^{-1} phosphate buffer (pH 7.8) 0.1 mmol L^{-1} EDTA, 13 mmol L^{-1} methionine, 75 μmol L^{-1} nitroblue tetrazolium (NBT), 2 μmol L^{-1} riboflavin and 50 μL of the supernatant which had been diluted 5 times. The production of blue formazan was followed by monitoring the increase of the absorbance at 560 nm. A non-irradiated reaction mixture did not develop color and served as control.

POD (EC1.11.1.7) activity was assayed as the increase in absorbance due to the formation of tetra-guaiacol recorded at 470 nm (Castillo *et al*., 1984). The 3 mL reaction mixture contained 16 mmol L^{-1} guaiacol, 2 mmol L^{-1} H_2O_2, 70 mmol L^{-1} phosphate buffer (pH 6.1) and 20 μL enzyme extract which had been diluted twice.

CAT (EC 1.11.1.6) was assayed by measuring the initial rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm according to the method of Chance & Maehly (1955) as modified by Dhindsa & Matowe (1981) and Cakmak & Marschner (1992). The 3 mL reaction mixture contained 50 mmol L^{-1} phosphate buffer (pH 7.0) 15 mmol L^{-1} H_2O_2, and 50 μL enzyme extract which had been diluted 10 times. Enzyme solution containing hydrogen peroxide-free phosphate buffer was used as control.

Photosynthetic enzymatic extraction and assay: Enzymes were extracted from 0.1 g tissue which was ground at 4°C in a pre-cold mortar and pestle with 1 mL of grinding buffer containing 50 mmol L^{-1} Tris-HCl (pH 7.0), 20 mmol L^{-1} $MgCl_2$, 2 mmol L^{-1} $MnCl_2$, 1 mmol L^{-1} EDTA, 5 mmol L^{-1} DTT, 10% glycerol and 1% PVP (MW 40,000). The crude extract was immediately centrifuged at 15,000×g for 15 min at 4°C and then used to assay different enzymatic activities at 25°C.

PEPCase, NADP-ME and NAD-ME activities were monitored spectrophotometrically by the change of pyridine nucleotide at 340 nm. PEPCase (EC 4.1.1.31) activity was assayed as described by Ting & Osmond (1973). The buffer for PEPC (1 mL) contained 50 mmol L^{-1} Tris-HCl (pH 8.0), 5 mmol L^{-1} $MgCl_2$, 2 mmol L^{-1} DTT, 1 mmol L^{-1} $NaHCO_3$, 0.2 mmol L^{-1} NADH, 0.1 mmol L^{-1} EDTA, 3 units malate dehydrogenase and 20 μL extract. The reaction was initiated with 2 mmol L^{-1} PEP.

NADP-ME (EC 1.1.1.40) and NAD-ME (EC 1.1.1.38) activities were assayed as described by Sayre *et al*., (1979). Assays of NADP-ME activity were carried out in 1 mL buffer containing 50 mmol L^{-1} Tris-HCl (pH 8.0), 1 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} $MnCl_2$, 1 mmol L^{-1} EDTA, 0.33 mmol L^{-1} NADP, 5 mmol L^{-1} L-malate and 20 μL extract. NAD-ME activity were assayed in 1 mL buffer containing 25 mmol L^{-1} Tris-HCl (pH 7.4), 5 mmol L^{-1} $MnCl_2$, 5 mmol L^{-1} L-malate, 2 mmol L^{-1} NAD, 7.5 mmol L^{-1} $MgSO_4$, 5 mmol L^{-1} DTT, 0.2 mmol L^{-1} EDTA and 20 μL extract.

Protein content of the crude enzyme extract was detected according to Bradford (1976) using bovine serum albumin as a standard.

Results

Influence of PFD, T_{Air}, soil moisture and RWC to desert plants: The diurnal variations of PFD and T_{Air} were shown on sunny days in the early ten days of August 2003 in Minqin (Fig. 1). PFD reached its highest value (1,638 μmol m^{-2} s^{-1}) at 13:00 and the highest $T._{Air}$ is 42.4°C at 15:50. While the values of PFD and $T._{Air}$ were all much higher at 15:00, they reached 1,568 μmol m^{-2} s^{-1} and 40°C respectively.

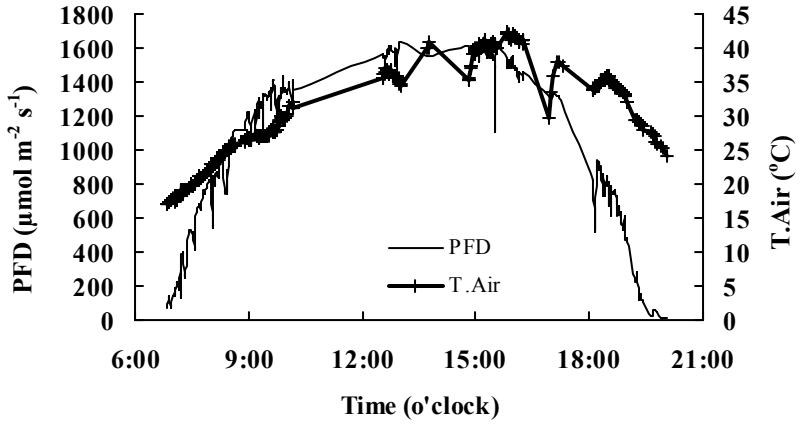

Fig. 1. Diurnal changes of photon flux density PFD (μmol m^{-2} s^{-1}) and air temperature $T_{.Air}$ (^oC) on sunny days in the early ten days of August 2003 in Minqin, northwest China

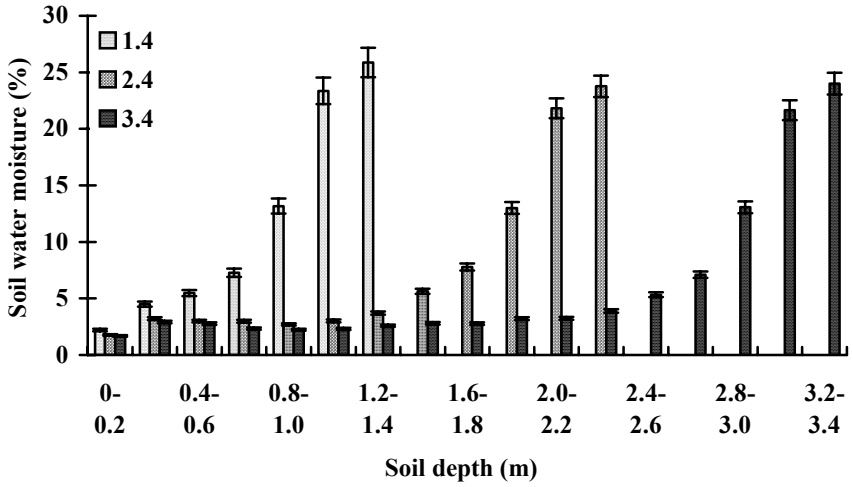

Fig. 2. Soil water moisture at different soil depths for three depths of ground water (DGW) which were 1.4 m, 2.4 m, and 3.4 m, respectively. Each value represents mean (± S.E.) of three replications ($p < 0.05$)

Soil moisture gradually increased with the increase of soil depths for the 3 depths of ground water, resulting in different effects on plant growth and development at different DGW (Fig. 2).

As shown in Table 1, RWC in leaves and assimilating shoots of *H. scoparium* were very low. Slightly higher RWC was recorded in the 1.4 m DGW with the lowest in the 3.4 m at 15:00. RWC was lower in the leaves than in the assimilating shoots and the changes of RWC in leaves were gentler under the same condition.

Table 1. Effects of different depths of ground water (DGW) on the relative water content (RWC, %) in leaves and assimilating shoots of *Hedysarum scoparium* at 15:00. Each value represents mean (± S.E.) of three replications.

Organs	DGW		
	1.4 m	2.4 m	3.4 m
Assimilating shoots	50.0 ± 3.85	45.78 ± 2.89	42.43 ± 3.09
Leaves	47.58 ± 2.05	42.62 ± 2.10	38.87 ± 2.39

Antioxidant enzymes system of *H. scoparium*: In order to compare the activities of antioxidant enzymes in leaves and in assimilating shoots, the changes in activities of antioxidative enzymes were investigated at different DGW at 15:00 when plants were exposed to high irradiance and temperature. SOD activity in leaves and assimilating shoots gradually increased with the increase of DGW. Among the enzymes responsible for the scavenging of active oxygen species, SOD activity was obviously much higher in leaves than in assimilating shoots of *H. scoparium* at 3 DGW (Fig. 3A), and the ratio of SOD activity of leaves/assimilating shoots gradually decreased.

Some different trend in POD activity in leaves of *H. scoparium* was observed (Fig. 3B). POD activity in leaves increased slightly from 1.4 m to 2.4 m but declined at 3.4 m DGW. POD activity in assimilating shoots gradually increased with the increase of DGW, as well as the ratio of POD activity of leaves / assimilating shoots gradually decreased with the increase of DGW.

CAT activity was found to be higher in leaves than in assimilating shoots of *H. scoparium* at 3 DGW (Fig. 3C). However, increased CAT activity was observed either in leaves or in assimilating shoots from 1.4 m to 2.4 m but declined at 3.4 m DGW. CAT activity increased subjected to mild stress but declined during severe water stress.

Activities of C_4 photosynthetic key enzymes: The activities of C_4 enzymes in *H. scoparium* were lower than that in C_4 plant. The activities of C_4 photosynthetic key enzymes were investigated in *Hedysarum scoparium* and *Haloxylon ammodendron* (a C_4 desert shrub) at 2.4 m DGW where represents moderate water stress (Table 2).

The activities of C_4 photosynthetic key enzymes in leaves were all higher than that in assimilating shoots of *H. scoparium* at different DGW. The activity of PEPCase in leaves reached the top at 15:00 (ca. 7 μmol PEP $min^{-1}mg^{-1}$ protein) while it kept at a lower level in assimilating shoots (ca. 3 μmol PEP $min^{-1}mg^{-1}$ protein). The activity of PEPCase in leaves increased slightly with the increase of DGW, and the activity of PEPCase in assimilating shoots increased in the same time, even the ratio of PEPCase activity of leaves / assimilating shoots was more and more lower (Fig. 4A), which was consistent with the changes of RWC. NADP-ME were also detected in leaves and in assimilating shoots of *H. scoparium*, NADP-ME activity in leaves gradually declined, but the changes of NADP-ME activity in assimilating shoots was similar to the changes of PEPCase. The activity of NAD-ME in leaves and assimilating shoots increased gradually with the increase of DGW, and the activity of NAD-ME was about 3 times higher in leaves than in assimilating shoots (Fig. 4B, C). The activity of NADP-ME was lower than that of NAD-ME.

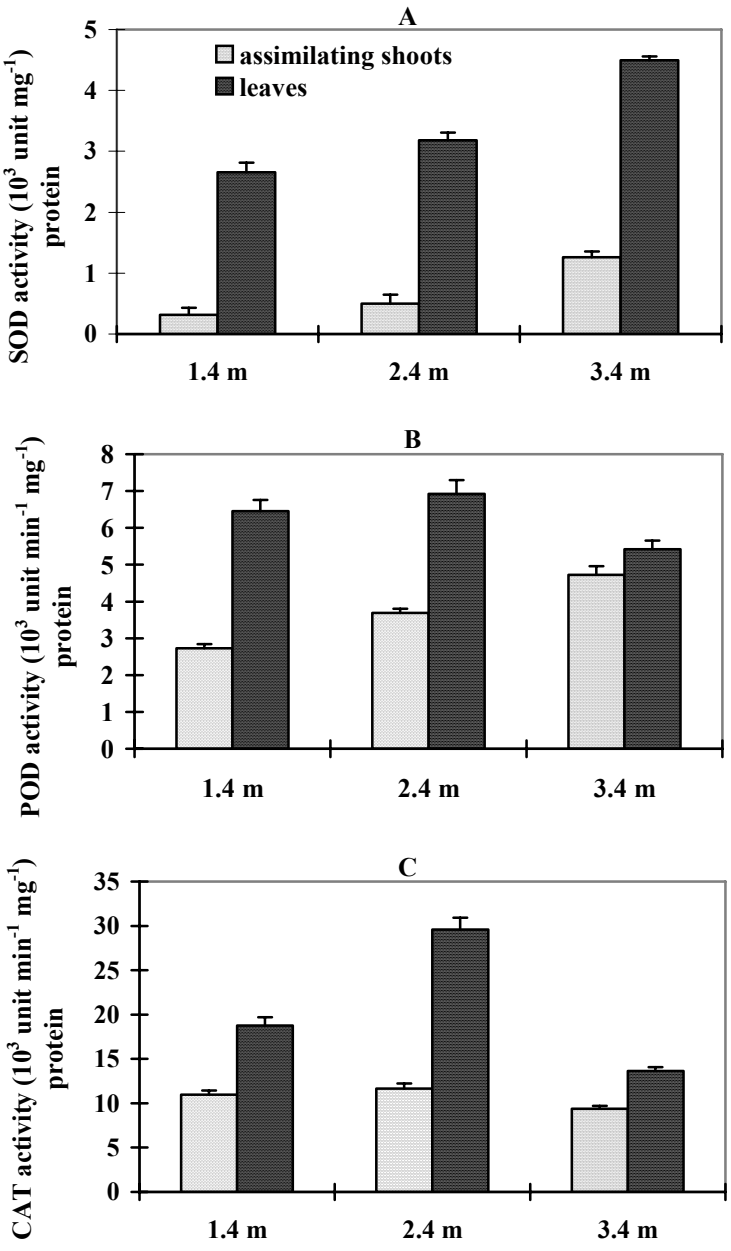

Fig. 3. Variations of SOD, POD and CAT activities in leaves and assimilating shoots of *Hedysarum scoparium* at different DGW of 1.4 m, 2.4 m, and 3.4 m at 15:00. Each value represents mean (± S.E.) of three replications

Table 2. Comparisons of PEPCase, NADP-ME and NAD-ME activities in *Hedysarum scoparium* and *Haloxylon ammodendron* at 2.4 m DGW where water stress was moderate. Each value represents mean (± S.E.) of three replications.

Species	Organs	PEPCase (μmol PEP min^{-1} mg^{-1} protein)	NADP-ME (μmol min^{-1} mg^{-1} protein)	NAD-ME (μmol min^{-1} mg^{-1} protein)
Hedysarum scoparium (C_3)	Assimilating shoots	3.40 ± 0.35	0.75 ± 0.06	1.38 ± 0.21
	Leaves	6.98 ± 0.55	1.24 ± 0.06	3.38 ± 0.53
Haloxylon ammodendron (C_4)	Assimilating shoots	53.39 ± 1.91	24.21 ± 1.52	7.37 ± 0.70

Discussion

Antioxidant protection in *Hedysarum scoparium*: Protection might be performed by the increased activities of enzymes involved in the destruction of free radicals and oxidants, but their responses depended on the water status of the plant. RWC either in leaves or in assimilating shoots of *H. scoparium* was very low, which corresponded with the water use efficiency (WUE) of *H. scoparium* (Su 2003). Lower RWC of *H. scoparium* suggested that water availability was the key environmental factor. Lower RWC and gentler change were found in leaves than in assimilating shoots of *H. scoparium* under the same condition, indicating that assimilating shoots played an important role in water storage.

On exposure to high light, there was an increase in synthesis of protective antioxidant enzymes (Mishra *et al*., 1993; Sharma & Singhal, 1992). They also observed that water stress altered the membrane organization and the absorbance/fluorescence properties of chloroplasts (Singh & Singhal, 1984). Among the enzymes responsible for the scavenging of active oxygen species, the changes of antioxidant enzymes were all undulated violently either in leaves or assimilating shoots in process of a day (data not shown). Activities of antioxidative enzymes were higher at noon and lower in the early morning and evening. In general, the activities of antioxidative enzymes were higher in leaves than in assimilating shoots. SOD activity was obviously much higher in leaves than in assimilating shoots of *H. scoparium* at 3 DGW (Fig. 3A), and SOD activity increased gradually both in leaves and in assimilating shoots, depending upon the increase of water stress. The ratio of SOD activity of leaves / assimilating shoots gradually decreased with the increase of DGW, demonstrating that SOD activity in assimilating shoots played a more significant role than that in leaves when plants grow in the prolonged water stress and explaining why the appearance of assimilating shoots of *H. scoparium* was essential to defend oxidative stress in exacerbated water environment. Lyshede (1979) pointed out the formation of assimilating shoots was the summit of evolution under drought condition. Exogenous antioxidants stimulated shoot organogenesis (Gupta & Datta, 2003). Maybe the evolution of shoot was supplemented and complemented each other with the increasing stress.

As SOD activity increased, the quantity of H_2O_2 formed through SOD catalysis might also increase. The action modes of these two enzymes were essentially different. CAT catalyzed the dismutation of two molecules of H_2O_2 to water and molecular O_2 at near diffusion-controlled rates, whereas POD used the substrates to reduce H_2O_2 to water. APx had a much higher affinity for H_2O_2 than CAT, but CAT had a much higher *V*max (Asada, 1992). There was a similar effect in POD activity of *H. scoparium* with in SOD activity of *H. scoparium*. Induction of antioxidant enzymes, such as SOD, POD and CAT, could lead to the increase in antioxidant protection and the decrease in oxidative

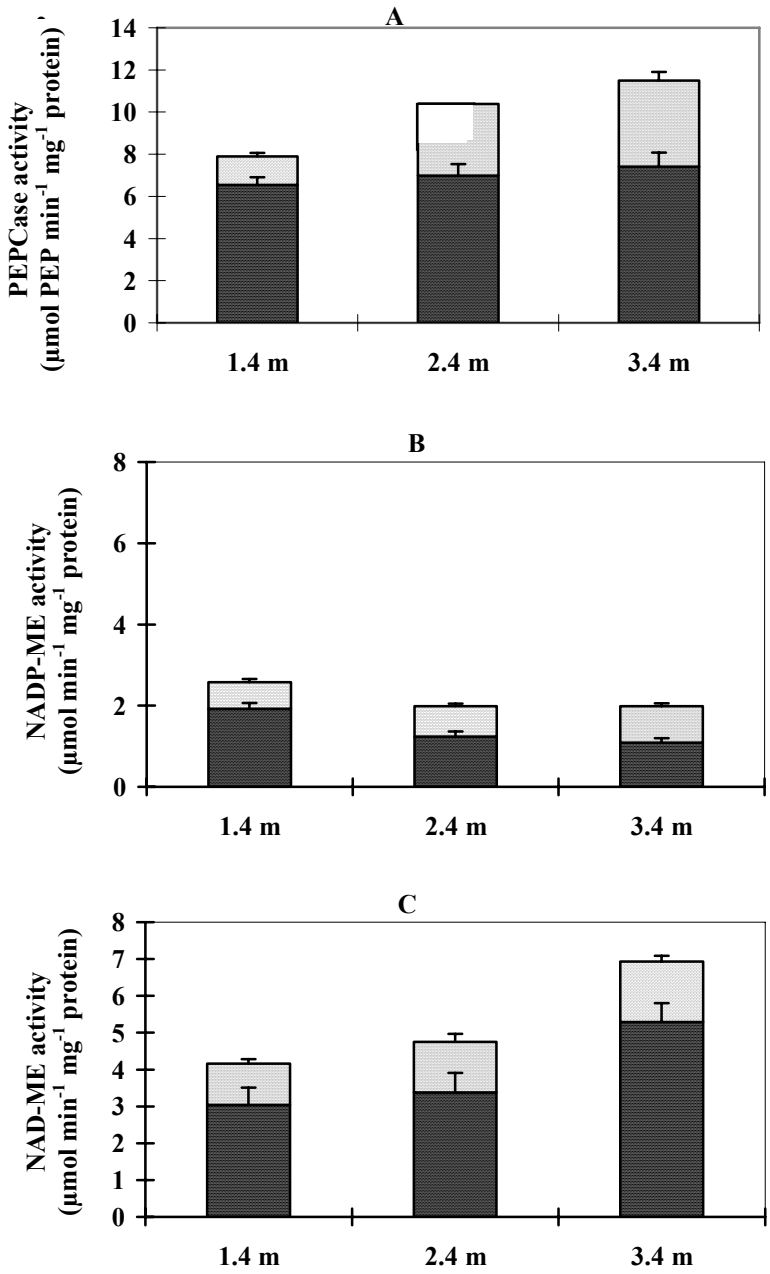

Fig. 4. Variations of PEPCase, NADP-ME and NAD-ME activities in ■ leaves and □ assimilating shoots of *Hedysarum scoparium* at different DGW of 1.4 m, 2.4 m, and 3.4 m at 15:00. Each value represents mean (± S.E.) of three replications

damage. High activity of antioxidative enzymes could be correlated to the process of differentiation that occurred during shoot induction (Thakar & Bhargava, 1999). At the more drought-tolerant 3.4 m DGW, the lower POD activity in leaves and the higher in assimilating shoots were found, suggesting that POD might not greatly contribute to drought tolerance mechanism in leaves at 3.4 m DGW. In scavenging H_2O_2, maybe APX played a more important role in leaves (Willekens *et al*., 1995). Therefore, further studies on other antioxidative enzymes are needed.

CAT was the enzyme capable of detoxifying H_2O_2 in the cell (Lin & Kao, 2000). We considered the performance of some enzyme activities from the perspective of H_2O_2 scavenging. So, we measured CAT activity. There was only minor losses of CAT activity occurred in assimilating shoots of *H. scoparium* at 3 DGW, but a marked apparent water deficit inactivation of CAT was observed in leaves of *H. scoparium* in 3.4 m DGW. CAT activity was suppressed in severe water stress, which was an indication of a reduced capacity of the leaves to decompose H_2O_2. This result was consistent with CAT activity in stems of *R. raetam* and in leaves of *A. halimus* in natural daylight (Streb *et al*., 1997). The present findings further confirmed that CAT activity was subjected not only to high irradiance but also to water deficit. Furthermore, mild water stress could stimulate CAT activity and severe water stress inactivated CAT activity, supporting that drought stress inactivated the key enzyme activity of light respiration-ethanol acid oxidative enzyme 44% (Morgan *et al*., 1994). The inactivation of the key enzyme activity resulted in the accumulation of more NADPH and ATP, and the increase of production of O_2^-, H_2O_2 in chloroplast. More NADPH and ATP may supply more energy to recapture CO_2 released by respiration performing C_4 photosynthesis (Ku *et al*., 1991; Li *et al*., 2000) in C_3 plant. The result was also supported by the changes of C_4 enzymatic activities (Fig. 4). So, it was concluded that PSII might avoid photoinhibition by supplying more energy to ensure performance of C_4 pathway at high temperature and irradiance under severe water stress.

C_4 photosynthesis in *Hedysarum scoparium*: The variations in C_4 photosynthetic key enzymes were consistent with the changes of protein content of enzymes (data not shown), indicating that the increase of enzymatic activities was the synthesis of enzymatic protein but not the activation of enzymes.

The intrinsic link between photosynthesis and biomass production suggested that photosynthetic response to drought probably played a major role in determining the ability of these species to persist in drought areas (Lambers *et al*., 1998; Gulías *et al*., 2002), also in consideration of global change. Water availability was an important factor affecting photosynthetic activity of plant species (Llorens *et al*., 2003). The lack of water that occurred during the dry season was the important limiting environmental factor that triggered the plant to change their photosynthetic strategy. Liu *et al*. (2004) suggested that the occurrence of C_4 species was common in the more arid region of China, so it was considered that environmental conditions played significant roles in the distribution and ecophysiological features of different photosynthetic types and even changed the photosynthetic pathways. Wang *et al*. (2001) reported the presence of PEPCase in all green organs of wheat. Furthermore, this activity was significantly higher in each non-leaf organ (Xu *et al*., 2003). As PEPCase activity was positively and negatively regulated by metabolites (Kai *et al*., 2003), the ubiquity of these C_4 cycle enzymes in C_3 plants strongly indicated that these 'C_3 isoforms' served as the starting point for the evolution of the C_4 genes (Monson, 1999). C_4 enzymatic activities were measured on *H. scoparium* and demonstrated the existence of C_4 photosynthesis both in leaves and assimilating

shoots, although lacking Kranz-anatomy in desert areas. Meanwhile, determination of enzymatic activity showed that leaves and assimilating shoots of *H. scoparium* had the properties of NAD-ME biochemical subtypes. C_4 plants of NAD-ME subtypes were known to be more resistant to stress than the species of the NADP-ME subtypes (Pyankov & Vakhrusheva, 1989, Gamaley *et al*., 1992), suggesting the trend of evolution of C_4 pathway in C_3 desert plant was more resistant to arid stress.

The activity of PEPCase in leaves had a minor elevation, while the activity of NADP-ME was declined faintly with the increase of water stress. The activity of NAD-ME in leaves increased and was higher than that of NADP-ME. However, both of them in assimilating shoots were all increased with the increase of water stress, which was consistent with the variations of antioxidative enzymes in assimilating shoots. In addition, photosynthetic key enzymatic activities of leaves add assimilating shoots almost increased with the increase of water stress, suggesting that photosynthetic latent capacity may be advanced in *H. scoparium* with the increase of water stress. Although the photosynthetic rate of *H. scoparium* declined and stomatal conductance decreased in midday (Su, 2003), the activities of C_4 photosynthetic key enzymes increased, suggesting that the decline of photosynthetic rate was caused by stomata factor but not the activities of photosynthetic enzymes. Furthermore, plants may perform C_4 pathway by supplied malate from root systems (Hibberd & Quick, 2002) to improve photosynthetic inactivation at midday.

Water, heat and light as stress factors interacted to modulate photosynthesis (Jagtap *et al*., 1998). C_4 photosynthetic key enzymes and antioxidative enzymes in leaves were always higher than those in assimilating shoots. The presence of the more severe water stress in 3.4 m DGW (Table 1) and the relative high C_4 photosynthetic carbon assimilating activities (Fig. 4) in leaves might lead to the production of active oxygen species and induce the high activities of active oxygen detoxifying enzymes. High irradiance results in an increase of high respiration rate in leaves (Yamazaki *et al*., 1999), higher CO_2 concentration in cellular result in an increase of PEPCase activity. It was emphasized that water sensitivity was different between the two photosynthetic organs, so an increase of the metabolic pathway and the degradation of many scavenging enzymes induced the accumulation of active oxygen species, and especially, the production of H_2O_2 during drought stress may also result from the catalytic activity of glycolate oxidase in peroxisomes during photorespiration (Osmond, 1981) inactivated enzymatic activities of C_3 pathway (Wang, 2001), became a survival reason for the occurrence of C_4 pathway under stress. But the changes of photosynthetic contribution in leaves and assimilating shoots were dramatically different. Although the activities of C_4 photosynthetic key enzymes in leaves were always higher than that in assimilating shoots of *H. scoparium*, indicating that leaves of *H. scoparium* were the main photosynthetic and antioxidative organs at actual water gradient. However, C_4 pathway was presented with enhanced activities as the increase of water stress in assimilating shoots of *H. scoparium*, it was proposed that C_4 photosynthetic response in assimilating shoots was more positive than that in leaves as water condition became deteriorated rapidly. It might be the adaptable mechanism and the evolutional direction of the organs of the C_3 desert species in exacerbated water circumstance in desert areas.

In conclusion, the findings demonstrated that the desert specie of Leguminosae was characterized by performing C_4 biochemical pathways of CO_2 fixation, not only in leaves, but also in assimilating shoots. Both photosynthetic pathway types appeared adaptation to

desert environments and this specie showed environmentally induced changes in their photosynthetic responses consistent with desert adaptation. The multiple independent origins of C_4 photosynthesis suggested that the evolution of a C_3 into a C_4 species must have been relatively easy in genetic terms (Westhoff & Gowik, 2004). Kellogg (1999) and Monson (1999) provided strong evidence that even within a single taxon, i.e., the Gramineae, the transition from C_3 to C_4 may have occurred more than once. The results indicated that the occurrence of C_4 photosynthetic pathway was an adaptation in many of these species. In addition, the organ diversity of C_4 photosynthesis in C_3 desert species was an important factor for plant growth, survival and reproduction in arid regions of China. Simultaneity, the antioxidative enzymes played an important role in the protection of plants from oxidants during the C_3-CAM shift induced by water stress (Castillo, 1996). This study demonstrated that antioxidative enzymatic system seemed to be very important in the protection against oxidants, both increasing considerably antioxidative enzymes and C_4 photosynthetic enzymes under increasing water stress in C_3 plant.

Abbreviations: APX, ascorbate peroxidase; **CAT,** catalase; **DGW**, depth of ground water; **DM**, dry mass; **DTT**, 1,4-dithiothreitol; **FM,** fresh mass; **NAD-ME**, NAD^+-malate enzyme; **NADP-ME**, $NADP^+$-malate enzyme; **NBT**, nitroblue tetrazolium; **PEPCase**, phosphoenolpyruvate carboxylase; **PFD**, photon flux density; **POD,** peroxidase; **PVP**, polyvinylpyrrolidone; **RWC,** relative water content; **SM**, saturated mass; **SOD,** superoxide dismutase; **WUE**, water use efficiency.

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