



Physiological and biochemical regulation mechanisms for drought adaption in typical desert shrubs

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ABSTRACT

The physiological and biochemical mechanisms for different photosynthetic pathways desert shrubs was not completely understood and raises the question if C_4 plants are better adapted for severe drought than C_3 plants. We selected two C_4 plants *Calligonum mongolicum* and *Haloxylon ammodendron*, and three C_3 plants *Caragana korshinskii*, *Nitraria sphaerocarpa* and *Tamarix ramosissima* in their natural environments, and to systematically study the water status, antioxidant enzyme activity, osmotic adjustment substances content and photosynthetic physiological characteristics. Our results suggested that two C_4 species showed higher leaf water potential and superoxide dismutase activity compared to the other three C_3 species. Higher light saturation point and apparent quantum yield were also found in *C. mongolicum* and *H. ammodendron* suggested they had more efficient solar energy utilization. *C. korshinskii* had higher catalase and peroxidase activities to auxiliary antioxidant activity, *N. sphaerocarpa* showed a greater capability for free amino acid and proline accumulation. *T. ramosissima*, as a non-belt plant, was accumulates soluble sugars to maintain internal osmotic balance. It was concluded that two C_4 species would be more competitive than the C_3 species in desert areas as for the higher antioxidant defenses capacity and light use efficiency.

Keywords: Desert plants, Antioxidant enzyme, Osmotic adjustment, Photochemical activity, Chlorophyll fluorescence

INTRODUCTION

The desert of central Hexi Corridor is a typical arid desert climate region, rare rainfall, high temperatures and high evaporative demand, as desert shrubs are generally the dominant vegetation types and have a unique growth advantage of its adaptability. Desert plants have evolved special physiological and morphology traits in the process of adapting to the environmental stresses, and exhibit a much higher drought tolerance [1]. The activities of desert plants are tightly coupled to water availability and can induce the physiological and biochemical responses. These responses of plants to environmental stresses have been well documented, and ranging from photosynthesis to antioxidant defenses and solute accumulation as components of drought tolerance [2, 3].

It is well known that photosynthetic systems in higher plants are extremely sensitive to drought stress and increased photooxidation was observed at extreme temperature [4]. High temperatures inhibit photosynthetic CO_2 fixation and damage photosynthetic electron transport, particularly at the site of PSII [5]. Chlorophyll fluorescence is closely related to photosynthesis, it reflects the photosynthetic efficiency and utilization of light energy. The parameters of chlorophyll fluorescence analysis can give insights into the ability of a plant to tolerate environmental stresses and

the extent to which stresses damage the photosynthetic apparatus [6].

It has also been suggested that increase in photosynthetic electron flux to O₂ will result in the increased production of superoxide radicals and H₂O₂ [7]. Tolerant plants generally have a better capacity to protect themselves against drought-induced oxidative stress, which can also be achieved via the enhancement of the activities of antioxidant enzymes. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), are known to increase in response to drought and high temperature. SOD converts O²⁻ into H₂O₂ and O₂, and CAT and POD scavenge H₂O₂ into H₂O [8]. On the other hand, the other potentially important mechanism of drought tolerance is osmotic adjustment, which can be achieved from the accumulation of compatible solutes in protoplasm [9]. Osmotic adjustment has been considered as an important physiological adaptation character associated with drought tolerance, which facilitate extracting water from dry soils and maintaining cell turgor, gas exchange and growth in very dry environments [4]. Meanwhile, there are a closely relationship between PSII light energy conversion and osmotic adjustment under drought stress. Accumulation of osmotic adjustment substances and antioxidant capacity enhancements are two important mechanisms through which plants can survive under adversity. In a physiological context, plants have developed three types of photosynthetic pathways: C₃, C₄ and CAM. C₄ pathway is considered to be an advantage survival pathway of plant under high temperature, strong light and drought conditions, and have superior water use efficiency (WUE) than C₃ plant [10]. In the desert regions of China, C₄ plants, especially C₄ woody plants, have an important position and role in the desert ecosystem. Wentworth [11] predicted that with the climate change, it is likely to have a large effect on the abundance and distribution of plant species in desert areas, and C₄ species should be proportionally more successful in desert areas. While, the photosynthetic physiological and biochemical mechanisms for different photosynthetic pathways is not completely understood and raises the question if C₄ plants are better adapted for severe drought than C₃ plants. Thus, the main objective of this study was to compare the photosynthetic characteristics and protective mechanisms in desert plants with different photosynthetic pathway. Understanding the ecological strategies of drought tolerance in desert plants would be better know the drought adaption mechanisms and predict plants response to climate change.

EXPERIMENTAL SECTION

Study site and plants material

The experiment was carried out in the middle reaches of Heihe river in northern Linze country, Gansu province (39°19'-39°21' N, 100°02'-100°21' E), at an altitude of 1370 m. It is a typical drought desert climate with an average annual precipitation of 117 mm. This area is one of the most seriously desertified areas of China [12].

Five desert shrubs *Caragana korshinskii* Kom., *Nitraria sphaerocarpa* Maxim., *Calligonum mongolicum* Turcz., *Haloxylon ammodendron* (C.A.Mey.) Bge. and *Tamarix ramosissima* Ledeb. were selected to study their photochemical and biochemical activities in natural environments. All of these species are widely distributed in desert areas, the leaves of *H. ammodendron* and *C. mongolicum* were all degradation, by the assimilating shoots for photosynthesis. *C. korshinskii* has a very strong ecological adaptability, *N. sphaerocarpa* displays tenacious growth in soils with extremely low moisture content and *T. ramosissima* is a non-zonal plant with a large distribution across desert-oasis ecotone. All plant specimens were collected at growing and developing stages in late July, 2011. Samples of plant photosynthetic organs (leaves or assimilating shoots) were immediately frozen in liquid nitrogen and stored at -70 °C until required.

Measurement of the δ¹³C values

Take plant leaves or assimilating shoots back to the laboratory and the stable carbon isotope ratio was analyzed by MAT-252 mass spectrometer using the formula:

$$\delta^{13}\text{C} (\text{‰}) = [(\text{R}_{\text{sample}} - \text{R}_{\text{standard}}) / \text{R}_{\text{standard}}] \times 10^3 \quad (1)$$

Where: R_{sample} and R_{standard} were the abundance ratios, ¹³C/¹²C, of the sample and the standard, Pee Dee belemnite (PDB), respectively.

Determination of the relative water content (RWC) and leaf water potential (LWP)

The RWC of leaves and assimilating shoots were measured in early August, 2011 and calculated with the following formula:

$$\text{RWC} (\%) = [(\text{FW} - \text{DW}) / (\text{SW} - \text{DW})] \times 100 \quad (2)$$

LWP was measured using a WP4-T Dew point water meter (Decagon, American).

Photosynthesis response to light intensity

Light response curves were made by using LI-6400 gas exchange system (LI-COR, Lincoln, USA). Afterwards, all the leaves were collected, and their areas were measured using the LI-3100 Area Meter (LI-COR), then recomputes the date through the LI-6400.

Measurement of chlorophyll fluorescence and chlorophyll content

Chlorophyll fluorescence was measured with a PAM-2100 portable modulated chlorophyll fluorometer (WALZ, Germany). Leaves were dark-adapted before measurement and from 8:00 to 18:00 with 1-h intervals in the same plant.

Chlorophyll (Chl) and carotenoids (Car) were extracted by 95% ethanol and the calculation of pigment content was performed according to the method by Lichtenthaler [13].

Antioxidant enzymes activity and membrane lipid peroxidation determination

SOD activity was assayed according to Giannopolitis and Ries [14]. CAT activity was assayed according to the method of Aebi [15]. POD activity was assayed according to Rao [16]. Lipid peroxidation was estimated by measuring the MDA content [17].

Measurement of osmotic adjustment solute content

Soluble sugar was extracted and determined following the phenol method. Soluble protein content was assayed by using the Coomassie Brilliant Blue G-250 chromogenic method. Total free amino acids were determined following the ninhydrin coloration method by Lee et al. [18]. Proline was determined following the method of Bates et al. [19]. All absorbance measurements for enzyme activity and content determination were conducted using a GeneQuant 1300 spectrophotometer (Britain) and all the measurements were repeated at least three times.

Statistical analysis

Values presented are means \pm one standard error (SE) of three replicates. The significance of the same index for different plants was determined using Duncan's new multiple range test. All the statistical analyses were carried out on SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS

Stable carbon isotope ratio ($\delta^{13}\text{C}$) and water status with desert shrubs

There was a positive correlation between $\delta^{13}\text{C}$ and WUE, so the $\delta^{13}\text{C}$ value can indicate plants WUE. From table 1 we can see that *H. ammodendron* and *C. mongolicum* had a higher $\delta^{13}\text{C}$ values than other three species. It also indicated that *H. ammodendron* and *C. mongolicum* had higher WUE in arid environment.

There were obvious differences between RWC in the five desert shrubs (Table 1). The average of RWC in two C_4 plants *C. mongolicum* and *H. ammodendron* (71.7%) were lower than in other C_3 plants (75.2%). *N. sphaerocarpa* had the highest RWC than other species, and had significantly differences ($F=18.394$, $P<0.05$) among of them.

LWP reflects the ability of plants to absorb and hold water and contributes to drought tolerance. Among the five desert shrubs, the C_4 plants had higher LWP than the C_3 plants ($F=8.598$, $P<0.05$), and *C. mongolicum* had the highest LWP of -1.5 MPa. C_3 plants *C. korshinskii* had the lowest value of LWP with -4.7 MPa.

Table 1. Stable carbon isotope ratio ($\delta^{13}\text{C}$) and water status with desert shrubs

Values represent means \pm SE of three replications per plant. Different lowercase in the same rows indicate significant differences ($P<0.05$). $\delta^{13}\text{C}$, Stable carbon isotope ratio; RWC, Relative water content; LWP, Leaf water potential

Parameters	<i>C. korshinskii</i>	<i>N. sphaerocarpa</i>	<i>T. ramosissima</i>	<i>C. mongolicum</i>	<i>H. ammodendron</i>
$\delta^{13}\text{C}$ (‰)	-26.89 \pm 0.03 ^c	-26.19 \pm 0.03 ^c	-26.36 \pm 0.02 ^c	-15.99 \pm 0.01 ^a	-17.08 \pm 0.01 ^b
RWC (%)	71.5 \pm 0.2 ^d	80.8 \pm 0.1 ^a	73.4 \pm 0.2 ^b	70.9 \pm 0.3 ^e	72.5 \pm 0.2 ^c
LWP (MPa)	-4.7 \pm 0.3 ^e	-4.3 \pm 0.1 ^d	-3.9 \pm 0.1 ^c	-1.5 \pm 0.1 ^a	-2.3 \pm 0.2 ^b

The response of PN to different photosynthetic photon flux density (PPFD)

There were significant differences in the responses of P_N to PPFD in the five plants. Of the five shrubs, two C_4 plants had higher light saturation point (LSP), apparent quantum yield (AQY) and lower light compensation point (LCP) compared to three C_3 shrubs. *C. mongolicum* had the highest LSP and AQY values, which were 2040 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and 0.059 $\text{mol}\cdot\text{mol}^{-1}$, respectively (Table 2), while *N. sphaerocarpa* had the lowest LSP and AQY values.

Table 2. Photosynthetic physiological parameters with five desert shrubs

Values represent means \pm SE of three replications per plant. Different lowercase in the same rows indicate significant differences ($P < 0.05$). LCP, Light compensation point; LSP, Light saturation point; AQY, Apparent quantum yield

Parameters	<i>C. korshinskii</i>	<i>N. sphaerocarpa</i>	<i>T. ramosissima</i>	<i>C. mongolicum</i>	<i>H. ammodendron</i>
LCP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	100.2 \pm 3.1 ^a	106.1 \pm 3.0 ^a	90.2 \pm 6.9 ^b	53.6 \pm 2.9 ^c	44.4 \pm 4.1 ^d
LSP ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	1690 \pm 85 ^{bc}	1580 \pm 92 ^{cd}	1500 \pm 44 ^d	2040 \pm 131 ^a	1825 \pm 51 ^b
AQY ($\text{mol}\cdot\text{mol}^{-1}$)	0.040 \pm 0.003 ^c	0.036 \pm 0.001 ^c	0.037 \pm 0.002 ^c	0.059 \pm 0.006 ^a	0.050 \pm 0.002 ^b

Chlorophyll fluorescence characteristics

It can be seen from table 3 that lower maximum photochemical efficiencies of PSII (F_v/F_m), apparent electron transport rate (ETR) and effective photochemical efficiency (Yield) were observed in two C_4 plants *H. ammodendron* and *C. mongolicum*, and had significantly differences than C_3 plants. *N. sphaerocarpa*, on the other hand, had the highest values of the three parameters. Although two C_4 plants *C. mongolicum* and *H. ammodendron* showed the lower daily average (8:00-18:00) of F_v/F_m , but the difference between the maximum and minimum values were smaller than three C_3 plants (data not shown).

Non-photochemical quenching (qN) is a good indicator to reflect plants against photoinhibition (Demmig-Adams et al. 1996), and photochemical quenching (qP) reflect the light adaptation state of the photochemical reaction of PS II capacity. In our study, *N. sphaerocarpa* had higher qP values but lower qN value, while *H. ammodendron* had the highest qN in the five plants, and the differences was significantly ($F=7.647$, $P < 0.05$).

Table 3. Chlorophyll fluorescence parameters of the five desert shrubs

Values represent means \pm SE of three replications per plant. Different lowercase in the same rows indicate significant differences ($P < 0.05$). F_v/F_m , maximum photochemical efficiency of photosystem II; ETR, relative electron transport rate; qP, photochemical quenching; qN, non-photochemical quenching; Yield, effective photochemical efficiency

Parameters	<i>C. korshinskii</i>	<i>N. sphaerocarpa</i>	<i>T. ramosissima</i>	<i>C. mongolicum</i>	<i>H. ammodendron</i>
F_v/F_m	0.794 \pm 0.009 ^a	0.802 \pm 0.005 ^a	0.799 \pm 0.015 ^a	0.744 \pm 0.006 ^c	0.774 \pm 0.011 ^b
ETR	86.850 \pm 4.533 ^b	88.710 \pm 10.983 ^b	125.310 \pm 6.794 ^a	81.320 \pm 3.562 ^b	61.790 \pm 5.147 ^c
qP	0.952 \pm 0.025 ^a	0.971 \pm 0.033 ^a	0.968 \pm 0.022 ^a	0.939 \pm 0.028 ^a	0.958 \pm 0.029 ^a
qN	0.738 \pm 0.012 ^b	0.689 \pm 0.015 ^c	0.742 \pm 0.037 ^b	0.745 \pm 0.007 ^b	0.784 \pm 0.023 ^a
Yield	0.542 \pm 0.037 ^a	0.543 \pm 0.041 ^a	0.521 \pm 0.023 ^a	0.505 \pm 0.022 ^a	0.439 \pm 0.030 ^b

Chlorophyll content with desert shrubs

Chlorophyll a (Chl a), Chl b, total Chl (a + b) contents and Car in *C. korshinskii* was highest than other species (Table 4). *H. ammodendron*, on the other hand, had the lowest chlorophyll content among the five plants. In the five desert plants, *H. ammodendron* had the highest Chl a/b ratio, while *C. korshinskii* had lowest value of 2.15, which is contrary to the chlorophyll content results, but the differences in the five plants were not significantly ($F=2.626$, $P > 0.05$).

Table 4. The chlorophyll (Chl) content and Chl a/b ratio with desert plants

Values represent means \pm SE of three replications per plant. Different lowercase in the same rows indicate significant differences ($P < 0.05$). Chl a, chlorophyll a; Chl b, chlorophyll b; Car, carotenoid; chlorophyll a/ chlorophyll b, Chl a/b

Parameters	<i>C. korshinskii</i>	<i>N. sphaerocarpa</i>	<i>T. ramosissima</i>	<i>C. mongolicum</i>	<i>H. ammodendron</i>
Chl a ($\text{mg}\cdot\text{g}^{-1}\text{FW}$)	1.76 \pm 0.02 ^a	0.79 \pm 0.14 ^b	0.83 \pm 0.01 ^b	0.64 \pm 0.05 ^c	0.41 \pm 0.01 ^d
Chl b ($\text{mg}\cdot\text{g}^{-1}\text{FW}$)	0.82 \pm 0.02 ^a	0.36 \pm 0.07 ^b	0.38 \pm 0.02 ^b	0.29 \pm 0.07 ^b	0.15 \pm 0.03 ^c
Chl (a+b) ($\text{mg}\cdot\text{g}^{-1}\text{FW}$)	2.59 \pm 0.04 ^a	1.14 \pm 0.19 ^b	1.12 \pm 0.03 ^b	0.93 \pm 0.11 ^c	0.56 \pm 0.03 ^d
Car ($\text{mg}\cdot\text{g}^{-1}\text{FW}$)	0.35 \pm 0.01 ^a	0.17 \pm 0.04 ^b	0.20 \pm 0.01 ^b	0.12 \pm 0.01 ^c	0.09 \pm 0.01 ^c
Chl a/b	2.15 \pm 0.04 ^a	2.19 \pm 0.32 ^a	2.16 \pm 0.13 ^a	2.20 \pm 0.40 ^a	2.74 \pm 0.58 ^a

Antioxidant enzymatic activity and MDA content

The antioxidant enzymes activities in the five desert plants are show in Fig. 1. The average SOD activity was 144.71 $\text{U}\cdot\text{g}^{-1}\text{FW}$ and 179.46 $\text{U}\cdot\text{g}^{-1}\text{FW}$ in C_3 and C_4 plants, respectively. Among the five shrubs, *C. mongolicum* had the highest SOD activity (193.74 $\text{U}\cdot\text{g}^{-1}\text{FW}$) while *N. sphaerocarpa* had the lowest SOD activity. CAT is a high capacity enzyme with low oxygen affinity that destroys hydrogen peroxide. *C. korshinskii* is distinguished from the other four plants by its high CAT activity of 9.35 $\text{U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ (Fig. 1b). The average POD activity for the C_3 plants was higher than for the two C_4 plants (Fig. 1c). Among the three C_3 plants, the highest POD activity was exhibited by *C. korshinskii* (257.53 $\text{U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$), while the POD activity of *C. mongolicum* was too small to be detected. MDA is the product of lipid peroxidation and its content can reflect the level of lipid peroxidation. The highest MDA content, which was measured in *C. korshinskii*, was 32.27 $\text{nmol}\cdot\text{g}^{-1}\text{FW}$, and significantly greater than the content of all other plants (Fig. 1d, $P < 0.05$). The MDA content of the other four plants were similar to each other. Of the five desert plants, the lowest MDA content was observed in *H. ammodendron* (6.81 $\text{nmol}\cdot\text{g}^{-1}\text{FW}$).

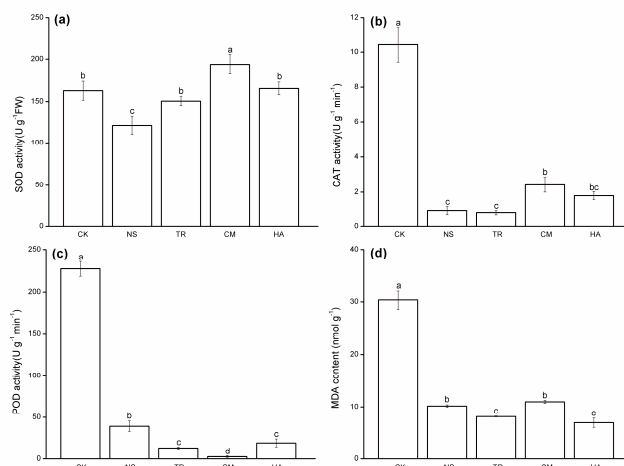


Fig. 1. Antioxidative enzymatic activity and MDA content in five desert plants

Comparison among the different species, the different lowercase in the same index means significant differences ($P < 0.05$). CK, *C. korshinskii*; NS, *N. sphaerocarpa*; CM, *C. mongolicum*; HA, *H. ammodendron*; TR, *T. ramosissima*

The content of osmotic adjustment solute

The soluble sugar content of the C₃ and C₄ plants did not differ significantly (Fig. 2a). *T. ramosissima* had the largest soluble sugar content (76.72 mg·g⁻¹) and *H. ammodendron* had the lowest content. The soluble protein content was higher in *C. korshinskii* than other desert plants (Fig. 2b), and *T. ramosissima* had the lowest soluble protein content among the five shrubs. The total free amino acid varied significantly among the five desert shrubs (Fig. 2c). *N. sphaerocarpa* had the highest free amino acid contents with value of 208.57 mg·g⁻¹. Proline is the main osmotic adjustment substance in plants. Generally, plants with higher proline content are more adapted to drought. The highest proline content was found in *N. sphaerocarpa*, which was significant difference from other four plants (Fig. 2d, $P < 0.05$). The lowest values were observed in *T. ramosissima*, with a value of 25.26 ug·g⁻¹.

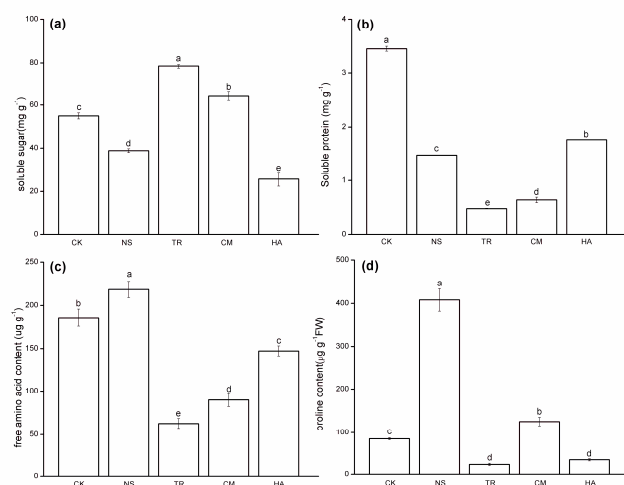


Fig. 2. The osmotic adjustment solute content in the five desert plants

Comparison among the different species, the different lowercase in the same index means significant differences ($P < 0.05$). CK, *C. korshinskii*; NS, *N. sphaerocarpa*; CM, *C. mongolicum*; HA, *H. ammodendron*; TR, *T. ramosissima*

DISCUSSION

Desert plants in harsh environments, with different photosynthetic pathways respond differently to environmental factors, and therefore have dissimilar environmental adaptation strategies. Water deficit caused by drought and high temperature changes in morphology, water status, gas exchange and chlorophyll content which are connected with the onset of protective mechanisms in the plant [20]. RWC are physiological processes very susceptible to drought stress, and can reflect the water-holding capacity of leaves. The leaf of *N. sphaerocarpa* is fleshy leaf, with higher RWC to resist drought. High RWC may be an adaptive feature for *N. sphaerocarpa* to overcome the effect of drought and water deficit environments. LWP is an important physiological indicator predicting plant water stress and drought tolerance, and higher LWP value were observed in the two C₄ plants *C. mongolicum* and *H.*

ammodendron. While, *C. korshinskii* had the lowest value of LWP. Lower LWP in *C. korshinskii* and *N. sphaerocarpa* was beneficial to absorb soil water, which is a physiological response of plants to withstand drought.

Photosynthesis is a biochemical process and acts as an index to determine plant growth and resistance to adverse conditions. Extensive reviews of leaf-level research suggests that C_4 species should outperform C_3 individuals at higher temperatures given inherent properties associated with their photosynthetic pathways [21]. In the present studies, highly significant differences in photosynthetic capacity were found among C_3 and C_4 plants. Two C_4 plant *H. ammodendron* and *C. mongolicum* with lower LCP, higher LSP and AQY (Table 2), thus had more efficient solar energy utilization than other three C_3 plants, and suggested that they have a stronger adaptation tolerance for high temperature and dry environments. Ehleringer et al. [22] proposed that C_4 plants are typically found in warmer and drier habitats and with climate warming, is therefore likely to favour C_4 species at the expense of C_3 species. Comparatively speaking, the two C_4 plants were adaptable to a wider range of light than the C_3 plants.

Two C_4 plants *C. mongolicum* and *H. ammodendron* had lower F_v/F_m , ETR, and Yield values, while *N. sphaerocarpa* had the highest F_v/F_m , qP and Yield values. F_v/F_m represents the photosynthetic institutions absorbed light to chemical reaction for maximum efficiency. In drought environmental, the F_v/F_m values of dark-adapted plants are above 0.83. Results from this research showed that the daily average of F_v/F_m in five plants were all below 0.83, suggesting all plants investigated may suffer severe photoinhibition of PS II.

Earlier study proved that chlorophyll contents usually decreased under drought stress due to their slow synthesis or fast breakdown. In this study, the chlorophyll content of three C_3 plants was higher than that of two C_4 plant, while the Chl a/ Chl b ratios of the two C_4 shrubs were higher. Li and Cao [23] found that under drought environment, the chlorophyll content in *Terminthia paniculata* seedlings is reduced and Chl a/ Chl b ratio tended to increase. The reduction in chlorophyll contents may be a strategy of photo-protection reducing light absorption and thus decreasing energetic pressure at the PSII level. Studies have shown that Chl a/ Chl b increases with adversity [24]. The higher Chl a/ Chl b ratios in the two C_4 plants suggested that they are better adapted to drought than C_3 shrubs, and also considered as a decreased emphasis on light collection in relation to the rates of PSII photochemistry.

There are many processes affecting P_N performance in plants, including increased photorespiration, damage to the CO_2 fixed reaction mechanism, as well as an occurrence of reactive oxygen species (ROS). Under drought environments, generation of excessive ROS in the chloroplasts can result in photoinhibition and photooxidation damages. Schwanz and Polle [25] suggested that increase the drought tolerance of plants is related to increase biochemical protection at the tissue level. Our findings revealed that both the plant species had varying ability to deal with oxidative stress that might govern their differential sensitivity to drought environments (Fig. 1). The higher levels of SOD activities in *C. mongolicum* and *H. ammodendron* reflect their greater involvement in C_4 plants to counter oxidative stress as compared to C_3 plants like *C. korshinskii*, which had relatively more activity of CAT and POD. Streb et al. [26], who investigated the antioxidant protection mechanism of two desert plants (*Atriplex halimus* and *Retama raetam*), suggested that desert plants employ different strategies to protect themselves against high light intensity and temperature. Similar to our findings, Caravaca et al. [27] pointed that SOD activity was also increased by drought stress in shoots of Mediterranean plants such as *Myrtus communis* and *Phillyrea angustifolia*. Relative to C_3 shrubs, the higher SOD activity in C_4 plants lends a defense against free radical formation and photo-inhibition triggered by high temperature environment. Significantly, In this research, the activities of CAT ($9.35U \cdot g^{-1} \cdot min^{-1}$) and POD ($257.53 U \cdot g^{-1} \cdot min^{-1}$) were higher in *C. korshinskii* compared to the other four desert plants, and increase in CAT and POD activity helps to overcome the damage of tissue metabolism by reducing the toxic level of H_2O_2 .

The increasing of osmotic adjustment substances content could be lowering the cell osmotic potential and maintenance of leaf turgor under water-limited conditions. Plants with strong drought tolerance have higher accumulation of osmotic adjustment substances. Soluble sugars might play a central role in photochemical apparatus protection and ROS scavenging in stressed plants. In the present study, soluble sugar content had greater difference in the five species (Fig. 2a). *T. ramosissima* as a non-belt plant, had the highest soluble sugar content differ from other four desert plants. Higher soluble sugars content would be involved in dehydration tolerance by protecting the macro-molecules through preferential hydration during water loss. Higher accumulates soluble sugars of *T. ramosissima* accumulates is closely related to their tolerant of high salinities, and could be maintain internal osmotic balance and compensates for loss of other osmotic adjustment substances, which was also demonstrated in Cui et al. [28]. Studies by Rajendrakumar et al. [29] indicated that proline may protect protein structures by maintaining their structural stability. *N. sphaerocarpa* had the highest content of free amino acids and proline contents among the five species (Fig. 2d). Higher accumulation of proline is a result of long-term adaptation to a drought environment. Tobe et al. [30] reported that the xerophytes accumulated very high levels of proline, and proline serves as an alternative compatible solute for osmotic adjustment.

Numerous papers showed that global change will affect the relative distribution of C₃ and C₄ plants by affecting the key physiological and biochemical parameters. Physiologically, C₃ and C₄ species do not inherently differ in the degree to which they withstand severe drought, and the main difference in performance in response to aridity is greater water use efficiency, higher solar energy utilization and carbon fixation capacity in C₄ plants. Meanwhile, C₄ plants *C. mongolicum* and *H. ammodendron*, with higher LWP and SOD activity, is drought tolerant by postponement of dehydration with high tissue water potential. Whereas, *C. korshinskii* triggered an efficient enzymatic auxiliary antioxidant system (higher in CAT and POD activity) and an effective protection against photochemical damages. *N. sphaerocarpa* was succulent xerophytes, higher accumulation of total free amino acid and proline could increase the water-holding capacity and tolerate drought environments. Higher soluble sugar content in *T. ramosissima* would be improve its osmotic regulation ability, and that's maybe the reason why *T. ramosissima* would widely surviving in adverse environments such as salinity and drought areas. Finally, the results of this study suggest that different species developed different photosynthetic characteristics and protective mechanisms for drought adaption. The advantages of the C₄ plants could lead to higher drought resistance due to higher SOD activity and higher light use efficiency, and would be more abundant in desert areas with higher temperatures, higher irradiance and lower moisture.

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REFERENCES

- [1] DW Lawlor; G Cornic; *Plant. Cell Environ.*, **2002**, 25, 275–294.
- [2] N Smirnov; *Plant Sci.*, **1993**, 125, 27–58.
- [3] A Wahid; S Gelani; M Ashraf; MR Foolad; *Environ. Exp. Bot.*, **2007**, 61, 199–223.
- [4] MM Chaves; J Flexas; C Pinheiro; *Ann Bot-London*, **2009**, 103, 551–560.
- [5] Y Yamane; Y Kashino; H Koike; *Photosynth. Res.*, **1997**, 52, 57–64.
- [6] K Maxwell; GN Johnson; *J. Exp. Bot.*, **2000**, 51, 659–668.
- [7] K Asada; *Annu. Rev. Plant. Physiol. Mol. Bio.*, **1999**, 50, 601–639.
- [8] JG Scandalios; *Plant Physiol*, **1993**, 101, 7–12.
- [9] N Misra; AK Gupta; *Plant Sci.*, **2005**, 169, 331–339.
- [10] MD Hatch; *Plant. Cell. Physiol.*, **1992**, 33, 333–342.
- [11] TR Wentworth; *Vegetatio* **1983**, 52, 21–34.
- [12] PX Su; XM Liu; LX Zhang; AF Zhao; WR Li; HS Chen; *Isr. J. Plant Sci.*, **2004**, 52, 87–97.
- [13] HK Lichtenthaler. *Method. Enzymol.*, **1987**, 148, 350–382.
- [14] C Giannopolitis; N Ries; Superoxide dismutases I. *Plant Physiol.*, **1977**, 59, 309–314.
- [15] H Aebi; Catalase in vitro. *Method. Enzymol.*, **1984**, 105, 121–126.
- [16] MV Rao; C Paliyath; DP Ormrod; *Plant Physiol.*, **1996**, 110, 125–136.
- [17] P Aravind; MNV Prasad; *Plant. Physiol. Bioch.*, **2003**, **41**, 391–397.
- [18] SW Lee; JM Lim; SH Bhoo; YS Paik; TR Hahn; *Analytica Chimica Acta.*, **2003**, 480, 267–274.
- [19] LS Bates, RP Waldren, ID Teare; *Plant Soil*, **1973**, 39, 205–207.
- [20] TA Mansfield; WJ Davies; Stomata and stomatal mechanisms. In: Paleg LG, Aspinall D (eds) *The physiology and biochemistry of drought resistance in plants*. Academic Press, New York, **1981**.
- [21] RF Sage; DS Kubien; *Plant. Cell. Environ.*, **2007**, 30, 1086–1106.
- [22] JR Ehleringer; TE Cerling; BR Helliker; *Oecologia*, **1997**, 112, 285–299.
- [23] W Li; KF Cao; *Acta Botanica Boreal-Occidentalia Sinica* **2006**, 26, 266–275.
- [24] DR Carter; JM Cheeseman; *Plant. Cell. Environ.*, **1993**, 16, 215–222.
- [25] P Schwanz; A Polle; *Environ. Exp. Bot.*, **2001**, 45, 43–53.
- [26] P Streb; E Telor; J Feierabend; *Funct. Ecol.*, **1997**, 11, 416–424.
- [27] F Caravaca; MM Alguacil; JA Hernández; A Roldán; *Plant. Sci.*, **2005**, 169, 191–197.
- [28] BS Cui, QC Yang; KJ Zhang; XS Zhao; ZY You; *Plant Ecol*, **2010**, 209, 279–290.
- [29] CSV Rajendrakumar; BVB Reddy; AR Reddy; *Biochem. Bioph. Res. Co.*, **1994**, 201, 957–963.
- [30] K Tobe; XM Li; K Omasa; *Aust. J. Bot.*, **2000**, 48, 455–460.