Responses of Antioxidative Protection to Varying Drought Stresses Induced by Micro-Ecological Fields on Desert C_4 and C_3 Plants in Northwest China

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Abstract: Desert plants are exposed to a combination of environmental stress conditions, including water deficit, high temperature and high irradiance. We focused on antioxidative protection systems of C_4 desert plant *Haloxylon ammodendron* and C_3 desert plant *Hedysarum scoparium* within arid dune ecosystem with artificial controlling water availability. The activities of antioxidative enzymes (SOD, POD and CAT) in *H. scoparium* were significantly higher than those in *H. ammodendron* under the same conditions; the activities of antioxidative enzymes in leaflets of *H. scoparium* were higher than those in rachis. With the increasing drought, the accumulation of the antioxidant (flavonoids) was increased in *H. ammodendron*. However, the amount of flavonoids was decreased in *H. scoparium*. Flavonoid contents in the leaflets and rachis of *H. scoparium* were higher than those in assimilating shoots of *H. ammodendron*. In conclusion, *H. ammodendron* and *H. scoparium* applied different strategies of protection against water deficit, high irradiance stress and high temperature stress during daily process in summer, and inducing high activities of antioxidative enzymes in C_3 desert plant was an important safeguard to live in desert condition, and the advantages of C_4 metabolism of *H. ammodendron* was that it was not easily influenced physiologically to the same level of stress as *H. scoparium*.

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Keywords: antioxidative enzymes; antioxidant; desert C_3 and C_4 plants; *Haloxlon ammodendron*; *Hedysarum scoparium*; drought stress.

1. Introduction

Plants are subjected to several harsh environmental stresses that adversely affect growth, metabolism, and yield (Almaghrabi, 2012; Metwali, 2012). Especially, the desert plants are often exposed to all kinds of stressful conditions such as high irradiance, extreme temperature, water deficit and air-dryness. Water deficit is the principal impact on plants growth and development, causing physiological and biochemistrical responses. Generation of reactive oxygen species (ROS) is considered to be a primary event under a variety of stress conditions (Posmyk *et al*., 2009). There is overwhelming evidence that many antioxidants play a key role in plant adaptation to both abiotic and biotic stresses (Burritt and MacK-enzie, 2003; Vranova´ *et al*., 2002). The sequence of events in the plant tissue subjected to drought stress are: (1) increased production of ROS and of oxidized target molecules; (2) increasing in the levels of antioxidative enzymes and antioxidants; and (3) increased scavenging capacity for ROS, resulting in tolerance against the drought stress (Mano, 2002). During the long term of evolution, higher plants have developed various defending systems to scavenge ROS, which helps them to survive under unfavourable conditions (Bowler *et al*., 1992). Mechanisms of ROS detoxification exist in all plants and can be categorized as enzymatic [superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), glutathione reductase (GR) and monodehydroascorbate reductase (MDAR)] and non-enzymatic (flavanones, anthocyanins, carotenoids and ascorbic acid) (Reddy *et al*., 2004).

Antioxidant enzymes are known to increase in response to drought (Menconi *et al*., 1995; Zhang and Kirkham, 1996; Sairam and Saxena, 2000; Sairam and Srivastava, 2001), high temperature (Upadhyaya *et al*., 1990; Sairam *et al*., 2000). High SOD activity has also been reported during the treatment of low temperature, high irradiance stress and drought (Dhindsa and Matowe, 1981; Burke *et al*., 1985; Baisak *et al*., 1994). CAT is irradiance-sensitive and easy to be inactivated by irradiating (Feierabend and Engel, 1986). Meanwhile plants typically produce a diverse group of antioxidants as a protective mechanism against oxidative compounds which are produced in response to various stresses and known to have a damaging effect on membranes, organelles and macromolecules (Mittler, 2002; Noctor and Foyer, 1998). A significant part of antioxidants produced by plants in response to stress is secondary metabolites, including a vast array of

simple and complex phenolic compounds derived primarily via the phenylpropanoid pathway (Dixon and Paiva, 1995). Flavonoids are a vast group of polyphenolic substances, with high antioxidant properties, found in most plant species (Moure *et al*., 2001).

Studying the response of desert plants to the combined effect of these stresses within their natural habitat may therefore unravel complex relations between known mechanisms, and possibly reveal novel mechanisms and strategies that enable plants to resist stressful conditions. Mittler *et al*. (2001) found in response to prolonged exposure to extreme conditions, the upper part of a C_3 desert plant such as *R. raetam* enters a state of `dormancy' that may protect the entire plant during stress. The two main strategies employed by desert plants to withstand the extreme environmental conditions of their natural habitat are avoidance, as with growth of winter annuals or resurrection plants only during the rainy season; and resistance, as in the survival of evergreens throughout the different seasons (Cushman *et al*., 1989; Raven *et al*., 1992). The level of response depends on the species, the development, and the metabolic state of the plant, as well as the duration and intensity of the stress. Many stress situations cause an increase in the total foliar antioxidant activity (Pastori *et al*., 2000), the anatomical and physiological adaptations of evergreen desert plants have been the subject of numerous studies (Fahn and Cutler, 1992; Mittler *et al*., 1991; Streb *et al*., 1997), but little is known about the protected mechanisms of different carbon metabolism pathway of desert plants that enable these plants to withstand harsh desert environments.

In this research we aimed to investigate the anti-oxidative response of desert plants which were subjected to increasing water stress in natural combined conditions. In order to discuss environmental adaptation of the different carbon metabolism pathway of desert plants we have investigated respectively the change of protecting components such as SOD, POD, CAT and flavanones in desert C_3, C_4 plants. *Haloxlon ammodendron* is a C_4 plant with assimilating shoots as photosynthetic organ, and *Hedysarum scoparium* is a desert legume C_3 plant with leaflets and rachis performing photosynthesis (Su *et al*., 2003). *H. ammodendron* and *H. scoparium* are common in arid ecosystems in the desert regions of China. They are important components of the desert vegetation and even as a dominant population in arid areas in Northwest China. *H. ammodendron* and *H. scoparium* are distributed over areas where there is air-dryness, adequate sunshine and precipitation less than 130 mm. *H. ammodendron* is a priori species of desert vegetation in Northwest China. In the present paper we will report putative different defense responses of *H. ammodendron* and *H. scoparium*. We detected activities changes of antioxidative emzymes in different organs at three depths of ground water (DGW) every three hours in *H. ammodendron* and *H. scoparium*, attempting to compare different mechanisms in the antioxidative system defensing against high temperature, water deficit and high irradiance between C_4 and C_3 desert plants.

2. Material and Methods

Habitat of trial site: The study was carried out near the town of Minqin, northwestern China, which locates in the joint 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 which is in the Desert Plantation of Gansu Provincial Institute of Desert Control Research. The trial spot is in natural state where there are many sand dunes correlating to *Nitraria tangutorum* and *Reaumuria soongorica* communities in an enclosed and protected area. The average precipitation a year is 115 mm, with main precipitation converging in 7-9 month; dry degree is 5.3, and the average evaporation a year is 2643.9 mm which is 23 times to the average precipitation a year. The average temperature a year is 7.8℃, with the highest temperature 38.1℃, the lowest temperature –28.8℃. The non- frost season is 165 days, and the period of blowing sand is 139 days. the process of sandstorm is 37 days. The soil is sandy soil. The depth of ground water is 18 m (Zhao *et al*., 2003). With the diurnal changes of photon flux density (PFD) and air temperature ($T._{Air}$) of sunny day in early August, 2003 in Minqin, the highest value of photon flux density (PFD) increases at 13:00, which reaches 1638 μmol $m^{-2}s^{-1}$, and the highest temperature is 42.4 ℃, appearing at 15:50 (Figure 1) (Gong *et al*., 2006).

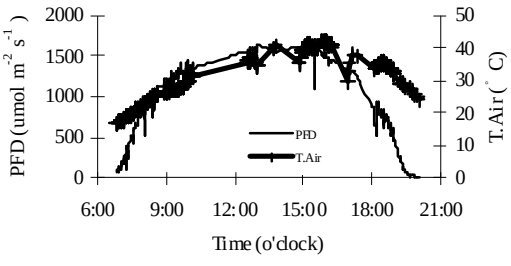

Figure 1. Diurnal changes of photon flux density (PFD) and air temperature **($T._{Air}$)** on sunny days in early ten days of August 2003 in Minqin, northwest China (Gong *et al*., 2006)

Plants growth conditions and sampling procedures: Description of the Plant Transpiration Consume Water Observation Station and transplant and treatment of *H. ammodendron* and *H. scoparium* was detailed by Gong *et al*. (2006). Water was supplied by groundwater eternal compensatory apparatus, which could balance water level of storage in a stable water level. The Plant Transpiration Consume Water Observation Station established 3 depths of ground water (DGW), 1.4 m, 2.4 m and 3.4 m. Soil water content increased gradually as soil depth increase at every DGW (Figure 2), resulting in light, moderate and severe water stress for plant growth (Gong *et al*., 2006). There was no negative effect for plant's growth and development at 3 depths of groundwater in the Plant Transpiration Consume Water Observation Station (Gong *et al*., 2006).

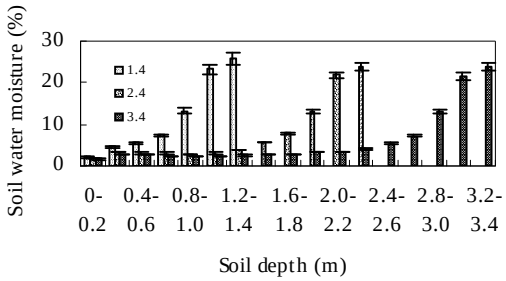

Figure 2. Soil water moisture at different depths of ground water (DGW) in root areas of *Haloxlon ammodendron* and *Hedysarum scoparium*. Each value represents mean (±s.e.) of three replications ($P < 0.05$) (Gong *et al*., 2006)

The sample of collected leaves and assimilating shoots was frozen in liquid nitrogen. We did this investigation in early August, 2003. The collected samples were all in the growing and developing stage.

Determination of water content: Relative water content (RWC) of the assimilating shoots in H. ammodendron and the leaflets and rachis in H. scoparium were estimated by recording the fresh mass (FM) and the saturated mass (SM) of 0.5 g fresh leaf samples by keeping in water for 24 h, followed by drying in hot air oven till constant dry mass (DM) is achieved according to the method of Barrs et al. （1962）.

RWC（%）=（（FM-DM）/（SM-DM））×100%

Enzyme assays: Leaf tissue, 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 mmolL-1 phosphate buffer, pH 7.8, containing 1 % insoluble polyvinylpyrrolidone and 10 mmolL^{-1} sulfhedryl alcohol. The homogenate was centrifuged at 15000 × 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 ℃. Determination of all enzyme activities was conducted through UV-751 ultraviolet spectrophoto meter. An aliquot of the extract was used to determine its protein content by Bradford (1976) method 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 and Fridovich (1971) as modified by Dhindsa and Matowe（1981）. The reaction mixture (3 ml) contained 50 mM phosphate buffer, pH 7.8 , 0.1 mM EDTA, 13 mM methionine, 75 µM NBT, 2 µM riboflavin and 50 µl of the supernatant diluted 5 times. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under two 15 W fluoroscent lamps. The reaction was terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. Reaction product was measured at 560 nm. The volume of the supernatant corresponding to 50% inhibition of the reaction was assigned a value of 1 enzyme unit. A non-irradiated reaction mixture did not develop colour and served as control. Enzyme activity was indicated unit mg-1protein.

POD (EC1.11.1.7) activity was assayed as increase in absorbance due to the formation of tetra-guaiacol was recorded at 470 nm (Castillo *et al*., 1984). The 3 ml reaction mixture contained 16 mM guaiacol, 2 mM H_2O_2, 70 mM phosphate buffer (pH 6.1) and 20 µl enzymatic extract diluted 2 times.

CAT (EC 1.11.1.6) was assayed by measuring the initial rate of disappearance of hydrogen peroxide at 240 nm according the method of Chance and Maehly (1955) as modified by Dhindsa and Matowe（1981）and Cakmak and Marschner（1992）. The 3 ml contained 50 mM phosphate buffer, pH 7.0, 15 mM hydrogen peroxide, and 50µl enzyme extract diluted 10 times. The decrease in hydrogen peroxide was followed as a decline in 240nm. The activity was expressed in units where one unit of catalase converted one µmole of hydrogen peroxide per minute. Enzyme solution containing hydrogen peroxide-free phosphate buffer was used as control.

Flavonoids contents assays: The relative content of flavinoids was detected at 305 nm treating with acidified methanol according Mirecki *et al*.（1984）. The fully expanded green assimilating shoots, leaflets and rachis (0.1 g) were cleaned and air-dried and

extracted with 20 ml acid methyl alcohol (methanol: water: hydrochloric acid = 79:1:20) to deal with 3000 × g centrifuge, the supernatants in the 305 nm light absorption value means the relative concentration of flavonoids.

Statistical analyses: Tukey's HSD (honest significant difference) tests were used to analyze the differences between activities of enzyme. Correlations between parameters were determined using least-squares linear regression. Data presented in the figures are means of three replications (± s.e.).

3. Results and discussions

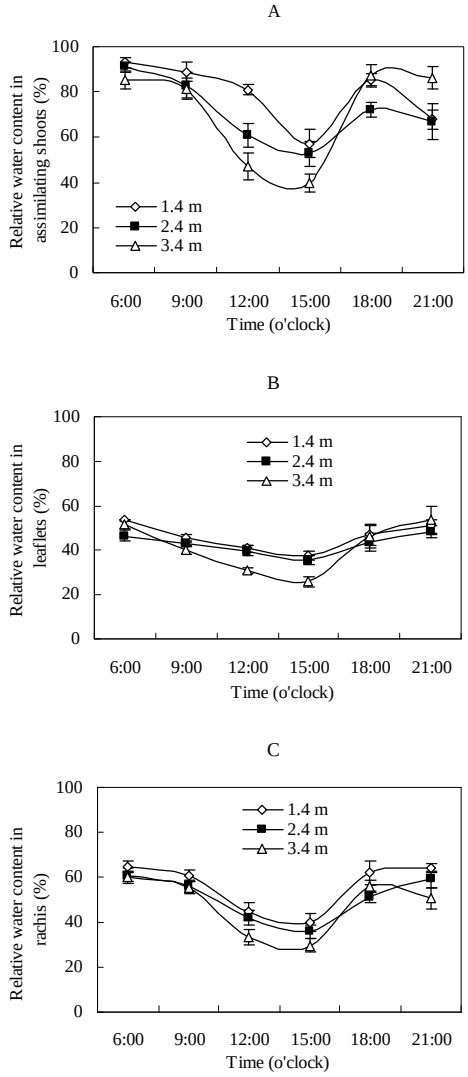

Figure 3. RWC in assimilating shoots of *Haloxlon ammodendron* (A), and in leaflets (B) and rachis (C) of *Hedysarum scoparium*. Vertical bars represent s.e. Each point represents mean±s.e. (n=3, $P < 0.05$)

The higher RWC in *H. ammodendron* than in *H. scoparium*: *H. ammodendron* true leaves are quite reduced and the cortex of young annual cylindrical shoots was the main photosynthetic tissue. *H. scoparium* contained two different assimilating organs (leaflets and rachis). As is shown in Figure.3, the RWC either in leaflets or in rachis of *H. scoparium* was very low compared with that of *H. ammodendron*. The high resistance of *H. ammodendron* to hot summer, drought conditions may be attributed in part to assimilating shoots which are succulency and fleshy (Pyankov *et al.*, 1999). Even higher RWC was recorded at 15:00 in the 1.4 m, RWC in 2.4 m higher than that in 3.4 m in assimilating shoots of *H. ammodendron* (Figure 3A). The lower RWC in *H. scoparium* may be one of survived mechanism of C_3 plant in desert environment. Drought-related physiological changes, such as decrease in leaf water content and the accompanying stomatal closure, resulted in limited CO_2 availability and the channeling of reducing equivalents from the photosynthetic apparatus to the production of ROS, rather than to CO_2 fixation (Krause and Cornic, 1987). It will trigger the antioxidative protection. By controlling the availability of water, we were able to show that the lack of water, and high temperatures, excess light, or low relative humidity which occured in the dry season, was the limiting environmental factor that triggered the plant to defend oxidative damage. The diurnal changes of RWC in leaflets were gentler and lower than those in rachis of *H. scoparium* in same condition (Figure 3B and C). It was more sensitive for RWC of cylindrical assimilating shoots in *H. ammodendron* and cylindrical rachis in *H. scoparium* to increase drought than that of rachis in *H. scoparium*.

Antioxidative enzymes system of *H. ammodendron*: SOD activity tended to increase at a low level in a short period treatment of the combined stress (Ye *et al.*, 2000). We also detected that SOD activity unremarkably enhanced during early morning to afternoon and reached peak in 15:00 when in high irradiance and high temperature, the lowest RWC whatever any DGW (Figure 4A). *H. ammodendron* at 3 DGW from 1.4 m to 3.4 m showed increased SOD activity. Plants were resistant to single high irradiance, and many evidences showed that photochemical apparatus could keep their integrity and the recovery from photoinhibition is fast. Due to high temperature sustain from 10:00 which simplified conclusion that SOD activity in response to high irradiance was relatively high compared to water deficit, it was possible that the increase in SOD activity provided another pathway for the removal of

excess electrochemical energy (Ye *et al.*, 2000). High SOD activity has also been reported during the treatment of drought (Baisak *et al.*, 1994). Although activity of SOD in 3 DGW in response to high irradiance stress were probably sufficient to withstand the amount of oxidative stress, our results also clearly showed the ability of tolerant water deficit in *H. ammodendron*.

POD and CAT appeared to play an essential protective role in the scavenging processes when coordinated with SOD activity (Massacci *et al.*, 1995). Our data also indicated that, the activities of guaiacol peroxidase (POD) increased almost coordinately with SOD activity in all treatments (Figure 4B). POD activity increased at 12:00 when plants faced with high irradiance (> 1500 μmol $m^{-2}s^{-1}$) and high temperature (<35℃) but decreased at 15:00 when plant resisted to high irradiance(>1500 μmol $m^{-2}s^{-1}$) and high temperature(>39℃) at 3.4 m. These indicated not only moderate irradiance could produce more H_2O_2 under high temperature (Ye *et al.*, 2000), but also high irradiance could produce more H_2O_2 under higher temperature. We thus found that POD activity was more inactive to temperature than to irradiance. In addition, a significant increase in the POD activity in *H. ammodendron* assimilating shoots accompanied the increase of water stress at noon, indicated that *H. ammodendron* is capable of effectively scavenging the ROS for the production of certain secondary metabolites to withstand during stresses of high irradiance and water deficit.

An increase in the activity of *H. ammodendron* CAT was observed from 1.4 m to 2.4 m treatment and a decline in 3.4 m from morning to afternoon (Figure 4C). There was no significant change in the activity of CAT at 3.4 m treatment from 12:00 to 15:00 in spite of high irradiance and temperature during this period. This further suggested that the responses of CAT activity to water deficit proved to be very complex, involving nearly every major function of plant growth. Under the double effect of irradiance and temperature, most decrease of CAT activity was caused by temperature, and irradiation exacerbated the inactivation of CAT. There was sustained increased in CAT activity at 3 DGW from 6:00-12:00 when temperature and irradiance were all moderate, however, CAT activity increased at 1.4 m, 2.4 m, but more decreased at 3.4 m. Moreover, during 12:00 (high irradiance) -15:00 (high temperature) only 7.8% of CAT activity were decreased at 2.4 m, far less than that at 1.4 m. This result supported that CAT activity was more sensitive to temperature than to irradiance (Ye *et al.*, 2000). After high temperature at 18:00, apparently, there was no significant difference in CAT activity change at 3 DGW under different irradiances. This further suggested that concomitant resynthesis of catalase compensates for the loss of catalase and maintains a constant level under irradiation when plants were not exposed to stress (Feierabend and Engel, 1986).

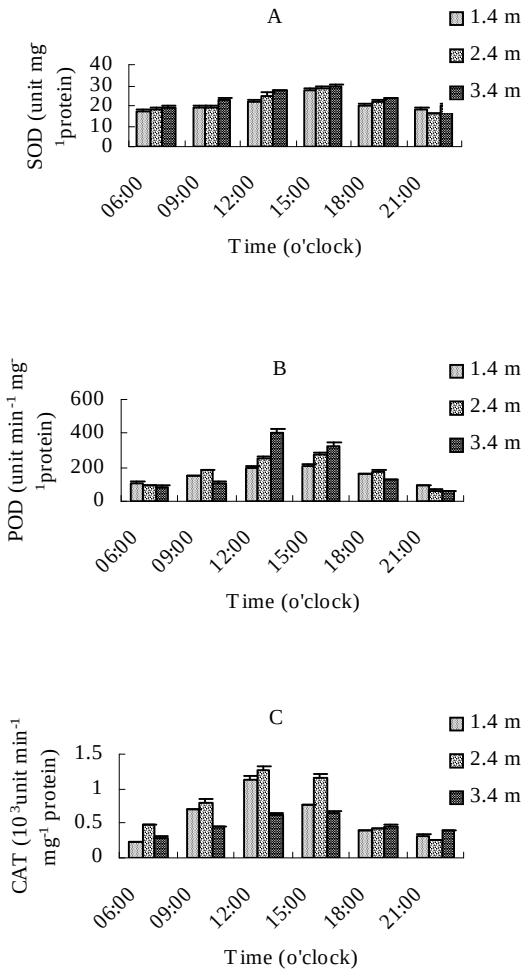

Figure 4. Diurnal changes of antioxidant enzymes activities in assimilating shoots of *Haloxylon ammodendron* at different DGW. (A)SOD activity, (B) POD activity and (C) CAT activity. Vertical bars represent s.e. Each point represents mean±s.e. (n=3, $P < 0.05$)

As is shown in Figure 4, the activities of the SOD, POD and CAT was upregulating during 6:00-15:00, suggesting that the intracellular level of ROS may increase in plants during these time points. It was also possible that changes in the activities level of these enzymes reflected a specific diurnal rhythm. Induction of oxidative stress in drought-stressed plants had also been well known (Ramachandra Reddy *et al.*, 2000; Chaitanya *et al.*, 2002; Mano, 2002). Antioxidant components were also known to

be distributed among all photosynthetic cells in higher plants. The distribution of antioxidative enzymes between mesophyll and bundle sheath cells of C_4 plants has been described (Foyer, 2002). In maize leaves, most of the SOD and APX were localized in mesophyll and bundle sheath cells. CAT was approximately equally distributed between mesophyll and bundle sheath cells. H_2O_2 was found to accumulate only in mesophyll cells (Doulis *et al*., 1997; Foyer, 2001). These localization studies are very interesting, because enzymes of the PCR cycle, which are very sensitive to H_2O_2, are found only in bundle sheath chloroplasts. Such studies on C_4 plants indicate that oxidative damage was not uniformly distributed between mesophyll and bundle sheath cells of C_4 plants. Kingston-Smith and Foyer (2000) suggested that oxidative damage under stressful conditions in C_4 plants is restricted to bundle sheath tissue because of inadequate antioxidant protection in this tissue. Regretfully, very little mechanistic information is available on drought-induced antioxidative metabolism between the two cell types in *H. ammodendron*.

Antioxidative enzymes system of *H. scoparium*: In general, activities of antioxidative enzymes were higher in leaflets than in rachis in *H. scoparium*, they are all higher than that in *H. ammodendron.* Changes of antioxidant enzymes were all undulated violently either in leaflets or in rachis in process of a day. Activities of antioxidative enzymes were higher at noon but lower in the early morning and evening. In order to compare activities of antioxidant enzymes in leaflets to in rachis we discuss specially the changes in activities of anti-oxidative enzymes at different DGW at 15:00 when plants exposure to high irradiance and high temperature.

SOD activity in leaflets and in rachis gradually increased with the deeper of DGW at 15:00, SOD activity was found to be much higher in leaflets than in rachis of *H. scoparium* at 3 DGW (Figure 5A), and the ratio of SOD activity of leaflets/rachis gradually decreased, which demonstrated that SOD activity in leaflets play a more significant role than in rachis when plants grow in the relative sufficient water condition and explained why appearance of assimilating shoots of *H. scoparium* is essential to defend oxidative stress in environment of water deficit.

A different trend in POD activity in leaves of *H. scoparium* was observed, POD activity in leaves increased slightly from 1.4 m to 2.4 m but declined at 3.4 m at 15:00. POD activity in assimilating shoots gradually increased with the deeper of DGW at 15:00, as well as the ratio of POD activity of leaves/assimilating shoots gradually decreased with the deeper of DGW (Figure 5B). There was a familiar effect in POD activity of *H. scoparium* with in SOD activity of *H. scoparium*. High activity of antioxidative enzymes could be correlated to the process of differentiation that occurred during shoot induction (Thakar and Bhargave, 1999).

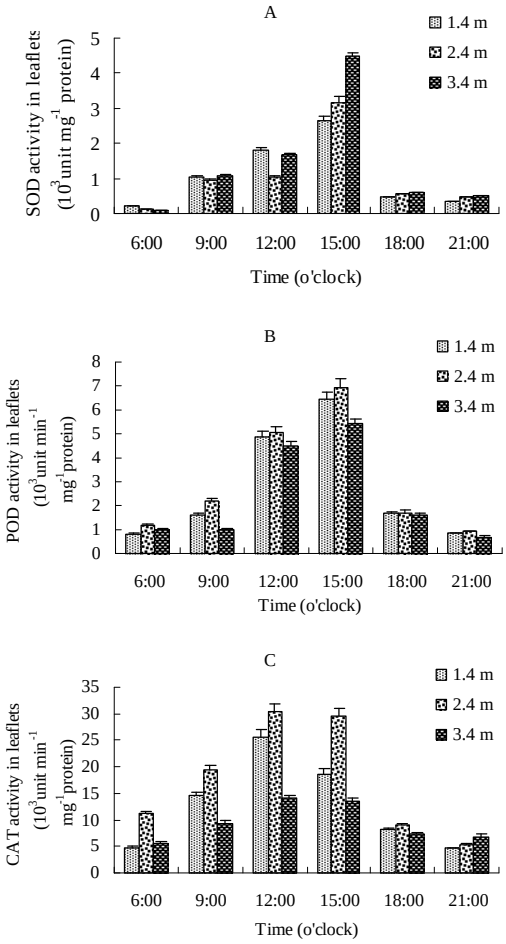

Figure 5. Diurnal changes of antioxidant enzymes activities in assimilating shoots of *Haloxlon ammodendron* and in leaflets and rachis of *Hedysarum scoparium* at different DGW. (A)SOD activity, (B) POD activity and (C) CAT activity. Vertical bars represent s.e. Each point represents mean±s.e. (n=3, $P < 0.05$).

There was only minor losses of CAT activity occurred in assimilating shoots of *H. scoparium*, but a marked apparent water deficit inactivation of catalase was observed in leaves of *H. scoparium* . This result consisted with CAT activity in stems of *R. raetam* and in leaves of *A. halimus* in natural daylight (Streb *et al*., 1997). CAT activity was found to be higher in leaves than in assimilating shoots of *H. scoparium* under 3 DGW at 15:00 ,

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 at 15:00 (Figure 5C). The present findings further convinced that CAT activity not only subjected to high irradiance but also water deficit, moderate water stress could stimulate CAT activity but stronger water stress inactivated dramatically CAT activity. This finding supported that drought stress inactivated the key enzyme activity of light respiration-ethanol acid oxidative enzyme 44% (Morgan, 1994), leading to accumulating more NADPH and ATP，increasing the possibility of production of O_2^-、H_2O_2 in chloroplast. Production of H_2O_2 during drought stress may also result from the catalytic activity of glycolate oxidase in peroxisomes during photorespiration (Osmond, 1981). CAT is also involved in H_2O_2 scavenging during stress (Comba *et al*., 1998). The activity of SOD increased during somatic embryogenesis while POD and CAT activities decreased (Gupta and Datta, 2003). The change of CAT activity in rachis is more smoothed than that in leaflets, suggesting that rachis was not subjected to a similar level of stress to these leaflets during the midday stressful hours.

Antioxidant in *H. ammodendron* and *H. scoparium*: Plants containing flavonoids have been reported to possess strong antioxidant and hypolipidemic properties (Aviram, 2004; Sudheesh and Vijayalakshmi, 2005; Sweedy *et al*., 2007). Previously, methanolic extract of this whole plant extract exhibited strong antioxidant activity (Surveswaran *et al*., 2007). Flavonoids content was determined in *H. ammodendron* and *H. scoparium*, which may be how the plants to resist high temperatures, drought, ultraviolet radiation and removal of reactive oxygen species.

Flavonoid contents in the leaflets and rachis in *H. scoparium* was higher than that of assimilating shoots under the same DGW (Figure 6A, B and C). Flavonoids content in a different trend both in *H. ammodendron* and in *H. scoparium* under different DGW, in that other environmental conditions are the same, different DGW influence the accumulation of flavonoids. Flavonoid contents was the lowest and most stable at 1.4 m and was highest at 2.4 m in *H. ammodendron*. The drought increase, the accumulation of flavonoids was increase. Flavonoid content was higher at 1.4 m and was the lowest at 3.4 m in *H. scoparium.* The drought increase, the amount of flavonoids was decrease. But in the same environmental conditions C_3 plants *H. scoparium* need to produce more amounts of flavonoids in order to protect them from higher temperature, stronger light and higher drought, to absorb ultraviolet light, to prevent the evaporation of water, to regulate metabolism. This is also one of the C_3 plants mechanisms adapting to adversity in the desert conditions. The flavonoid-rich fraction of *S. hispida* seed possesses strong antioxidant properties as evidenced by a significant increase in the levels of enzymic and non-enzymic antioxidants (Kaviarasan *et al*., 2008).

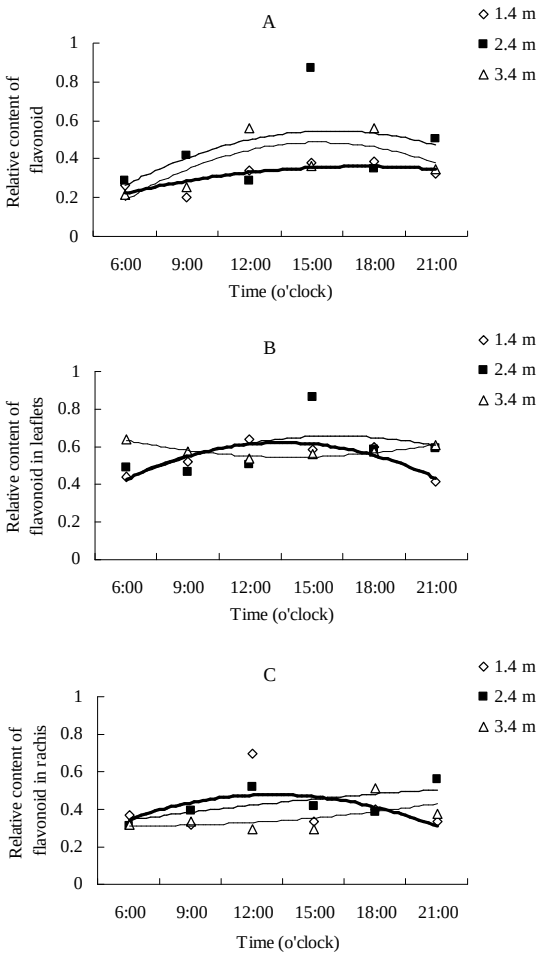

Figure 6. Diurnal changes of flavonoid in assimilating shoots of *Haloxylon ammodendron* (A) and in leaflets (B) and rachis (C) of *Hedysarum scoparium* at different DGW

Antioxidant levels and the activities of ROS scavenging enzymes have been correlated with tolerance to several different environmental stresses (Massacci *et al*., 1995), because one of the biochemical changes subjected to environmental stresses in plants is the production of ROS (Dat *et al*., 2000). As plants acclimatise or experience sub-lethal levels of stress like high temperature, potential to scavenge free radicals often increases (Bridger *et al*., 1994). From the foregoing discussion it can be

concluded that antioxidant enzymes such as SOD, CAT play an important role in the regulation of senescence processes. So we prefer to conclude that antioxidant enzymes in naturally growing desert plants would respond to naturally occurring stresses and artificial controlling water content within their natural habitat, explaining the detail between the activities of ROS scavenging enzymes and several different environmental stresses. It seems quite plausible to assume that much of the increase in SOD activity observed in mature leaves could be increased in mitochondrial SOD (Sairam *et al*., 2003). SOD activity has also been reported during high irradiance stress (Burke *et al*., 1985). Certain POD isomers utilise the phenolic compounds and H_2O_2 to initiate the biosynthesis of several secondary metabolites required for the plant growth, development and differentiation. H_2O_2 of lower concentration will be scavenged by POD in the oxidant of somatic such as phenols (Mittler, 2002). Chowdhury and Choudhuri (1985) reported a larger decrease in the activities of SOD and CAT in a drought-sensitive line of jute than in a drought-tolerant line. CAT is irradiance-sensitive and easy to be inactivated by irradiating (Feierabend and Engel, 1986). Despite its restricted localization and photolability, CAT may play a significant role in scavenging H_2O_2, which can readily diffuse across the membrane (Bowler *et al*., 1992). In addition, CAT was sensitive to longer daily exposures, evidenced by a decrease in its activity (Casati *et al*., 2002).

Here we show that all these different antioxidative protection responses of the desert plants to combined environmental stress, which were tested in the laboratory, can be found in naturally growing desert plants that respond to naturally occurring stresses and artificial controlling water content within their natural habitat. The increased activities of ROS scavenging enzymes should have a greater significance as invaluable tools in the elucidation of plant metabolic regulation under stressful environment (Chaitanya *et al*., 2001). As the results have shown, activities of antioxidant enzymes either in leaflets or in rachis of *H. scoparium* were higher than those of *H. ammodendron* (Figure 5 and Figure 6). In supporting of our observation, higher activity of SOD is one of the most important guarantees which defend high irradiance (Rabinowitch and Sklan, 1980). Leaves of plant in light were more sensitive than in dark either in high temperature or in low temperature (Feierabend *et al*., 1992). Due to C_4 plant tending to occupy places which were in high irradiance, high temperature and water deficit but C_3 plant did not acclimatize well. However, we found C_4 pathway of photosynthetic carbon assimilation in leaflets and in rachis of *H. scoparium* (Gong *et al*., 2006), which explained why *H. scoparium* as a kind of C_3 shrub could survive in desert environment. The observation that high temperatures stimulated the mulberry leaf chloroplasts to reduce oxygen to superoxide due to high temperature-induced electron transport system, prompted us to postulate that the over-expression of SOD might have improved the tolerance to high temperature (Chaitanya *et al*., 2001), over-expression of SOD might have improved the adaptation of C_3 plants in desert conditions. It is conceivable that acquisition of the stress tolerance in any plant is a multi-factorial function and amelioration of ROS^-scavenging systems is an important index to assess the abilities of mulberry cultivars to tolerate the stressful conditions like high temperature. We presume that the metabolism of the reactive oxygen species under stressful environment is dependent on different functionally interrelated antioxidant enzymes. This finding may suggest that, physiologically, *H. ammodendron* is not subjected to the same level of stress as *H. scoparium*. It is possible that the additional anatomical and physiological adaptations of a C_4 plant better protect it from the stressful conditions in the desert ecosystem (Edwards and Walker, 1983).

4. Conclusions

The above results suggested that the desert plants *H. ammodendron* and *H. scoparium* applied different strategies of protection against water deficit, high irradiance stress and high temperature stress damage. In the assimilating shoots of *H. ammodendron* antioxidative protection appears to play the major role, while *H. scoparium* appears to avoid oxidative damage in the field by leaflets more than rachis. The drought increase, the amount of flavonoids was increase in *H. ammodendron*. Whereas, the drought is increase, the accumulation of flavonoids is decrease in *H. scoparium*. Flavonoid content in the leaflets and rachis of *H. scoparium* were higher than those in of assimilating shoots of *H. ammodendron*. Our findings may provide the advantages of C_4 metabolism of *H. ammodendron* is not subjected physiologically to the same level of stress as *H. scoparium*. It is essential for us to provide with a theoretical foundation of rational exploitation and optimizable distribution for desert plants in Northwest China.

In conclusion, the present work compared the oxidative protection system of the C_3 desert plant with that of C_4 desert plant during the daily process in summer, and demonstrated high activities of antioxidative enzymes in C_3 desert plant was important safeguard of living in desert condition.

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References

1. Almaghrabi OA (2012) Impact of Drought Stress on Germination and Seedling Growth Parameters of Some Wheat Cultivars. Life Science Journal 9(1):590-598.
2. Aviram M (2004) Flavonoids-rich nutrients with potent antioxidant activity prevent atherosclerosis development: the licorice example. International Congress Series 1262: 320-327.
3. Baisak R, Rana D, Acharya PBB, Kar M (1994) Alterations in the activities of active oxygen scavenging enzymes of wheat leaves subjected to water stress. Plant & Cell Physiology 35: 489-495.
4. Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for the estimating of water deficits in leaves. Australian Journal of Biology Science 15: 413-428.
5. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry 44: 276-287.
6. Bowler C, Montagu MV, Inze DD (1992) Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology 43: 83-116.
7. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.
8. Bridger GM, Yang WD, Falk E, McKersie BD (1994) Cold acclimation increases tolerance of active oxygen in winter ce-reals. Journal of Plant Physiology 144: 235-240.
9. Burritt DJ, MacKenzie S (2003) Antioxidant metabolism during acclimation of Begonia_erythrophylla to highlight levels. Annuals of Botany 91: 783-94.
10. Burke JJ, Gamble PE, Hatfield JL, Quisenberry JE (1985) Plant morphological and biochemical responses to field water deficits. I. Reponses of glutathione reductase activity and paraquat sensitivity. Plant Physiology 79: 415-419.
11. Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity on enhance activities of superoxide dismutase, ascorbate peroxidase and glatation reductase in bean leaves. Plant Physiology 98: 1222-1227.
12. Casati P, María VL, Carlos SA (2002) Regulation of enzymes involved in C4 photosynthesis and the antioxidant metabolism by UV-B radiation in Egeria densa, a submersed aquatic species. Photosynthesis Research 71: 251-264.
13. Castillo FI, Penel I, Greppin H (1984) Peroxidase release induced by ozone in Sedum album leaves. Plant Physiology 74: 846-851.
14. Chaitanya KV, Sundar D, Masilamani S, Ramachandra RA (2001) Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Regulation 00: 1-6.
15. Chaitanya KV, Sundar D, Masilamani S, Ramachandra Reddy A (2002) Variation in heat stress-induced antioxidant enzyme activities among three mulberry cultivars. Plant Growth Regulation 36: 175-80.
16. Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Methods in Enzymology 2: 764-817.
17. Chowdhury SR, Choudhuri MA (1985) Hydrogen peroxide metabolism as an index of water stress tolerance in jute. Plant Physiology 65: 503-507.
18. Cushman JC, Meyer G, Michalowski CB, Schmitt JM, Bohnert HJ (1989) Salt stress leads to differential expression of two isogenes of phosphoenolpyruvate carboxylase during Crassulacean acid metabolism induction in the common ice plant. Plant Cell 1: 715-725.
19. Dat J, Vandenabeele S, Vranova E, Van Montagu M, Inze D, Van Breusegem F (2000) Dual action of the active oxygen species during plant stress responses. Cellular and Molecular Life Sciences 57: 779-795.
20. Dhindsa RS, Matowe W (1981) Drought tolerance in two mosses: correlated with enzymatic defense against lipid peroxidation. Journal of Experimental Botany 32: 79-91.
21. Dixon RA, Paiva NL (1995) Stress-inducedphenylpropanoid metabolism. Plant Cell 7: 1085-1097.
22. Doulis AG, Debian N, Kingston-Smith AH, Foyer CH (1997) Differential localization of antioxidants in maize leaves. Plant Physiology 114: 1031-1037.

23. Edwards G, Walker D (1983) C_3, C_4 Mechanisms, and Cellular and Environmental Regulation of Photosynthesis. University of California Press, Berkeley, CA.
24. Fahn A, Cutler DF (1992) Xerophytes. Gebruder Borntraeger, Berlin.
25. Feierabend J, Engel S (1986) Photoinactivation of catalase in vitro and in leaves. Archives of Biochemistry and Biophysics 251: 567-576.
26. Feierabend J, Schaan C, Hertwig B (1992) Photoinactivation of catalase occurs under both high and low temperature stress conditions and accompanies photoinhibition of photosystem II. Plant Physiology, 100: 1554-1561.
27. Foyer CH (2001) Prospects for enhancement the soluble antioxidants ascorbate and glutathione. Biol. Fac. 15: 75-78.
28. Foyer CH (2002) The contribution of photosynthetic oxygen metabolism to oxidative stress in plants. In Inze D, Montago MV, editor. Oxidative stress in plants. Taylor and Francis Publishers, New York, USA, pp 33-68.
29. Gong CM, Gao XW, Cheng DL, Wang GX (2006) C_4 photosynthetic characteristics and antioxidative protection of C_3 desert shrub Hedysarum scoparium in northwest china. Pakistan Journal of Botany 38: 647-661.
30. Gupta SD, Datta S (2003) Antioxidant enzyme activities during in vitro morphogenesis of gladiolus and the effect of application of antioxidants on plant regeneration. Biologia Plantarum 47: 179-183.
31. Kaviarasan K, Kalaiarasi P, Pugalendi V (2008) Antioxidant efficacy of flavonoid-rich fraction from Spermacoce hispida in hyperlipidemic rats. Journal of Applied Biomedicine 6: 165-176.
32. Kingston-Smith AH, Foyer CH (2000) Bundle sheath proteins are more sensitive to oxidative damage than those of the mesophyll in maize leaves exposed to paraquat or low temperature. Journal of Experimental Botany 51:123-130.
33. Krause GH, Cornic G (1987) CO2 and O2 interactions in photoinhibition. In Kyle DJ, Osmond CB, Arntzen CJ, eds, Photoinhibition. Elsevier Science Publishers, Amsterdam, the Netherlands, pp 169-195.
34. Mano J (2002) Early events in environmental stresses in plants-induction mechanisms of oxidative stress. In Inze D, Montago MV, editors. Oxidative stress in plants. Taylor and Francis Publishers, New York, USA, pp 217-245.
35. Massacci A, Ianelli MA, Pietrini F, Loreto F (1995) The effect of growth at low temperature on photosynthetic characteristics and mechanisms of photoprotection of maize leaves. Journal of Experimental Botany 46: 119-127.
36. Menconi M, Sgherri CLM, Pinzino C, Navari-Izzo F (1995) Activated oxygen production and detoxification in wheat plants subjected to a water deficit programme. Journal of Experimental Botany 46: 1123-1130.
37. Metwali E. M. R (2012) Molecular studies on some barley genotypes in relation to salt stress tolerance] Life Science Journal 9(1):111-123.
38. Mirecki RM, Teramura AH (1984) Effects of ultraviolet B irradiance on soybean. V. The dependence of plant sensitivity on the photosynthesis photon flux density during and after leaf expansion. Plant Physiology 74: 475-480.
39. Mittler R, Nir M, Tel-Or E (1991) Antioxidative enzymes in Retama and their seasonal variation. Free Radical Research Communications 14: 17-25.
40. Mittler R, Merquiol E, Hallak-Herr E, Rachmilevitch S, Kaplan A, Cohen M (2001) Living under a `dormant' canopy: a molecular acclimation mechanism of the desert plant Retama raetam. The Plant Journal 25: 407-416.
41. Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7: 405-409.
42. Morgan JA, Hunt HW, Monz CA, LeCain DR (1994) Consequences of growth at two carbon dioxide concentrations and two temperatures for leaf gas exchange in Pascopyrumsmithii (C_3) and Bouteloua gracilis (C_4). Plant, Cell & Environment 17: 1023-1033.
43. Moure A, Cruz JM, Franco D, Dominquez JM, Sinero J, Dominquez H, Nunez MJ, Parajó JC (2001) Natural antioxidants from residual sources. Food Chemistry 72: 145-171.
44. Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology 49: 249-79.
45. Osmond CB (1981) Photorespiration and photoinhibition: some implications for the energetics of photosynthesis. Biochimeca et Biophysica Acta 639, 77-98.
46. Pastori G, Mullineaux P, Foyer CH (2000) Post transcriptional regulation prevents accumulation of glutathione reductase protein and activity in the bundle sheath cells of maize. Implication on the sensitivity of maize to low temperatures. Plant Physiology 122: 667-75.
47. Posmyk MM, Kontek R, Janas KM (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicology and Environmental Safety 72: 596-602.

48. Pyankov VI, Black Jr CC, Artyusheva EG, Voznesenskaya EV, Ku MSB, Edwards GE (1999) Features of photosynthesis in Haloxylon species of Chenopodiaceae that are dominant plants in central Asian deserts. Plant Cell Physiology 40: 125-134.
49. Rabinowitch, HD, Sklan D (1980) Superoxide dismutase: a possible protective agent against sunscald in tomatoes. Planta 148: 162-167.
50. Ramachandra Reddy A, Chaitanya KV, Sundar D (2000) Water stress mediated changes in antioxidant enzyme activities of mulberry (Morus alba. L). Journal of Sericulture Science of Japan 69: 169-75.
51. Raven PH, Evert RF, Eichhorn SE (1992) Biology of Plants. Freeman WH. Worth Publishers, New York.
52. Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of Plant Physiology 161: 1189-1202.
53. Sairam RK, Singh DV, Srivastava GC (2003) Changes in activities of antioxidant enzymes in sunflower leaves of different ages. Biologia Plantarum 47: 61-66.
54. Streb P, Tel-or E, Feierabend J (1997) Light stress effects and antioxidative protection in two desert plants. Functional Ecology 11: 416-424.
55. Su PX, Chen HS, Li QS (2003) Characteristics of δ13C values of desert plants and their water utilization efficiency indicated by δ13C values in the desert of central hexi corridor region. Journal of Glaciology and Geocryology 25: 597-602 [In Chinese with English abstract]
56. Sudheesh S, Vijayalakshmi NR (2005) Flavonoids from Punica granatum–potential antiperoxidative agents. Fitoterapia 76: 181-186.
57. Sairam RK, Srivastava GC, Saxena DC (2000) Increased antioxidant activity under elevated temperature: a mechanism of heat stress tolerance in wheat genotypes. Biologia Plantarum 43: 245-251.
58. Sairam RK, Saxena DC (2000) Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. Journal of Agronomy and Crop Science 184: 55-61.
59. Sairam RK, Srivastava GC (2001) Water stress tolerance of wheat (Triticum aestivum L.): Variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. Journal of Agronomy and Crop Science 186, 63-70.
60. Surveswaran S, Cai Y, Corke H, Sun M (2007) Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chemistry 102: 938-953.
61. Sweedy ME, Hamid NA, Moselhy ME (2007) The role of a mixture of green tea, turmeric and chitosan in the treatment of obesity-related testicular disorders. Journal of Applied Biomedicine 5: 131-138.
62. Thakar J, Bhargava S (1999) Seasonal variation in antioxidant enzymes and the sprouting response of Gmelina arborea Roxb. Nodal sectors cultured in vitro. Plant Cell Tissue Organ Cultivation 59: 181-187.
63. Upadhyaya A, Davis TD, Larsen MH, Waker RH, Sankhla N (1990) Uniconazole-induced thermo-tolerance in soybean seedling root tissues. Plant Physiology 79: 78-84.
64. Vranova´ E, Atichartpongkul S, Villarroel R, VanMontagu M, Inze´ D, Van Camp W (2002) Comprehensiveanalysisof gene expressionin Nicotiana tabacum leaves acclimated to oxidative stress. Proceedings of the National Academy of Sciences of the United States of America 99: 10870-10875.
65. Ye L, Gao HY, Zou Q (2000) Responses of the antioxidant systems and xanthophyll cycle in Phaseolus vulgaris to the combined stress of high irradiance and high temperature. Photosynthetica 38: 205-210.
66. Zhang J, Kirkham MB (1996) Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytologist 132, 361-373.
67. Zhao GD, Liu SR, Ma QL (2003) Ecophysiological responses of two xerophytes atraphaxis frutescens and elaeagnus angustifolia to the change of groundwater depth in arid area I. Changes in leaf nutrient, chlorophyll, soluble sugar and starch contents. Acta Phytoecologica Sinica 27: 228-234 [In Chinese with English abstract].

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