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Effects of sand burial on the survival and physiology of three psammophytes of Northern China

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Corispermum macrocarpum, Setaria viridis* and *Agriophyllum squarrosum* are dominant psammophytes with strong environmental adaptability in Horqin Sandy Land, a typical agro-pastoral ecotone with frequent and intense sand activity in Northern China. We studied the survival rates, activity of antioxidant enzyme (superoxide dismutase, peroxidase and catalase), content of osmotic substances (soluble sugar, soluble protein and proline), and extent of lipid peroxidation and membrane permeability of the three psammophytes under ten treatments of sand burial with different depths to understand the effects of burial stress on survival and physiology of these species. The results show that *A. squarrosum* had the strongest adaptability to sand burial with the highest survival rate in all treatments except treatment C (buried to 3/4 of seedling height), compared to the other two species. The role of different enzymes varied among the three psammophytes. Peroxidase plays a protective role in scavenging reactive oxygen species of all three psammophytes. Superoxide dismutase and catalase are effective in *A. squarrosum* and *S. viridis*, *C. macrocarpum* and *S. viridis*, respectively. The osmotic substances also increased to keep Malondialdehydeand Relative electrolyte leakage at a low level (except the soluble protein content of *A. squarrosum* and *S. viridis*). However, because stress increased consistently in this study due to the enhanced level of sand burial depth, the antioxidant enzyme system and osmotic substances were not able to repair the damage leading to death of the plants.

Key words: Sand burial, psammophytes, survival rate, lipid peroxidation, membrane permeability, antioxidant enzyme activity, osmotic substances.

INTRODUCTION

Over 1.68 million km^2 or 17.6% of total land area of China has experienced severe degradation, or desertification (Wang, 2008). In Northern China, where the majority of the impact has occurred, large areas of natural grassland and farmland have experienced significant wind erosion. Roads, water conservation facilities, and essential communication networks are buried and destructed by sand. The direct economic losses amounted to 50 billion yuan annually (Ci and Liu, 2000). Horqin Sandy Land located in a typical agro-pastoral transitional area with frequent wind sand activities in Northern China. Burial of plants by sand is a common phenomenon in this area (Liu et al., 1992).

In Horqin Sandy Land, *Corispermum macrocarpum, Setaria viridis* and *Agriophyllum squarrosum* are impor-

tant annual species due to their strong adaptability in sand land habitats, and these annuals play important roles in different desertification restoration stages. *A. squarrosum* is a dominant psammophyte in mobile dunes, *C. macrocarpum* is a common species in semi-mobile dunes, and *S. viridis* is a common species in fixed dunes. All three, however, are susceptible to stress caused by sand burial (Luo et al., 2009).

Numerous studies have been conducted on the stress tolerance of plants experiencing drought (Luo et al., 2011), freezing (Yoshida et al., 1997), water deficit (Qayyum et al., 2011), and the impact of salt (Chen et al., 2011; Bybordi, 2011). Those studies which focused on physiology have shown that when plants are subjected to these harmful stresses, reactive oxygen species (ROS) which induce lipid peroxidation, membrane injuries, protein degradation and enzyme inactivation will be accumulated and then cause cell death (Yoshida et al., 1997; Zhou and Zhao, 2004; Luo et al., 2011). Research on the relationship between sand burial and plant survival has primarily been concentrated on how seed germination and seedling emergence were affected. While sand burial can have a significant effect on seed germination, different species respond differently, depending on seed size. Generally, seed germination and seedling emergence are greater for large seeds due to more energy reserves (Zhang and Maun, 1990; Benvenuti et al., 2001). In addition, species from the habitats with lighter burial had smaller survival rate than those from the habitats with intensive burial when buried under same depth (Liu et al., 2008). Research on the effects of sand burial on growth of older plants suggests that mor-phology, growth and reproduction may show some plasticity when psammophytes are buried under sand (Manu, 1996; Harris and Davy, 1987; Luo et al., 2009). Moderate sand burial can change the distribution of matter and energy in plants and is conducive to growth and reproduction of plants (Liu et al., 2008). Some plants adapt to sand burial stress by generating adventitious roots (Sykes and Wilson, 1990; Shi et al., 2004; Harris and Davy, 1988). However, there is little information about effect of sand burial on the physiological charac-teristics of psammophytes. Moreover, the resistant ability of plants to stress is closely related to their physiological characteristics such as antioxidant enzymes activities and osmotic adjustment substances (Tan et al., 2006).

Impacts of sand burial depth on survival, antioxidant enzymes activities, lipid peroxidation and osmotic adjustment substance of three dominant psammophytes in Horqin Sandy Land were investigated experimentally in order to determine (1) the adaptability of three psam-mophytes to sand burial; (2) the effects of sand burial on lipid peroxidation and membrane permeability of these psammophytes; and (3) the protective role of antioxidant enzymes system (SOD, POD and CAT) and osmotic adjustment substances (soluble sugar, proline and soluble protein) among three psammophytes under sand burial stress. We tested two hypotheses that (1) *A squarrosum*

from mobile dune habitat with intensive burial has higher survival rate than the other two species from semi-mobile dune habitat; (2) activity of SOD, POD and CAT and content of osmotic substances (soluble sugar, proline and soluble protein) increase play a protective role when psammophytes were buried.

MATERIALS AND METHODS

Study area

The study area was located in Naiman county (42°55′ N, 120°42′ E; altitude approx. 360 m), which is located in the southwestern part of Horqin Sandy Land, Inner Mongolia, China. Pressures related to grazing, cultivation and collection of fuelwood have made this area one of the most severely desertified regions of China. The climate is temperate, semi-arid continental and monsoonal. The annual precipitation is about 360 mm, with 75% of the precipitation in the growing season of June to September. The mean annual temperature is 6.4°C, and the mean annual pan-evaporation is 1935 mm. The mean annual wind velocity ranges from 3.2 to 4.1 m s^{-1}, the dominant winds are southwest to south in summer and autumn and northwest in winter and spring. Wind erosion often occurs from April to mid-June before the rainy season begins.

The distribution pattern of main natural vegetation is characterized by a mosaic of lowland grassland, fixed dune, semi-fixed, semi-mobile dunes and mobile dune. The dunes tend to be covered at varying levels with native plants, generally dominated by psammophytes including some grasses (example, *Setaria viridis* L., *Ixetis denticulate* L., *Cleistogenes squarrosa* L., *Corispermum macrocarpum* L., *Digitaria ciliaris* L.), forbs (example, *Salsola collina* L., *Agriophyllum squarrosum* L.) and shrubs (for example, *Caragana microphylla* L., *Lespedeza davurica* L., *Salix gordejevii* L. *Artemisia halodendron* L.) (Luo et al., 2010).

Experimental design

This experiment was conducted from April to May in 2010, the time of year that plants are frequently buried by deposited sand in Horqin Sandy Land. In April, seeds of three dominant psammophytes, *C. macrocarpum, S. viridis* and *A. squarrosum* were separately sown in cement pools of 1×1×1 m^3, filled with sand from a mobile dune. After germination, we thinned out seedlings to avoid death due to competition. 75 seedlings having the similar growth were left and marked in each cement pool. Seedlings were kept vertical after thinning, and the average height for the seedlings is 6.0 ± 0.2 cm. There were nine buried treatments: buried to 1/4 (A), 1/2 (B), 3/4 (C), 4/4 (D) of seedling height and 2 cm (E), 4 cm (F), 6 cm (G), 8 cm (H), 10 cm (I) above seedling height, and one control treatment (CK, no burial). For each species, every treatment consisted of four replicates, and there were 120 cement pools in total. Unmarked seedlings that emerged after sand burial were removed, no additional water or fertilizer was added at any time during the experiment in order to keep the soil condition as close to natural conditions as possible. After 20 days of sand burial, live seedlings were counted (to document survival rates) and leaves from 10 seedlings were sampled randomly in each treatment to measure enzyme activity (SOD, POD and CAT), lipid peroxidation and membrane permeability (measured in terms of MDA content and REL), and content of osmotic substances (soluble sugar, soluble protein and proline).

Analytical methods

For survival rates, the number of live seedlings in each treatment was recorded after the experiment. For physiological indices, only

TABLE 1. ANOVA RESULTS ON EFFECTS OF DIFFERENT SAND BURIAL TREATMENTS ON SURVIVAL RATES OF THREE PSAMMOPHYTES.

Sand burial treatment	Survival rate (%)		
	S. viridis	*C.macrocarpum*	*A. squarrosum*
CK	37.00 ± 9.82aA	52.00 ± 15.59abA	63.00 ± 6.04aA
A	76.00 ± 20.78aA	66.00 ± 7.90aA	85.00 ± 4.52aA
B	58.33 ± 0.88aA	27.00 ± 13.78abA	54.33 ± 4.17abA
C	59.00 ± 3.86aA	8.67 ± 2.93bB	54.41 ± 6.52aA
D	0bA	0cA	56.00 ± 15.44aB
E	0bA	0cA	45.67 ± 9.28aB
F	0bA	0cA	44.33 ± 1.81aB
G	0bA	0cA	19.33 ± 4.41cB
H	0bA	0cA	19.67 ± 6.54cB
I	0bA	0cA	9.00 ± 2.70cB
F value	1.83	5.03	3.18
P	0.282	0.076	0.043

CK: (CONTROL TREATMENT, NO BURIAL). A: BURIED TO 1/4 OF SEEDLING HEIGHT. B: BURIED TO 1/2 OF SEEDLING HEIGHT. C: BURIED TO 3/4 OF SEEDLING HEIGHT. D: BURIED TO 100% OF SEEDLING HEIGHT. E: BURIED TO 2 CM ABOVE SEEDLING HEIGHT. F: BURIED TO 4 CM ABOVE SEEDLING HEIGHT. G: BURIED TO 6 CM ABOVE SEEDLING HEIGHT. H: BURIED TO 8 CM ABOVE SEEDLING HEIGHT. I: BURIED TO 10 CM ABOVE SEEDLING HEIGHT. n=4 (EVERY TREATMENT CONSISTED OF FOUR REPLICATES FOR EACH SPECIES, AND 75 SEEDLINGS WERE LEFT IN EACH REPLICATES BEFORE BURIED). VALUES WERE ASSIGNED AS MEAN ± SE. MEAN VALUES WITH DIFFERENT LOWER CASE LETTERS IN THE SAME COLUMN ARE SIGNIFICANTLY DIFFERENT AMONG THE DIFFERENT SAND BURIAL DEPTHS. MEAN VALUES WITH DIFFERENT UPPER CASE LETTERS IN THE SAME ROW ARE SIGNIFICANTLY DIFFERENT AMONG THE THREE KINDS OF PSAMMOPHYTES ($P < 0.05$, LSD TEST).

seedlings that survived the burial treatment were measured. In treatments D through I, all seedlings of *C. macrocarpum* and *S. viridis* were dead, thus no physiological data were recorded for these treatments of these two species.

Enzymes were extracted from 1 g of leaf material using mortar and pestle with 5 ml of an ice-cold medium containing 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, and 2% (w/v) PVP. The homogenate was centrifuged at 15,000 g for 20 min and supernatants were used for protein content determination, or otherwise stored at 4°C for further analyses. SOD activity was determined with the method of Beauchamp and Fridovich, (1971). POD activity was determined spectrophotometrically by measuring the oxidation of methyl catechol at 470 nm (Srivastava and Huystee, 1973). CAT activity was measured spectrophotometrically using the method of Aebi (1974).

MDA content was measured using the method described by Hernandez and Almansa, (2002). Membrane permeability was estimated by measuring the leakage of electrolytes (conductivity) according to Shanahan et al. (1990). Soluble sugar was extracted and analyzed according to the method of Moing et al. (1992). Proline content was determined as described by Bates et al. (1973). Soluble protein content was determined using the method of Bradford (1976). All physiological measurements were carried out at 0 to 4°C

Statistical analysis

Data were analyzed and summarized using Microsoft Excel and Origin 8.0 software. Values were presented as mean ± SE. The significant differences between different treatments and species were determined through one-way and two-way analysis of variance (ANOVA) procedure. The Fisher's least significant difference ($P < 0.05$, LSD test) was performed to compare the differences among mean values.

RESULTS

Effects of sand burial on survival rates

We found that for all species, the highest survival rates occurred in treatment A, and *S. viridis*, *C. macrocarpum*, *A. squarrosum* seedlings were 39, 14 and 22% higher than CK, respectively (Table 1). However, the difference was not significant. Survival rates decreased gradually with an increase in the depth of sand burial. Although, moderate burial did not affect survival of *C. macrocarpum* and *S. viridis* seedlings, no seedlings survived when they were buried completely (treatment D). *A. squarrosum* seedlings showed a very strong ability to endure sand burial stress. However, its survival rate was significantly reduced to 19.33% when the burial depth was increased to the treatment G level, declining sharply to 9% in treatment I.

The difference among survival rates of three species was not significant, except *C. macrocarpum* seedling in treatment C was significantly lower (Table 1). However, the survival rate of *A. squarrosum* seedling was the highest among the three species in all treatments except treatments B and C. When not buried by sand, the survival rate of *C. macrocarpum* seedling was higher than
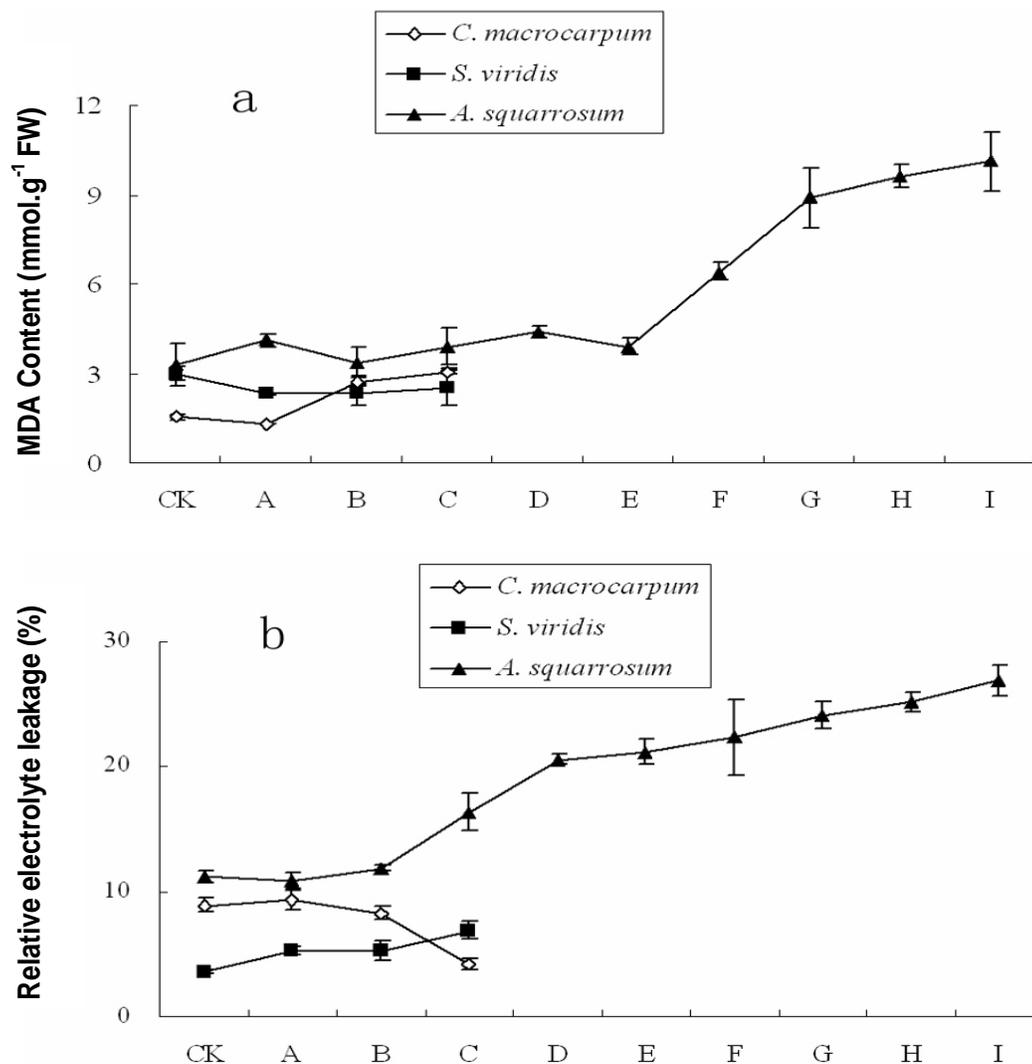
Figure 1. Effects of different sand burial depth on lipid peroxidation (a) and membrane permeability (b) in three psammophytes. CK: (Control treatment, no burial). A: Buried to 1/4 of seedling height. B: Buried to 1/2 of seedling height. C: Buried to 3/4 of seedling height. D: Buried to 100% of seedling height. E: Buried to 2 cm above seedling height. F: Buried to 4 cm above seedling height. G: Buried to 6 cm above seedling height. H: Buried to 8 cm above seedling height. I: Buried to 10 cm above seedling height. Values were assigned as mean ± SE. No seedlings of *C. macrocarpum* and *S. viridis* survived after the burial depth beyond treatment C.

that of *S. viridis* seedling. However, *S. viridis* seedling survived more than *C. macrocarpum* seedling under sand burial treatments.

Effects of sand burial on lipid peroxidation and membrane permeability

MDA

The MDA content of the three species after 20 days buried by sand is depicted in Figure 1-a. MDA content of *S. viridis* was higher than *C. macrocarpum* in CK and treatment A. However, the situation was reverse in treatment B and C. The difference between CK and

plants buried by sand of these two species were not significant ($P > 0.05$). On the other hand, MDA content of *A. squarrosum* was the highest among three species and it increased significantly when the burial depth reached the level of treatment F.

REL

The REL of the three species after 20 days buried by sand is depicted in Figure 1b. REL of *S. viridis* was lower than *C. macrocarpum* in CK, treatment A and treatment B. However, the situation was reverse in treatment C. The difference between CK and plants buried by sand of
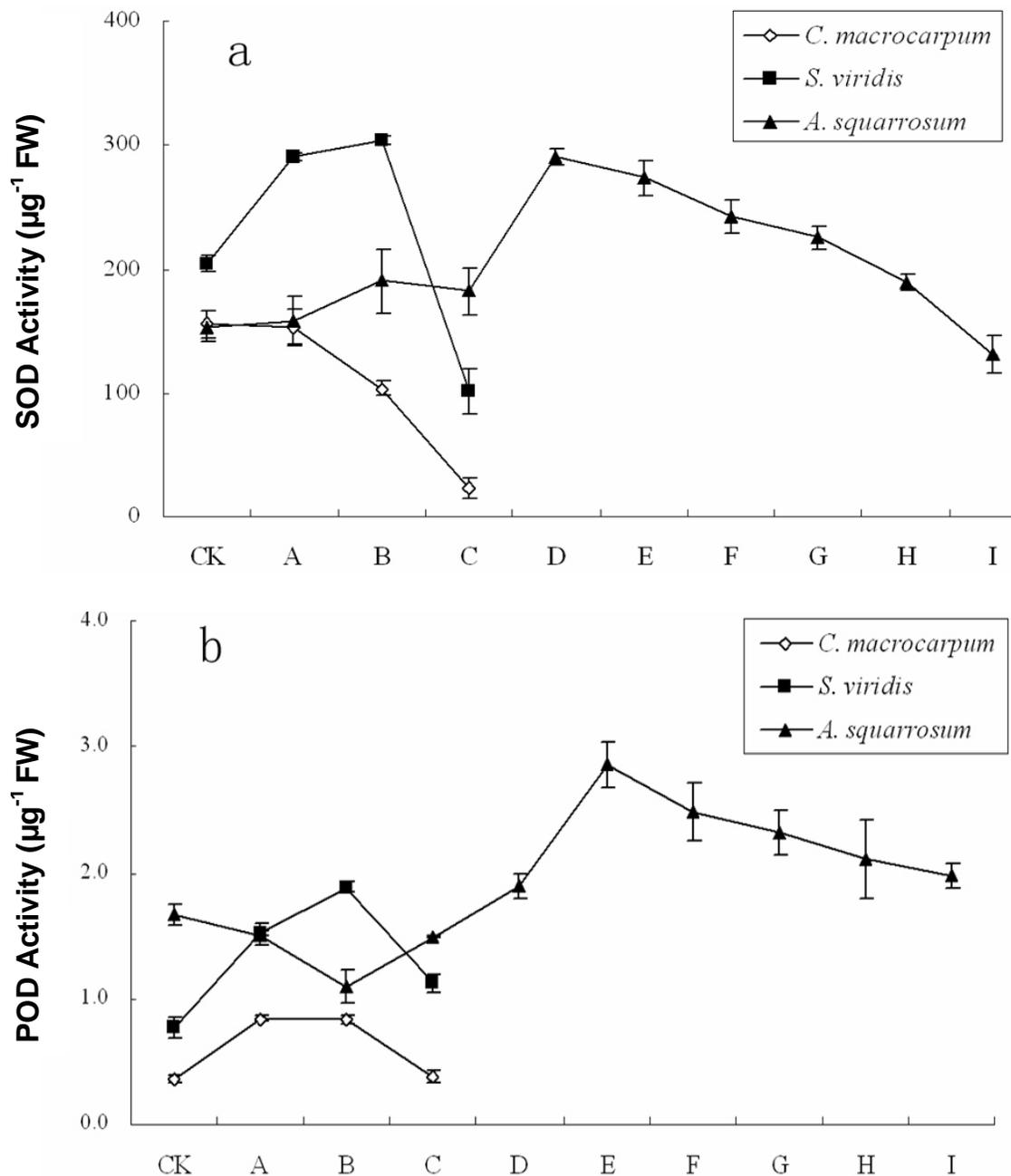
Figure 2. Effects of different sand burial depth on SOD (a), POD (b) and CAT (C) activities in three psammophytes. CK: (Control treatment, no burial). A: buried to 1/4 of seedling height. B: Buried to 1/2 of seedling height. C: Buried to 3/4 of seedling height. D: Buried to 100% of seedling height. E: Buried to 2 cm above seedling height. F: Buried to 4 cm above seedling height. G: Buried to 6 cm above seedling height. H: Buried to 8 cm above seedling height. I: Buried to 10 cm above seedling height. Values were assigned as mean ± SE. No seedlings of *C. macrocarpum* and *S. viridis* survived after the burial depth beyond treatment C.

these two species was not significant ($P > 0.05$). Similar to MDA content, REL of *A. squarrosum* was the highest one among the three species and increased significantly when the burial depth reached the level of treatment C. Compared to CK, its value significantly increased by 140% in treatment I. ($P < 0.05$).

Effects of sand burial on antioxidant enzyme activity

SOD

The SOD activity of the three species after 20 days buried by sand is shown in Figure 2-a. *S. viridis* showed a
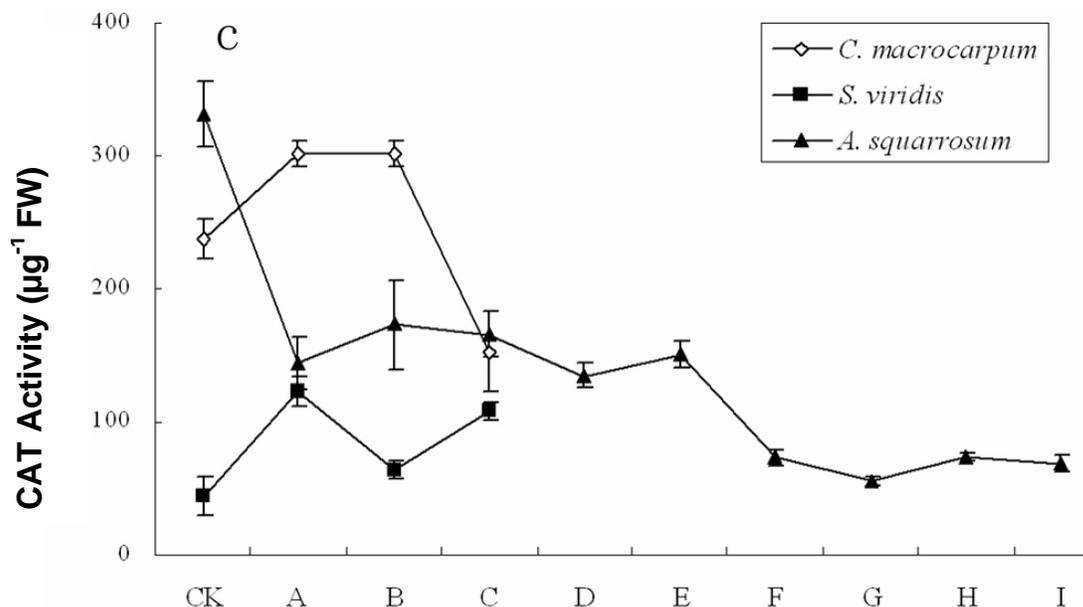
Figure 2. Contd.

significant increase in SOD activity with increasing depth of sand burial. The maximum value appeared in treatment B, and increased by 49% when compared with CK. Then it decreased sharply when the burial depth reached over 50% of the seedling height ($P < 0.05$). *C. macrocarpum* exhibited a continuing decline in SOD activity with increasing depth of sand burial. The SOD activity in treatment C was just about 1/3 of that in CK, a significant difference ($P < 0.05$). The SOD activity of *C. macrocarpum* was the lowest among the three species after sand burial treatment. Change of SOD activity of *A. squarrosum* was similar to *S. viridis*, increasing with a low level of burial depth and then decreasing gradually with increasing burial depths. The maximum and minimum value appeared in treatment D and treatment I, respectively. The difference between CK and plants buried by sand was significant when burial depth reached the level of treatment B of *A. squarrosum* ($P < 0.05$).

POD

Figure 2-b shows the effects of increasing level of sand burial depth on POD activity of the three species. The variation tendency of *S. viridis* and *C. macrocarpum* is similar, increasing when the sand depth was shallow and decrea-sing when burial depth increased. The POD activity of *C. macrocarpum* was the lowest among the three species whether it was exposed to sand burial stress or not. The change of POD in *A. squarrosum* along the burial depth, on the other hand, showed a different trend. Unlike *S. viridis* and *C. macrocarpum*, it decreased with increasing burial depth initially, but increased after the burial depth reached the level of treatment B, peaking

at the level in treatment E. Compared to CK, it was increased by 70.7%. Afterwards, it decreased again with increasing depth of sand burial.

CAT

There was a significant difference of CAT activity in response to sand burial for the three species (Figure 2-c). CAT activity of *S. viridis* showed an increasing-decreasing-increasing change following with the increase in depth of sand burial, and its value was at a minimum regardless of buried by sand among the three species. An increasing-stable-decreasing changing tendency was found in *C. macrocarpum*. CAT activity of *A. squarrosum* showed an opposite trend, decreasing dramatically when burial depth was shallow and increasing slightly until the burial depth reached to the level of treatment E level. There were significant differences between CAT activity of CK and buried plants of these three species ($P < 0.05$).

Effects of sand burial on osmotic substances

Soluble sugar

Changes in soluble sugar content in *S. viridis*, *C. macrocarpum* and *A. squarrosum* are depicted in Figure 3-a. With increasing depth of sand burial, the soluble sugar content of the three species initially increased and then decreased. The maximum values for each of the three species were 70.43 mg^{-1} FW of *S. viridis* in treatment A, 67.03 mg^{-1} FW of *C. macrocarpum* in treatment B and 1 43.87 mg^{-1} FW of *A. squarrosum* in
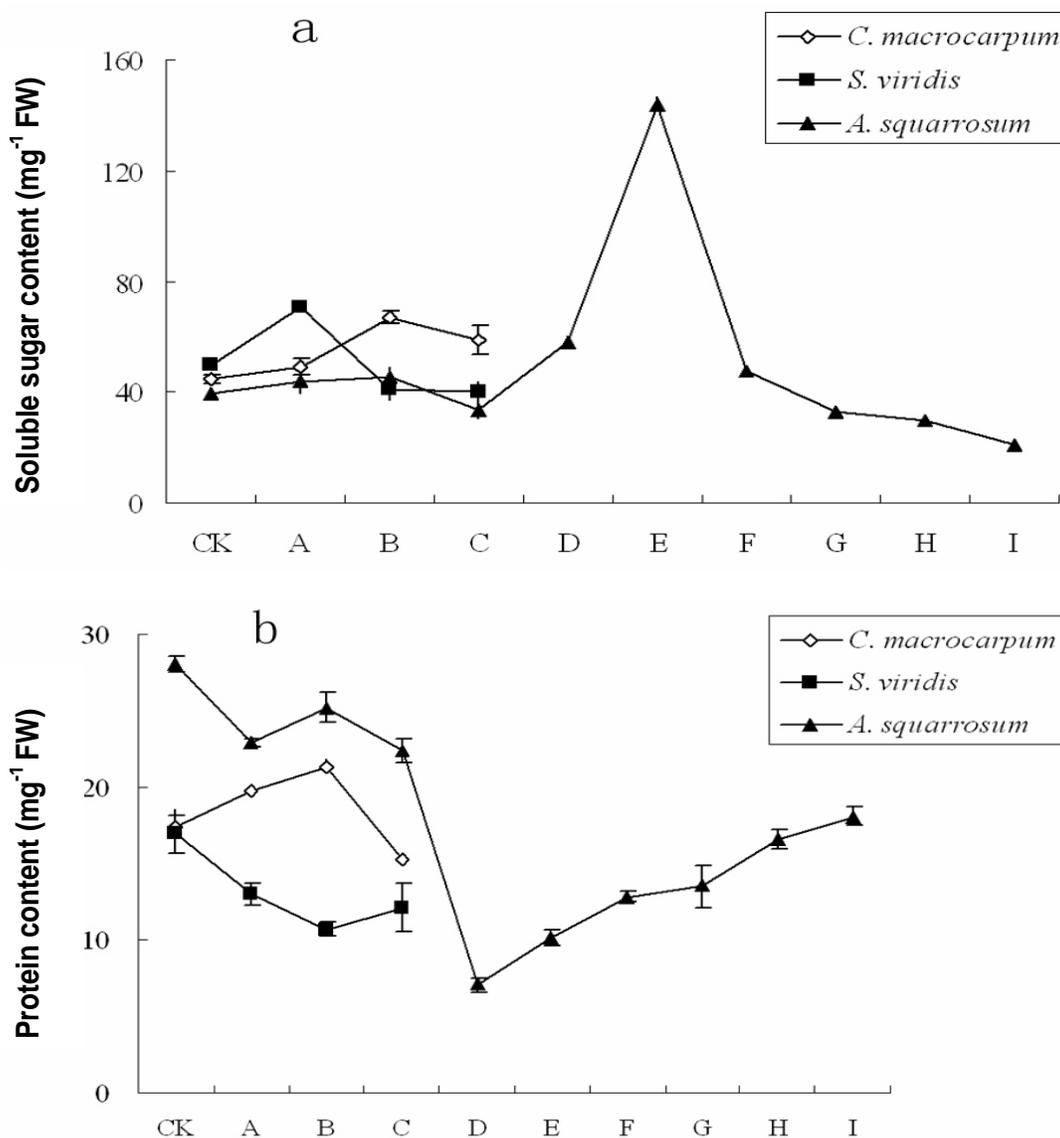
Figure 3. Effects of different sand burial depth on soluble sugar (a), soluble protein (b) and proline (c) content in three psammophytes. CK: (Control treatment, no burial). A: Buried to 1/4 of seedling height. B: Buried to 1/2 of seedling height. C: Buried to 3/4 of seedling height. D: Buried to 100% of seedling height. E: Buried to 2 cm above seedling height. F: Buried to 4 cm above seedling height. G: Buried to 6 cm above seedling height. H: Buried to 8 cm above seedling height. I: Buried to 10 cm above seedling height. Values were assigned as mean ± SE. No seedlings of *C. macrocarpum* and *S. viridis* survived after the burial depth beyond treatment C.

treatment E. Compared with CK, they increased by 40.3, 49.7 and 265.8%. The soluble sugar content of *A. squarrosum* increased substantially (146.4%) from treatment D to treatment E.

Soluble protein

The soluble protein content of the three species was measured on the 20th day after exposure to the sand burial treatment (Figure 3-b). Same content of soluble protein was detected in no sand burial stress (CK) of *S. viridis* and *C. macrocarpum*, although their trends were opposing after the seedlings were buried by sand. Sand burial treatment had negative effect on the soluble protein content of *S. viridis* and a positive effect on *C. macrocarpum*. Soluble protein content of *A. squarrosum* decreased before the burial depth reached the level of treatment D, and then increased gradually with increasing of sand burial depth. There were significant differences between soluble protein content of CK and buried plants of these three species ($P < 0.05$).
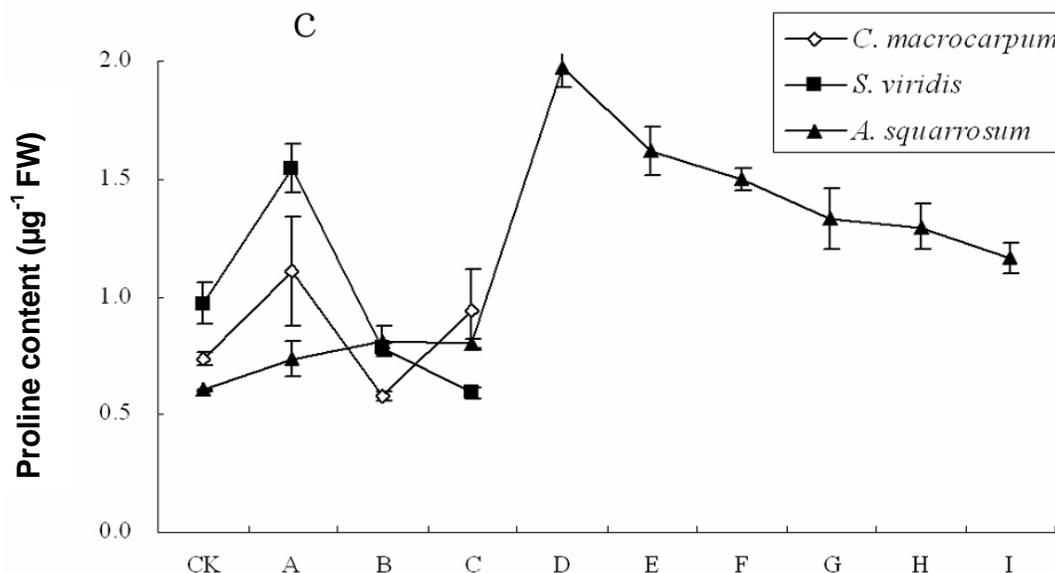
Figure 3. Contd.

Proline

Changes in proline content in *S. viridis*, *C. macrocarpum* and *A. squarrosum* after exposure to the sand burial are depicted in Figure 3-c. Similar with soluble sugar, with increasing level of sand burial depth, the proline content of these three species were increased firstly and then decreased. The maximum value of *S. viridis* and *C. macrocarpum* emerged in treatment A, reached to 1.545 µg^{-1} FW and 1.105 µg^{-1} FW, respectively. Compared with CK, they increased by 51 and 59%. The maximum value of proline content of *A. squarrosum* was 1.970 µg^{-1} FW appearing in treatment D, compared with CK, increased by 227%. Unlike the other two species, no significant differences of proline content were observed between CK and the buried *C. macrocarpum*.

DISCUSSION

Effects of sand burial on survival of three psammophytes

Sand burial is a very complex process which alters all aspects of survival conditions of plants, such as soil temperature, soil moisture, bulk density, nutrient status, soil pH and oxygen levels, and it acts as a filter that eliminates species when burial exceeds their threshold of survival (Poulson, 1999). The Horqin Sandy Land region in China is highly susceptible to wind-blown sand and an average time affected by strong wind as high as 47 d every year (Huang et al., 2007). *S. viridis*, *C. macrocarpum* and *A. squarrosum* play important roles in a variety of sand dune restoration efforts in Horqin Sandy

Land (Luo et al., 2009). Sand movement can increase the vulnerability of psammophytes by burying their seeds, seedlings and adult plants (Maun and Riach, 1981). Nevertheless, several studies have indicated that many sand dune species can adapt well to sand burial stress, and these species may actually require burial in sand to maintain high vigor, as short-term and relatively shallow sand burial may promote plant growth (Van, 1993; Olson, 1958). However, each species has an upper threshold limit above which it is unable to survive the burial stress: deep burial is ultimately fatal to seedlings because it creates a physical barrier for vertical growth, reduces the photosynthetic area, and limits oxygen availability to roots (Harris and Davy, 1988; Maun, 1994). For example, Sykes and Wilson (1990) reported that only a few species survived complete burial in sand but all species survived partial burial to 66 % of their height. In our study, similar results were observed. Shallow burial had positive effects on survival rates, and seedlings buried to 1/4 of seedling height (treatment A) had the maximum survival rates in all the three species we studied. This may be due to an increase in the amount of soil moisture and a decrease in soil temperature in the root zone corresponding to increased soil depth (Shi et al.,2004), as dry soil conditions and high temperatures are major factors limiting plant growth in arid regions (Niu et al., 2003). Furthermore, Sand burial can provide extra nutrient to plants, it is favorable for plant growth (Poulson, 1999). Deeper burial, however, is fatal to the psammophytes in Horqin Sandy Land, which can be explained by more limited access to oxygen and more limited potential for photosynthesis under conditions of complete burial (Kurz, 1939; Shi et al., 2004). The upper threshold limit of *S. viridis* and *C. macrocarpum* is complete burial. Seedlings

of *A. squarrosum* can withstand the stress much further along the sand burial gradient, and there were still 9% of *A. squarrosum* seedlings remained alive even after the burial depth reached 10 cm higher than seedling height. This may explain why *A. squarrosum* is a pioneer plant of mobile dunes. *A. squarrosum* suffers sand burial stress more frequently compared to the other two species, and thus tolerance to burial seems to be a requisite for its survival (Zhang et al., 2005). During this experiment, *A. squarrosum* continued to grow after burial to rapidly recover lost leaf area, which helped the seedlings maintain photosynthetic tissue. Further investigation should be conducted to determine the upper limit of the capacity of *A. squarrosum* seedlings to endure sand burial stress.

Effects of sand burial on physiology of three psammophytes

The extent of lipid peroxidation, indicated by MDA, is related to the levels of antioxidant activity in plant cells (Demiral and Turkan, 2005). MDA content of *A. squarrosum* and *C. macrocarpum* remained at lower levels under more limited sand burial depths, and then increased after the sand burial depth extended the level of treatment E and A, respectively suggesting that the cell membranes were not damaged because antioxidant enzyme system of these species was effective when the burial depth was shallow. However, the extent of lipid peroxidation in both species are very serious due to the protective role of antioxidant enzyme system of have been less effective. It is agreed with the changes of SOD, POD and CAT. Concerning *S. viridis*, the content of MDA was lower in the sand burial treatments than CK, which indicates a greater protection from oxidative damage. This may result from a more efficient anti-oxidative system, consistent with the results that SOD, POD and CAT all play protective role in *S. viridis*.

Relative electrolyte leakage indicates the extent of membrane permeability (Bai et al., 2006), REL increased with the increasing of sand burial depth in *A. squarrosum* and *S. viridis*, while it decreased in *C. macrocarpum*. In addition, both the highest MDA content and REL were found in *A. squarrosum*, showing that *A. squarrosum* was subjected to more oxidative damage than the other two species after being buried by sand, which may have stimulated an increase in the activities of various antioxidant enzymes such as SOD, CAT, and POD as a survival mechanism in response to the burial stress (Asada, 1999).Higher plants have a wide range of defensive mechanism systems that help them adapt various environmental stresses (Yu and Tang, 2004; Chaves et al., 2003). SOD, POD and CAT are common and important indices for evaluating the anti-oxidative ability of plants, an aspect of their capability to resist stress (Saba et al., 2001; Dhanda et al., 2004). Many researchers have found that when exposed to stress, the

enzyme activities of plants rapidly increased improving the antioxidant capacity of the cells and inhibiting ROS formation (Gao et al., 2008; Liu et al., 2010).

SOD catalyzes the dismutation of O^{2-} to H_2O_2 and O_2 (McCord and Fridovich, 1969; Monk et al., 1989; Zhang et al., 2004). Theoretically, high SOD activity indicates low membrane lipid peroxidation because SOD is the first line of defense against lipid membrane damages caused by ROS (Mittler, 2002). In our study, SOD activity of *A. squarrosum* and *S. viridis* showed a positive response to the increase in sand burial depth. However, SOD activity decreased sharply when the burial depth of *A. squarrosum* and *S. viridis* extended beyond 100% of seedling height (treatment D) and 1/2 of seedling height (treatment B), respectively. This suggests that after sand burial, the SOD activity of these two species increased to improve the antioxidant capacity of the cells and inhibit ROS formation. However, the enhanced SOD activity was unable to repair ROS-induced damage with increased burial depth. Other researchers have also reported that the protective ability of enzymes is limited in higher plants (Sunita et al., 2008). In terms of *C. macrocarpum*, SOD activity decreased gradually with increased sand burial depth, indicating it not play a protective role against sand burial stress.

POD can catalyze H_2O_2 and ROOH· into H_2O and R-OH, a mechanism to avoid cell damage (Liu et al., 2010), and it played a protective role in response to sand burial stress in all these three species studied. In terms of *C. macrocarpum* and *S. viridis*, POD activity showed a positive response to the increase in sand burial depth initially, and then decreased when the burial depth reached 3/4 of seedling height (treatment C). In terms of *A. squarrosum*, POD activity did not respond when the burial depth was shallow, and when the burial depth extended beyond 2 cm above seedling height (treatment E), its protective ability to cell damage was reduced. This suggests that POD is an effective antioxidant enzyme which can play a protective role against sand burial stress in all the species studied. However, similar to SOD, its protective ability is also limited. After exposure to a stressful environment, H_2O_2 can be converted to HO impairing the electron transport chain in chloroplasts and mitochondria (Jiménez et al., 1997; Meneguzzo et al., 1998), lipid peroxidation (Quartacci et al., 1995; Navari-Izzo et al., 1996), protein denaturation (Baccio et al., 2004) and DNA damage (Conte et al., 1996). Fortunately, CAT can effectively remedy those damages by quenching H_2O_2 in higher plants. It played a protective role in *C. macrocarpum* and *S. viridis* in our study. The peak value of CAT activity occurred in treatment A (burial at 1/4 of seedling height) for these two species. However, CAT did not play a protective role in *A. squarrosum*, as it decreased continuously with the increasing sand burial depth. Gao et al. (2008)'s study on *Alternanthera philoxeroides* and *Oryza sativa* subjected to drought stress showed that different psammophytes responded to stress differently in terms of the enzyme activities of

POD, SOD and CAT. We also found that SOD, POD and CAT alone have a limited contribution to tolerance of sand burial stress, and their protective role in different species is not same. POD is a protective enzyme of all the three species, SOD plays a protective role in *A. squarrosum* and *S. viridis*, and CAT has a protective effect in *C. macrocarpum* and *S. viridis*.

The function of proline as an osmoprotectant under stress has been widely reported. It can stimulate the CAT and SOD activity to scavenge ROS which may damage the membrane (Parida et al., 2008; Taschler et al., 2004). In our study, the accumulation of proline of *A. squarrosum* and *S. viridis* increased dramatically with increasing of burial depth, and then decreased gradually when the burial stress became serious. This observation is similar with the results reported by Delauney and Verma (1993) who found that the increase of proline may allow plants to overcome stress and to enhance their ability of survival and tolerance under harmful conditions. However, the contribution of proline to stress resistance is limited (Wang et al., 2005), which may explain why proline levels in *A. squarrosum* and *S. viridis* decreased at more extreme burial depths.

In terms of *A. squarrosum* and *S. viridis* in our study, soluble protein content decreased at shallow burial depths and then increased, however, the value in all burial treatments was lower than in the control plants. The situation is the opposite for *C. macrocarpum*. This is evidence that the effects of stress on soluble protein are different in different species. Some researchers have reported that soluble protein content is induced strongly by environmental stress in order to protect plants from further dehydration (Jiang and Huang, 2002; Han and Kermode, 1996). On other hand, drought-induced decrease in soluble protein has also been reported (Parida et al., 2008).

Soluble sugar is also an important osmotic substance in plants. It has been reported that soluble sugar content increases under drought, freezing, heat and saline stress to reduce the extent of membrane damage (Greenway and Munne, 1980). This was observed in our study, soluble sugar content increased in all three species with the greatest increase found in *A. squarrosum*. This may, at least in part, explain why *A. squarrosum* had the strongest ability to adapt to sand burial stress among the three psammophytes.

Conclusion

In conclusion, hypothesis 1 (*A. squarrosum* from mobile dune habitat with intensive burial has higher survival rate than the other two species from semi-mobile dune habitat) is supported by our results. We found that the survival rate of *A. squarrosum* seedling was greatest among the three species in both the control (CK, no burial) and all of the sand burial treatments, except for treatment C (buried to 3/4 of seedling height). In addition,

A. squarrosum seedlings were more tolerant of sand burial than the other two species: about 9% of the seedlings were able to survive even when the burial depth reached 10 cm higher than seedling height. Hypothesis 2 (Activity of enzymes and content of osmotic substances increase to play a protective role when psammophytes were buried) was partly supported. After exposure to sand burial stress, the enzyme activities and osmotic substances content of psammophytes increased to keep the MDA content and REL at a low level in order to improve antioxidant capacity and inhibit ROS formation. This is the reason why the psammophytes can survive or even grow better at moderate sand burial depth. However, the different enzymes worked differently in the different psammophytes to protect them from sand burial stress. POD is effective in all three psammophytes, while SOD and CAT played protective role in *A. squarrosum* and *S. viridis*, *C. macrocarpum* and *S. viridis*, respectively. In addition, both antioxidant enzyme systems and osmotic substances have a limited ability to help avoid plant death when the sand burial stress is severe. When the burial depth was moderate, MDA content and REL of *A. squarrosum* seedlings were maintained at a low level. However, they increased sharply with the burial depth increasing continually. This indicates that the injury of cell was very seriously as burial depth increased. Unexpectedly, MDA content and REL of *C. macrocarpum* and *S. viridis* seedlings did not change much in the whole experiment. For the time being, we do not know why these remained at a low level under sand burial stress. No doubt, further studies are needed. Our study developed an understanding of the tolerance of three dominant psammophytes in Horqin Sandy Land to sand burial. Our finding related to *A. squarrosum*, in particular, suggests its potential as a target sand dune stabilization species for areas suffering frequent sand burial (for example, mobile dune).

REFERENCES

Asada K (1999). The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Biol. 50: 601-639.
Aebi H (1974). Catalase. Method Enzymol Analysis 2 - Academic Press, New York, pp. 673-684.

Baccio DD, Navari-Izzo F, Izzo R (2004) Seawater irrigation: antioxidant defence responses in leaves and roots of a sunflower (*Helianthus annuus* L.) ecotype. J. Plant Physiol. 161: 1359-1366.

Bai LP, Sui FG, Ge TD, Sun ZH, Lu YY, Zhou GS (2006). Effect of soil drought stress on leaf water status, membrane permeability and enzymatic antioxidant system of maize. Pedosphere, 16: 326-332.

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39: 205-207.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamidegel. Anal. Biochem. 44: 276-287.

Benvenuti S, Macchia M, Miele S (2001). Light, temperature and burial depth effects on *Rumex obtussifolius* seed germination and emergence. Weed Res. 41: 177-186.

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

Bybordi A (2011). Effect of ammonium: nitrate ratio on fatty acid composition and proline accumulation of canola cultivars grown under salinity stress. Afr. J. Biotechnol. 10: 16826-16832.

Chaves MM, Maroco J, Pereira J (2003). Understanding plant responses to drought-from genes to the whole plant. Funct. Plant Biol. 30: 239-264.

Chen LN, Yin HX, Xu J, Liu XJ (2011). Enhanced antioxidative responses of a salt-resistant wheat cultivar facilitate its adaptation to salt stress. Afr. J. Biotechnol. 10: 16887-16896.

Ci LJ, Liu YP (2000). Driving Action of Population Increase on Desertification. J. Arid, Land, Resour. Environ. Chinese, 14: 28-33.

Conte D, Narindrasorasak S, Sarkar B (1996). *In vivo* and *in vitro* iron-replaced zinc finger generates free radicals and causes DNA damages. J. Biol. Chem. 271: 5125-5130.

Delauney A, Verma D (1993). Proline biosynthesis and osmoregulation in plants. Plant J. 4: 215-223.

Demiral T, Turkan I (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53: 247-257.

Dhanda SS, Sethi GS, Behl RK (2004). Indices of drought tolerance in wheat genotypes at early stages of plant growth. J. Agron. Crop Sci. 190: 6-12.

Gao JM, Xiao Q, Ding LP, Chen MJ, Yin L, Li JZ, Zhou SY, He GY (2008). Differential responses of lipid peroxidation and antioxidants in *Alternanthera philoxeroides* and *Oryza sativa* subjected to drought stress. Plant Growth Regul. 56: 89-95.

Greenway H, Munne R (1980). Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 31: 149-190.

Han B, Kermode A (1996). Dehydrin-like proteins in castor bean seeds and seedlings are differentially produced in response to ABA and water-deficit-related stresses. J. Exp. Bot. 47: 933-939.

Harris D, Davy AJ (1987). Seedling growth in *Elymus farctus* after episodes of burial with sand. Ann. Bot. London 60: 587-593.

Harris D, Davy AJ (1988). Carbon and nutrient allocation in *Elymus farctus* seedlings after burial with sand. Ann. Bot. London, 61: 147-157.

Hernandez JA, Almansa MS (2002). Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plantarum, 115: 251-257.

Huang G, Zhao XY, Zhao YP, Su YG (2007). Roots Distribution of two Typical Shrubs in Singal or Mixture Circumstance in Horqin Sandy Land. J. Desert Res. Chinese, 27: 50-59.

Jiang Y, Huang B (2002). Protein alterations in tall fescue in response to drought stress and abscisic acid. Crop Sci. 42: 202-207.

Jiménez A, Hernández JA, Del Rýo LA, Sevilla F (1997). Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea (*Pisum sativum* L.) leaves. Plant Physiol. 114: 275-284.

Kurz H (1939). The reaction of magnolia, scrub live-oak, slash-pine, palmetto and other plants to dune activity on the western Florida coast. Proceedings Florida Acad. Sci. 4: 195-203.

Liu B, Liu ZM, Guan DX (2008). Seedling growth variation in response to sand burial in four *Artemisia* species from different habitats in the semi-arid dune field. Trees, 22: 41-47.

Liu JG, Zhang XL, Sun YH, Lin W (2010). Antioxidative capacity and

enzyme activity in *Haematococcus pluvialis* cells exposed to superoxide free radicals. Chin. J. Oceanol. Limnol. 28: 1-9.

Liu XM, Zhao HL, Xu B (1992). Destrction Causes of Korqin Sandy Land and Approaches to its Restoration Chinese J. Ecol. 11: 38-41 (in Chinese).

Luo YY, Zhao XY, Huang YX, Zuo XA, Wang SK, Zhang YF (2009). Seedling emergence of three Chenopodiaceae annuals in response to different sand burial depth and irrigation regimes. Acta Prataculturae Sinica Chinese, 18: 122-129

Luo YY, Zhao XY, Zuo XA, Zhang JH, Liu RT, Wang SK (2010). Leaf nitrogen resorption pattern along habitats of semi-arid sandy land with different nitrogen status. Pol. J. Ecol. 58: 707-716.

Luo YY, Zhao XY, Zhou RL, Zuo XA, Zhang JH, Li YQ (2011). Physiological acclimation of two psammophytes to repeated soil drought and rewatering. Acta Physiol. Plant, 33: 79-91.

Maun MA, Riach S (1981). Morphology of caryopses, seedlings and seedling emergence of the grass Calamovilfa longifolia from various depths in sand. Oecologia. (Berl.) 49: 137-142.

Maun MA (1994). Adaptations enhancing survival and establishment of seedlings on coastal dune systems. Vegetation. 111: 59-70.

Maun MA (1996). The effects of burial by sand on survival and growth of *Calamovilfa longifolia*. Eco. Sci. 26: 93-100.

McCord JM, Fridovich I (1969). Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244: 6049-6055.

Meneguzzo S, Sgherri CLM, Navari-Izzo F, Izzo R (1998). Stromal and thylakoid-bound ascorbate peroxidases in NaCl treated wheat. Physiol. Plantarum, 104: 735-740.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 9: 405-410.

Moing A, Carbonne F, Rashad MH, Gaudille`re JP (1992). Carbon fluxes in mature peach leaves. Plant Physiol. 100: 1878-1884.

Monk SL, Fagerstedt KV, Crawford RMM (1989). Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. Physiol. Plantarum, 76: 456-459.

Navari-Izzo F, Quartacci MF, Sgherri CLM (1996). Superoxide generation in relation to dehydration anrehydration. Biochem. Soc. Trans. 24: 447-451.

Niu SL, Jiang GM, Li YG, Gao LM, Liu MZ, Peng Y, Ding L (2003). Comparison of photosynthetic traits between two typical shrubs: legume and non-legume in Hunshandak Sandland. Photosynthetica, 41: 237-242.

Olson JS (1958). Rates of succession and soil changes on southern Lake Michigan dunes. Bot. Gaz. 119: 125-170.

Parida AK, Dagaonkar VS, Phalak MS, Aurangabadkar LP (2008). Differential responses of the enzymes involved in proline biosynthesis and degradation in drought tolerant and sensitive cotton genotypes during drought stress and recovery. Acta Physiol. Plant, 30: 619-627.

Poulson TL (1999). Autogenic, Allogenic and individualistic mechanisms of dune succession at Miller, Indiana. Nat. Area J. 19: 172-176.

Qayyum A, Razzaq A, Ahmad, M, Jenks MA (2011). Water stress causes differential effects on germination indices, total soluble sugar and proline content in wheat (*Triticum aestivum* L.) genotypes. Afr. J. Biotechnol. 10: 14038-14045.

Quartacci MF, Pinzino C, Sgherri CLM, Navari-Izzo F (1995). Lipid composition and protein dynamics in thylakoids of two wheat cultivars differently sensitive to drought. Plant Physiol. 108: 191-197.

Saba J, Moghaddam M, Ghassemi K (2001). Genetic properties of drought resistance indices. J. Agric. Sci. Technol. 3: 43-49.

Shanahan JF, Edwards IB, Quick JS, Fenwick JR (1990). Membrane thermostability and heat tolerance of spring wheat. Crop Sci. 30: 247-251.

Shi L, Zhang ZJ, Zhang CY (2004). Effects of sand burial on survival, growth, gas exchange and biomass allocation of *Ulmus pumila* seedlings in the Hunshandak Sandland, China. Ann. Bot. London, 94: 553-560.

Srivastava OP, Huystee PB (1973). Evidence for close association of POD Polyphenol oxidase and IAA oxidase isoenzyme of peanut suspension culture medium. Can. J. Bot. 51: 2207-2214.

Sunita S, Anita K, Veena S (2008). Effect of nitric oxide and putrescine on antioxidative responses under NaCl stress in chickpea plants. Physiol. Mol. Biol. Plants, 14: 355-362.

Sykes MT, Wilson JB (1990). An experimental investigation into response of New Zealand sand dune species to different depths of burial by sand. Acta Botanica Neerlandica, 39: 171-181.

Tan Y, Liang ZS, Shao HB, Du F (2006). Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of Radix Astragali at seeding stage. Colloids and Surfaces B: Biointerfaces, 49: 60-65.

Taschler D, Beikircher B, Neuner G (2004). Frost resistance and ice nucleation in leaves of five woody timberline species measured in situ during shoot expansion. Tree Physiol. 24: 331-337.

Van Der P, Van Dijk C, Peters BAM (1993). Plant-specific soil-borne diseases contribute to succession in foredune vegetation. Nature, 362: 53-55.

Wang FZ, Wang QB, Kwon SY (2005). Enhanced drought tolerance of transgenic *O.sativa* plants expressing a pea manganese superoxide dismutase. J. Plant Physiol. 162: 465-472.

Wang T (2008). Strategic consideration on desert and destification sciences development in China. J. Desert Res. Chinese, 28: 1-7

Yoshida M, Abe J, Moriyama M, Shimokawa S, Nakamura Y (1997). Seasonal changes in the physical state of crown water associated with freezing tolerance in winter wheat. Physiol. Plant, 95: 363-370.

Yu SW, Tang KX (2004). MAP kinase cascades responding to environmental stress in plants. Acta Bot. Sin. 46: 127-136.

Zhang F, Guo GK, Yang YL, He WL, Zhang LX (2004). Changes in the pattern of antioxidant enzymes in wheat exposed to water deficit and rewatering. Acta Physiol. Plant, 26: 345-352.

Zhang J, Zhao H, Zhang TH (2005) Community succession along a chronosequence of vegetation restoration on sand dunes in Horqin Sandy Land. J. Arid. Environ. 62: 555-566.

Zhang JH, Maun MA (1990). Effects of sand burial on seed germination, seedling emergence, survival, and growth of *Agropyron psammophilum*. Can. J. Bot. 68: 304-310.

Zhou RL, Zhao HL (2004). Seasonal pattern of antioxidant enzyme system in the roots of perennial forage grass grown in alpine habitat, related to freezing tolerance. Physiol. Plantarum. 121: 399-408.