Australian Journal of Crop Science AJCS
AJCS 6(5):869-876 (2012) ISSN:1835-2707

Effects of sand burial stress on maize (*Zea mays* L.) growth and physiological responses

Hao Qu[1,2*], Halin Zhao[1], Ruilian Zhou[3], Xiaoan Zuo[1], Jin Wang[3], Jin Li[1,2], Barron J. Orr[4]

[1]Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences Lanzhou 730000, China
[2]Graduate University of Chinese Academy of Sciences Beijing 100049, China
[3]School of Life Science, Ludong University Yantai 264025, China
[4]Office of Arid Lands Studies, University of Arizona, 1955 E. 6th Street, Tucson, AZ 85719, USA

[*]Corresponding author: quhao66@163.com

Abstract

We examined the effects of sand burial on the growth and the physiological response of maize (*Zea mays* cv. Zhengdan958) in the Horqin sandy lands. We had five treatments that varied in the amount of sand burial: control (no burial), A (burial to 1/4 of plant height), B (burial to 1/2 of plant height), C (burial to 3/4 of plant height), and D (burial to 100% of plant height). We then measured survival rate, plant height, above-and belowground biomass, yield, and activity of antioxidant enzymes: superoxide dismutase, peroxidase, catalase, and malondialdehyde content. Results show that the survival rate (91.67%) and plant height (230.3 cm) of maize under Treatment A at harvest time are significantly higher ($P < 0.05$) than those under other treatments; sand burial stress does not change the specific value of above and belowground biomass. Shallow burial (Treatment A) had no negative effects on the survival and growth of maize, but does decrease maize crop yield. Malondialdehyde content in shallow sand burial treatments (Treatments A and B) remained at low levels, but under sand burial ≥ 3/4 plant height (Treatments C and D) they increased significantly. Superoxide dismutase activity increased significantly 12 days after sand burial and the increase under Treatment A is the highest (203%). Under all treatments, peroxidase activity increased 6 days after sand burial, but decreased significantly 12 days after sand burial. Catalase activity under all burial treatments was lower than that in the control. Sand burial should be avoided in the seedling stage of maize in our study area because even shallow burial can decrease yield.

Keywords: Horqin sandy land; survival rate; plant height; biomass; antioxidant enzyme activity; malondialdehyde.
Abbreviations: CAT- catalase; EDTA- ethylene diaminetetraacetic acid; FW- fresh weight; MDA- malondialdehyde; POD-peroxidase; PVP- polyvinyl-pyrrolidone; ROS- reactive oxygen species; SOD- superoxide dismutase; CRD- completely randomized design.

Introduction

Horqin sandy land is one of the most severely desertified regions in northern China because of heavy grazing, cultivation and the fuelwood gathering. It is also one of the major ecologically fragile zones in the world with strong winds and sand activity (Zhao et al., 2008). In spring, the average wind speed is 4.3 ms^{-1}. The average wind speed in this area is 3.5 ms^{-1} annually and flying sand occurs approximately 20 to 30 days a year. The geomorphology of Horqin sandy land is mainly based on slowly undulating sands, with alternating flat, sandy meadows and farmland distributed throughout the area (Zhao et al., 2009). Plants and crops growing in this area are often subjected to stress caused by sand burial. Sand burial, and the elasticity of plant response are closely linked to concepts of plant succession and pedogenesis; understanding its effect on plants is important at the level of individual plant ecophysiology as well community ecology (Kent et al. 2001). Most of the recent work on the impact of sand burial on the growth of plants and the response of plants to sand burial stress mainly focus on seed germination and emergence (Zhang et al., 1990; Benvenuti et al., 2001). These studies show that seed germination and seedling emergence are related to seed mass after sand burial where seeds with larger relative mass show higher rates of seed germination and seedling emergence. Studies investigating the effect of sand burial on plant growth have found that shallow burial is favorable to plant growth by changing the distribution of substance and energy in plants, however, deep burial is fatal to plant growth (Sykes et al., 1990, Shi et al., 2004, Harris et al., 1988). The physiological response of plants and crops to stress is mainly focused on high temperature (Xu and Zhou, 2006), cold (Pan et al., 2011), drought (Zhang et al., 2004), salt (Pan et al., 2006), and other primary stresses. These studies show that plants exposed to stress had elevated antioxidant enzyme activity in plants and osmotic substances in order to resist or repair damage caused by stress. When stress exceeds tolerance limits in plants, damage such as cell membrane lipid peroxidation, inactivation of antioxidant enzymes, and protein denaturation occurs via the build up of reactive oxygen species (ROS) and leads to plant death. The effects of sand burial are very complicated because it causes mechanical damage, reduction in photosynthetic area, and changes in soil temperature and humidity (Poulson, 1999). Thus far, there are limited studies of the effects of sand burial on physiological responses of plant species. Zhang (1996) found that plants partially buried by sand had higher leaf chlorophyll concentration than those unburied at the early stages of development, especially under low soil moisture content. Kent et al. (2005) determined that there were no differences between the effects of short- and long-term burial on the photosynthetic efficiency of machair sand dune vegetation. However, studies on the response of crops to sand burial stress are very limited, and the antioxidant enzymes activities which are closely related to the resistant ability of plants to stress (Tan et al., 2006), are rarely discussed. Furthermore, how crops respond to sand burial stress in growth and physiology, and whether it is in accordance with other stresses, must be determined. Thus, a study on the sand burial of maize, a crop planted widely in Horqin sandy land, a region known for strong winds and blowing sands will lead to insights into the future of agricultural cultivation and environmental protection in this and other arid regions. The objectives of our study are as follows: (1) to know the effects of sand burial on the growth and production of maize and determine ability of maize to resist sand burial by measuring plant height, above and belowground biomass, and yield. (2) Characterize the physiological response of maize to sand burial stress by comparing the extent of injury as well as physiological indices of stress. We specifically examined the physiological role that different antioxidant enzymes play under different sand burial depths. (3) To propose rational suggestions for cultivation of local crops based on what is learned. The following are two hypotheses drawn based on previous studies of other plant species under sand burial and other stresses: (1) shallow sand burial is favorable to the growth and production of maize, but deep sand burial inhibits the growth of maize high crop mortality and low yields. (2) Shallow sand burial stimulates the antioxidant enzyme activity of maize, thereby maintaining lower MDA content and protecting plants from injury, but deep sand burial beyond the adjustable range of maize causes a significant MDA increase and serious damage to maize.

Results

Effects of sand burial on the growth of maize Survival rate

When exploring the survival rate of maize seedlings relative to burial depth, the highest rate (91.67%) was under Treatment A (burial to 1/4 of plant height) (Table 1). However, it was not significantly different ($P > 0.05$) than the survival rate in the control (no burial) (89.58%). The increase in sand burial depth markedly decreased the survival rate of maize. The survival rates under Treatments B, C, and D (45.83%, 29.17%, and 12.50%, respectively) are significantly lower than those of the control and those under Treatment A ($P < 0.05$). The differences in the survival rates under Treatments B and C, and Treatments C and D were insignificant ($P > 0.05$). The difference in the survival rates under Treatments B and D was significant ($P < 0.05$)

Plant height

The changes in the heights of maize plants under different sand burial treatments are shown in Table 1. We found that the difference in plant height between each treatment is not significant before burial treatment was conducted ($P > 0.05$). Plant height was greatest in treatments that had sand burial ≤ 1/2 of plant height (control, treatment A and B), and Treatment A with minimal sand burial (1/4 of plant height) had the tallest maize plants with an average plant height of 230.3 cm, Treatment A was significantly higher than that of the maize under Treatment C (211 cm) and Treatment D (191.3 cm) ($P < 0.05$). However, the difference between the height of the maize under Treatment A and that of the control (227.8 cm) and under Treatment B (217.3 cm) is not significant ($P > 0.05$). Treatment D (burial to 100% of plant height) was significantly shorter than all other treatments ($P < 0.05$).

Biomass

The aboveground biomass of maize decreases with an increase in sand burial depth (Table 1). Aboveground biomass under Treatment A is the highest (2268 g/m^2), whereas that under Treatment D is the lowest (676 g/m^2). The differences in the aboveground biomass of the maize in the control, Treatment A, and Treatment B are not significant ($P > 0.05$). Treatments with sand burial ≤ 1/2 of plant height (control, treatment A and B) had significantly more aboveground biomass than those with sand burial ≥ 3/4 of plant height ($P < 0.05$), and the difference between Treatments C and D is not significant ($P > 0.05$). Similarly, the belowground biomass of the maize under Treatment A (1137 g/m^2) is the highest, whereas that under Treatment D (365 g/m^2) is the lowest. The difference in the belowground biomass between the control and Treatment A is not significant ($P > 0.05$), and as the amount of sand burial increased to ≥1/2 of plant height there was a significant decline in the amount of belowground biomass investment (Table 1). The belowground biomass under Treatment D (burial to 100% of plant height) is significantly smaller than under all other treatments and the control ($P < 0.05$). Table 1 also reveals patterns of investment into above and belowground biomass is similar regardless of sand burial as the ratio of belowground biomass to aboveground biomass remains within the range 0.45 to 0.56.

Yield

Maize yield decreases as sand burial depth increases (Table 1). The highest yield (1399 g/m^2) is observed in the control, whereas the lowest yield (257 g/m^2) is observed under Treatment D (burial to 100% of plant height): the former is five fold larger than the latter. The statistical analysis shows that the differences between the yield of the control and that of all treatments are significant ($P < 0.05$). The yield differences between the treatments are also significant ($P < 0.05$).

Physiological response of maize to sand burial Antioxidant enzyme activity

The difference of SOD activity between every treatment and the control are not significant (Fig 1-a), with the value between 32.2 and 39.1 μg^{-1} FW before sand burial ($P > 0.05$). Six days after sand burial, the differences in SOD activity under the control and Treatments C and D are not significant with the period before sand burial ($P > 0.05$). Relative to the period before the sand burial, SOD activity decreased significantly under Treatments A and B ($P < 0.05$), by 55.4% and 35.1%, respectively. The comparison of all treatments reveals that the difference between Treatments A and B is not significant ($P > 0.05$), nor is the difference between Treatments C and D ($P > 0.05$). However, the SOD activity under Treatments A and B are significantly lower than that under Treatments C and D ($P < 0.05$). Twelve days after sand burial, the SOD activity under the control (32.64 μg^{-1} FW) changed slightly. By contrast, the SOD activity under all other sand burial treatments increased markedly and was significantly higher than the SOD activity before and six days after sand burial ($P < 0.05$). The SOD activity under Treatments A, B, C, and D twelve days after sand burial was 118.47, 96.94, 93.72, and 90.13 μg^{-1} FW, respectively. Compared with the SOD activity before sand burial, these treatments increased 203%, 175%, 191%, and

Table 1. Effects of sand burial on survival and growth of maize.

Sand burial treatments	Biomass (g/m^2)			Yield (g/m^2)	Survival rate (%)	Plant height (cm)
	Aboveground	Belowground	Belowground/ Aboveground			
Control	2210±31a	1137±33a	0.51±0.02a	1399±10a	89.58±5.51a	227.8±5.7ab
A	2268±35a	1263±65a	0.56±0.03a	1233±28b	91.67±4.17a	230.3±8.4a
B	1841±164a	819±49b	0.45±0.01a	736±21c	45.83±7.51b	217.3±2.8ab
C	1324±172b	666±27b	0.53±0.09a	564±82d	29.17±7.51bc	211±6.7b
D	676±186b	365±87c	0.55±0.03a	257±58e	12.50±3.61c	191.3±0.7c
F value	23.95	40.86	0.92	106.4	36.65	7.85
P	< 0.05	< 0.05	0.49	< 0.05	< 0.05	< 0.05

Values were assigned as mean ± SE. Mean values with different letters in a column are significantly different at $P < 0.05$ (one-way ANOVA and Fisher's LSD test). Control: no burial; A: burial to 1/4 of plant height; B: burial to 1/2 of plant height; C: burial to 3/4 of plant height; D: burial to 100% of plant height.

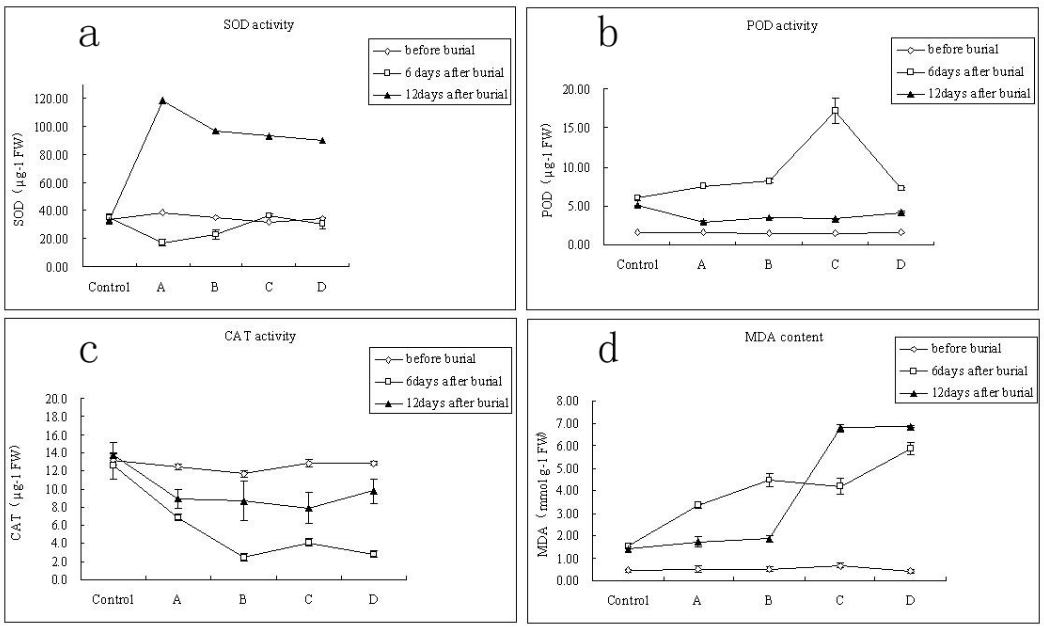

Fig 1. The physiological response of maize to sand burial stress. Values were assigned as mean ± SE (one-way ANOVA and Fisher's LSD test at $P < 0.05$). a: SOD activity; b: POD activity; c: CAT activity; d: MDA content. Control: no burial; A: burial to 1/4 of plant height; B: burial to 1/2 of plant height; C: burial to 3/4 of plant height; D: burial to 100% of plant height.

162%, respectively. The difference between Treatment A and the other three sand burial treatments is significant ($P < 0.05$), whereas the difference between the other three treatments is not significant ($P > 0.05$). The differences in the SOD activity between all sand burial treatments and the control are significant ($P < 0.05$). Changes of POD activity of maize after sand burial are depicted in Fig 1-b. We found that before sand burial, the POD activity of the control and Treatments A, B, C, and D are confined between the values of 1.42 and 1.59 μg^{-1} FW, and these differences are not significant ($P > 0.05$). Six days after sand burial, POD activity in the control and Treatments A, B, C, and D markedly increased ($P < 0.05$). The increase in the control group was lowest, and was 3.8 fold of what was measured before sand burial. The increase under Treatments C is the maximum, was the highest, and was 12 times of what was measured, reaching 17.19 μg^{-1} FW. The statistical analysis shows that the difference of POD activities between Treatments A and D and the control is not significant ($P > 0.05$). POD activity of maize under Treatment B is significantly higher than that in the control ($P < 0.05$), but its difference with Treatments A and D is not significant ($P > 0.05$). POD activity of maize under Treatment C is significantly higher than that in the control and the other treatments ($P < 0.05$). In the 6 to 12 days after sand burial, POD activity of maize under all sand burial treatments decreased significantly ($P < 0.05$). However, POD activity in the control plots essentially remained the same over this time period. Treatment C decreased the most, falling from 17.19 to 3.31 (80.7% decrease), whereas Treatment D had the lowest decrease, falling from 7.21 to 4.19 (41.9% decrease). Changes in CAT activity of maize after sand burial are depicted in Fig 1-c. We found that CAT activity in the control remained basically unchanged during the 12-day physiological experiment ($P > 0.05$). Six days after sand burial, the CAT activity measured under Treatments A, B, C, and D was lower than that before burial, with reductions of 44.8%, 78.6%, 68.2%, and 78.1%, respectively. The CAT activity under Treatment A was much higher than that under the other treatments, but the differences between the other three treatments are not significant ($P > 0.05$). Twelve days after sand burial, the CAT activity of all treatments began to increase. The CAT activity measured under Treatments A, B, C, and D were 8.9, 8.7, 7.9, and 9.8 μg^{-1} FW, respectively. However, these were still lower than the levels before sand burial and of the control. The difference in the CAT activity between all treatments is not significant ($P > 0.05$). We found that the CAT activity under Treatment D from 6 days to

Table 2. Changes in soil properties of experimental site in maize growing season.

	Depth	pH	Temperature (℃)	Water content (volumetric, %)
April	0 cm	8.18±0.27	12.9±1.3	4.2±2.0
	10 cm	8.33±0.34	5.6±1.1	16.5±5.3
	20 cm	8.26±0.36	6.4±0.9	20.5±1.9
May	0 cm	8.64±0.29	28.3±1.2	5.6±3.1
	10 cm	7.47±0.35	14.6±1.0	21.9±6.2
	20 cm	8.06±0.09	14.2±0.8	26.8±4.5
June	0 cm	8.31±0.13	33.2±1.4	4.5±1.2
	10 cm	8.45±0.12	21.2±1.4	16.2±2.6
	20 cm	8.29±0.08	20.8±1.8	20.7±2.7
July	0 cm	8.33±0.05	27.9±2.7	5.3±1.9
	10 cm	8.3±0.13	22.5±3.2	18.3±3.3
	20 cm	8.43±0.06	22.5±1.6	21.2±3.2
August	0 cm	8.7±0.06	33.3±2.3	5.5±3.8
	10 cm	8.69±0.17	23.4±3.1	16.5±2.1
	20 cm	8.51±0.11	23.7±1.4	21.6±2.0
September	0 cm	8.55±0.07	25.5±1.5	9.2±2.6
	10 cm	8.53±0.10	14.2±2.1	15.9±1.8
	20 cm	8.47±0.08	16.0±1.6	20.1±2.9

Values were assigned as mean ± SE. Measurements were made every 10 days during the growing season of maize.

12 days after sand burial achieved the highest increase (250%).

MDA content

Changes of the MDA content of maize after sand burial are depicted in Fig 1-d. We found that the difference in MDA content between all treatments, and the difference in MDA content between the treatments and the control, are not significant before sand burial (within 0.41 to 0.67 mmol g^{-1} FW). MDA content in the control and in all the treatments increased six days after the sand burial ($P < 0.05$). The increase in the control was the lowest (3.4 fold), whereas that under Treatment D was the highest (14.3 fold). The statistical analysis shows that the difference in the MDA content between all treatments and the control is significant ($P < 0.05$), whereas the difference between Treatments B and C is not significant ($P > 0.05$). MDA content in Treatment D is significantly higher than that of the other treatments ($P < 0.05$); whereas MDA content in Treatment A is significantly lower than in the other treatments ($P < 0.05$). Between 6 - 12 days after sand burial, MDA content in the control did not change markedly ($P > 0.05$). However, MDA content under Treatments A and B decreased significantly, while that under Treatments C and D increased significantly ($P < 0.05$). The greatest decrease was under Treatment B (58.2%), while the highest increase was under Treatment C (61.5%). Twelve days after sand burial, the difference in MDA content between the control and Treatments A and B is not significant ($P > 0.05$). The difference between Treatments C and D is not significant ($P > 0.05$), and MDA content under Treatments C and D is significantly higher than that of the control and under Treatments A and B ($P < 0.05$).

Discussion

Effects of sand burial on the growth of maize

Maize demonstrates similar trends in survival rate, plant height and biomass after undergoing sand burial stress: the difference between the shallow burial (burial to 1/4 of plant height) and the control is not significant ($P > 0.05$), and all indices decreased significantly with increased sand burial stress. Our study indicates that shallow sand burial in Horqin sandy land has no negative effects on the survival and growth of maize, nor does it have obvious beneficial effects. These results differ from those of previous studies that indicated that shallow sand burial can improve the growth rate of plants to a certain degree, and that it may be a survival strategy adopted by plants to avoid full sand burial (Van Der Putten 1993; Olson 1958). In his study on *Cakile edentula*, Wagner (1964) found that the growth rate of both flowers and seeds production is higher after shallow sand burial than in a no burial control. Shallow sand burial can be favorable to the growth of plants, probably because humidity and the nutrients of soil around the root increase and the temperature of soil decreases when plants are buried to some degree in sand (Shi et al., 2004; Brown 1997; and Liu 2008). Dry soil and high temperature are the key factors limiting the growth of plant (Niu et al., 2003). Our research indicates that an increase in sand burial depth has a serious negative impact on the growth of maize, reducing its survival rate and height. This finding is consistent with the results of a study on the relationship between accumulating sand and the growth and production of maize (Zhao 2006; Zhao 2007). Sykes and Wilson (1990) showed that only a few species are able to resist sand burial stress with more than 2/3 of plant height in their study of the resistance to sand burial stress of 29 sand plant species in New Zealand. The results of our study showed that the adaptability of maize to sand burial stress is limited, similar to other plants. Deep sand burial causes plants to die because of the direct mechanical obstruction to plant growth: plants have insufficient lift force (especially in the completely buried treatment) to grow continuously through and above the sand. In addition, sand burial inhibits the growth of plants or causes plant death by reducing the photosynthetic area of leaves and causing the roots of the plants to become hypoxic (Harris and Davy 1988; Maun 1994). Oxygen and photosynthesis are necessary for plant growth, and both are inhibited by sand burial (Kurz 1939; Shi et al., 2004). In our study, the specific value of aboveground to belowground biomass remained unchanged before and after the different sand burial treatments. The result is the same as found by Zhao (2006), which indicated that the difference between the specific value of the aboveground biomass and that of the belowground biomass of maize under light, middle, and serious sand burial treatments is not significant. However, Martínez and Moreno-Casasola (1996), found that five of six tropical plant species investigated showed increased above and belowground biomass after suffering sand burial stress, apparently to obtain more photosynthetic area. Sykes and Wilson (1990) proved that the root/shoot ratio of 4 in 29 plant species studied did not change after sand burial stress, while 19 plants showed an increase, and 6 others decreased. These results showed that different kinds of plants adopt different strategies in the distribution of biomass and other adaptable methods after being subjected to sand burial stress. In our study, the plant height and biomass of maize were not affected by shallow sand burial (burial to 1/4 of plant height), but the yield of the maize under shallow sand burial depth is significantly lower than that in the control ($P < 0.05$). A possible reason is that after being subjected to sand burial, maize uses the substance and energy which would have otherwise allotted to reproductive growth to accelerate its growth in order to survive the stress of sand burial. As described by Maun (1998), after suffering from environmental stress, plants can produce more substances and energy to distribute to the organs necessary to their survival by adjusting the distribution of substances and energy in their systems (Maun 1998).

Physiological response of maize to sand burial

When plants suffer from stress, ROS in their systems accumulates to a certain degree, resulting in metabolic disorder and cell membrane lipid peroxidation which leads to oxidative stress (Sheokand et al., 2008). MDA is the first product in membrane lipid peroxidation. Therefore, the measurement of its content is applied widely to judge the degree of injury of plant cells (Demiral and Turkan 2005). In our study, MDA contents of maize in different burial depths on the sixth day following burial were significantly increased compared with those of the control, indicating that sand burial causes injury to plants. Under deep sand burial (Treatment D), the increase of MDA content of maize is highest, which shows that maize under Treatment D suffered the most serious injury. This finding coincides with the result on the survival rate of maize under Treatment D, in which a number of maize plants died. With prolonged sand burial stress, MDA content of maize under Treatments C and D increased continuously up to the 12th day, whereas that under Treatments A and B it decreased significantly. This suggests that maize under Treatments A and B, the plants received better protection to avoid cell damage. This is probably because sand burial depth under Treatments A and B was very shallow, hence, the stress was mild and the maize plants generated a certain adaptable mechanism (e.g., stimulate antioxidant enzyme activity or osmotic substances) 12 days after sand burial. This may explain why Treatment A had no negative effects on the survival and growth of maize. However, the degree of stress under Treatments C and D was very serious and beyond the range tolerable by the maize plants, causing increased mortality, inhibited growth, and reduced the yield of maize. SOD can serve as a catalyst in the disproportionation of O^{2-} and convert it into H_2O_2 and O_2 (McCord and Fridovich 1969; Monk et al., 1989; Zhang et al., 2004). SOD is the first defense material to resist membrane lipid peroxidation caused by ROS. Theoretically, higher SOD activity can reflect a lower degree of membrane lipid peroxidation with more limited damage to plants (Mittler 2002). In our study, SOD activity in the control remained basically unchanged. SOD activities under all treatments did not increase significantly until twelve days after sand burial; Treatment A showed the highest increase. This suggests SOD is a very effective antioxidant enzyme for maize. After suffering from sand burial stress, maize increased its SOD activity as a mechanism for adapting to sand burial. The highest SOD activity, noted in Treatment A, suggests that this mechanism plays an important role under shallow sand burial. This phenomenon may explain why MDA content of maize is maintained at a lower level under Treatment A and why no influence on the growth is observed. Perhaps SOD activity of maize under all treatments did not increase six days after sand burial because SOD activity in maize needs a longer amount of time to respond to environmental stress. POD can protect plants from injury by catalyzing H_2O_2 and ROOH· into H_2O and R-OH (Liu et al., 2010). In our study, the performance of POD differs from that of SOD. POD activity increased six days after sand burial, and its response time to stress was less than that of SOD. However, the difference between Treatment A and D and the control are not significant ($P > 0.05$). This is probably because the sand burial stress under Treatment A is not serious, and thus POD activity was not triggered, whereas the stress under Treatment D is excessively high, causing POD inactivation. The increase of POD activity under Treatment C was the highest among all treatments, which may be attributed to this depth of sand burial (3/4 of the plant height) stimulating POD activity very well, in order to induce resistance to stress. Twelve days after sand burial, POD activity decreased under all treatments, indicating that the resistance of maize to stress through POD activity is limited. POD activity cannot play a highly protective role when the stress is very serious or when the duration of stress is prolonged. When plants suffer from environmental stress, the H_2O_2 in their systems changes into HO, leading to the destruction of the electron transfer chain in the mitochondrion and chloroplasts (Jiménez et al., 1997; Meneguzzo et al., 1998), membrane lipid peroxidation (Quartacci et al., 1995), protein inactivity (Baccio et al., 2004), DNA damage (Conte et al., 1996), and other consequences. CAT in higher plants can repair these injuries by inhibiting H_2O_2 (Navari-Izzo et al., 1996). In our study, CAT activity in maize decreased significantly six days after sand burial ($P < 0.05$). CAT activity under Treatment A was significantly higher than those under other treatments ($P < 0.05$). CAT activity after sand burial was always lower than in the control probably because maize cannot stimulate CAT activity to resist the stress resulting from sand burial. However, 12 days after sand burial, CAT activity under all treatments increased, and the increase was highest under Treatment D (100% burial of plant height). However, CAT activities under all treatments were still lower than that of the control ($P < 0.05$). This finding may be explained by the short test time, which consequently did not allow sufficient time within which CAT activity in maize could respond to sand burial stress. Further investigation is necessary to determine the role of CAT in maize. Higher plants have a wide range of defenses to adapt to all kinds of environmental stresses (Yu and Tang 2004; Chaves et al., 2003). As important components of the antioxidant enzyme system of a plant, SOD, POD, and CAT are often used to indicate the capacity of plants to resist environmental stress (Saba et al., 2001; Dhanda et al., 2004). When plants suffer from stress, they can improve resistance to oxidation by increasing enzyme activity, thus preventing the formation of ROS (Liu et al., 2010). Other studies found that not all kinds of enzymes provide protective action after plants suffer from stress. Different plants and different stresses cause a certain difference (Gao et al., 2008). Our study proved that after sand burial, SOD played an active role in protecting maize from stress, POD has a limited effect, and further investigation is required to determine the role of CAT.

Materials and methods

Study area

The study area was located in Naiman county (42°55′ N, 120°42′ E; altitude approx. 360 m) in the South-Western part of

Horqin sandy land, Inner Mongolia, China. The climate is temperate, semi-arid continental and monsoonal, receiving annual 360 mm in precipitation, with 75% of the precipitation in the growing season of June to September. The mean annual temperature is 6.4℃, and the mean annual pan-evaporation is 1935 mm. The mean annual wind velocity ranges from 3.2-4.1 m s-1, and 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 arrives. The distribution pattern of mainly natural vegetation is characterized by a mosaic of lowland grassland, fixed dune, semi-fixed and mobile dune. Most of the cropland is in the lowland area. Thickness of the soil layer in the cropland is about 30-45 cm, and the soil consists mainly of coarse sand and silt. Changes in some soil properties (soil pH, soil temperature and soil water content) in Horqin sandy cropland (maize) as affected by sand burial are provided in Table 2. Maize (*Zea mays* cv. Zhengdan958) monoculture dominates the cultivated land, accounting for 70% of the total farm fields (Zhang et al., 1999; Zhao et al., 2006).

Experimental design

The experiment was performed during the entire maize growing period of 2011 (end of April to mid-September). The sand was added in the middle of May, which is the period with the strongest wind and sand activities in Horqin sandy land and the period at which maize was in the seedling stage with little resistance to the outside environment. At the end of April, maize seeds were sown in a 2 m × 2 m × 2 m cement plot filled with farmland soil. After the seedlings emerged, thinning was performed. Following local farming habits, 16 maize seedlings with similar growth were kept and marked in each plot. About 20 days after thinning, sand burial treatments were applied. All sand used in the burial treatments was from local mobile dunes. The treatments were divided into a control (no burial), A (burial to 1/4 of plant height), B (burial to 1/2 of plant height), C (burial to 3/4 of plant height), and D (burial to 100% of plant height). A completely randomized design (CRD) was used to conduct this experiment. Each treatment consisted of three replicates, and there were 15 plots in total. Six and twelve days after sand burial, the surviving leaves were selected to measure the physiological indices (superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and malondialdehyde (MDA)). In mid-September, the survival rates, plant height, yield, as well as above and belowground biomass of maize were measured. During the experimental period, water and fertilizer were strictly done according to local farming methods (Li et al., 2003). Any unmarked seedlings that emerged were removed during the experimental period.

Analytical methods

Soil properties

Soil pH was determined with a pH meter (Multiline F/SET-3, Germany) in 1:1 soil-water slurry. Soil temperature (at depths of 0, 10, 20 cm) and volumetric soil water content (at depths of 0, 10, 20 cm) were measured every 10 days during the growing season with geothermometers (HH82, Exphil Calibration Labs, Bohemia, NY, USA) and hygrometers (TRIME-FM, IMKO, GmbH, Ettlingen, Germany).

Survival and growth

The ratio of the number of living plants at harvest time to that before sand burial was obtained to determine the survival rate. The heights of the plants were measured by ruler at harvest time. All living plants were dug out at harvest time to determine the biomass, and the above and belowground parts were measured after they were dried to constant weight. The yield was calculated based on seed weight of one square meter.

Physiological indices

For physiological indices, only seedlings that survived the burial treatment were measured. Enzymes were extracted from 1 g of seedling leaves 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 minutes and supernatants were used for protein content determination, or otherwise stored at 4℃ for further analyses. SOD activity was determined with the method of Beauchamp (1971). POD activity was determined spectrophotometrically by measuring the oxidation of methyl catechol at 470 nm (Srivastava et al., 1973). CAT activity was measured spectrophotometrically using the method of Patra et al. (1978). MDA content was measured using the method described by Hernandez et al. (2002).

Statistical analysis

Data were analyzed and described with Microsoft Excel and Origin 8.0 software. Values were presented as mean ± SE, and significant differences between mean values were analyzed by one-way analysis of variance (ANOVA) procedures. Fisher's least significant difference ($P < 0.05$, LSD test) was performed to determine the significance of different treatments.

Conclusions

Both hypothesis 1 and hypothesis 2 were partially proven. Shallow sand burial (i.e. Treatment A) had no negative effects on survival rate, height, biomass, but also did not promote growth either. Deep sand burial had negative effects on these indices. The response of three antioxidant enzymes was not consistent. After sand burial, SOD activity of maize increased significantly, the increase under shallow sand burial is higher than that under deep sand burial. POD activity also increased to a certain degree. However, CAT activity is still lower than the control at 12 days after sand burial. On the one hand, MDA content is kept at a lower level under shallow sand burial, which shows that maize receives protection from the antioxidant enzyme system; hence, the injury is slight. On the other hand, MDA content is significantly higher under deep sand burial than in the control, indicating serious cell injury. Plants and crops in the Horqin sandy land, an area with strong wind and sand activity, inevitably suffer from sand burial. Understanding the impact of sand burial on maize in particular is important, as this crop accounts for 70% of the total cultivated area in Horqin sandy land. Our study demonstrates that shallow sand burial (Treatment A) has no negative effects on the growth of maize. However, yield decreased significantly even under shallow burial. Therefore, we recommend that those farming under these conditions consider the use of protection practices in this area. The construction of sand barriers, such as fences and grass grids, can help avoid sand burial of maize plants. This is particularly important in the season which has the strongest wind and sand activity and corresponds to the seedling stage of maize phenology (and thus limited resistance) in Horqin sandy land. In the longer term, the construction of windbreak forests is the major measure that could contribute to the protection of crops from sand burial stress, something that has the potential to increase yields, and correspondingly the income of farmers.

Acknowledgments

We thank all members of Naiman Desertification Research Station, Chinese Academy of Sciences (CAS), for their help in field and laboratory work. Thanks for Amy Kochsiek from School of Biological Sciences, University of Nebraska-Lincoln, USA to do the language revision. This research was funded by one of Chinese National Fund Projects (30972422) and one of the Chinese National Key Projects for Basic Scientific Research (2009CB421303) and one of the Chinese National Support Projects of Science and Technology (2011BAC07B02-06).

References

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

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

Brown JF (1997) Effects of experimental burial on survival, growth and resource allocation of three species of dune plants. J Ecol 85: 151-158

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

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

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

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.

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

Kent M, Owen NW, Dale MP, Newnham RM, Giles TM (2001) Studies of vegetation burial: a focus for biogeography and biogeomorphology? Progress in Geography 25(4):455-482

Kent M, Owen NW, Dale MP (2005) Photosynthetic Responses of Plant Communities to Sand Burial on the Machair Dune Systems of the Outer Hebrides, Scotland. Ann Bot-London 95: 869-877

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 of the Florida Academy of Science 4: 195-203

Li YL, Cui JY, Zhang TH (2003) Measurement of evapotranspiration of irrigated spring wheat and maize in a semi-arid region of north China. Agr water manage 61: 1-12

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

Martínez ML, Moreno-Casasola P (1996) Effects of burial by sand on seedling growth and survival in six tropical sand dune species from the Gulf of Mexico. J Coastal Res 12: 406-419

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

MaunMA (1998) Adaptations of plants to burial in coastal sand dunes. Can J Bot 76: 713-738

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

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 Transact 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

Pan Y, Wu LJ, Yu ZL (2006) Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis Fisch*). Plant Growth Regul 49: 157-165

Pan YJ, Wang WS, Zhao XQ, Zhu LH, Fu BY, Li ZK (2011) DNA methylation alterations of rice in response to cold stress. Plant Omics J 4: 364-369.

Patra HL, Kar M, Mishra D (1978) Catalase activity in leaves and cotyledons during plant development and senescence. Biochem Physiol Pflanzenphysiol 172: 385-390

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

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

Sheokand S, Kumari A, Sawhney V (2008) Effect of nitric oxide and putrescine on antioxidative responses under NaCl stress in chickpea plants. Physiol Mol Biol Plant 14: 355-362

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.

Sykes MT, Wilson JB (1990) An experimental investigation into response of New Zealand sand dune species to different depths of burial by sand. Acta Bot Neerl 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 Surfaces B 49: 60-65.

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

Wagner RH (1964) The ecology of Uniola paniculata in the dune-strand habitat of North Carolina. Ecol Monogr 34: 79-96

Xu ZZ, Zhou GS (2006) Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. Planta 224: 1080-1090

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 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

Zhang JH (1996) Interactive effects of soil nutrients, moisture and sand burial on the development, physiology, biomass and fitness of *Cakile edentula.* Ann Bot-London 78: 591-598.

Zhang TH, Zhao HL, Zhao XY, Zhao AF (1999) Study on Water Consumption of Spring Maize in Horqin Sandy Land. Journal of Desert Research 19: 137-139 (in Chinese)

Zhao HL, Zhou RL, Zhang TH, Zhao XY (2006) Effects of desertification on soil and crop growth properties in Horqin sandy cropland of Inner Mongolia, north China. Soil Till Res 87: 175-185

Zhao HL, Zhou RL, Sam Drake (2007) Effects of aeolian deposition on soil properties and crop growth in sandy soils of northern China. Geoderma 142: 342–348

Zhao HL, Okuro T, Li YL, Zuo XA, Huang G, Zhou RL (2008) Effects of human activities and climate changes on plant diversity in Horqin sandy grassland, Inner Mongolia. Acta Prataculturae Sinica 17: 1-8 (in Chinese)

Zhao XY, Zhang CM, Zuo XA, Huang G, Huang YX, Luo YY, Wang SK, Qu H (2009) Challenge to the desertification reversion in Horqin Sandy Land. Chinese Journal of Applied Ecology 20: 1559-1564 (in Chinese)