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# Growth and physiological responses of *Agriophyllum squarrosum* to sand burial stress

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**Abstract:** *Agriophyllum squarrosum* is an annual desert plant widely distributed on mobile and semi-mobile dunes in all the sandy deserts of China. We studied the growth and physiological properties of *A. squarrosum* seedlings under different sand burial depths in 2010 and 2011 at Horqin Sandy Land, Inner Mongolia to understand the ability and mechanism that *A. squarrosum* withstands sand burial. The results showed that *A. squarrosum* had a strong ability to withstand sand burial. Its survival rate, plant height and biomass increased significantly at a burial depth 25% of seedling height and decreased significantly only when the burial depth exceeded the height of the seedlings; some plants still survived even if the burial depth reached 266% of a seedling height. The malondialdehyde (MDA) content and membrane permeability of the plant did not change significantly as long as the burial depth was not greater than the seedling height; lipid peroxidation increased and cell membranes were damaged if the burial depth was increased further. When subjected to sand burial stress, superoxide dismutase (SOD) and peroxidase (POD) activities and free proline content increased in the seedlings, while the catalase (CAT) activity and soluble sugar content decreased. Sand burial did not lead to water stress. Reductions in photosynthetic area and cell membrane damage caused by sand burial may be the major mechanisms increasing mortality and inhibiting growth of the seedling. But the increases in SOD and POD activities and proline content must play a certain role in reducing sand burial damage.

**Keywords:** *Agriophyllum squarrosum*; psammophyte; sand burial stress; growth inhibition; physiological response

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Sand movement by wind is a common event in coastal and inland dunes as well as desertification areas around the world (Danin, 1996; Maun, 1996). As a result, plants growing in these areas may experience various degrees of sand burial, including partial or complete burial (Maun, 1998; Liu et al., 2008). Thus, the stress caused by frequent and unpredictable sand burial may be a major limiting factor in plant survival, growth, development and distribution in these areas (Maun, 1994, 1996; Brown, 1997). The ability of plant seedlings to withstand burial is crucial for the successful establishment and population maintenance of dune species (Brown, 1997; Zhao et al., 2007; Chen et al., 2010).

Numerous field and greenhouse studies have demonstrated that sand burial may affect seed germination and emergence, seedling survival and growth, plant biomass and reproduction (Maun, 1994, 1996; Chen and Maun, 1999; Zhao et al., 2007; Zhao, 2012). It may also modify the physiology and morphology of plants (Disraeli, 1984; D'Hertefeldt and Putten, 1998), because sand burial can change both abiotic and biotic conditions such as photosynthetically active radiation, soil temperature, moisture, organic matter, rhizosphere oxygen content and the activity of soil microorganisms (D'Hertefeldt and Putten, 1998; Maun, 1998; Zhao et al., 2007; Zhao, 2012). Studies have suggested that the effects of sand burial on plants may change with the depth of burial (Maun, 1994; Brown, 1997;

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Liu et al., 2008). Relatively shallow and short periods of sand burial may enhance plant growth, total biomass and/or biomass allocation (Maun, 1996, 1998; Zhao et al., 2010). However, deep and long-term sand burial is likely to impose severe stress on plant growth and result in increased mortality and growth inhibition (Zhao et al., 2007; Zhao et al., 2010; Zhao, 2012). However, so far, mechanisms of physiological adaption by plants to sand burial are not clear, because few studies have tested physical changes in plants affected by sand burial (Wang et al., 2012).

*Agriophyllum squarrosum* is an annual psammophyte, naturally distributed in all the sandy deserts of China, and widely used in restoration and reconstruction of vegetation because it is very good at adapting to the dune environment (Zhao, 2012). The objectives of this study are: 1) to examine changes in survival rate, plant height, biomass, osmotic regulation substances, protective enzyme activities and membrane permeability of *A. squarrosum* at different sand burial depths; 2) and to analyze the ability and mechanism by which *A. squarrosum* as a psammophyte withstands sand burial.

## 1 Materials and methods

### 1.1 Study area

The study area was located in Naiman county (42°15′N, 120°42′E; 345 m asl), in the eastern part of Inner Mongolia, China. Naiman is located in the hinterland of the Horqin Sandy Land, which is under a continental semiarid monsoon climate in the temperate zone. Mean annual precipitation is 364 mm, mean annual potential evaporation is 1,920 mm, and annual mean temperature is 6.3°C. Annual frost-free period is approximately 141 days. Annual average wind speed is 3.4–4.5 m/s and the mean wind speed in the wind erosion season (spring) is 5.0–6.0 m/s. The landscape in this region is characterized by the alternation between dunes and lowland areas. Most of the lowland areas are reclaimed by cropland and the dunes are used as pasture. The soils are identified as degraded sandy Chestnut soils according to the Chinese soil classification system, which are equivalent to Orthi-sandic Entisols in the FAO-UNESCO system. The sandy soil mainly consists of coarse sand and silt. The natural vegetation mainly consists of *Artemisia halodendron*, *Caragana microphylla*, *Lespedeza davurica*, *Agriophyllum squarrosum*, *Setaria viridis* and *Corispermum marocarpum* (Zhao et al., 2010).

### 1.2 Experiment design

A field experiment was conducted from April to August in 2010. The experiment was set in a water balance field of the Naiman Desertification Research Station (NDRS), Chinese Ecosystem Research Network, which consisted of a number of 2 m×2 m×2 m bottomless cement pools filled with sandy soil. Seeds of *A. squarrosum* were collected in August 2009 at sand dune areas near NDRS. The seeds were sown in the pools in rows 30 cm apart in April 2010. After germination, we thinned out the seedlings. Three hundred seedlings having similar growth were left and marked in each cement pool. Seedlings were kept vertical after thinning, and the average height of the seedlings was 6.0±0.2 cm when they were buried with sand in mid-May. There were ten burial treatments: buried to 0 (CK, no burial), 25% (A), 50% (B), 75% (C), 100% (D), 133% (E), 166% (F), 200% (G), 233% (H) and 266% (I) of seedling height. Every treatment consisted of four replicates and there were 40 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.

### 1.3 Data collection and analysis

After 10 days of sand burial, leaves from 10 live seedlings were sampled randomly in each treatment to measure enzyme activities (SOD, POD and CAT), lipid peroxidation (measured in terms of MDA content) and membrane permeability, and contents of osmotic substances (soluble sugar and free proline) (Bao, 2000). The survival rate, plant height and above-ground biomass of all seedlings in every treatment were measured in August 2010.

All data were analysed using program SPSS version 11.5 for Windows. Multiple-comparison and one-way analysis of variance (ANOVA) procedures were used to compare differences among the treatments. Least significant difference (LSD) tests were performed to determine pairwise significant differences among treatment means at $P<0.05$. Pearson correlation coefficient was used to evaluate relationships between the corresponding variables.

## 2 Results

### 2.1 Changes in seedling survival rate, height and biomass

The survival rate, height and biomass of the seedlings increased significantly in treatment A compared with that in CK ($P<0.05$) (Fig. 1). Except under the treatment A, the survival rate and the plant height decreased with the increase of sand burial depth. In comparison with the treatment of CK, there was no significant difference in survival rate and plant height under treatments B to F ($P<0.05$). However, the biomass was significantly high in treatment C, extremely low in treatments B, F, G and I ($P<0.05$) and no difference in treatments D, E and H ($P>0.05$).

### 2.2 Changes in RWC, MDA and membrane permeability

In comparison with that in CK, the relative water content (RWC) of the leaves did not change significantly in treatments A to C and I ($P>0.05$). But it was significantly high in treatments D to H ($P<0.05$) (Fig. 2a). MDA contents in treatments A to E had no significant change compared with that in CK, but increased significantly in treatments F to I ($P<0.05$). Membrane permeability increased gradually with the increase of sand burial depth, and was significantly high in all the treatments compared to that in CK ($P<0.05$), except in treatments A and B (Fig. 2b).

### 2.3 Changes in SOD, CAT and POD activities

Superoxide dismutase (SOD) activity first increased and then decreased with the increase of sand burial depth. It achieved a maximal value in treatment D and was significantly high ($P<0.05$) in all the treatments compared to that in CK except in treatments A and I ($P<0.05$) (Fig. 3a). Peroxidase (POD) activity first decreased and then increased with the increase of sand burial depth. The minimum value appeared in treatment B and the maximum value appeared in treatment E. But there was no significant change in treatments A to D in comparison with that in CK. However, it was higher in treatments E to I than that in CK ($P<0.05$) (Fig. 3a). Catalase (CAT) activity decreased with the increase of sand burial depth, and was significantly lower in all the burial treatments than that in CK ($P<0.05$) (Fig. 3b).

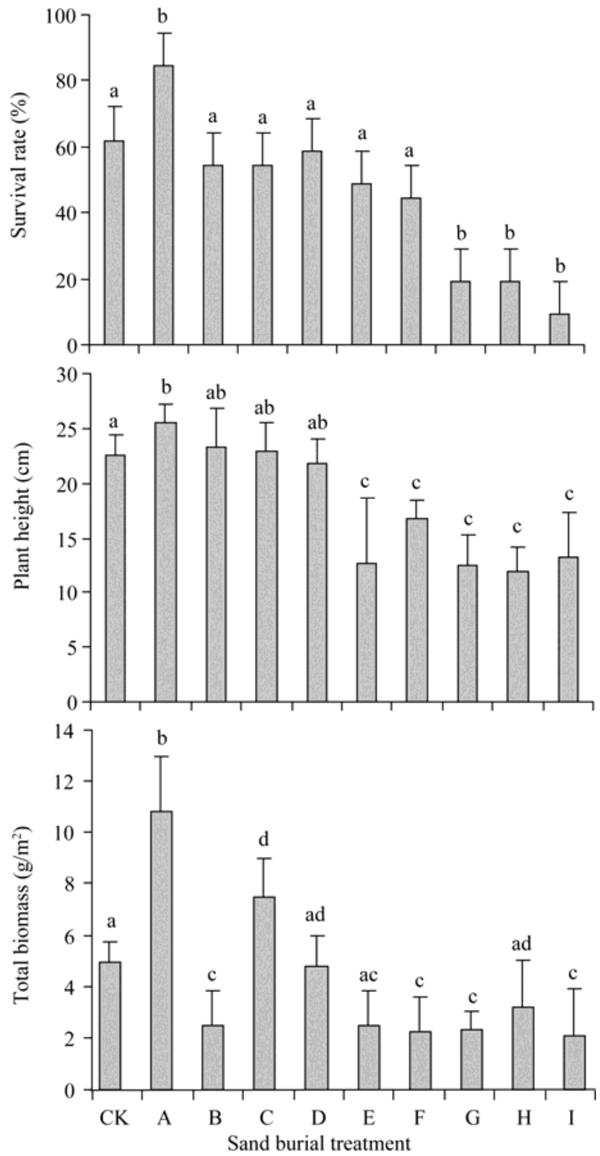


**Fig. 1** Effects of sand burial on seedlings survival rate, plant height and aboveground biomass. CK, no burial; A–I indicate sand burial treatments with 25%, 50%, 75%, 100%, 133%, 166%, 200%, 233% and 266% of seedling height. The symbols are the same as in Figs. 2–4. Bars represent mean±SD. Different lowercase letters indicate significant difference among treatments at $P<0.05$ level.

### 2.4 Changes in free proline and soluble sugar contents

The free proline content increased slowly in treatments A to C and then decreased slowly in treatments E to I after increased sharply in treatment D (Fig. 4).

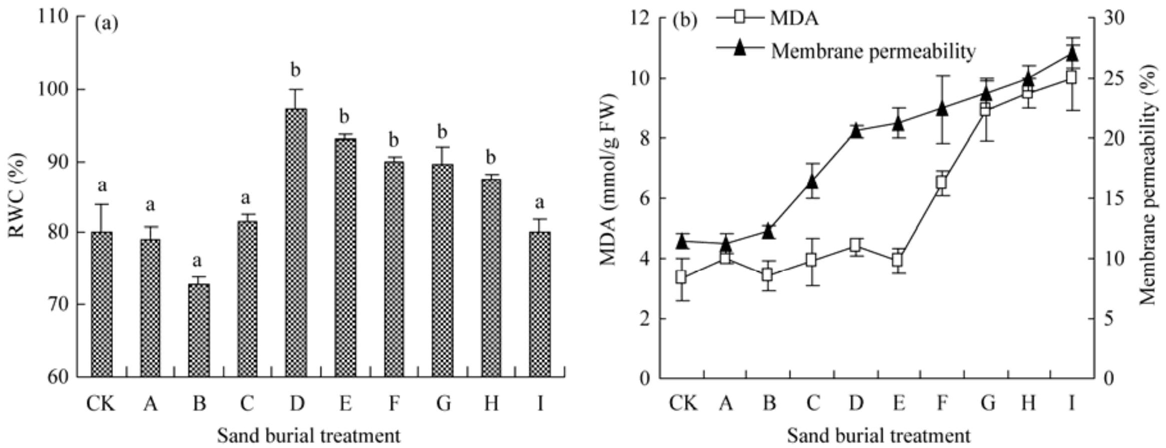


**Fig. 2** Effects of sand burial on RWC (a), and MDA and membrane permeability (b). Bars represent mean±SD. Different lowercase letters indicate significant difference among treatments at *P*<0.05 level.

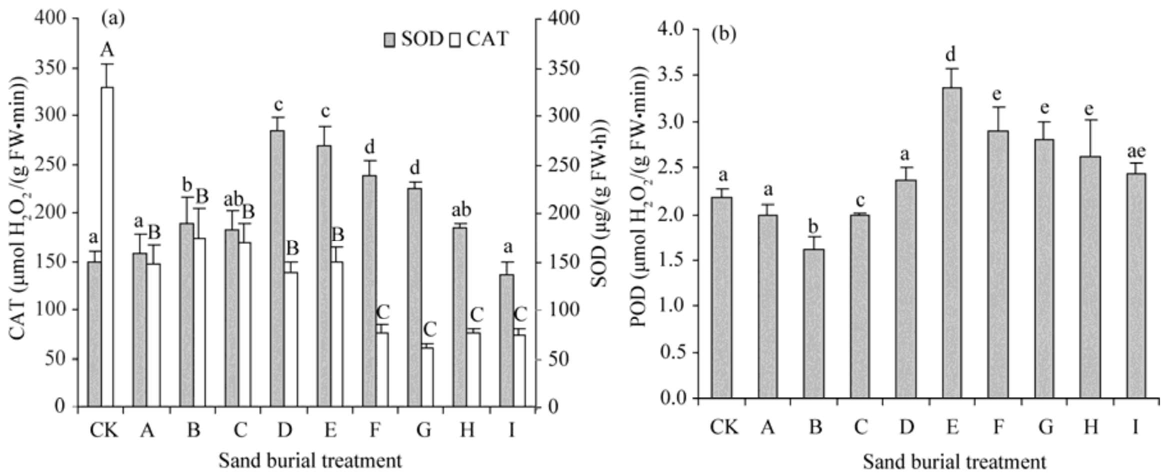


**Fig. 3** Effects of sand burial on the activities of SOD, CAT (a) and POD (b). Bars represent mean±SD. Different little and upper case letters indicate significant difference among treatments at *P*<0.05 level.

However, it was significantly higher in all treatments than that in CK ($P<0.05$). Although the soluble sugar content showed a similar pattern of changes as the free proline content, the soluble sugar content in all the treatments was equal to or less than that in CK, except in the treatments D to F.

### 2.5 Correlation analysis

The results from the correlation analysis showed there were significant positive correlations between plant height, aboveground biomass and survival rate ($P<0.05$) (Table 1), and significant negative correlations between survival rate, plant height, MDA and membrane permeability ($P<0.05$). There was no negative correlation between survival rate, plant height and RWC. MDA had a positive correlation with free proline content as well as SOD and POD activities, but had a weak negative correlation with soluble sugar and CAT ($P<0.05$). Membrane permeability had a negative correlation with CAT and positive correlations with free proline, soluble sugar, SOD, POD and MDA. But the correlations were insignificant except for free proline.

## 3 Discussion

### 3.1 The ability and mechanism by which *A. squarrosum* withstands sand burial

Sand burial is a common event faced by desert plants, and can affect the survival and growth of a plant because it changes the plant's surrounding environmental conditions, such as soil temperature, humidity, hardness and light transmission rate etc., but there are

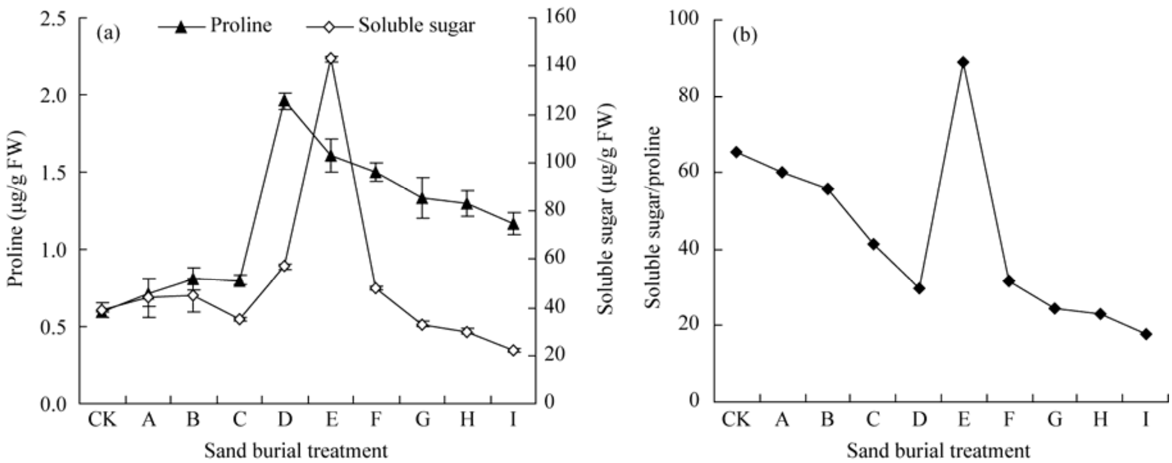


**Fig. 4** Effects of sand burial on soluble sugar and proline contents (a) and soluble sugar/proline (b). Bars represent mean±SD.

**Table 1** Correlation analysis between plant growth properties and physiological indices

| Item | Survival | Height | Biomass | Proline | Sugar | SOD | CAT | POD | MDA |
|---|---|---|---|---|---|---|---|---|---|
| Height | 0.861** | 1.000 | | | | | | | |
| Biomass | 0.736* | 0.720* | | | | | | | |
| MDA | –0.897** | –0.777** | –0.476 | 0.285 | –0.388 | 0.171 | –0.748* | 0.372 | 1.000 |
| MP | –0.862** | –0.880** | –0.626 | 0.687* | 0.011 | 0.282 | –0.781* | 0.674* | 0.842** |
| RWC | –0.175 | –0.457 | –0.262 | 0.902** | 0.457 | 0.841** | –0.365 | 0.762* | 0.175 |

Note: * and ** indicate significant difference at $P<0.05$ and $P<0.01$ levels, respectively; MP, membrane permeability.

great differences in the abilities of different plants to withstand sand burial (Maun, 1996; Liu et al., 2008; Wang et al., 2012). For example, when the sand burial was 33% of plant height, the survival rates of *Bromus inermis* and *Caragana microphylla* seedlings were 100%; plant height and biomass increased significantly compared to that without sand burial. But once the burial depth reached 100% of seedling height, *B. inermis* seedlings all died while *C. microphylla* seedlings were still 27.5% alive. *C. microphylla* seedlings died when the depth of the sand reached 133% of seedling height (Yang et al., 2007; Zhao et al., 2010). In this study, the survival rate, plant height and biomass of *A. squarrosum* seedlings increased significantly when they were buried to a depth of 25% of a seedling height. Not only did the relative survival rate remain as high as 94.8%, but also the plant height and biomass were still not significantly low compared with that in CK when the plants were buried to a depth of 100% of a seedling height. The survival rate, plant height and biomass kept at 14.8%, 58.7% and 42.9% of that in CK when sand burial was up to 266% of a seedling height. These results suggested that *A. squarrosum* had a very strong ability to tolerate sand burial compared with non-psammophytes of *B. inermis* and *C. microphylla* (Bao, 2000; Zhao et al., 2007; Zhao et al., 2010); Tolerable sand burial depth exceeded 266% of the seedling height. *A. squarrosum* also has a strong ability to adapt to light and moderate sand burial. Its survival and growth abilities remain unaffected as long as the burial depth not exceeding 100% of the seedling height. Light sand burial actually stimulated the survival and growth of *A. squarrosum* seedlings. As an annual psammophyte, *A. squarrosum* is one of the earliest species to invade mobile sand dunes in desert vegetation succession and has developed special properties to withstand drought, barren and sand burial due to long-term adaptation in a windy environment (Zhou and Wang, 1999; Zhao, 2012). There is sufficient energy reserved in seeds for the seedling. However, when the burial depth exceeded the height of the seedlings, more and more energy are required for breaking through the soil with the increase of sand burial depth. Beyond a point, it was often impossible for the seedlings to break from the ground when they were buried under thick sand, resulting in increased seedling mortality (Yang et al., 2007; Wang et al., 2012). On the other hand, even when their original energy reserves allowed them to emerge, seedling growth would still be inhibited because deep burial can create a physical barrier to vertical growth, reducing a plant's photosynthetic area and limiting oxygen availability to the roots (Maun, 1994; Wang et al., 2012; Zhao, 2012).

### 3.2 Physical response of the seedlings to sand burial stress

Previous studies suggested that when subjected to drought, cold or high temperature stress, the balance between generating and clearing reactive oxygen in plant cells could be destroyed, causing overproduction of reactive oxygen and lipid peroxidation, and resulting in a great accumulation of MDA (Zhou and Wang, 1999; Zhou et al., 2004; Bai et al., 2006). This increased membrane lipid peroxidation is one of the most important contributors to cell membrane oxidative damage and cell death (Demiral and Turkan, 2005; Chen et al., 2013). In the present study, when the burial depth was less than the height of the plants, changes in MDA contents and membrane permeability were not significant and the survival rate, plant height and biomass of the seedlings were not significantly different compared to CK. Once the burial depth reached 133% or 166% of a plant height, MDA contents and membrane permeability increased significantly and the plant mortality increased and growth was inhibited. The result from the correlation analysis showed that there was a significant negative correlation between survival rate, plant height, MDA and membrane permeability. This was consistent with the studies by Zhou and Wang (1999) on the growth and physiological responses of *A. squarrosum* seedlings to drought and high temperature stress. The results suggested that light or moderate degrees of sand burial had no significant influence on the survival rate, growth and biomass of the seedlings because cell membranes were not damaged by sand burial stress (Zhou and Wang, 1996; Bai et al., 2006; Zhao, 2012). The accumulation of MDA were sufficient to cause cell membrane damage under the extreme conditions when sand burial is a major physiological mechanism, which resulted in increased mortality and growth inhibition (Gao et al., 2008; Wang et al., 2012).

Many studies suggested that plants have evolved special enzymatic mechanisms under a long-time environmental stress, including SOD, POD and CAT, which play important roles in scavenging harmful oxygen species (Zhou et al., 2004; Demiral and Turkan, 2005). The activities of these antioxidant enzymes were altered when plants were subjected to the stress (Hernandez and Almansa, 2002; Gao et al., 2008). The present results showed significant differences in the changes in activities of the three enzymes with the increase of sand burial depth. Changes in SOD and POD activities showed similar tendencies, both of which increased first and then decreased. They were still significantly higher in the sand burial treatments than in CK even at a burial depth of 233% of a plant height while CAT activity showed a decreasing trend with the increase of the burial depth. The results of the correlation analysis indicated that MDA and membrane permeability had positive correlations with SOD and POD and a negative correlation with CAT. These results suggested that SOD and POD, as major antioxidant enzymes, together play an important role in inhibiting membrane lipid peroxidation, while CAT did not play a role (Zhou et al., 2004).

When subject to drought or salt stress, plants usually maintain turgor and growth of cells by increasing cell solute content and reducing cytoplasmic osmotic potential to prevent excessive loss of moisture (Delauney and Verma, 1993; Wang et al., 2012; Qayyum et al., 2013). However, the present results showed that sand burial stress did not cause water stress in the plant cells. At burial depths from 25% to 75% of seedling heights, RWC showed no significant difference compared to CK, while RWC was significantly higher compared to CK when the burial depth was over 100% of seedling heights. This was the main reason that sand burial could promote a moister rhizosphere and satisfy the needs of the plant to water (Yang et al., 2007; Wang et al., 2012). However, the content of osmoregulatory substances still showed significant changes with the increase of sand burial depth. Free proline content increased, soluble sugar fluctuated and decreased, and the soluble sugar/free proline ratio was significantly reduced (Fig. 4b). Free proline content had a significant positive correlation with membrane permeability. These results suggested that when subjected to sand burial stress, proline played a positive role as an osmoregulatory substance in increasing leaf water content, decreasing the osmotic potential of the cells and preventing leakage of cytoplasm (Delauney and Verma, 1993; Qayyum et al., 2013).

## 4 Conclusion

We concluded that *A. squarrosum* has a strong ability to withstand sand burial compared to *B. inermis* and its survival rate, plant height and biomass decreased significantly only when burial depth exceeded 133% of seedling height. Changes in MDA and membrane permeability were not significant until the burial depth exceeded 133% of seedling height. With the increase of sand burial depth, SOD and POD activities as well as free proline content increased, which played an important role in inhibiting membrane lipid peroxidation and reducing cytoplasmic osmotic potential. Decreases in photosynthetic area and cell membrane damage were the major mechanisms inhibiting seedling growth and increasing mortality under the severe stress of sand burial.

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