Acta Geochim (2017) 36(2):205–212
DOI 10.1007/s11631-017-0140-z

ORIGINAL ARTICLE

Responses of antioxidant defense system of epilithic mosses to drought stress in karst rock desertified areas

Xianqiang Zhang[1,2] · Yuzhong Zhao[3] · Shijie Wang[2]

Received: 12 October 2016 / Revised: 22 December 2016 / Accepted: 9 January 2017 / Published online: 17 February 2017

Abstract *Barbula fallax* Hedw., *Erythrodontium julaceum* (Schwaegr.) Par., and *Bryum argenteum* Hedw. are typical rock mosses growing on rocks in different terrestrial habitats. In this study, *B. fallax* and *E. julaceum*, which are epilithic mosses growing in rock desertification in Guizhou, China, were used as ecophysiological mosses in a combination of field investigations and laboratory experiments. We also investigated the reference moss *B. argenteum*, which is a widely distributed moss in habitats with soil as substrate. Our research focused on the response of the antioxidant defense system of epilithic mosses to drought stress. Most antioxidant defense indicators increased initially, then declined at later stages of drought stress. In contrast, the carotenoid content increased constantly. In addition, there was an initial increase (albeit variable) in relative membrane permeability, with this parameter showing a parabolic trend in all of the epilithic mosses. Among the three species, *E. julaceum* demonstrated the strongest resistance followed by *B. fallax* and then by *B. argenteum*. The epilithic mosses displayed stronger resistance compared to the native mosses; the increase in $O_2^{\cdot-}$ content and other reactive oxygen species (ROS) at the early stage of drought stress induced the enzymatic and non-enzymatic scavenging systems to sequester ROS. Moreover, the radical scavenging ability and strong drought tolerance was maintained. The long-term growth of bryophyte under drought conditions in a karst environment can help eliminate the intense response of mosses to drought stress as they adapt.

Keywords Rocky desertification of karst · Epilithic mosses · Antioxidase system · Drought stress

✉ Shijie Wang
wangshijie@vip.skleg.cn

Xianqiang Zhang
zhangxianqiang@126.com

1 Anshun University, Anshun 560000, China

2 State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550081, China

3 Guizhou Minzu University, Guiyang 550025, China

1 Introduction

Drought (water deficiency) is one of the major environmental stresses that seriously limit plant distribution, growth, and yield worldwide (Shi et al. 2014a). With changes in the environment and global climate, drought stress not only seriously affects plant growth and development, but also disrupts the ecological balance of the ecosystem (Zhao 2010). As a response to drought stress, complex biochemical and physiological strategies have evolved, allowing plants to adapt to sudden environmental changes (Cutler et al. 2010; Harb et al. 2010; Hirayama and Shinozaki 2010; Krasensky and Jonak 2012; Qin et al. 2011; Shi et al. 2014b).

Karst lands are widely distributed in China, particularly in Southwest China, producing a unique landscape. However, karst lands in this region have been severely degraded by prolonged human interference and the hot, humid climate; the desertified rocky karst lands in southern China currently cover a total land area of 1.2×10^5 km^2. Desertification has led to environmental deterioration and hampered social and economic development. The karst lands in southern China are characterized by thin soil, few soil nutrients, and low water-holding capacity (Cao et al. 2014).

The southern karst plateau is located in a zone with a subtropical monsoon humid climate. The characteristics of seasonal drought habitat formed in karst were shallow soil and exposed bedrock, a large infiltration coefficient of atmospheric precipitation, and severe leakage. This kind of drought is not caused by air-drying. Epilithic mosses grow on the surface of limestone, and drought occurs through rapid seepage of precipitation. Frequent rainfall allows for moss growth. While studies in China have been an important contributor to international academic knowledge on the mechanism of antioxidant defense systems under water stress, they have focused on seed plants, with few studies conducted among epilithic mosses in karst areas under drought stress. Bryophytes are a class of pioneer plants in desert ecosystem succession, as well as in extremely inhospitable environments. Therefore, studies on the response to drought stress of the antioxidant defense system of epilithic mosses in karst rocky desertification areas offer important theoretical significance and practical value in understanding drought resistance among mosses, as well as in restoring or rebuilding degraded ecosystems. By comparing the adaptability of *Erythrodontium julaceum*, *Barbula fallax*, and *Bryum argenteum* to various habitat conditions in rocky desertification environments, this study provides the theoretical foundation for the rehabilitation and management of rock desertification ecology.

Most bryophytes grow in humid environments. However, they can survive in extreme arid regions and in special matrices such as in leaves of vascular plants; their drought resistance is rendered by a special physiological mechanism that makes them strongly adaptable to the environment (Farrant and Moor 2011). As is commonly the case with pioneer plants, mosses are indispensable in the establishment of biological soil crusts (BSCs), which determine the vegetation type, hold the soil, fix the sand, and prevent erosion by water or wind in desert ecosystems. BSC is not only a research hotspot worldwide in terms of the processes of earth's surface in arid regions but is also related to both geographical and biological knowledge. Comparative physiological analysis among bryophytes suggests that changes in water availability and osmolyte accumulation during drought stress contribute to the natural variation in drought resistance among these plants (Zhang et al. 2012). However, the antioxidant defense mechanisms underlying the response of mosses to drought stress remain largely unknown. Drought stress–related plant damage is mainly caused by oxidation induced by reactive oxygen species (ROS). ROS demonstrate potential toxic effects on cells, and the extent of ROS' scavenging ability indirectly reflects the drought resistance of plants (Baxter et al. 2014). Under normal conditions, ROS metabolism in plants is in equilibrium; however, drought stress disrupts the steady-state production and scavenging of ROS, resulting in reduced activity and in disrupted membrane structure (Ajithkumar and Panneerselvam 2014). Consequently, the plant will either actively or passively mobilize its own antioxidant defense system, including enzymatic and non-enzymatic defense systems. Antioxidant defense systems protect the plants from stress-induced injuries (Gao and Zhang 2008). Mosses exhibit two types of antioxidant defense mechanism (Chobot et al. 2008). The first type removes or reduces free radicals, including enzymes and antioxidants, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and Vitamin C, carotenoids (Car), and Glutathione (GSH). The second type produces antioxidants, such as glutathione reductase and ascorbate peroxidase (Oliver et al. 2005).

2 Materials and methods

2.1 Plant materials

We collected *E. julaceum*, *B. reflexa*, and *B. argenteum*, which are dominant in the Huaxi District of Guiyang City where we collected rocks as experimental materials. The samples were collected on a sunny day with an average temperature of 25 ± 1 °C.

2.2 Material culture and treatment

For drought-stress treatment, three moss species grown in pots were subjected to normal (sufficiently watered) and drought (water-deficient) conditions in dishes for 7 days. The mosses were then cultured in 1/10 Hoagland medium for 1 week. Gametophytes of similar height were inserted in polyethylene sheets, which were placed in dishes filled 1/10 Hoagland and PEG-6000 (−2.0 Mpa) solution. The lower ends of the specimens were immersed in the solution, whereas the upper ends were exposed to air. Protoplast layers of plant cells have selective permeability and since inside and outside solutions had different concentrations, water molecules diffused through the protoplast layer. The osmotic potential of PEG-6000 was measured according to the methods described by Miehel and Kaufmann (1973). The specimens were divided into two groups: (1) the control group (CK), which was cultured in 1/10 Hoagland solution; and (2) the treatment group, which was cultured in 1/10 Hoagland and PEG-6000 (−2.0 Mpa) solution. Indexes were determined at 0, 12, 24, 48, and 72 h post-treatment. To investigate the effect of the antioxidant defense system on drought-stress resistance, the three mosses were watered with PEG-6000 (−2.0 Mpa) for 7 days prior to drought-stress treatment. At least three pots from each variety were used in each independent experiment, and all pots were rotated daily during drought-stress treatment to minimize environmental effect. The mosses obtained from different limestones were subjected to control and drought-stress conditions according to the method described by Zhang et al. (2010). All experiments were repeated five times.

2.3 Research methods

2.3.1 Determination of antioxidant enzyme activity

For enzyme extraction, each sample (0.2 g) was placed in a pre-cooling mortar and then quartz sand and sodium phosphate buffer (0.05 mol·L^{-1}) were added. The buffer contained 0.2 mM of EDTA (pH 7.8) and 2% PVP.

In many stressful conditions, the anti-stress ability of the protective enzyme system is a key factor in plant response to environmental stresses (Zhou et al. 2000). SOD, POD, and CAT are the most important antioxidases. The increase in their activities enhances their ability to eliminate oxygen free radicals and to provide antioxidant protection. The activities of three antioxidant enzymes, namely, CAT (EC 1.11.1.6), SOD (EC 1.15.1.1), and POD (EC 1.11.1.7) were assayed. SOD activity was determined using the nitrogen blue four triazole (NBT) light reduction colorimetric method (Giannopolitis and Ries 1977), whereas POD activity was determined according to the method described by Ya et al. (2009). The reaction system used in POD activity determination included 0.025 mol·L^{-1} of sodium phosphate buffer (pH 7.0 with 0.1 mM EDTA), 20 mM of H_2O_2, 1% guaiacol, and 0.1 mL of enzyme liquid; POD activity was measured at 470 nm wavelength. The changes in OD470 absorbance value per minute indicated the degree of enzyme activity expressed as μg min^{-1}·g^{-1} FW. CAT activity was determined according to the method described by Aebi (1984). The reaction system for CAT activity determination included 0.025 mol·L^{-1} of sodium phosphate buffer (pH 7.0 with 0.1 mM of EDTA), 100 mM of H_2O_2, and 0.1 mL of enzyme liquid; CAT activity was measured at 240 nm wavelength. Enzyme activity is expressed in units of 1 min OD_{240} to reduce the amount of enzyme 0.1, with units of U·g^{-1}·FW·min^{-1}.

2.3.2 Determination of superoxide anion ($O_2^{-\cdot}$)

Enzyme extract (2 mL) was placed in a 10-mL centrifuge tube and then 1.5 mL of phosphate buffer (pH 7.8) and 0.5 mL of hydroxylamine hydrochloride were added and mixed; the mixture was incubated for 20 min in a bath at 25 °C. Subsequently, 2.0 mL of amino acid (17 mmol·L^{-1}) and 2.0 mL of α-naphthylamine were added and allowed to react for 30 min in a bath at a constant temperature of 30 °C. Absorbance was measured at 530 nm after the reaction. The $O_2^{-\cdot}$ content of the mixture was calculated from the standard curve (Zhang et al. 2008).

2.3.3 Determination of malondialdehyde content and relative permeability of cell membrane

According to the thiobarbituric acid (TBA) colorimetric method, measurements were obtained under high-temperature acidic conditions (Davey et al. 2005). The above-mentioned enzyme extraction (1 mL), TBA (3 mL), and trichloroacetic acid were mixed in a test tube, allowed to react in a bath for 30 min, and then cooled to room temperature for centrifugation for 10 min at 1500 r·min^{-1}. The absorbance of the supernatant was measured at 532 and 600 nm. Distilled water (2 mL) was used instead of enzyme extraction to determine the absorbance of the control. Absorbance was determined by DDS-307A conductivity meter. The relative permeability of cell membrane represented the degree of cell damage. The relative conductivity was determined using the following equation: relative conductivity = initial boiling conductivity/final boiling conductivity (Zhang et al. 2012).

2.3.4 Determination of carotenoid content

The Car content was determined according to the method described by Bao and Leng (2005). Moss sample ends were clipped and sediments were removed along with impurities. Water on the surface was dried. Samples were cut into small pieces (about 2 mm) for mixing. Approximately 0.2 g of each sample was placed into a mortar and a small amount of quartz sand, calcium carbonate, and 2–3 mL of 95% ethanol added to homogenize the sample; this procedure was performed five times. Ethanol (10 mL) was added and the mixture was ground further. The mixture was allowed to stew for 3–5 min. The homogenate was then filtered into a brown 25 mL volumetric flask and rinsed several times with a small amount of ethanol. We used 95% ethanol to achieve constant volume to determine the absorbance at 665, 649, and 470 nm. The entire pigment extraction was performed under weak light and low temperature (4 °C) conditions. The pigment concentrations were calculated according to the following formulas:

Chl *a* content (mg·L^{-1}) $= 13.95A_{665} - 6.88A_{649}$
Chl *b* content (mg·L^{-1}) $= 24.96A_{649} - 7.32A_{665}$
Car content (mg·L^{-1}) $= (1000\ OD_{470} - 2.05 \times$ Chl*a* content $- 114.8 \times$ Chl*b* content)/245
Pigment content of samples (mg·g^{-1}) $= \rho \times V \times N/m \times 1000$

ρ: colorimetric liquid pigment content of sample (mg·L^{-1}); V: extraction volume (mL); m: sample fresh weight or dry weight (g); 1000: coefficient that converts mL into L.

2.4 Statistical analysis

All experiments were repeated at least five times. Data were processed using SPSS13.0 software and were presented as mean ± SD of three independent experiments.

3 Results

3.1 Drought stress influenced the protective enzyme system

SOD is the first line of defense against oxygen free-radical–induced injuries through a reaction ($2\ O_2^{-\cdot} + 2H^+ = H_2O_2 + O_2$) that removes excess superoxide anion ($O_2^{-\cdot}$) (Wilkins et al. 2011; Smirnoff 1993). SOD can inhibit NBT reduction in the light and thus can indicate enzyme activity. Under aerobic conditions, riboflavin may be deoxygenized by light but is easily re-oxidized to produce $O_2^{-\cdot}$. $O_2^{-\cdot}$ reduces NBT into blue methyl hydrazone, whose absorbance in this experiment peaked at 560 nm. By contrast, SOD can inhibit methyl hydrazone generation by removing $O_2^{-\cdot}$. In *B. argenteum*, SOD activity increased slowly under stress for 24 h, then increased quickly. SOD activity peaked after 48 h at 679.21 $U \cdot g^{-1} \cdot FW \cdot h^{-1}$ in the control and then decreased slowly (Fig. 1). In *B. reflexa*, SOD activity decreased initially, then increased, and decreased again. A stress for 24 h gradually reduced the SOD activity which dropped to a minimum value of 519.41 $U \cdot g^{-1} \cdot FW \cdot h^{-1}$, a reduction of 19.41%. In addition, SOD activity increased rapidly under stress for 24–48 h and peaked at 758.89 $U \cdot g^{-1} \cdot FW \cdot h^{-1}$, an increase of 17.8%. The SOD activity subsequently dropped to 573.67 $U \cdot g^{-1} \cdot FW \cdot h^{-1}$ (a reduction of 11.0%), which is lower than that of the control (644.65 $U \cdot g^{-1} \cdot FW \cdot h^{-1}$). In *E. julaceum*, stress for 24 h rapidly decreased SOD activity from 814.5 to 660.46 $U \cdot g^{-1} \cdot FW \cdot h^{-1}$. The activity remained unchanged at 12–48 h, having peaked at 814.5 $U \cdot g^{-1} \cdot FW \cdot h^{-1}$ at 0 h, and then declined slowly. Our results show that the increasing stress level did not weaken SOD activity, thereby demonstrating a sustained strong ability to clear $O_2^{-\cdot}$. SOD activities of the three moss species displayed a similar trend, where they first increased and then decreased, even if the amplitudes of their increase and decrease varied. The ability of the mosses to clear $O_2^{-\cdot}$ according to degree of reduction in SOD activity is as follows: *E. julaceum* > *B. reflexa* > *B. argenteum*.

The POD activity of the three moss species all increased first and then decreased. In *B. argenteum*, POD activity increased under stress for 12 h, peaked at 9.08 $\mu g \cdot g^{-1} \cdot FW \cdot min^{-1}$, a 1.63-fold increase, and then gradually decreased. In *B. reflexa*, POD activity peaked at 8.26 $\mu g \cdot g^{-1} \cdot FW \cdot min^{-1}$ at 24 h and then rapidly declined to 4.57 $\mu g \cdot g^{-1} \cdot FW \cdot min^{-1}$ (44.7% reduction). Compared with that in the control, the POD activity in *E. julaceum* increased gradually from 6.25 $\mu g \cdot g^{-1} \cdot FW \cdot min^{-1}$ at 0 h to a maximum value of 8.46 $\mu g \cdot g^{-1} \cdot FW \cdot min^{-1}$ at 24 h, and then decreased

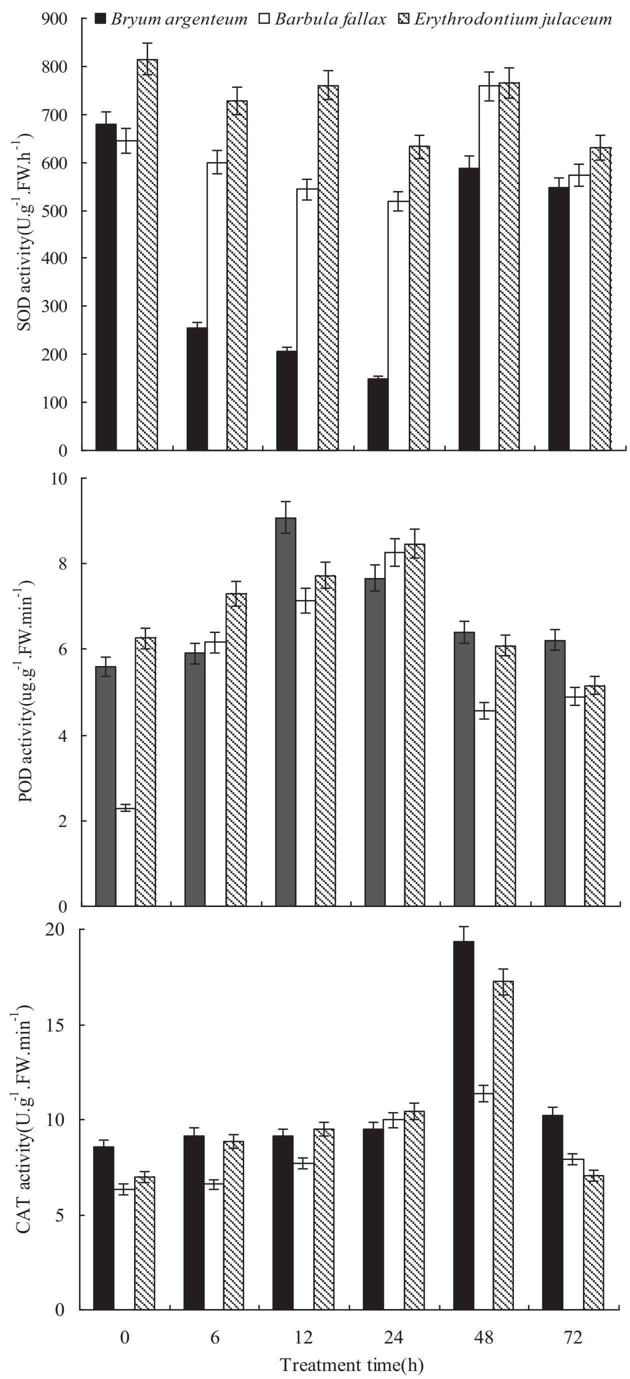

Fig. 1 Effect of drought stress on SOD, POD, and CAT activity of three epilithic mosses species

rapidly. These results show that POD changed significantly in the early stage of stress.

CAT is an important antioxidant enzyme that mainly catalyzes H_2O_2 decomposition into H_2O and O_2. The changes in CAT activity of the three moss species were similar to those of POD. With intensified osmotic stress, CAT activity increased first and reached its maximum value at 48 h, and then decreased. At 48 h, the CAT activity in *B. argenteum, B. reflexa*, and *E. julaceum* peaked at 19.35, 11.35, and 17.24 $U \cdot g^{-1} \cdot FW \cdot min^{-1}$, showing an increase by 2.56, 1.85, and 2.46 times, respectively, and then decreased rapidly.

3.2 Drought stress influenced carotenoid content

Found in animals, higher plants, fungi, algae, and bacteria of yellow, orange, or red pigment, Car, mainly β-carotene and γ-carotene, are important natural pigments. Car are also important antioxidants that effectively remove free radicals and other harmful substances produced by stress, thereby reducing damage to plant cells (Gomez and Carpena 2014). The Car contents in the three moss species were significantly different (Fig. 2). Car content in the CK was as follows: *B. argenteum* > *B. reflexa* > *E. julaceum*. Following drought stress, the Car contents in *B. reflexa* and *B. argenteum* increased gradually within 0–24 h to 0.266 $mg \cdot g^{-1} \cdot FW$ relative to that of the CK (0.180 $mg \cdot g^{-1} \cdot FW$), and then decreased rapidly after 48 h. In *B. reflexa*, Car content also increased gradually within 0–24 h to 0.180 $mg \cdot g^{-1} \cdot FW$ relative to that of the CK (0.147 $mg \cdot g^{-1} \cdot FW$). After 24 h, Car content increased rapidly, peaked at 0.302 $mg \cdot g^{-1} \cdot FW$ (an increase by 2.38 compared with the control) after 48 h, and then stabilized.

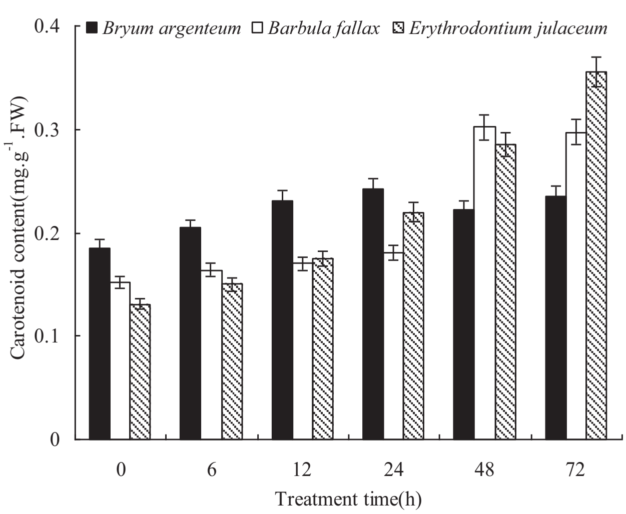

Fig. 2 Effect of drought stress on carotenoid content of three epilithic mosses species

Car content rapidly increased, accumulated, and then decreased slowly, indicating that Car sequestered the active oxygen free radicals caused by drought stress. In *E. julaceum*, Car levels increased to 0.356 $mg \cdot g^{-1} \cdot FW$ relative to those in CK (0.131 $mg \cdot g^{-1} \cdot FW$) at 72 h (increased by 2.72 times). Car slightly accumulated and then decreased rapidly in *B. argenteum*; increased rapidly, considerably accumulated, and decreased slowly in *B. reflexa*; and consistently increased, continued to accumulate, and were maintained at a high level in *E. julaceum*. The decreasing ability of the species to eliminate active oxygen free radicals was as follows: *E. julaceum* > *B. reflexa* > *B. argenteum*.

3.3 Drought stress influenced active oxygen metabolism

Free radical production and active oxygen elimination in plant cells usually occur in a dynamic balance. Low concentrations of free radical and active oxygen do not cause cell damage. This balance is disrupted when plants are subjected to stress, and the free radical and active oxygen contents will both increase sharply, increasing membrane permeability and ion leakage through peroxidation, and leading to plant death or injury (Proctor and Smirnoff. 2011).

Researchers have confirmed that drought stress can induce generation of ROS, which induces the formation of an antioxidant defense system (Uzilday et al. 2012). The $O_2^{-\cdot}$ content increased gradually with intensified drought stress (Fig. 3). In *B. argenteum*, $O_2^{-\cdot}$ content initially increased significantly, peaked at 33.08 $\mu g \cdot g^{-1} \cdot FW$ after 24 h (an increase by 5.52 times relative to that of the control), dropped to 12.39 $\mu g \cdot g^{-1} \cdot FW$, and then recovered slightly after 72 h. *B. reflexa* showed a similar trend,

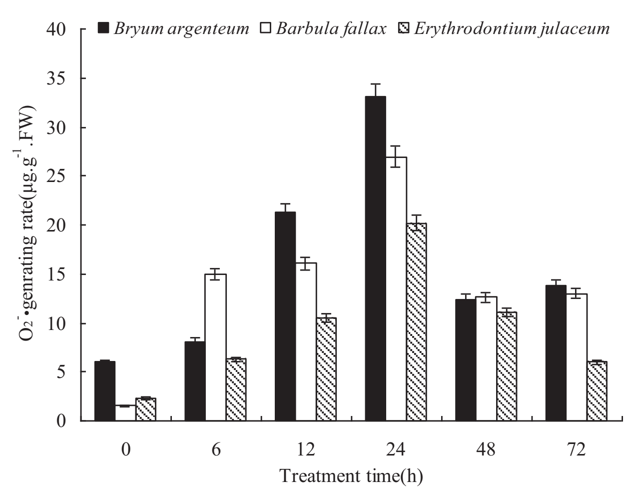

Fig. 3 Effect of drought stress on $O_2^{-\cdot}$ generation rate of three epilithic mosses species

although its maximum $O_2^{-\cdot}$ content was lower: 26.94 $\mu g \cdot g^{-1} \cdot FW$ at 24 h. In *E. julaceum*, $O_2^{-\cdot}$ also initially increased significantly, but peaked at 20.17 $\mu g \cdot g^{-1} \cdot FW$, declined sharply after 24 h, and returned to the control level at 72 h. The $O_2^{-\cdot}$ content of the three moss species all increased first and then decreased.

3.4 Drought stress influenced the malondialdehyde contents and the permeability of cell membrane

Excessive free radical production under stressful conditions can cause membrane lipid peroxide to poison plants, whose MDA content is often used as an indicator of the degree of injury (Liu et al. 2013). Drought stress caused membrane lipid peroxide to decrease, to form MDA, to destroy the membrane structure, and to injure the plant. With increasing stress level, MDA content first increased and then decreased. In *B. argenteum*, MDA content peaked at 17.65 $nmol \cdot g^{-1} \cdot FW$, 3.19 times greater that of the CK (5.54 $nmol \cdot g^{-1} \cdot FW$) within 24 h, and then declined sharply. In *B. reflexa*, MDA content increased from 8.74 to 21.12 $nmol \cdot g^{-1} \cdot FW$ within 12 h, an increase of 2.12 fold, and then declined gradually. In *E. julaceum*, MDA content increased from 6.69 to 16.33 $nmol \cdot g^{-1} \cdot FW$ within 24 h, an increase of 2.44 fold (Fig. 4). The MDA content in the three moss species all peaked within 12–24 h; however, some antioxidant enzymes and antioxidants played a protective role in succession, and MDA concentration began to reduce. With further increase in stress, the antioxidant defense system cannot remove excess ROS and might cause irreversible damage to the cells.

The cell membrane structure is highly sensitive to stress. When active oxygen production increases, peroxidation of membrane lipid may also increase and thus destroy the integrity of the membrane structure, leading to increased membrane permeability. Therefore, the relative permeability of the plasma membrane can reflect the degree of injury to cell structure. The changes in the relative membrane permeability of the three moss species showed a pattern similar to a parabola; moreover, these changes increased at different rates compared with the control. The membrane permeability of the three moss species all peaked at 24 h, an increase by 41.36% in *B. argenteum*, 39.45% in *B. reflexa*, and 55.84% in *E. julaceum*, and were 3.78, 2.03, and 3.65 times that of the control, respectively (Fig. 4).

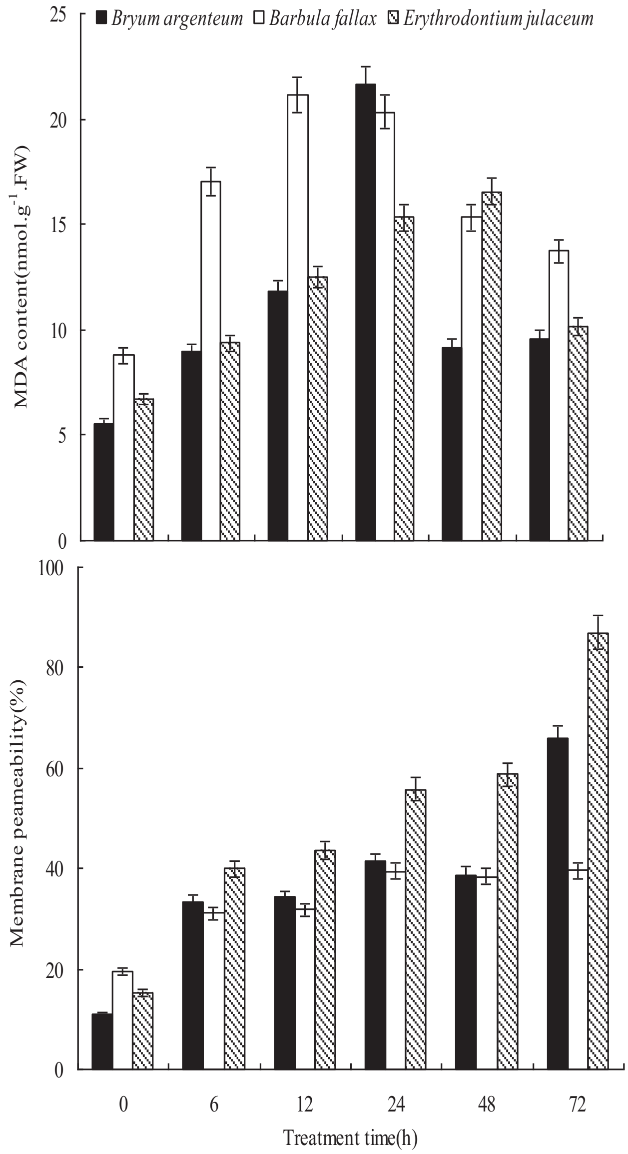

Fig. 4 Effect of drought stress on MDA content and membrane permeability rate of three epilithic mosses species

4 Discussion

Bryophytes can produce reactive oxygen free radical to induce oxidative stress in order to form complex enzymatic and non-enzymatic defense systems that will resist cell damage caused by drought stress and thus ensure their survival. SOD, POD, and CAT are the most critical enzymes in the enzymatic defense system. SOD catalyzes $O_2^{-\cdot}$ to form H_2O_2, which is removed and decomposed by POD and CAT. In the non-enzymatic defense system, Car are the most important quenchers of $O_2^{-\cdot}$, which can prevent peroxidation of unsaturated fatty acid, protecting the membrane system. In our study, antioxidant enzyme activities and Car content of the three moss species were positively correlated with stress intensity under non-drought stress. In the early stage of stress (i.e., low stress level), SOD, POD, and CAT can scavenge active oxygen free radicals by increasing their enzymatic activity to prevent self-inflicted injury. As stress levels increased (i.e., mosses suffering from severe drought damage for 48 h), the balance between ROS generation and the antioxidant system was disrupted, leading to damaged membrane structure and inhibition of enzyme activity. Car slightly accumulated and decreased rapidly in *B. argenteum*; increased rapidly and showed a high rate of accumulation and low rate of reduction in *B. reflexa*; and continuously increased and was maintained at a high level in *E. julaceum*. Mosses can accumulate a certain amount of Car to protect the membrane system, can reduce light inhibition, and can convert Car into xanthoxin by photolysis or oxidative decomposition, forming ABA, to improve their stress resistance (Oliveira et al. 2014).

The $O_2^{-\cdot}$ content increased gradually with intensified drought stress. In *B. argenteum*, $O_2^{-\cdot}$ content increased 5.52 fold within 0–24 h, dropped significantly, and then increased slightly after 72 h. *B. reflexa* showed a similar trend, although its $O_2^{-\cdot}$ content was lower. In *E. julaceum*, $O_2^{-\cdot}$ increased significantly within 0–24 h, declined sharply, and then returned to the control level after 72 h. Thus, the $O_2^{-\cdot}$ content of the three moss species all increased first and then declined.

MDA demonstrates strong cytotoxicity and is one of the main products of lipid peroxidation; MDA destroys many biological molecules, such as protein, nucleic acid, and enzymes, as well as the structure and function of biological membrane (Jiang and Zhang 2001). Under prolonged stress, the MDA content of the three moss species increased first and then declined. In *B. argenteum*, MDA increased 3.19 fold and then declined sharply, but remained slightly higher than that of the control. The MDA content in *B. reflexa* increased 2.12 fold and then declined gradually. In addition, the MDA content in *E. julaceum* increased 2.44 fold within 24 h. The changes in the relative membrane permeability of the three moss species showed a parabolic trend, where the maximum value was reached at 24 h and then declined. Compared with the control, they all increased, although to varying degrees.

The drought resistance of a plant is affected by many factors that are interrelated and mutually conditioned (Shi et al. 2014a, b). Under drought stress, the mosses growing on bare stone in the karst rocky desertification area coped with temporary drought through their special antioxidant defense system. Under water stress, the antioxidant enzyme activities and antioxidant content of mosses were positively correlated with antioxidant stress capacity. In the early stage wherein water stress was mild, the mosses removed excess active oxygen radicals by increasing their enzyme activity and antioxidant content. In the later stages, the mosses escaped extreme drought stress through physiological dormancy, which can be restored by rehydration. In the course of evolution, epilithic mosses have acquired a series of physiological and metabolic mechanisms to adapt to drought; these adaptations can reduce water loss through morphological changes, as well as allow the mosses to cope with harsh environmental conditions through adjustments to physiological activity (Oliver et al. 2002, 2013).

The results of this study reveal the resistance mechanism of mosses found in rocky desertification and arid environments. Moreover, this study contributes to the restoration of degraded rocky ecosystems through greater understanding of drought response.

5 Conclusion

Studies on the response to drought stress of the antioxidant defense system of epilithic mosses in karst rocky desertification areas offer important theoretical significance and practical value in revealing the mechanism of drought resistance among mosses, as well as in restoring or rebuilding degraded ecosystems. The superoxide dismutase, catalase, superoxide anion ($O_2^{-\cdot}$), and malondialdehyde contents, as well as peroxidase activity, of the epilithic mosses initially increased under drought conditions, although these parameters declined at later stages of drought stress. In contrast, Car content was constantly increasing. In addition, although the increase in relative membrane permeability varied, this parameter showed a parabolic trend in all of the epilithic mosses. Among the three species, *E. julaceum* demonstrated the strongest resistance followed by *B. fallax* and then by *B. argenteum*. Moreover, the epilithic mosses growing on rocks displayed stronger resistance compared with the native mosses. Our conclusion is that the increase in $O_2^{-\cdot}$ content and other ROS at the early stage of drought stress induced the enzymatic and non-enzymatic scavenging systems to sequester ROS. Moreover, the radical scavenging ability and strong drought tolerance was maintained. The long-term growth of bryophyte under drought in karst environments can help eliminate the intense response to drought as the mosses become adapted to drought stress.

Acknowledgments This work was supported by funds from the National Natural Science Foundation (Grant No. 41463006).

References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Ajithkumar IP, Panneerselvam R (2014) ROS scavenging system, osmotic maintenance, pigment and growth status of *Panicum sumatrense* roth. Under drought stress. Cell Biochem Biophys 68(3):587–595

Bao WK, Leng L (2005) Determination methods for photosynthetic pigment content of bryophyte with special relation of extraction solvents. Chin J Appl Environ Biol 11(2):235–237

Baxter A, Mittler R, Suzuki N (2014) ROS as key players in plant stress signalling. J Exp Bot 65(5):1229–1240

Cao KF, Fu PL, Chen YJ et al (2014) Implications of the ecophysiological adaptation of plants on tropical karst habitats for the ecological restoration of desertified rocky lands in southern China. Sci Sin Vitae 44:238–247

Chobot V et al (2008) Evaluation of antioxidant activity of some common mosses. Z Naturforsch C 63(7–8):476–482

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

Davey MW et al (2005) High-throughput determination of malondialdehyde in plant tissues. Anal Biochem 347(2):201–207

Farrant JM, Moor EJP (2011) Programming desiccation-tolerance: from plants to seeds to resurrection plant. Curr Opin Plant Biol 14(3):340–345

Gao Q, Zhang L (2008) Ultraviolet-B-induced oxidative stress and antioxidant defense system responses in ascorbate-deficient vtc1 mutants of *Arabidopsis thaliana*. J Plant Physiol 165(2):138–148

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: II. purification and quantitative relationship with water-soluble protein in seedlings. Plant Physiol 59(2):315–323

Gomez DA, Carpena RO (2014) Effect of 1-naphthaleneacetic acid on organic acid exudation by the roots of white lupin plants grown under phosphorus-deficient conditions. J Plant Physiol 171(15):1354–1361

Harb A, Krishnan A, Ambavaram MMR, Pereira A (2010) Molecular and physiological analysis of drought stress in Arabidopsis reveals early responses leading to acclimation in plant growth. Plant Physiol 154:1254–1271

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J 61:1041–1052

Jiang M, Zhang J (2001) Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol 42:1265–1273

Krasensky J, Jonak C (2012) Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. J Exp Bot 63:1593–1608

Liu M et al (2013) Regulation of flavanone 3-hydroxylase gene involved in the flavonoid biosynthesis pathway in response to UV-B radiation and drought stress in the desert plant, *Reaumuria soongorica*. Plant Physiol Biochem 73:161–167

Miehel BE, Kaufmann MR (1973) The osmotic potential of polyethylene glycol 6000. Plant Physiol 51(5):914–916

Oliveira MT et al (2014) Different mechanisms drive the performance of native and invasive woody species in response to leaf phosphorus supply during periods of drought stress and recovery. Plant Physiol Biochem 82:66–75

Oliver JE et al (2002) Bruchins, plant mitogens from weevils: structural requirements for activity. J Chem Ecol 28(12):2503–2513

Oliver MJ, Velten J, Mishler BD (2005) Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? Integr Comp Biol 45(5):788–799

Oliver JD et al (2013) Simple and robust determination of monosaccharides in plant fibers in complex mixtures by capillary electrophoresis and high performance liquid chromatography. J Chromatogr A 1291:179–186

Proctor MC, Smirnoff N (2011) Ecophysiology of photosynthesis in bryophytes: major roles for oxygen photoreduction and non-photochemical quenching? Physiol Plant 141(2):130–140

Qin F, Shinozaki K, Yamaguchi-Shinozaki K (2011) Achievements and challenges in understanding plant abiotic stress responses and tolerance. Plant Cell Physiol 52:1569–1582

Shi H, Ye T, Chen F, Cheng Z, Wang Y, Yang P, Zhang Y, Chan Z (2014a) Modulation of auxin content in Arabidopsis confers improved drought stress resistance. Plant Physiol Biochem 82:209–217

Shi H, Ye T, Chan Z et al (2014b) Comparative proteomic responses of two bermudagrass (*Cynodon dactylon* (L). Pers.) varieties contrasting in drought stress resistance. Plant Physiol Biochem 82:218–228

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficits and desiccation. New Phytol 125:27–58

Uzilday B et al (2012) Comparison of ROS formation and antioxidant enzymes in *Cleome gynandra* (C4) and *Cleome spinosa* (C3) under drought stress. Plant Sci 182:59–70

Wilkins KA et al (2011) Reactive oxygen species and nitric oxide mediate actin reorganization and programmed cell death in the self-incompatibility response of papaver. Plant Physiol 156(1):404–416

Ya J, Jing ZG, Jin GX, Li Z (2009) Brassinosteroids alleviate chilling induced oxidative damage by enhancing antioxidant defense system in suspension cultured cells of *Chorispora bungeana*. J Plant Growth Regul 59(3):207–214

Zhang HX, Xia Y, Wang GP, Shen ZG (2008) Excess copper induces accumulation of hydrogen peroxide and increases lipid peroxidation and total activity of copper-zinc superoxide dismutase in roots of *Elsholtzia haichowensis*. Planta 227:465–475

Zhang XQ, Zhang L, He YJ et al (2010) Water uptake mechanism and desiccation-tolerant adaptation of *Taxiphyllum aomoriense* crust in karst rocky desertification. Acta Ecol Sin 30(12):3108–3116

Zhang XQ, Zeng JJ, Chen JW, Luo ZW, Sun M (2012) The epilithic moss's features of absorbing water and its structural adaptability in the heterogeneous environment with rock desertification. Acta Ecol Sin 32(12):3902–3911

Zhao Y (2010) Auxin biosynthesis and its role in plant development. Annu Rev Plant Biol 61:49–64

Zhou A et al (2000) Renal protective effects of blocking the intrarenal renin-angiotensin system: angiotensin II type I receptor antagonist compared with angiotensin-converting enzyme inhibitor. Hypertens Res 23(4):391–397