***Pak. J. Bot.*, 50(3): 921-928, 2018.**

EXOGENOUS SPERMIDINE IMPROVES DROUGHT TOLERANCE IN *PHYLLOSTACHYS EDULIS* SEEDLINGS

SHANLU SHAO[1], MESERETCHANIE ABATE[1], XUHUA DU[2] AND YEQING YING[1*]

[1]State Key Laboratory of Subtropical Silviculture, Zhejiang A & F University, Lin'an, Hangzhou, China
[2]Key Laboratory of High Efficient Processing of Bamboo of Zhejiang Province,
China National Bamboo Research Center, Hangzhou, China
[*]Corresponding author's email: yeqing@zafu.edu.cn

Abstract

Phyllostachys edulis is an endemic and dominant bamboo species in southern China. It suffers often from seasonal drought during the cultivation process. In the present study, the effect of exogenous spermidine (Spd) on the following parameters of leaves of *Phyllostachys edulis* seedlings under drought stress were studied: The generation rate of superoxide free radical, proline content, REC, MDA content, RWC, Chlorophyll content; and activities of POD, SOD and CAT. The drought stress was caused by 20% polyethyle glycol (PEG) and three concentrations of spermidine were applied (0.0l mmol/LSpd, 0.1 mmol/LSpd, 1.0 mmol/LSpd). Drought stress caused a decrease in growth rate which was related to several physiological changes: decreasing RWC and chlorophyll content and increasing generation rate of superoxide free radical, increasing MDA and REC, and increasing activities of POD and CAT. Foliar application with 0.01mM exogenous Spdincreased POD activity and RWC, but had no effect on SOD activity, Chl(a+b) or Chla/Chlb and also no significant effect on proline content, activity of P5CS, or generation rate of superoxide free radical. The 0.1 mM application with exogenous Spd significantly increased the chlorophyll content and RWC, inhibited the generation rate of superoxide free radical, reduced membrane lipid per-oxidation, returned the activities of antioxidant enzymes to the normal level, and minimized the increases in REC, proline, and MDA content. Application with the highest spermidine concentration (1.0mM Spd) has the same effects apart from the increase of H_2O_2 content. In conclusion, application with 0.1 mM exogenous Spd can effectively alleviate a series of physiological and biochemical changes caused by drought stress in the bamboo trees. This may provide more insight to the possible mechanisms of how exogenous polyamines can influence the drought resistance of *Phyllostachys edulis* and in this way facilitate the cultivation of this bamboo species under drought stress.

Key words: *Phyllostachys edulis*, Drought stress, Polyamine (PA), Drought resistance.

Abbreviations: CAT – Catalase; MDA– Malondialdehyde; PA– Polyamine; POD – Peroxidase; ROS– Reactive oxygen species; RWC– Relative water content; SOD– Superoxide Dismutase; Spd– Spermidine

Introduction

Drought stress is a major factor restricting plant growth, development and productivity in many terrestrial ecosystems (Reddy, 2004). This is because drought stress alters many essential physiological and biochemical processes of plants, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, and nutrient metabolism (Farooq *et al.*, 2008; Jaleel *et al.*, 2008). Drought affects also the degree of stomatal closure, which can restrict the amount of available CO_2, and finally reduce the photosynthetic rate (Flexas *et al.*, 2006). Various abiotic stresses are major causes of plant loss all over the world. So, improving the drought resistance of plants, for instance by exploring various compounds to reduce the losses caused by stress, is essential for the cultivation of many plant species.

Phyllostachys edulis is a native temperate species of giant timber bamboo of China and Taiwan and it has been introduced for cultivation also elsewhere (Yuming, 2003). By providing many ecological, social and economic benefits, *Phyllostachys edulis* is one of the most important bamboo species in China. From summer to autumn, this plant, mostly grown in the southern part of China, frequently suffers from drought. From July to October the precipitation is 120 mm , which is only 10% of the average annual precipitation in the region, which varies from 1, 000 to 1, 500 mm (Feng & Hong, 2007). The growth and development of this bamboo species, especially at young age, is greatly hindered by water stress during the dry season, leading to the decrease of vegetative and reproductive growth. Therefore, it is crucial to investigate influence of water stress on the physiological and biochemical characteristics of *Phyllostachys edulis* seedlings.

The early stages of plant development (*i.e.*, seedling emerging and establishment) are more sensitive to environmental stresses than the developmental stages of the adult plant. Furthermore, the seedling stage is very important for forest regeneration, and species composition and succession dynamics of the forest (Li *et al.*, 2004).

Under drought stress, plants can acclimate to the continuous environmental changes by adjusting the physiological and biochemical processes related to drought tolerance. In addition to the physiological and biochemical changes discussed above, polyamines, which act as signal molecules in plants, are also affected by environmental stresses (Alcázar *et al.*, 2006). The polyamines are ubiquitous aliphatic amines related to the regulation of osmotic adjustment, free radical scavenging, and regulation of cation-anion balance in plants under stress conditions (Bouchereau *et al.*, 1999). Putrescine (Put), spermidine (Spd) and spermine (Spm) are generally the most common PAs in plants, but instead of Spd, thermospermine (Tspm) exists in some plants. Polyamines are located in various organelles which have simple structures, and they may participate in the physiological mechanisms which stabilize the molecular structure of the cell membrane, allowing the membranes to maintain their biological functions of their metabolic components (Hussain *et al.*, 2011; Shi & Chan, 2014; Hina *et al.*, 2016).

Increased plant drought resistance caused by exerting exogenous polyamines, such as exogenous Spd, which can effectively cope with oxidative stress by increasing antioxidant enzyme activity, has been reported. Furthermore, the content of endogenous polyamines has been shown to be higher in resistant plants than in plants sensitive to environmental stresses (Lee, 1997). Improving drought resistance through the application of exogenous polyamines in plants has been reported (Yiu *et al.*, 2009). For example, the application of exogenous Spd can effectively enhance the activities of antioxidant enzymes in response to oxidative stress (Roychoudhury *et al.*, 2011). Increasingly many researches have suggested that drought-induced oxidative stress in plants is mainly caused by the accumulation of ROS due to the stomatal closure (Ozkur *et al.*, 2009; Asada, 1999). Accumulation of excess ROS can damage cellular components of plants, such as lipids, photosynthetic pigments, and related enzymes (Smirnoff, 2010). To cope with the oxidative stress, the active oxygen content is reduced and the photosynthetic complexes are stabilized through the antioxidant enzyme system involving SOD, POD, CAT (Ozkur *et al.*, 2009). Under drought stress, the decrease of ROS in spring wheat leaves was found to be caused by ethylene (ETH) produced by polyamines (PAs). As compatible osmotic agents or ionic constituents properties of cells, the exogenous polyamines (PAs) protect the cells mainly by directly associating with the cell membranes and by reducing the oxidative activity (Afzal *et al.*, 2009; Wang *et al.*, 2007). *Gupta et* reported that compared to the control plants, photosynthetic attributes were enhanced, the contents of Pro, soluble sugars and total amino acids were increased significantly, and membrane injury was mitigated by the application of exogenous polyamines (PAs) This lead eventually to increased crop yields (Li *et al.*, 2015). The studies reviewed above show that the antioxidants including enzymatic and non-enzymatic components are the basis of plant stress resistance (Li *et al.*, 2004).

However, it has also been reported that polyamine (PA) catabolism caused damage in tobacco cells, by producing large amounts of H_2O_2 and acrolein (Mano, 2012; Takano *et al.*, 2012). However, whether polyamine (PAs) can alleviate the damage caused by drought in *Phyllostachys edulis* seedlings and in this way improve their drought resistance, remains unclear.

The objective of the present study was to examine the effect of exogenous spermidine on physiological responses of *Phyllostachys edulis* seedlings to drought stress. The information obtained will 1) improve our understanding about the mechanisms by which exogenous polyamines can increase the drought resistance of *Phyllostachys edulis* and in this way 2) facilitate the cultivation of this bamboo species under drought stress.

Materials and Methods

Plant material: The experiment took place under a controlled environment room of Zhejiang Agriculture and Forestry University, China. On 23 September 2014, homogeneous one-year old *Phyllostachys edulis* seedlings were transplanted into pots (10 cm diameter * 8 cm high, 4 holes in the bottom) containing a sterilized 1:2 (v/v) mix of peat and perlite soil. The seedlings were kept in an artificial climate box in under a 16-h photoperiod with day/night air temperatures of 28/25°C and relative humidity of 80-85%. On 17 December 2014, when the seedlings had 6 leaves, the seedling population in each pot was thinned to the density of ten seedlings per pot and the seedlings were transferred to a culture room with same photoperiod and day/night air temperatures as in the climate box.

In the culture room, the pots with their seedlings were pad with a plastic box. Each plastic box contained 200mL yoshita nutrient solution and each seedling was casted in 100 mL yoshita nutrient solution with daily water of 50 ml and nutrient solution renewal in every 2 days. The experiments were started on 22 December 2014

Experimental design: Effects of exogenous spermidine (Spd) in leaves of *Phyllostachys edulis* seedlings under drought stress caused by polyethylene glycol (PEG) application were studied in an experiment including five treatments. The experiment was arranged in a completely randomized design with 3 replicates per treatment, each was repeated with two pots containing ten seedlings, so that for each treatment, a total of 60 seedlings were used. The five treatments were implemented as follows: T1 (control, Yoshita nutrition solution without PEG or Spd added); T2 (added 20% PEG); T3 (added 20% PEG + 0.0l mmol/L Spd); T4 (added 20% PEG + 0.1 mmol/L Spd); T5 (added 20% PEG + 1.0 mmol/L Spd). The plants were collected for sampling 20 days after treatments. The Yoshita nutrient solution was prepared following Zou *et al.*, (2012).

Measurement of relative water content (RWC): After collecting the fresh leaves they were immediately weighted (Wf), rehydrated for 24h in darkness and weighted again (Wt), and subsequently oven-dried to a constant weight at 85 °C (Wd). The relative water content (RWC) was calculated as RWC= (Wf-Wd)/(Wt-Wd)x100 %

Measurement of active oxygen: The activity of oxygen was detected by measuring the change of oxyradical and H_2O_2 after the following procedures: 1 ml of 1 mmol/L hydroxylamine hydrochloride and 0.5 mmol/L phosphate buffer (pH7.8) was added in a 0.5 ml sample extraction, which was then shaken at 25°C thermal insulation for one hour. Subsequently, 1 ml of 17 mmol/L of amino benzene sulfonic acid and 1 ml/L alpha naphthylamine (acetic acid: water = 3:1) were added and the extraction was mixed. Finally, the spectrophotometric meter wavelength of 530 nm OD values were determined after insulating the extraction in 25°Cfor 20 min.

The standard curve for the spectrophotometric analysis was prepared as follows: 1 ml of 17 mmol/L of amino benzene sulfonic acid and 1 ml/L alpha naphthylamine (acetic acid: water = 3:1) were added to a 1 ml series concentration of $NaNO_2$ (0, 4, 8, 12, 16, 20 umol/L). The solution was mixed in 25℃ insulation for 20 min, then the concentration of $[NO_2^-]$ and the OD_{530} value were measured and a mutual mapping function was prepared to make the standard curve. In a separate set of procedures 10 ml ice acetone was added in a 0.2 g grinded leaf sample, and the mixture was centrifuged at the speed of 3000 r/min for 10 min. The supernatant was extracted and 0.1 ml of 5 % titanium sulfate was added to a 0.1 ml sample extraction solution which was then shaken. 0.2 ml concentrated ammonia was added and the solution was centrifuged at 3000 r/min for 10 min after it formed precipitation. The supernatant was removed and repeatedly washed with acetone precipitation for 3-5 times until plant pigments were removed. 2 mol/L 5 ml sulfate was added to the washed precipitation, and the precipitation was completely dissolved, and optical absorption value was determined at 415 nm. The content of H_2O_2 in the sample was calculated by using the standard curve. The concentrations of oxyradical and H_2O_2 were measured using the methods of Zou *et al.,* (2012).

Measurement of photosynthetic pigments: 0.1 g of leaves was weighed, and then dissolved using 8 mL of 95% (V/V) ethanol repeatedly. Chlorophyll was extracted after incubating the solution for 48 h in darkness. Absorbance was measured at 470, 649 and 664 nm. The chlorophyll concentrations (including Chla, Chlb and Chl) in the leaves was calculated using to the Arnon formula (Zou *et al*., 2012).

Measurement of antioxidative enzyme activities: 0.2g tissue sample was taken into a precooled mortar. 2 ml cold preextraction medium (pH 7.8 0.1 mol/L phosphate buffer) was added to ice bath polishing slurry, and then used the extraction medium wash mortar to make the final volume into 8 ml. The solution was centrifuged in 4℃ at 10000 rpm for 15 min. The supernatants were used as the crude enzyme extract. The enzyme activities (POD, SOD, CAT) were determined after storing the supernatants in 0-4℃. The POD, SOD, CAT activities in the leaves were estimated using the method of Zou *et al.,* (2012).

Measurement of cell damage with REL: Approximately 0.2 g leaf samples were rinsed with deionized water 1-2 times and then cut into small pieces. The samples were put in a test tube, and the leaves were immersed in the liquid by adding 30 ml distilled water. After that, with a vacuum dryer pump working for 10 min and slowly pressing into air, the water was pressed into the blade until the blade sinks. After allowing the solution to become steady for 20 minutes the electrical conductivity C1 was determined by using carefully a glass rod at the constant temperature of 20-25℃. Subsequently, the test tube was treated with boiling water for 10 min, and then cooled, and the electrical conductivity C2 was measured at 20-25℃(Zheng, 2006). Relative electrolyte leakage REC was calculated as follows:

$$\text{REC (\%)} = C1/\, C2 \times 100\%$$

Measurement of lipid peroxidation: Using the method of Zheng *et al,* thelipid peroxidation level was assessed based on the malondialdehyde (MDA) contents. 1 mL supernatant was combined with 1 mL 0.6% thiobarbituric acid (TBA) and 3 mL 10% TCA. The solution was incubated in boiling water for 30 min and then cooled in an ice bath quickly. After centrifuging for 10 min at 5000 rpm, the absorbance of the supernatant was measured at 532, 600 and 450 nm.

Statistical analyses: Least significant difference tests and analysis of variance (ANOVA, GLM procedure) were used to analyse all of the studied parameters. The data were presented as the mean ± SD. The statistical significances of the differences among the treatments were examined at $p<0.05$. All statistical analyses were made using the software SAS 9.2 (2008).

Results

The 20% PEG drought stress decreased the RWC of the leaves by 20.6 % (Fig. 1). However, the effect of drought stress on the RWC decreased with the application of Spd. Compared with the control, with the application of 0.01 mM ,0.1mM and 1.0 mMSpd, drought stress decreased the RWC only by 19.6, 12.3, and 5.3% respectively.

The leaves had the highest accumulation of MDA under 20% PEG drought stress (Fig. 2b). The drought stress increased the MDA by 34.0%. However, compared with the control, under 0.01 mM, 0.1 mM and 1.0 mMSpd treatments, the amount of MDA was only increased by 29.75, 6.49 and 11.92%, respectively. Similar results were observed for REC (Fig. 2a). Compared with the control, REC increased by 33.3% under 20% PEG drought stress; but with the 0.01 mM, 0.1 mM and 1.0 mMSpd treatments REC increased only by 29.2,12.5 and 8.3%, respectively.

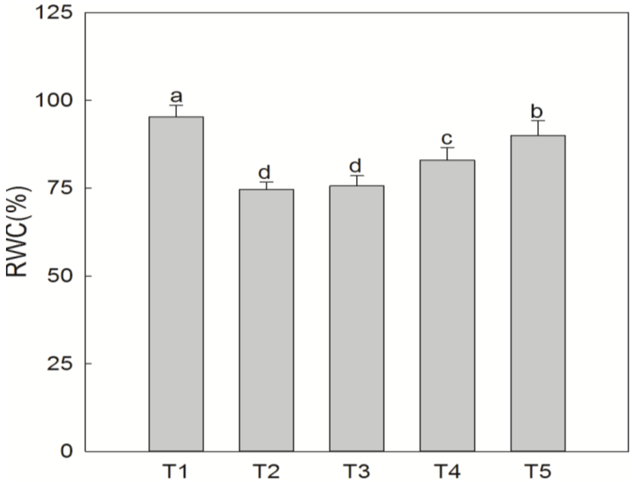

Fig. 1. Effects of exogenousspermidine (Spd) on relative water content (RWC) in leaves of *Phyllostachys edulis* seedlings under drought stress caused by polyethylene glycol (PEG) application. T1 (control, nutrition solution without PEG or Spd added); T2 (added 20% PEG); T3 (added 20% PEG + 0.0l mmol/L Spd); T4 (added 20% PEG + 0.1 mmol/L Spd); T5 (added 20% PEG + 1.0 mmol/L Spd). For each treatment mean ± SD of three replicates is indicated (n = 3). Bars with different letters indicate significant differences at $p<0.05$.

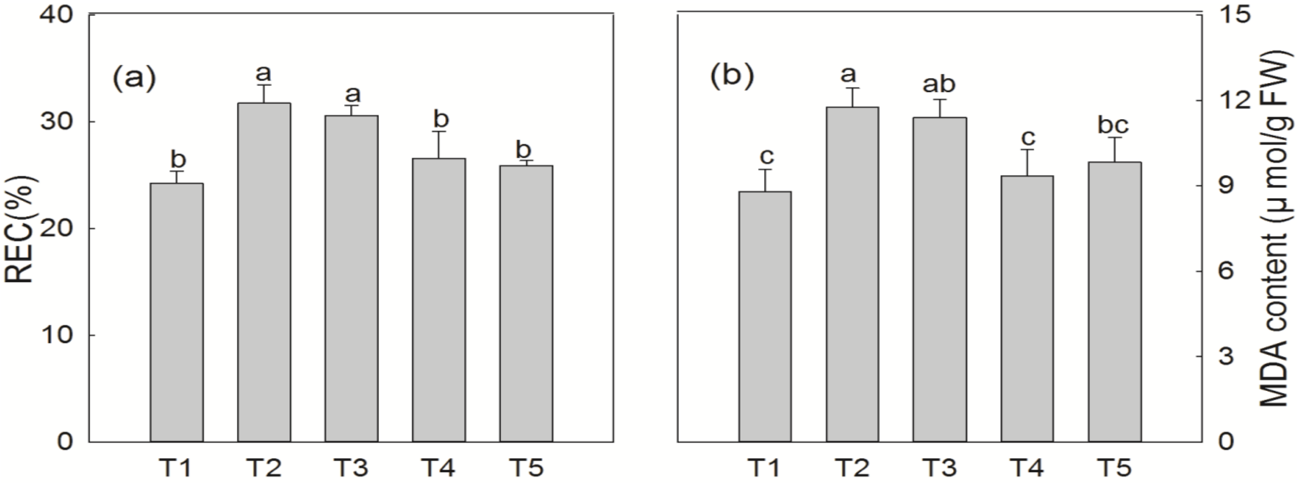

Fig. 2. Effects of exogenousspermidine (Spd) on relative electric conductivity (REC) (a) and MDA content (b) of *Phyllostachys edulis* seedlings under drought stress caused by polyethylene glycol (PEG) application. T1 (control, nutrition solution without PEG or Spd added); T2 (added 20% PEG); T3 (added 20% PEG + 0.0l mmol/L Spd); T4 (added 20% PEG + 0.1 mmol/L Spd); T5 (added 20% PEG + 1.0 mmol/L Spd). For each treatment mean ± SD of three replicates is indicated (n = 3). Bars with different letters indicate significant differences at $p<0.05$.

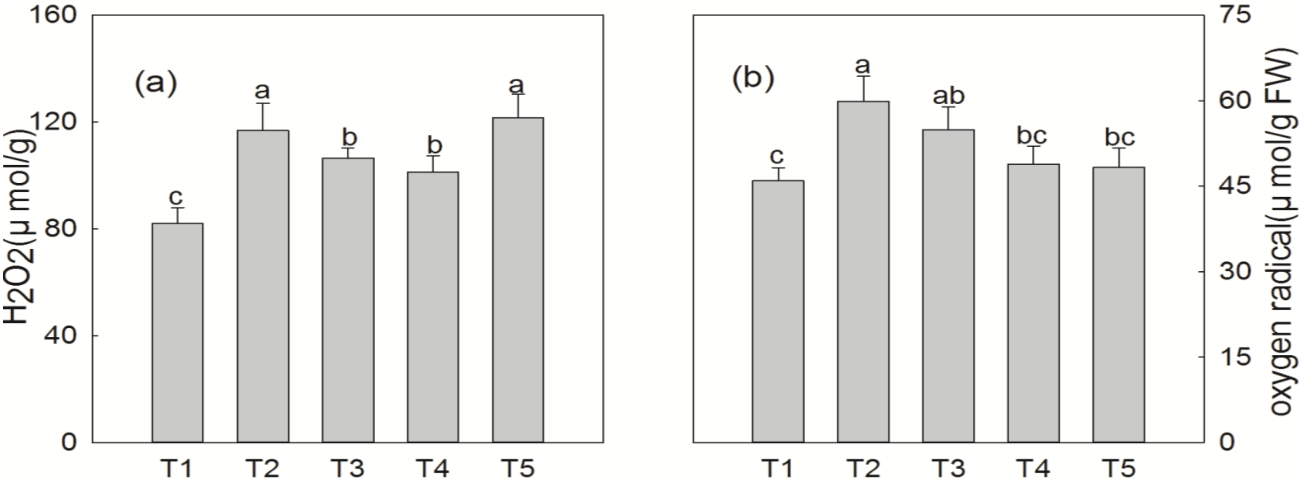

Fig. 3. Effects of exogenousspermidine (Spd) on H_2O_2 content (a) and oxygen radical content (b) in leaves of *Phyllostachys edulis* seedlings under drought stress caused by polyethylene glycol (PEG) application. T1 (control, nutrition solution without PEG or Spd added); T2 (added 20% PEG); T3 (added 20% PEG + 0.0l mmol/L Spd); T4 (added 20% PEG + 0.1 mmol/L Spd); T5 (added 20% PEG + 1.0 mmol/L Spd). For each treatment mean ± SD of three replicates is indicated (n = 3). Bars with different letters indicate significant differences at $p<0.05$.

Compared with the control, under 20% PEG drought stress, Chl (a+b) decreased by 28.0%, Chla/Chlb decreased by 12.7%, and Car increased by 18.0% (Table 1). However, with the exception of the lowest applied concentration of 0.01 mM the Spd treatments mitigated the influence of drought stress. Compared with the control, the 0.1 mM and 1.0 mMSpd decreased Chl (a+b) only by 16.9% and 14.94%, and Chla/Chlb by 0.30% and 4.5%, respectively. For Car, all of the applied concentrations of Spd mitigated the effects of the drought stress, and there were no significant differences among the different Spd treatments (Table 1).

Compared with the control, the 20% PEG drought stress increased the contents of H_2O_2 and the oxygen radical by 42.45% and 30.39%, respectively (Fig. 3). Compared with the control, with the application of Spd the contents of H_2O_2 were increased by only 29.93% and 23.45% under the 0.01 mM, 0.1 mMSpd treatments, respectively, but the 1.0 mMSpd increased it by 48.24% (Fig. 3a). Compared with the control, the lowest concentration 0.01 mM of Spd increased the oxygen radical only by 19.44%, whereas with the application of the two other concentrations there was no statistically significant effect on the oxygen radical (Fig. 3b).

Compared with the control the drought stress increased the POD (Fig. 4b) and CAT activities (Fig. 4c) of the leaves, the increase being bigger in POD (72.46%) than in CAT (12.46%); but the drought stress had no statistically significant effect on the SOD activity (Fig. 4a). The effect of Spd treatment on the enzyme activities was different with different enzymes. Compared with the control, no significant effect on SOD activity was found under 0.01 mM and 0.1 mMSpd treatments, while it was decreased by 8.3% under the 1.0 mMSpd treatment. Moreover, compared with the control the POD activity was increased under the 20% PEG drought stress by 59.03, 50.59 and 74.12% under the 0.01 mM, 0.1 mM and 1.0 mMSpd treatments, respectively (Fig. 4b). In the activity of CAT, compared with the control there was no statistically significant effect of any of the Spd treatments, and no statistically significant difference was found either among the different Spd treatments, even though they decreased significantly the activity when compared with the treatment with drought stress alone (Fig. 4).

Compared with the control, the content of Proline (Fig. 5a) and the activity of P5CS (Fig. 5b) were increased under drought stress by 119.37% and 25.67%, respectively. However, compared with the 20% PEG drought treatment almost all Spd treatments induced decrease of the proline content (Fig. 5a) and the activity of P5CS (Fig. 5b), but the degree of decrease was higher for proline content than for P5CS activity. Compared with the 20% PEG drought stress treatment, 0.01 mM, 0.1mM and 1.0 mMSpd decreased the proline content by 22.65, 40.64 and 56.15%, respectively (Fig. 5a); but the P5CS activity only by 8.1%, 15.89% and 20.24%, respectively (Fig. 5b) . For both the proline content (Fig. 5a) and the activity of P5CS (Fig. 5b), there was no statistically significant difference between the control and the treatments with the 0.1 mM and 1.0 mMSpd.

Table 1. Effects of exogenousspermidine (Spd) on Chl(a+b), chlorophyll a:b ratio (Chla/b), and carotenoids (Car) in leaves of *Phyllostachys edulis* seedlings under drought stress caused by polyethylene glycol (PEG) application.

Treatments	Photosynthetic pigments		
	Chl	Chl a/Chl b	Car
	(mg/g)		(mg/g)
T1	2.61 ± 0.09^{a}	3.32 ± 0.16^{a}	0.61 ± 0.06^{b}
T2	1.88 ± 0.13^{c}	2.90 ± 0.16^{b}	0.72 ± 0.03^{a}
T3	1.86 ± 0.05^{c}	2.83 ± 0.20^{b}	0.72 ± 0.04^{a}
T4	2.17 ± 0.13^{b}	3.31 ± 0.23^{a}	0.79 ± 0.05^{a}
T5	2.22 ± 0.18^{b}	3.17 ± 0.08^{ab}	0.72 ± 0.02^{a}

T1 (control, nutrition solution without PEG or Spd added); T2 (added 20% PEG); T3 (added 20% PEG + 0.0l mmol/L Spd); T4 (added 20% PEG + 0.1 mmol/L Spd); T5 (added 20% PEG + 1.0 mmol/L Spd).

For each treatment mean ± SD of three replicates is indicated (n = 3). Bars with different letters indicate significant differences at $p<0.05$.

Discussion

Plant water deficit disturb many cellular and whole plant functions, thus affecting plant growth and reproduction (Liu *et al.*, 2005). Plants grown under water deficiency have reduced biomass accumulation, CO_2 assimilation, and activities of photosynthesis-related enzymes accompanied with increased H_2O_2 accumulation, lipid per-oxidation and altered antioxidant enzymes. Polyamines play a pivotal role in regulating plant growth, development and drought stress response (Alcázar *et al.*, 2006; Bouchereau *et al*., 1999). In the present study, we intended to examine whether spermidine can induce a set of specific physiological responses including changes in RWC, chlorophyll content, ROS, antioxidant enzyme activities, photosynthesis, MDA and REC that enable *Phyllostachys edulis* to alleviate water stress.

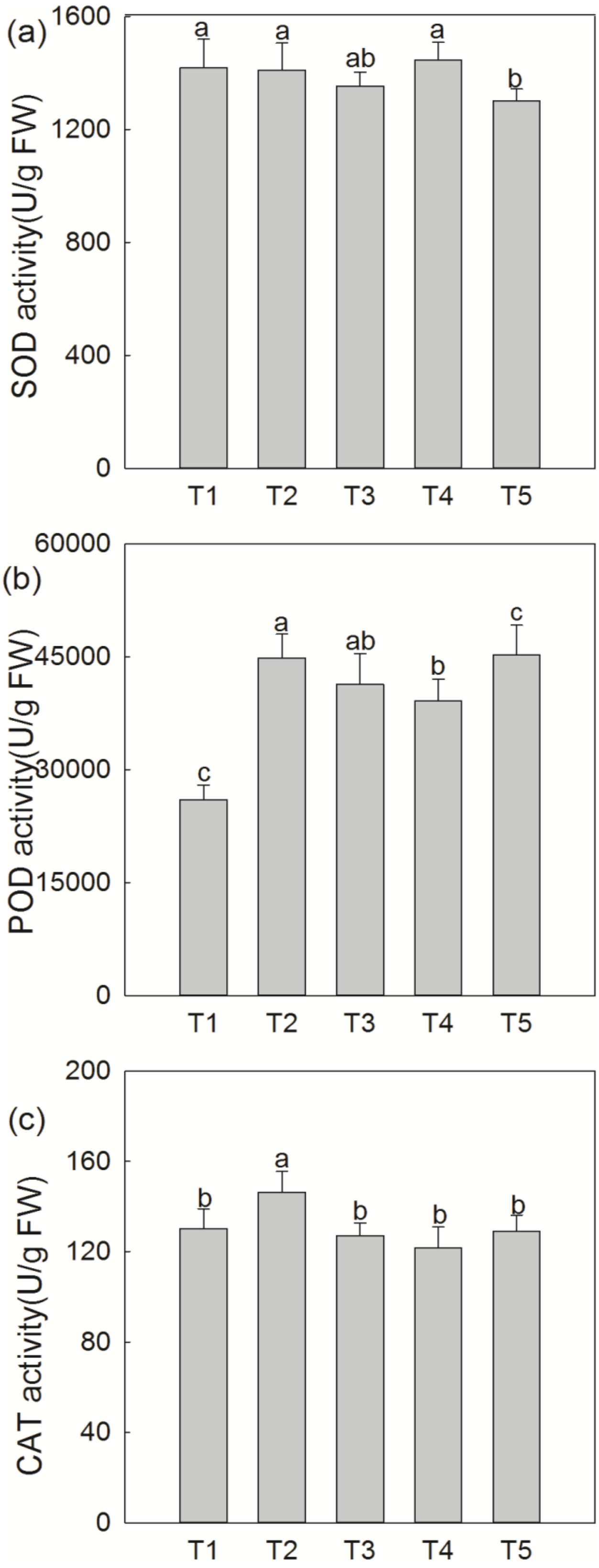

Fig. 4. Effects of exogenousspermidine (Spd) on the activities of SOD (a), POD (b), and CAT (c) in leaves of *Phyllostachys edulis* seedlings under drought stress caused by polyethylene glycol (PEG) application. T1 (control, nutrition solution without PEG or Spd added); T2 (added 20% PEG); T3 (added 20% PEG + 0.0l mmol/L Spd); T4 (added 20% PEG + 0.1 mmol/L Spd); T5 (added 20% PEG + 1.0 mmol/L Spd). For each treatment mean ± SD of three replicates is indicated (n = 3). Bars with different letters indicate significant differences at $p<0.05$.

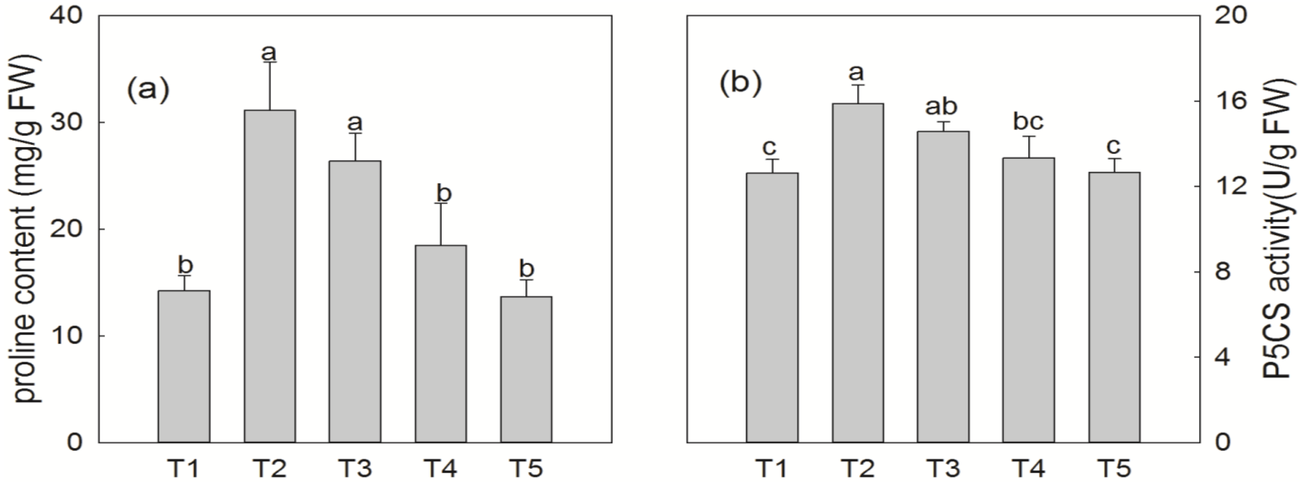

Fig. 5. Effects of exogenousspermidine (Spd) on proline content (a) and activity of P5CS (b) in leaves of *Phyllostachys edulis* seedlings under drought stress caused by polyethylene glycol (PEG) application. T1 (control, nutrition solution without PEG or Spd added); T2 (added 20% PEG); T3 (added 20% PEG + 0.0l mmol/L Spd); T4 (added 20% PEG + 0.1 mmol/L Spd); T5 (added 20% PEG + 1.0 mmol/L Spd). For each treatment mean ± SD of three replicates is indicated (n = 3). Bars with different letters indicate significant differences at $p<0.05$.

Relative water content (RWC) is an important plant characteristic that describe plant water relations. In the present study, exposure of *Phyllostachys edulis* seedlings to drought stress substantially decreased RWC. Similar findings were reported by *Egilla et al.*, who found that RWC, water use efficiency (WUE) and turgor potential of *Hibiscus rosa-sinensis* were decreased under water stress (Egilla *et al*., 2005). Our results showed that exogenous Spd could significantly increase the *Phyllostachys edulis* seedlings leaf RWC under drought stress conditions, and the degree of increase increased with the increase of Spd concentration. This was in agreement with the results of Li *et al.*, who found that the application of exogenous spermidine (Spd) could increase the RWC of creeping bent grass and maintain its chlorophyll content under drought stress, showing that the application of Spd delayed leaf senescence caused by drought stress (Gomes *et al.*, 2010). Under drought stress, plants maintain water absorption by osmotic adjustments, such as proline accumulation (Gomes *et al*., 2010). In the present study, with the 0.1 mM and 1mM Spd treatments, the content of proline was decreased to the control level, which was inconsistent with the results found for the P5CS activity (Fig. 5b). Furthermore, the RWC of the Spd-treated plants was increased under water stress. This effect of Spd may have been mediated by an osmotic adjustment caused by the application of Spd.

Changes in the photosynthetic pigments provide an important physiological index which can be used for observing the physiological changes of plants under drought conditions. In the present study, the content of chlorophyll (a+b) and chla/chlb of *Phyllostachys edulis* seedlings were significantly reduced under drought conditions, while no statistically significant effect was found for the content of Car (Table 1). No significant differences in the photosynthetic pigments could be found with the application of low concentrations of Spd, but for drought stressed plants, the addition of 0.1 mM exogenous Spd significantly increased the chlorophyll content. This suggests that the increase of the chloroplast pigment contents caused by Spd treatment might be due to increase of the activity of enzymes related to photosynthesis, leading either to stimulation of biosynthesis of chlorophyll, or reduction of its degradation, so that as an overall result the photosynthetic performance of the plants was improved (Liu *et al*., 2005).

Under conditions of water stress, reactive oxygen species, such as super-oxide anion radicals, hydrogen peroxide (H_2O_2) and alkoxy radicals (RO), are produced in plants (Yang *et al.*, 2015). Excessive accumulation of reactive oxygen species (ROS) will lead to membrane lipid peroxidation, damage to proteins, nucleic acids other molecular components of the cell, and to damage of the biological membranes. These changes finally lead to the destruction of the structure and function of the cell (Zhao *et al.*, 2008). Plants can protect themselves against the harmful effects of drought by activating the activities of antioxidant enzymes relieving the oxidative stress. The major antioxidant enzymes, such as POD, SOD and CAT, can fundamentally reduce the accumulation of ROS in plants during drought stress. In the present study, unlike SOD, the activities of POD and CAT of the *Phyllostachys edulis* seedlings were increased drastically under drought stress. Under drought stress, the seedlings treated with the exogenous spermidine (Spd) showed lower activities of CAT and POD than the seedlings without such application, so that in the treated seedlings the CAT activity was returned to the control level. Yiu *et al.*, (2009) also reported that exogenous polyamines were able to maintain the activities of antioxidant enzymes through adjusting radical scavenging system in welsh onion (*Allium fitsulosum*) subjected to moderate peroxide stress. In the present study, *Phyllostachys edulis* seedlings with Spd treatments had an increased antioxidant defense capability, resulting in lower production rates of ROS and MDA content, caused the increase of membrane permeability or lose of membrane integrity. The damage of the plasma membrane, the MDA, and the relative electrical conductivity all increased with the increase of drought intensity and duration. In this case, the increase of REC and MDA suggested the conspicuous increase of membrane damage (Fig. 2). Moreover, drought stress increased the contents of H_2O_2 and oxygen radical (Fig. 3). However, Spd treatments reduced the REC and the contents of MDA, H_2O_2 and oxygen radical significantly. Treatments with 0.1 mM and 1 mMSpd decreased REC, MDA and oxygen radical to the normal level, but the 1 mMSpd increased further the H_2O_2 content, suggesting that this highest concentration of Spd caused damage to the plants. We found that under drought stress, exogenous spermidine (Spd) significantly reduced the MDA and relative conductivity of the *Phyllostachys edulis* leaves (Fig. 2). Therefore, any potential increase of growth under drought stress with Spd treatment might result from the increased activities of SOD, CAT and CAT, and this can sustain the membrane integrity to improve the drought resistance of the *Phyllostachys edulis* seedlings. It has also been found that the enhancement of drought resistance was accompanied by the decrease of relative electrical conductivity and the increase of polyamine levels in the plants (Zou *et al*., 2012).

In conclusion, drought leads to a decrease in growth rate, which is related to the reduction of water content, chlorophyll content, and increased generation rate of active oxygen, increased content of MDA, increased activities of POD and CAT, and increased REC. However, exogenous Spd can effectively alleviate a series of changes in the water status, osmotic regulation substances, antioxidant enzyme system and membrane system caused by drought stress through significantly inhibiting the generation of superoxide free radical, reducing the accumulation of proline, lipid per-oxidation and the relative electric conductivity of *Phyllostachys edulis* seedling leaves. This returning in this way the activity of the antioxidant enzymes to the normal level and increasing the Chl(a+b) content. However, the different concentrations of Spd have different adjustment effects, the 0.1 mMSpd being the most suitable concentration. Our experimental results will facilitate further studies of stress resistance mechanisms in *Phyllostachys edulis* seedlings. In this way our results also facilitate the protection and yield improvement of *Phyllostachys edulis*.

Acknowledgments

The authors wish to express their appreciation to State Key Laboratory of Subtropical Silvi culture of Zhejiang A & F University for financial support of this work.

References

Afzal, I., F. Munir, C.M. Ayub, S.M.A. Basra, A. Hameed and A. Nawaz. 2009. Changes in antioxidant enzymes, germination capacity and vigour of tomato seeds in response of priming with polyamines. *Seed Sci. Technol*., 37(2): 765-770.

Alcázar, R., F. Marco, J.C. Cuevas, M. Patron, A. Ferrando, P. Carrasco, A.F. Tiburcio and T. Altabella. 2006. Involvement of polyamines in plant response to abiotic stress. *Biotechnol. Lett.*, 28(23): 1867-1876.

Asada, K. 1999. The water-water cycle in chloroplasts Scavenging of active oxygens and dissipation of excess photons. *Plant Physiol*., 50: 601-639.

Bouchereau, A., A. Aziz, F. Larher and J. Martin-Tanguy. 1999. Polyamines and environmental challenges: recent development. *Plant Sci*., 140(2): 103-125.

Egilla, J.N., F.T.D. Jr and T.W. Boutton. 2005. Drought stress influences leaf water content, photosynthesis, and water-use efficiency of Hibiscus rosa-sinensis at three potassium concentrations. *Photosynthetica*., 43(1): 135-140.

Farooq, M.A., S.M.A. Basra, A. Wahid, Z.A. Cheema, M.A. Cheema and A. Khaliq. 2008. Physiological role of exogenously applied glycinebetaine to improve drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *J. Agron. Crop Sci*., 194: 325-333.

Feng, L.H. and W.H. Hong. 2007. Characteristics of drought and flood in Zhejiang Province, East China: Past and future. *Chin. Geogr. Sci*., 17(3): 257-264.

Flexas, J., J. Bota, J. Galmes, H. Medrano and M. Ribas-Carbo. 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. *Physiol. Plant*., 127(3): 343-352.

Gomes, F.P., M.A. Oliva, M.S. Mielke, A.A.F. Almeida and L.A. Aquino. 2010. Osmotic adjustment, proline accumulation and cell membrane stability in leaves of *Cocos nucifera* submitted to drought stress. *Sci. Hort*., 126(3): 379-384.

Hina, F., Z.K. Shinwari, N. Ahmad and H. Abbasi. 2016. Factors influencing in vitro seed germination, morphogenetic potential and correlation of secondary metabolism with tissue development in *Prunella vulgaris* L. *Pak. J. Bot.*, 48(1): 193-200.

Hussain, S.S., M. Ali, M. Ahmad and K.H. Siddique. 2011. Polyamines: Natural and engineered abiotic and biotic stress tolerance in plants. *Biotechn. Adv*., 29(3): 300-311.

Jaleel, C.A., R. Gopi, B. Sankar, M. Gomathinayagam and R. Panneerselvam. 2008. Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *C R Biol*., 331(1): 42-47.

Lee, T.M. 1997. Polyamine regulation of growth and chilling tolerance of rice (*Oryza sativa* L) roots cultured *In vitro*. *Plant Sci*., 122(2): 111-117.

Li, C.Z., J. Jiao and G.X. Wang. 2004. The important roles of reactive oxygen species in the relationship between ethylene and polyamines in leaves of spring wheat seedlings under root osmotic stress. *Plant Sci*., 166(2): 303-315.

Li, Z., H. Zhou, Y. Peng, X.Q. Zhang, X. Ma, L.K. Huang and Y.H. Yan. 2015. Exogenously applied spermidine improves drought tolerance in creeping bentgrass associated with changes in antioxidant defense, endogenous polyamines and phytohormones. *Plant Growth Regul*., 76(1): 71-82.

Liu, J., Y. Zhou, W. Zhang and Y. Liu. 2005. Effects of exogenous polyamines on chloroplast-bound polymine content and photosynthesis of corn suffering salt stress. *Acta Bot. Boreal-Occid. Sin*., 26(2): 254-258.

Mano, J. 2012. Reactive carbonyl species: Their production from lipid peroxides, action in environmental stress, and the detoxification mechanism. *Plant Physiol. Biochem*., 59: 90-97.

Ozkur, O., F. Ozdemir, M. Bor and I. Turkan. 2009. Physiochemical and antioxidant responses of the perennial xerophyte Capparis ovata Desf. to drought. *Environ. & Exp. Bot*., 66(3): 487-492.

Reddy, A.R., K.V. Chaitanya and M. Vivekanandan. 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *J. Plant Physiol*., 161(11): 1189-1202.

Roychoudhury, A., S. Basu and D.N. Sengupta. 2011. Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of indica rice differing in their level of salt tolerance. *J. Plant Physiol*., 168(4): 317-328.

Shi, H. and Z. Chan. 2014. Improvement of plant abiotic stress tolerance through modulation of the polyamine pathway. *J. Integr Plant Biol*., 56(2): 114-121.

Smirnoff, N. 2010. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol*., 125(1): 27-58.

Takano, A., J.I. Kakehi and T. Takahashi. 2012. Thermospermine is Not a Minor Polyamine in the Plant Kingdom. *Plant & Cell Physiol*., 53(4): 606-616.

Wang, X., G. Shi, Q. Xu and J. Hu. 2007. Exogenous polyamines enhance copper tolerance of *Nymphoides peltatum*. *J. Plant Physiol*., 164(8): 1062-1070.

Yang, L.M., J.C. Fountain, H. Wang, X.Z. Ni, P.S. Ji, R.D. Lee, R.C. Kemerait, B.T. Scully and B.Z. Guo. 2015. Stress sensitivity is associated with differential accumulation of reactive oxygen and nitrogen species in maize genotypes with contrasting levels of drought tolerance. *Int. J. Mol Sci*., 16(10): 24791-24819.

Yiu, J.C., C.W. Liu, D.Y.T. Fang and Y.S. Lai. 2009. Waterlogging tolerance of Welsh onion (*Allium fistulosum* L.) enhanced by exogenous spermidine and spermine. *Plant Physiol. Biochem*., 47(8): 710-716.

Yuming, H.C.Y. 2003. Studies on the bamboo diversity and its conservation in yunan, China. *Sci Silvae Sin*., 1: 025.

Zhao, C.X., L.Y. Guo, C.A. Jaleel, H.B. Shao and H.B. Yang. 2008. Prospectives for applying molecular and genetic methodology to improve wheat cultivars in drought environments. *C R Biol*., 331(8): 579-586.

Zheng, B.S. 2006. *Modern plant physiology and biochemistry research technology*. Chinese Meteoro Press, Beijing.

Zou, J., C.F. Liu, A.L. Liu, D. Zou and X.B. Chen. 2012. Overexpression of OsHsp17.0 and OsHsp23.7 enhances drought and salt tolerance in rice. *J. Plant Physiol*., 169(6): 628-635.

(Received for publication 26 July 2017)