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ORIGINAL PAPER

Phytotoxicity of dimethyl sulfoxide (DMSO) to rice seedlings

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Abstract The physiological responses of dimethyl sulfoxide (DMSO) exposure were investigated in rice (*Oryza sativa* L. cv. XZX 45) seedlings. The seedlings were hydroponically exposed to different concentrations of DMSO for 72 h. Results showed that a linear decrease in relative growth rate and water use efficiency was observed with rice seedlings with increasing DMSO concentrations. The estimation of cell death measured by Evans blue uptake also indicated DMSO-induced damage in root tissues. Negligible decrease in chlorophylls was noted, while significant reduction in carotenoids content was only observed at 13.54 mM DMSO. Although DMSO did not have any significant effect on protein content in roots, the protein content in shoots was significantly decreased in a dose-dependent manner. Proline content in both plant tissues was positively affected by DMSO exposure, responding an inverted U-shaped curve with DMSO concentrations. Results also showed that DMSO-induced accumulation of hydrogen peroxide (H_2O_2) was evident in roots rather than shoots. DMSO did not result in any significant changes in superoxide dismutase and peroxidase activities as well as malondialdehyde content. Catalase (CAT) activity in both roots and shoots was quite sensitive to changes in DMSO treatments than other enzymes, suggesting that CAT may play central role in the detoxification of H_2O_2 in rice seedlings under DMSO exposure. Results suggest that growth inhibition and cell death of rice seedlings caused by DMSO exposure were largely related to the accumulation of H_2O_2 in plant tissues.

Keywords Antioxidant enzyme · DMSO exposure · Growth inhibition · Lipid peroxidation · Rice

Introduction

Over the last three decades, the rapid development of industrial activities has resulted in a substantial increase in the production and utilization of organic chemicals with various physicochemical properties in mainland China. Due to the increasing reliance on these non-desirable chemicals and insufficient waste disposal, pollution derived from anthropogenic activities has imposed a serious risk on environmental and human health. Dimethyl sulfoxide (DMSO), an organosulfur compound having two C–S bonds in its molecular structure, has been widely used in a range of industrial processes since it easily dissolves many organic and inorganic substances (Simo 1998; Murakami et al. 2002; Zhu et al. 2013). Indeed, it has been estimated that the annual world production of DMSO was approximately 5.6 million tons (Murakami et al. 2002; Hwang et al. 2007). In China, the annual consumption is about 7000–8000 tones (Han and Han 2002) and majority has been used in the opto-electronic and integrated circuit packaging industries (Hwang et al. 2007). DMSO was frequently detected in industrial wastewater from the washing or rinsing processes in the manufacture of semiconductors or liquid crystal displays at concentrations of 500–800 mg/L (Murakami et al. 2003; He et al. 2011). Microbial degradation of DMSO through the reduction pathway is a suggestive removal process involved in wastewater treatment of DMSO-containing effluents.

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However, odorous intermediate compounds (i.e., dimethyl sulfide (DMS) and methane thiol (MT)) and the final malodorous hydrogen sulfide (H_2S) are also problematic (Murakami et al. 2002, 2003; Hwang et al. 2007).

It is known that DMSO can be easily accumulated in the water body without any indication of its presence due to its high polarity through anthropogenic inputs (Murakami et al. 2002; Zhu et al. 2013). Therefore, uptake of DMSO by plants may be a possible removal process during the treatment of DMSO-containing wastewater, which may subsequently cause variation of physiological and biochemical process and finally affects growth, nutrient absorption and yield of plants (Kumar et al. 1976; Qiu et al. 2013). It has been reported that responses of plants to various stresses involve a variety of different mechanisms, which may serve to improve and/or control plant functions in multiple ways (Gholami et al. 2012). It has been well documented that increase in reactive oxygen species (ROS), which leads to oxidative stress, is a sensitive phenomenon in plants in response to numerous biotic and abiotic stress conditions (Smeets et al. 2009). In non-stress oxidative conditions, levels of ROS in plant cells are balanced through enzymes as well as metabolites (Halliwell 2006) and plants are able to maintain redox equilibrium (Foyer and Noctor 2005). However, over accumulation of ROS in plants can result in decomposition of cell membrane lipids in plants, chlorophyll bleaching, protein oxidation and damage to nucleic acids (Terzi and Kadioglu 2006; Pandey et al. 2009). Plants have evolved enzymatic defense system and non-enzymatic antioxidants for scavenging and detoxifying ROS. Superoxide dismutase (SOD, EC 1.1.5.1.1) scavenges and catalyzes the conversion of superoxide into hydrogen peroxide (H_2O_2). Peroxidase (POD, EC 1.11.1.7), catalase (CAT, EC 1.11.1.6) and the ascorbate–glutathione pathway are responsible for detoxification of H_2O_2 (Apel and Hirt 2004; Vanhoudt et al. 2010). Additionally, plants possess non-enzymatic antioxidants, including ascorbic acid (AsA), glutathione (GSH), phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols that protect against potentially cytotoxicity of ROS (Gholami et al. 2012). Indeed, increases in antioxidative enzyme activities have been extensively described in a number of phytotoxicity tests with different pollutants (Foyer and Noctor 2005; Smeets et al. 2009; Vanhoudt et al. 2010; Yu and Zhang 2013). It is evident that DMSO has toxic effects on many organisms due to its high osmolarity (Murakami et al. 2002). In our previous work, acute phytotoxicity assay was undertaken using relative growth rate and water use efficiency as variables to determine effective concentration (EC) of DMSO to rice seedlings, in which EC_{50} values for a 50 % inhibition of the relative growth rate of rice seedlings were estimated to be 11.93 mM (48 h) and 5.48 mM (96 h) (Yue et al. 2014). To our knowledge, available studies on DMSO-induced phytotoxicity are still few. Hence, the aim of this study is to investigate metabolic responses of rice seedlings to DMSO by determining growth parameters, photosynthetic pigments, protein and proline content, lipid oxidation, cell viability and key enzyme activities. This work was conducted at the College of Environmental Sciences and Engineering, Guilin University of Technology, P. R. China, from April 2014 to December 2014.

Materials and methods

Test chemicals and experiment design

Plant materials and exposure regime were identical to our previous work (Yu et al. 2014a). Fifteen-day-old rice seedlings (*Oryza sativa* L. cv. XZX 45) with similar height and weight were transplanted to a pre-treatment solution containing 1 mM $CaCl_2$ + 2 mM MES-Tris buffer (pH 6.0) for 4 h to clear the ions from cell wall space (Ebbs et al. 2008), and then ten rice seedlings were transferred into a 50-ml Erlenmeyer flask filled with 50 mL modified ISO 8692 nutrient solution (Yu et al. 2014a) with addition of 10 μM Fe-EDTA. The plants were first conditioned for 24 h to allow adaptation to the new environmental conditions. The flasks were all wrapped with aluminum foil up to the flask mouth to prevent escape of water and to inhibit potential growth of algae inside. All flasks were housed in a plant growth chamber with constant temperature of 25 ± 0.5 °C and a relative humidity of 60 ± 2 % under continuous artificial light. Then, the nutrient solution in each flask was replaced by respective spiked solution, except control.

Dimethyl sulfoxide (CAS No. 67-68-5, 99 % purity) was purchased from Sinopharm Chemical Reagent Co., Ltd. Shanghai, PR China. Nominal concentrations were used in this study.

Six different concentrations were employed. Each selected parameter was measured in four independent biological replicates. Exposure periods were 72 h.

Relative growth rate

Rice seedlings were weighed prior to application and at termination of exposure. The relative growth rate (RGR, %) was calculated using the formula

$$\mathrm{RGR} = \frac{M_{(\mathrm{F})} - M_{(\mathrm{I})}}{M_{(\mathrm{I})}} \times 100$$

where $M_{(\mathrm{I})}$ and $M_{(\mathrm{F})}$ are the initial and final weight (g) of rice seedlings, respectively.

Water use efficiency

Water use efficiency (WUE) (mg biomass/mL water) is the ratio between produced biomass and water transpired (Trapp et al. 2000).

$$\mathrm{WUE} = \frac{M_{(\mathrm{F})} - M_{(\mathrm{I})}}{V_{\mathrm{transpired}}}$$

where $M_{(\mathrm{I})}$ and $M_{(\mathrm{F})}$ are the initial and final weight (mg) of rice seedlings, respectively. $V_{\mathrm{transpired}}$ is the transpiration (mL water) of seedlings calculated by the weight loss of the plant–flask system.

Measurement of pigments and soluble proteins

The content of chlorophylls and carotenoids in shoots was estimated after extraction in 80 % acetone (0.2 g fresh weight in 25 mL) as described previously (Gholami et al. 2012). Soluble proteins in plant materials were measured at 595 nm using bovine serum albumin as standard after trituration in 65 mM phosphate buffer solution (0.2 g FW in 2.5 mL, pH 7.8) (Yu et al. 2014b).

Estimation of lipid peroxidation and hydrogen peroxide

Lipid peroxidation in plant materials was determined from the content of malondialdehyde (MDA), which was estimated by the amount of thiobarbituric acid reactive substances (TBARs) (Wang et al. 2010). Hydrogen peroxide (H_2O_2) content in plant materials was determined using the method (Kumar et al. 2013) with slight modifications. Fresh plant tissues (0.2 g) were precisely weighted and homogenized in a triturator with 5.0 mL of 0.1 % (m/v) trichloroacetic acid (TCA). Trituration was ground in liquid N_2 and then centrifuged at 12,000×g for 15 min at 4 °C. The 1.0 mL of collected supernatant was added to 1.0 mL of 10 mM potassium phosphate buffer (pH 7.0) and 2.0 mL of 1.0 M potassium iodide. The absorbance of the reaction solution was measured at 390 nm. H_2O_2 content was calculated by using a standard curve with known concentrations and expressed as micromoles per gram FW. Soluble sugar content in plant materials was also determined after exposure (Li 2006).

Measurement of proline content

The proline content in plant tissues was determined according to the method (Li 2006; Kumar et al. 2013) with some modifications. An aliquot (0.2 g) of fresh plant materials was homogenized on ice bath in 5 mL of 3 % aqueous sulfosalicylic acid. The homogenate was incubated in water bath at 100 °C for 10 min and then centrifuged at 3000×g for 10 min after cooling. Two milliliters of supernatant was reacted with 2 mL glacial acetic acid and 2 mL of freshly prepared acid ninhydrin reagent (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid). The mixture was incubated in water bath at 100 °C for 30 min, leading to the color change to red. After cooling, the reaction mixture was terminated with addition of 4 mL of toluene. The toluene-chromophore absorbance was measured at 520 nm using toluene as a blank.

Measurement of antioxidative enzyme activities

Plant tissues (0.2 g, fresh weight) were precisely weighted and homogenized in a triturator with 1.8 mL prechilled extraction medium (pH 7.8, containing NaH_2PO_4, Na_2HPO_4, PVPP, EDTA and mercaptoethanol). Trituration was ground in liquid N_2 and then centrifuged at 15,000×g for 15 min at 4 °C (Kumar et al. 2013). The supernatant was collected and assayed. Commercial kits of SOD, CAT and POD were purchased from Nanjing Jiancheng Bioengineering Institute (NJBI, China).

Measurement of root cell death

Cell death was determined spectrophotometrically according to the method (Kumar et al. 2013) with slight modification. Plant roots (0.2 g, fresh weight) were precisely weighted and incubated in Evans blue solution (0.25 %, w/v) for 15 min. After washing with double-distilled water for 30 min, roots were excised and soaked with 3 mL of N,N-dimethyl formamide for 1 h at 25 °C until the trapped Evans blue was released from the excised roots (Kumar et al. 2013). The absorbance of the supernatant was measured at 600 nm.

Statistical analysis

Analysis of variance (ANOVA) and Tukey's multiple range test were used to determine the statistical significance at 0.05 between the treatments (Zar 1999).

Results and discussion

DMSO-induced changes in growth parameters

All DMSO-treated rice seedlings showed a positive growth response (Fig. 1a). However, a linear decrease in relative growth rate (%) was observed with increasing concentrations of DMSO treatments ($R^2 = 0.99$, Fig. 1a). A remarkable reduction in relative growth rate was only detected with rice seedlings exposed to DMSO at higher than or equal to 10.16 mM ($p < 0.05$) in comparison with control rice seedlings. Similarly, water use efficiency of

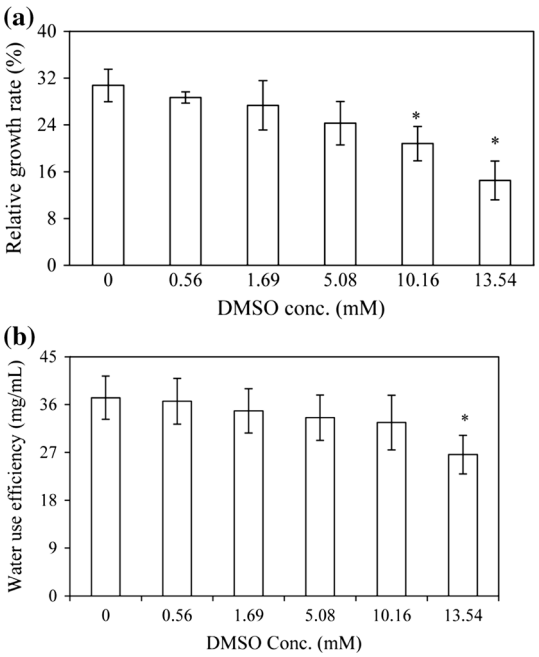

Fig. 1 Measured relative growth rate (**a**) and water use efficiency (**b**) of rice seedlings exposed to different treatments of DMSO. The exposure period was 72 h. Values are mean of four independent biological replicates. *Vertical lines* represent standard deviation. *Asterisk* symbol refers to the significance difference between DMSO treatments and control ($p < 0.05$)

DMSO-treated rice seedlings presented a dose-dependent decrease ($R^2 = 0.88$, Fig. 1b). Decrease in water use efficiency was significant only at 13.54 mM DMSO ($p < 0.05$).

DMSO-induced changes in photosynthetic pigments

Total chlorophyll concentrations in shoots of DMSO-treated rice seedlings decreased gradually ($p > 0.05$) with the increasing DMSO concentrations after 72 h of treatment period (Fig. 2a). Total chlorophylls decreased by 9.74 % at 0.56 mM to 16.74 % at 13.54 mM of DMSO, respectively, in comparison with control. Indeed, visible toxic symptoms of chlorosis were not observed in any of the treatments at the termination of 72-h exposure. On the other hand, carotenoid concentrations were slightly altered in rice seedlings exposed to DMSO concentrations $\leq$10.16 mM ($p > 0.05$) (Fig. 2b), while significant decrease in carotenoid content was detected at 13.54 mM ($p < 0.05$) in comparison with control.

DMSO-induced changes in protein content

Responses of protein content to DMSO exposure in shoots and roots were variable (Table 1). In roots of DMSO-treated rice seedlings, protein content was slightly increased at 0.56–1.69 mM DMSO ($p > 0.05$), but

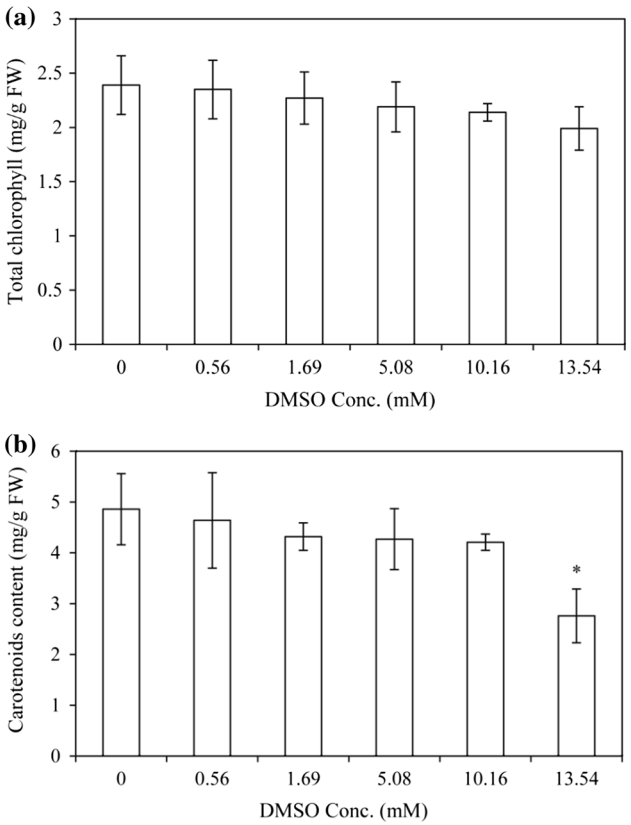

Fig. 2 Measured total chlorophyll content (**a**) and carotenoids content (**b**) in different parts of rice seedlings exposed to different treatments of DMSO. The exposure period was 72 h. Values are mean of four independent biological replicates. *Vertical lines* represent standard deviation. *Asterisk* symbol refers to the significance difference between DMSO treatments and control ($p < 0.05$)

gradually decreased at 5.08–13.54 mM ($p > 0.05$) when compared to control roots, respectively. However, DMSO induced significant decrease in the protein content in shoots ($p < 0.05$) at all DMSO treatments that accounted for 35.03–57.84 % decreases as compared to control.

DMSO-induced accumulation of H_2O_2

The result of H_2O_2 analysis showed that DMSO treatment caused the H_2O_2 accumulation in a dose-dependent fashion in roots ($R^2 = 0.88$; Table 1). Significant increase ($p < 0.05$) in H_2O_2 content in roots was observed at 5.08 mM or higher concentrations, while no differences in H_2O_2 content were noted between control and treatments below 1.69 mM ($p > 0.05$). It is also noted that DMSO induced H_2O_2 accumulation in shoots, but the change was negligible ($p > 0.05$).

DMSO-induced changes in lipid peroxidation

Lipid peroxidation in DMSO-treated rice seedlings was measured by the estimation of MDA content. Our

Table 1 Changes in selected parameters and antioxidative enzyme activities in different materials of rice seedlings exposed to DMSO

	DMSO concentrations (mM)					
	0 (T − 0)	0.56 (T − 1)	1.69 (T − 2)	5.08 (T − 3)	10.16 (T − 4)	13.54 (T − 5)
Shoots						
Soluble protein (μg/g FW)	1426.85 (62.41)	926.99* (214.75)	894.82* (230.67)	819.09* (158.62)	624.15* (101.77)	601.57* (56.03)
Soluble sugar (μmol/g FW)	31.30 (1.11)	30.67 (3.33)	30.97 (2.82)	31.88 (1.79)	32.08 (3.57)	33.06 (2.30)
H_2O_2 content (μmol/g FW)	2.53 (0.27)	2.72 (0.27)	2.75 (0.51)	2.84 (0.62)	2.87 (0.23)	2.63 (0.13)
MDA content (μmol/g FW)	3.17 (0.13)	3.27 (0.30)	3.25 (0.56)	3.27 (0.10)	3.36 (0.28)	3.91* (0.45)
Proline content (μg/g FW)	26.20 (1.35)	32.20* (2.42)	33.29* (1.96)	30.73* (1.46)	28.68 (2.51)	28.41 (2.25)
SOD (U/g FW)	361.34 (10.98)	374.70 (9.09)	392.22 (11.41)	379.19 (15.15)	353.09 (9.54)	347.11 (12.85)
CAT (U/g FW)	84.86 (5.81)	114.96* (5.80)	96.89 (7.31)	78.71 (5.98)	67.05* (2.07)	60.84* (5.24)
POD (U/g FW)	297.37 (5.26)	302.47 (9.88)	331.45 (12.59)	297.60 (6.79)	296.21 (4.87)	290.46 (5.75)
Roots						
Soluble protein (μg/g FW)	145.00 (6.93)	167.65 (15.84)	147.95 (21.71)	137.58 (15.22)	136.63 (29.35)	135.12 (46.33)
Soluble sugar (mmol/g FW)	6.53 (0.32)	6.60 (0.79)	6.69 (0.39)	6.83 (0.48)	6.74 (0.31)	7.68 (1.76)
H_2O_2 content (nmol/g FW)	84.96 (10.96)	97.33 (23.46)	101.24 (14.99)	104.04* (27.66)	124.16* (8.19)	168.12* (14.54)
MDA content (μmol/g FW)	1.98 (0.26)	2.10 (0.29)	2.10 (0.12)	2.25 (0.26)	2.22 (0.16)	2.26 (0.22)
Proline content (μg/g FW)	18.47 (1.81)	20.48 (2.02)	24.35* (2.38)	21.54* (1.19)	20.95 (1.72)	20.89 (0.72)
SOD (U/g FW)	31.84 (2.28)	33.19 (8.89)	34.41* (7.14)	29.25 (4.15)	27.45 (3.31)	24.94* (1.12)
CAT (U/g FW)	15.78 (1.16)	22.54* (1.10)	21.83* (1.43)	21.65* (2.79)	17.18 (1.33)	14.97 (0.37)
POD (U/g FW)	172.50 (8.08)	179.63 (4.92)	186.89 (9.88)	176.79 (8.32)	174.67 (7.48)	158.78 (4.72)

The exposure period was 72 h. Values are mean of 4 independent biological replicates. Numerical values in brackets represent standard deviation. Asterisk symbol refers to the significance difference between DMSO treatment and control ($p < 0.05$)

experimental results showed that DMSO treatments presented a dose-dependent increase in MDA content in both plant materials (Table 1). The MDA content in roots of DMSO-treated rice seedlings was increased insignificantly ($p > 0.05$) in all treated plants in comparison with control. 13.54 mM DMSO significantly increased the level of MDA in shoots compared to control ($p < 0.05$), while the effects of other concentrations of DMSO on the content of MDA in shoots were also negligible ($p > 0.05$).

DMSO-induced accumulation of proline

Out experimental results showed that DMSO enhanced the proline content in both plant materials of rice seedlings, but the changes presented an inverted U-shaped curve with the increasing DMSO concentration (Table 1). The proline content in roots was increased at 0.56 and 1.69 mM DMSO that accounted for 10.88 and 31.84 % increases as compared to control. However, when exposed to higher concentrations of DMSO, the proline concentrations in roots showed dose-dependent reduction. A similar change in proline content was also observed in shoots of DMSO-rice seedlings.

DMSO-induced changes in antioxidant enzyme activities

Activities of antioxidative enzymes such as SOD, POD and CAT were measured in both roots and shoots of rice seedlings exposed to different DMSO concentrations (Table 1). SOD activity showed different responses to DMSO in shoots and roots. The SOD activity in shoots remained almost unchanged in all DMSO treatments (mean 367.94 U/g FW, SD 16.09, $n = 6$). The SOD activity in roots increased at 0.56 and 1.69 mM DMSO that accounted for 4.29 and 39.48 % increases as compared to control. SOD activities in roots showed dose-dependent reduction when exposed to higher concentrations of DMSO (≥5.08 mM DMSO), but no significant difference between control and treatments was observed ($p > 0.05$). In comparison with control, CAT activities in roots significantly increased to 42.84, 38.34 and 37.2 % at 0.56, 1.59 and 5.08 mM DMSO, respectively. The CAT activity in shoots was increased at 0.56–1.59 mM DMSO, but decreased at 5.08–13.54 mM DMSO when compared to control, respectively. It is interesting to note that there was marginal decrease or increase in the POD activity in both plant materials.

DMSO-induced cell death in roots

DMSO-induced viability or cell death in root tissues was measured by Evan blue uptake. Evans blue can pass through ruptured membrane and stain dead cells (Baker and Mock 1994; Koodkaew et al. 2012; Kumar et al. 2013). Results of relative Evans blue uptake (%) showed a positive linear correlation with DMSO concentrations supplied ($R^2 = 0.92$; Fig. 3). Evans blue uptake by root tissues was significantly ($p < 0.05$) increased at 5.08 mM or higher concentrations of DMSO in comparison with control, while no significant differences in Evans blue uptake were observed between control and 1.69 mM DMSO or below ($p > 0.05$).

Biomass growth of young seedlings is one of the most sensitive bioindicators to chemical exposure because of their immature defense systems (Yu et al. 2014a), and hence inhibition of plant growth rates has been considered as a noticeable parameter for the assessment of phytotoxicity to various chemicals (Trapp et al. 2000; Yu et al. 2014a, b). In this study, although a clear decreased trend in relative growth rate and water use efficiency of rice seedlings was detected with increasing DMSO concentrations, neither negative growth in biomass nor visible toxic symptoms of chlorosis were observed in any of the DMSO treatments in comparison with control. Indeed, a negligibly negative correlation of DMSO concentrations with total chlorophyll content in shoot tissues also suggests that the doses of DMSO used have marginal effect on photosynthetic pigments. However, we observed a significant decrease in water use efficiency and carotenoids at the highest DMSO concentration of 13.54 mM, suggesting that 13.54 mM DMSO treatment can interfere with carotenoid biosynthesis and transpiration rates of plants (Kholodova et al. 2011; Mostafa et al. 2014).

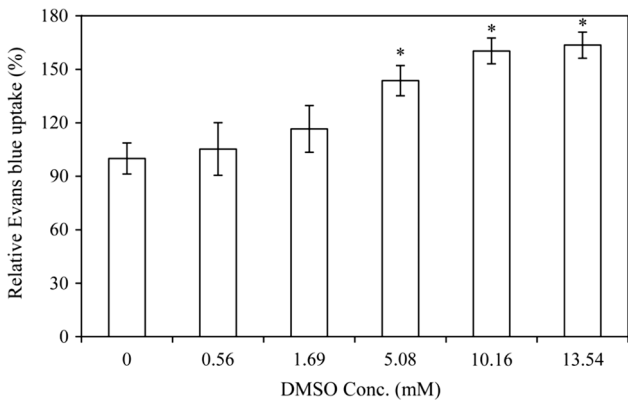

Fig. 3 Effects of different DMSO treatments on Evans blue uptake in roots of rice seedlings. The exposure period was 72 h. Values are mean of four independent biological replicates. *Vertical lines* represent standard deviation. *Asterisk* symbol refers to the significance difference between DMSO treatment and control ($p < 0.05$)

Decreases in soluble protein content in various species of plants under heavy metal stresses have been frequently reported (Singh and Sinha 2005; Ali et al. 2014), which is largely due to inhibition of protein synthesis and/or protein oxidation (Kumar et al. 2013). In this study, although changes in soluble protein in roots were negligible, a dose-dependent decline in protein content in DMSO-treated shoots was observed. MDA followed a similar trend as soluble protein in roots of DMSO-treated rice seedlings. MDA is a cytotoxic product of lipid peroxidation and an indicator of free radical production and consequent tissue damage (Sinha et al. 2007). It is evident that excess of heavy metals stimulated ROS burst which resulted in lipid peroxidation and increased production of MDA in plants (Wang et al. 2009). In this present study, we observed that DMSO even at 10.16 mM did not cause any significant increase in MDA production, suggesting an indirect or undetectable effect of DMSO on the plasma membrane of rice cells.

Proline is a multifunctional amino acid, which is frequently reported to accumulate in plant cells in response to various biotic and abiotic stresses (Kumar et al. 2013). Importantly, the role of proline in stress tolerance in terms of growth and physiology has been suggested, by which plant cells combat non-enzymatically against free radicals generated (Sharmila and Saradhi 2002). In our experiment, DMSO-treated rice seedlings showed enhanced proline accumulation in both plant tissues under any level of DMSO treatments in comparison with control. It is obvious that proline content showed an inverted U-shaped dose–response curve, where the maximum increase in proline accumulation was observed at 1.69 mM. Results of proline analysis suggest that increases of proline in plant materials may play an important role in DMSO tolerance of rice seedlings, and eventually rice seedlings are able to keep their normal function under DMSO exposure.

The carotenoids are a large family of fate-soluble antioxidant and are supposed to act as free radical scavengers by electron transfer to their double-bond structure and to play roles in the protection of chlorophylls in stressed plants (Wang et al. 2010). It has been proposed that elevated ROS levels can damage the photosynthetic apparatus (Apel and Hirt 2004). In this study, remarkable reduction in the content of carotenoids was only observed at 13.54 mM DMSO treatment with respect to control. As the imposed DMSO did not induce H_2O_2 accumulation in shoots of rice seedlings significantly, no direct evidence to proof the depletion of carotenoids in shoots is due to an oxidative damage.

Accumulation of ROS in plant cells is an alarming signal corresponding to oxidative stress (Smeets et al.

2009), and the levels of ROS are controlled by antioxidant enzymes or antioxidants in plants (Apel and Hirt 2004; Mi et al. 2014). In our experiment, the increased, decreased and unchanged antioxidant enzyme activities in both roots and shoots of rice seedlings were observed in this current work. It is known that SOD, CAT and POD are the main ROS-quenching enzymes in plant antioxidative defense systems. SOD can catalyze the dismutation of superoxide radicals, which act as precursors to other ROS (H_2O_2) (Alscher et al. 2002). We observed exposure to DMSO-induced negligible alternation of SOD activities in both roots and shoots of rice seedlings, with one exception of SOD activities in roots at 1.69 mM DMSO treatment, suggesting that superoxide radicals induced by DMSO were able to convert into H_2O_2 quickly without over load in plant cells. Indeed, the H_2O_2 accumulation in roots of rice seedlings exposed to DMSO was evident in our current experiment. CAT and POD catalytically scavenge H_2O_2 and provide the necessary defenses. POD is located in the cytosol, cell wall, and vacuolar and extracellular spaces, while CAT is mainly located in peroxisomes and mitochondria (Mishra et al. 2006; Mi et al. 2014). It is interesting to note that DMSO slightly affected POD activities in both plant materials, while CAT activities were altered significantly, suggesting that CAT may carry more weight for scavenging H_2O_2 associated with DMSO exposure in rice seedlings.

Conclusion

Our results showed that DMSO exposure induced oxidative stress in rice seedlings, resulting in accumulation of H_2O_2 in roots. Since induction of SOD and POD activities in response to DMSO exposure was independent of accumulation of H_2O_2, CAT probably plays a central role in the H_2O_2 detoxification in rice seedlings. Our results also suggested that accumulation of proline in plant materials may be an adaptive strategy for rice seedlings against DMSO toxicity. In conclusion, it is suggestive that DMSO exposure resulted in phytotoxicity on biomass growth and viability in roots, most likely due to the accumulation of H_2O_2.

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Compliance with ethical standard

Conflict of interest The authors declare no conflict of interest.

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