



**Full Length Article**

## Exogenous Selenium Alleviates Mercury Toxicity by Preventing Oxidative Stress in Rice (*Oryza sativa*) Seedlings

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### Abstract

The present study investigated the possible mediatory role of selenium (Se) in alleviating mercury stress on rice and its corresponding physiological mechanism. The effects of exogenous selenium of different concentrations (0, 1, 2, 3, 4  $\mu\text{mol/L}$ ) on growth, osmotic regulation, leaf protective enzymes and active oxidative damage of rice seedlings under 0.5 mg/L Hg stress were studied in nutrient solution hydroponics test. The results showed that low concentration of selenium (0~2.0  $\mu\text{mol/L}$ ) could promote dry matter accumulation of rice seedlings, increase chlorophyll content and superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), activity of ascorbic acid oxidase (APX), Glyoxalase I (GlyI), Glyoxalase II (GlyII), proline and soluble sugar content and decrease  $\text{Na}^+/\text{K}^+$  value. However, with the increase of selenium concentration, chlorophyll, proline and soluble sugar contents decreased and activities of SOD, POD, CAT, APX, GlyI and GlyII decreased, while contents of malondialdehyde (MDA) and superoxide anion ( $\text{O}_2^-$ ), plus  $\text{Na}^+/\text{K}^+$  began to increase. Exogenous selenium has a dose effect in alleviating the Hg stress of rice seedling. Appropriate amount of exogenous selenium could effectively alleviate mercury stress on rice and exogenous selenium of 2.0  $\mu\text{mol/L}$  has the best effect. The main mechanism is that selenium improves the activities of various protective enzymes in rice leaves, effectively reduces content of reactive oxygen species and malondialdehyde, reduces oxidative damage to rice activity, enhances antioxidant capacity and osmotic adjustment ability of rice and thus improves adaptability of rice seedling to mercury stress and reducing the negative consequences of oxidative stress caused by heavy metal toxicity. © 2017 Friends Science Publishers

**Keywords:** Selenium; Mercury; Oxidative stress; Physiology mechanism

### Introduction

Mercury (Hg) pollution of soil is one of the major problems in soil ecological environment degradation. Hg content in soil is 0.001~45.9 mg/kg in China with average content at 0.038 mg/kg. In China's major mercury mining areas such as Guizhou, Hunan, Guangdong, soil pollution around mercury mine is serious. For instance, mercury content in soil of Wanshan area, Guizhou is up to 130 mg/kg and mercury content of rice is up to 0.569 mg/kg far beyond the nationally specified maximum mercury content of food of 0.02 mg/kg. Mercury contamination not only increases selenium content of rice grains which damages the human body but also causes serious crop reduction (Luo *et al.*, 2015). It is because mercury stress causes water deficit in cell and damages chloroplasts and membrane lipid (Wani *et al.*, 2016), which in turn affects photosynthesis rate and supply of assimilation products leading to plant death.

Selenium (Se) is a micronutrient element essential for humans and animals, which is beneficial to growth and development of higher plants (Kaur *et al.*, 2014). Selenium can also enhance plant stress resistance (Feng *et al.*, 2013), improve plant adaptability to environmental stress of

drought (Proietti *et al.*, 2013), ultraviolet radiation (Yao *et al.*, 2013), high temperature (Djanaguiraman *et al.*, 2010), water (Wang, 2011) and desiccation (Pukacka *et al.*, 2011).

Under the condition of environmental stress a higher amount of methylglyoxal (Yadav *et al.*, 2008) will be accumulated in the plant. It has been reported that overexpression of glyoxalase and glyoxalase genes in tobacco and rice can significantly increase tolerance of crops to mercury stress (Singla-Pareek *et al.*, 2008). The plant can detoxify methylglyoxal with glyoxalase system. This system contains two enzymes, glyoxalase I (GlyI) and glyoxalase II (GlyII), which are responsible for conversion of methylglyoxal to lactic acid. Studies have shown that glyoxalase I must be combined with glutathione (GSH) to fulfill methylglyoxal detoxification under mercury stress (Diao *et al.*, 2014). Accordingly, we speculate that selenium may stimulate GSH synthesis, thus affecting the role of glyoxylase detoxification system of plant. However, so far, selenium regulation of plant ability to resist mercury stress through non-enzymatic and enzymatic channels is rarely reported and that exogenous selenium enhances plant tolerance to mercury by regulating glyoxylase system has not been reported, which means great significance for

expansion of selenium regulation of antioxidant defense system and for glyoxylase system mechanism in enhancing plant tolerance to mercury. In this study, rice was used as the test material to explore the role of exogenous selenium in reducing mercury stress of rice seedlings and its possible physiological mechanism, which could provide theoretical and technical support for improving mercury tolerance of plants.

## Materials and Methods

### Plant Material and Growth Conditions

The rice varieties tested were "Xiushui 48", which was provided by institute of soil science, Chinese Academy of Sciences. The rice seeds were surface sterilized by 10% H<sub>2</sub>O<sub>2</sub> for 30 min and then allowed to germinate on moist filter paper in the dark. After a true leaf appeared, it was transplanted to a 3 liter porcelain basin, with 4 seedlings per pot. Then, cultured with 1/2 Hoagland-Arnon nutrient solution, pH of the nutrient solution was adjusted to 5.8 the rice was treated into two concentrations of 0 and 0.5 mg/L for Hg after it grow into two leaves and one heart. To avoid the impact of mercury, mercury concentration increased by 0.1 mg/L Hg per day, until the predetermined concentration. The Hoagland-Arnon nutrient solution was subjected to mercury stress treatment (0.5 mg/L Hg) different concentrations of selenium solution (0, 1, 2, 3, 4 μmol/L) was added to the solution (Table 1) and each treatment was repeated four times. Replace nutrient solution daily during the treatment provide continuous oxygen supply with a ventilation pump and maintain the treatment for 8 days. Nutrient solution hydroponics was taken in the test and performed in plant nutrition laboratory of College of Resources and Environment, Southwest University, Peoples Republic of China.

The greenhouse conditions were as follows: daily illumination time was 16 h light intensity was 300 μmol m<sup>-2</sup>•s<sup>-1</sup>, daytime temperature was 25°C the nighttime temperature was 20°C, relative humidity was 67% and samples were harvested on the 8<sup>th</sup> day and immediately stored in liquid nitrogen. In each treatment group, four plants were examined for biochemical analysis.

### Determination and Methods

Determination of dry weight of rice: 8 days after treatment of hydroponic mercury, harvest and wash the plant, divide the plant into three parts of root, stem and leaf to be fixed at 105°C for 30 min dried at 60°C to constant weight.

Determination of chlorophyll in rice: treat the hydroponic mercury for 8 days completely expand the rice leaves was mixed acetone and ethanol in a ratio of 1: 1 for digestion and the extract liquid was subject to colorimetric determination at 645 and 663 nm with a spectrophotometer.

Plasma membrane permeability measurement refers to methods used by Min *et al.* (2004) to determine the relative conductivity.

Determination of protective enzyme activity in rice seedling leaves: Determination of POD activity was conducted with guaiacol method with change in absorbance per minute to indicate size of enzyme activity. Determination of CAT activity was assayed by the decomposition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) according to Aebi (1984). The activity of SOD was measured by nitroblue tetrazolium (NBT) photochemical reduction method with inhibition of 50% NBT photochemical reduction reaction as an enzyme activity unit (Beauchamp and Fridovich, 1971). Determination of APX activity followed method described by Nakano and Asada (1981).

Determination of MDA and O<sup>2-</sup>: MDA content was determined by thiobarbituric acid method (Li, 2000). According to the determination method of production rate of superoxide anion (O<sup>2-</sup>) (proposed by Wang and Luo, 1990), nmol•g<sup>-1</sup> FW•min<sup>-1</sup> was used to represent production rate of O<sup>2-</sup>.

Determination of proline content was conducted with sulfosalicylic acid method (Li, 2000) and determination of soluble sugar content was conducted with anthrone colorimetric method (Li and Wang, 2002).

Determination of glyoxalase I was conducted with method proposed by Hossain *et al.* (2010) and determination of glyoxalase II was conducted with method proposed by Principato *et al.* (1987).

### Data Analysis

All data were processed and mapped using Microsoft Excel and SPSS18.0 software was used for significant difference test (LSD method).

## Results

### Effects of Selenium on Growth of Rice Seedlings under Hg Stress

Dry weight of aboveground and underground part of rice seedlings treated alone with 0.5 mg/L Hg (T1) is lower than that of the control (T0) (Table 2). The dry weight of aboveground and underground parts of rice seedlings increased with the increase of selenium concentration after selenium treatment was added. When the selenium concentration was 2.0 μmol/L dry weight of aboveground and underground parts of rice seedlings reached the maximum value at the same time. When concentration of exogenous selenium was increased to 4.0 μmol/L dry weight of aboveground and underground parts of rice seedlings decreased gradually but still higher than that of (T1) treated with mercury alone.

**Table 1:** Experimental treatments and their codes

Code of treatment	Hg concentration (mg/L)	Se concentration ( $\mu\text{mol/L}$ )
T0	0	0
T1	0.5	0
T2	0.5	1.0
T3	0.5	2.0
T4	0.5	3.0
T5	0.5	4.0

**Table 2:** Effects of exogenous selenium application on the growth of rice seedlings under Hg stress

Code of treatment	Shoot dry weight (g/plant)	Root dry weight (g/plant)
T0	5.23 $\pm$ 0.15 a	1.34 $\pm$ 0.10 b
T1	3.36 $\pm$ 0.23 b	0.72 $\pm$ 0.05 c
T2	4.58 $\pm$ 0.21 ab	1.24 $\pm$ 0.10 b
T3	6.13 $\pm$ 0.22 a	2.24 $\pm$ 0.17 a
T4	4.75 $\pm$ 0.22 ab	1.26 $\pm$ 0.15 b
T5	4.25 $\pm$ 0.14 ab	1.06 $\pm$ 0.14 b

Note: Different letters within the same column indicate significant difference at 5% level. (mean $\pm$ SD; n=3)

**Table 3:** Effects of exogenous selenium on the chlorophyll content in leaves of rice seedlings under Hg stress

Code of treatment	Chlorophyll a content (mg/g FW)	Chlorophyll b content (mg/g FW)	Chlorophyll (a+b) content (mg/g FW)
T0	1.204 $\pm$ 0.003 a	2.289 $\pm$ 0.015 a	3.493 $\pm$ 0.018 a
T1	0.753 $\pm$ 0.002 b	1.856 $\pm$ 0.003 b	2.609 $\pm$ 0.005 b
T2	1.111 $\pm$ 0.022 a	2.204 $\pm$ 0.004 a	3.315 $\pm$ 0.026 a
T3	1.232 $\pm$ 0.012 a	2.345 $\pm$ 0.011 a	3.577 $\pm$ 0.023 a
T4	1.224 $\pm$ 0.011 a	2.325 $\pm$ 0.005 a	3.549 $\pm$ 0.016 a
T5	1.057 $\pm$ 0.013 a	2.071 $\pm$ 0.025 a	3.128 $\pm$ 0.038 a

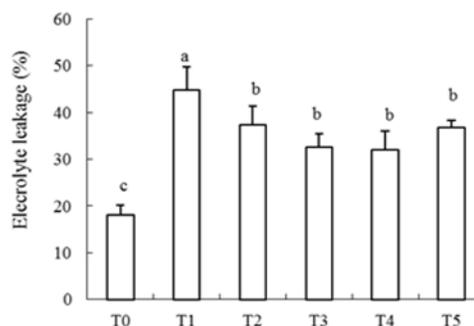
Note: Different letters within the same column indicate significant difference at 5% level. (mean $\pm$ SD; n=3)

### Effects of Selenium on Chlorophyll Content in Leaves of Rice Seedlings under Hg Stress

As shown in Table 3 chlorophyll a and chlorophyll b contents of each treatment show a consistent trend and the difference between treatments is insignificant. The chlorophyll content of 0.5 mg/L Hg alone (T1) was lower than that of the control (T0). When the selenium concentration was less than 2.0  $\mu\text{mol/L}$  chlorophyll content increased with the increase of selenium concentration. When the selenium concentration was 2.0  $\mu\text{mol/L}$  chlorophyll content was 2.3% higher than that of the control treatment (T0) and reached the maximum but then began to decrease with the increase of selenium concentration; chlorophyll content when selenium concentration was 3.0  $\mu\text{mol/L}$  was lower than that of the control treatment (T0). In addition, under the condition of mercury stress chlorophyll content of rice seedling leaves was higher after addition of selenium treatment than that treated with 0.5 mg/L Hg alone (T1).

### Effects of Selenium on Cell Membrane Permeability of Rice Seedlings under Hg Stress

The relative conductivity is an important indicator of cell

**Fig. 1:** Effects of exogenous selenium on cell membrane permeability of rice seedlings under Hg stress (mean $\pm$ SD; n=3, the same below)

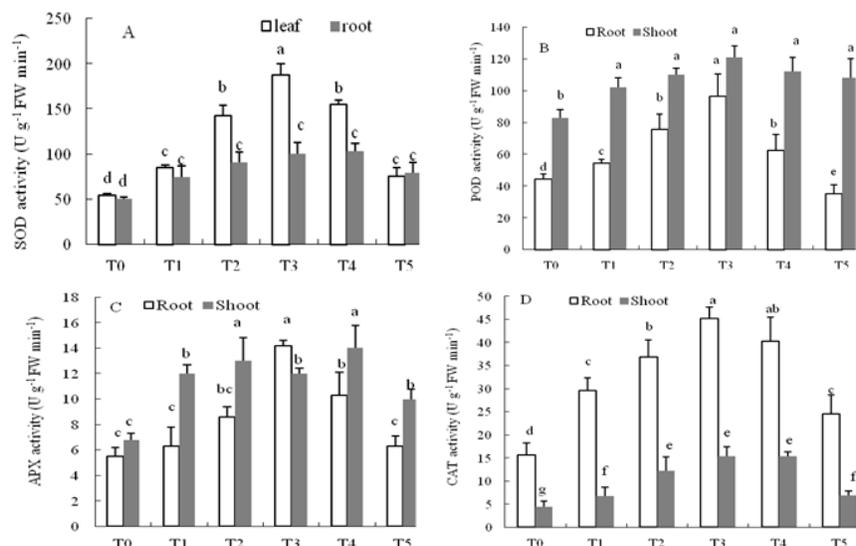
membrane permeability. Leaf relative conductivity of rice seedlings was significantly increased under Hg stress and that of mercury stress T1 was 2.49 times higher than that of the control. Selenium addition lowered electrolyte leakage rate, with T2, T3, T4 and T5 2.08, 1.80, 1.78 and 2.04 times respectively that of the control. Fig. 1 shows that appropriate increase in selenium concentration of solution can stabilize the cell membrane structure, inhibit external leakage of cell electrolyte and effectively alleviate the increase in rice membrane permeability under mercury stress.

### Effects of Selenium on Protective Enzyme Activities in Rice Seedlings under Hg Stress

Exogenous selenium treatment has significant effect on leaf SOD, POD, CAT and APX activities of rice seedlings. With the increase of selenium concentration, activities of four enzymes increased first and then decreased, indicating that selenium has dose effect on activity of the four enzymes in the leaves of rice seedlings, low concentration promotes enzyme activity, while high concentration inhibits enzyme activity (Fig. 2).

Leaf SOD activity of rice seedling treated with 0.5 mg/L Hg alone (T1) was significantly higher than that of (T0) untreated with Hg stress. Compared with T1, 1.0~3.0  $\mu\text{mol/L}$  selenium treatment (T2~T4) could significantly increase SOD activity. Wherein activity under selenium 2  $\mu\text{mol/L}$  treatment (T3) was the highest, increasing by 224.20% compared to T1. There was no significant difference between 4.0  $\mu\text{mol/L}$  selenium treatment (T5) and T1. SOD activity in root system of rice was lower than that of leaf and 1.0~3.0  $\mu\text{mol/L}$  selenium increased activity of SOD in roots, but the difference was insignificant (Fig. 2A).

The POD activity of rice roots was significantly higher than that of leaves and root POD activity of rice seedlings increased significantly after Hg stress, POD activity in roots continued to increase after selenium addition but there was no significant difference in POD activity between selenium treatments (Fig. 2B).



**Fig. 2:** Effects of exogenous selenium on SOD (A), POD (B), APX (C) and CAT (D) activities in leaves and roots of rice seedlings under Hg stress

The APX activity in rice roots was significantly higher than that in leaves. Hg stress significantly increased root APX activity of rice seedlings and APX activity in the root system continued to increase after selenium was added. Selenium treatments of T2, T3 and T4 had the greatest effect on APX activity in roots and difference among the three was insignificant (Fig. 2C).

The CAT activity of rice root was significantly lower than that of leaf. Hg stress increased CAT activity of root system of rice seedling and CAT activity of root system continued to increase after selenium was added. Selenium treatments of T2, T3 and T4 had the greatest effect on CAT activity in roots and difference among the three was insignificant (Fig. 2D).

### Effects of Selenium on Gly I and Gly II in Rice Seedlings under Hg Stress

As shown in Fig. 3 addition of exogenous selenium has a significant effect on activity of Gly I and Gly II in the leaves of rice seedlings. With the increase of selenium concentration activities of the four enzymes increased first and then decreased indicating that selenium has a dose effect on activities of the above two enzymes in leaves of rice seedlings low concentration promotes enzyme activity, while high concentration inhibits enzyme activity.

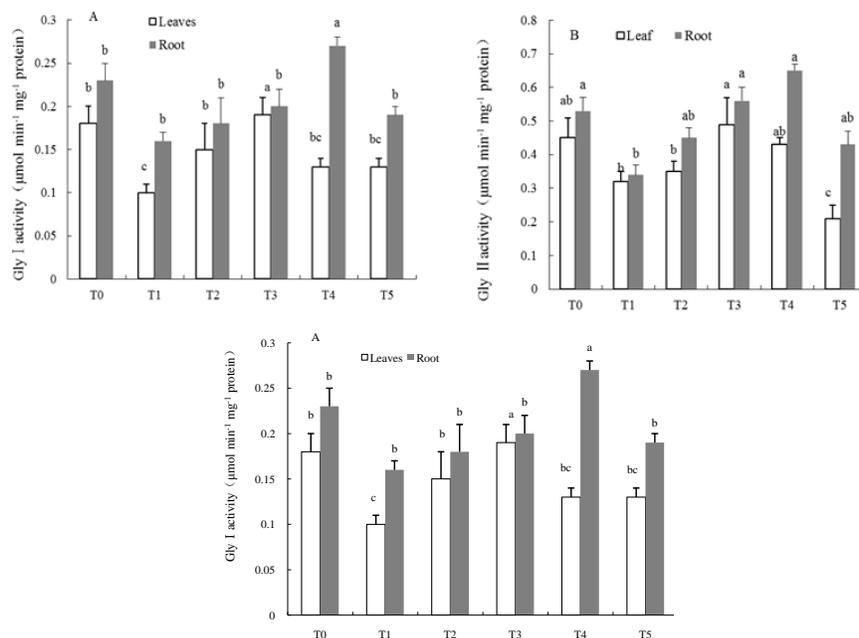
As shown in Fig. 3A leaf Gly I activity treated with 0.5 mg/L Hg alone (T1) was significantly lower than that untreated with Hg stress (T0). Compared with T1, 1.0~4.0  $\mu\text{mol/L}$  selenium treatment (T2~T5) could significantly improve Gly I activity and selenium 2.0  $\mu\text{mol/L}$  treatment (T3) had the highest activity, with no significant difference from that of treatment T0. Under Hg stress, Gly I activity of rice root was higher than that of leaves, Gly I activity was significantly decreased under Hg stress, which restored after

selenium was added. In particular, Gly I activity of T4 treatment was the highest.

As shown in Fig. 3B leaf Gly II activity treated with 0.5 mg/L Hg alone (T1) is significantly lower than that untreated with Hg stress (T0). Compared with T1, 1.0~4.0  $\mu\text{mol/L}$  selenium treatment (T2~T4) could significantly improve Gly II activity and selenium 2.0  $\mu\text{mol/L}$  treatment (T3) had the highest activity. Gly II activity of selenium treatment (T5) was significantly lower than that of T1 and T0 treatments. Under Hg stress, Gly II activity of rice root was higher than that of leaves, Gly II activity was significantly decreased under Hg stress, which restored after selenium was added. In particular, Gly II activity of T4 treatment was the highest.

### Effects of Selenium on MDA and O<sup>2-</sup> in Rice under Mercury Stress

As shown in Fig. 4A leaf MDA content of rice seedlings treated with 0.5 mg/L Hg alone (T1) is significantly higher than that untreated with Hg stress (T0), increasing by 116.11%. Compared with T1, selenium treatment of different concentrations could decrease MDA content under Hg stress. Wherein, T2 and T3 had the lowest contents, which were 56.30% and 55.01%, respectively lower than that treated with Hg alone (T1). After selenium concentration was higher than 2.0  $\mu\text{mol/L}$ , MDA content increased with the increase of selenium concentration. The degree of membrane lipid peroxidation in leaves and roots of rice was increased under Hg stress and damage to leaves was more serious than that to roots. Membrane lipid peroxidation products and membrane permeability were consistent in response to stress, showing increased cell membrane permeability, electrolyte extravasation, accumulation of membrane lipid peroxidation product.



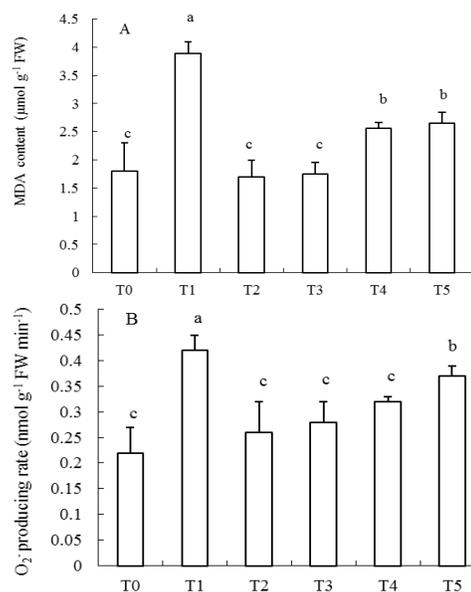
**Fig. 3:** Effects of exogenous selenium on Gly I (A), Gly II (B) activities in leaves and roots of rice seedlings under Hg stress

The determination results of  $O_2^-$  release rate shows that (Fig. 4B), (T1) treated with 0.5 mg/L Hg alone significantly promotes release of  $O_2^-$  compared to (T0) untreated with Hg stress. Compared with T1 selenium treatment of different concentrations could significantly reduce  $O_2^-$  release rate under Hg stress. Among the four selenium concentrations,  $O_2^-$  production rate of T2 and T3 was the lowest, which decreased by 38.10% and 33.33% respectively compared with (T1) treated with Hg alone. After selenium concentration was higher than 3.0  $\mu\text{mol/L}$  (T3),  $O_2^-$  release rate increased with the increase of selenium concentration.

#### Effects of Selenium on Proline and Soluble Sugar Contents in Rice Seedlings under Mercury Stress

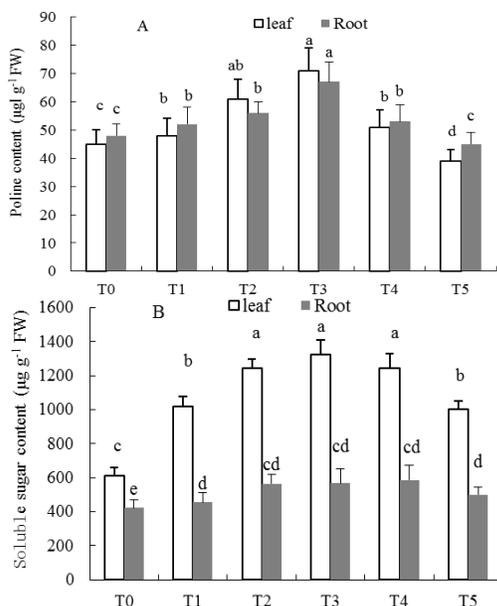
As can be seen from Fig. 5A content of proline treated with 0.5 mg/L Hg alone (T1) was significantly higher than that untreated with Hg stress (T0). Compared with T1, 1.0–3.0  $\mu\text{mol/L}$  selenium treatment (T2, T3 and T4) could significantly increase proline content under Hg stress and selenium 2.0  $\mu\text{mol/L}$  treatment (T3) had the highest proline content. When selenium concentration continued to increase proline content decreased significantly. Selenium 4.0  $\mu\text{mol/L}$  treatment (T5) had the lowest proline content. Hg stress increased proline content in roots, proline content increased significantly after selenium supplementation and T3 treatment had the highest proline content in root.

As shown in Fig. 5B soluble sugar content treated with 0.5 mg/L Hg alone (T1) was significantly higher than that untreated with Hg stress. Compared with T1, 1.0–3.0  $\mu\text{mol/L}$  selenium treatment had no significant difference in

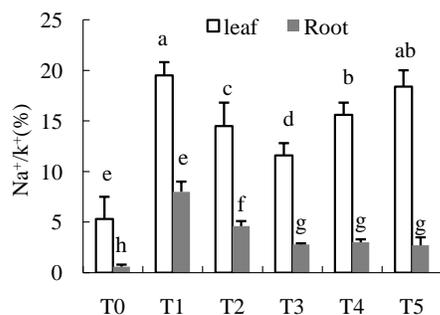


**Fig. 4:** Effects of exogenous selenium on the MDA (A) and  $O_2^-$  (B) contents in leaves and roots of rice seedlings under Hg stress

soluble sugar content. Wherein, selenium 0.5  $\mu\text{mol/L}$  treatment (T3) had the best effect, with increase of 29.42% compared with (T1) treated with Hg alone. The content of soluble sugar in root was significantly lower than that in leaves and soluble sugar increased under Hg stress. Soluble sugar content increased gradually after selenium supplementation but the difference was insignificant.



**Fig. 5:** Effects of exogenous selenium on proline (A) and soluble sugar (B) contents in leaves and roots of rice seedlings under Hg stress



**Fig. 6:** Effects of exogenous selenium on Na<sup>+</sup>/K<sup>+</sup> in leaves of rice seedlings under Hg stress

**Effects of Selenium on Na<sup>+</sup>/K<sup>+</sup> in Leaves and Roots of Rice Seedlings under Mercury Stress**

It can be seen from Fig. 6 that leaf Na<sup>+</sup>/K<sup>+</sup> value of rice seedling was only 5.3% under normal growth conditions, which, however significantly increased to 19.5% when subjected to mercury stress. The Na<sup>+</sup>/K<sup>+</sup> value was decreased after addition of exogenous selenium (1.0~4.0 µmol/L). Wherein, Na<sup>+</sup>/K<sup>+</sup> value was 11.6% when 2.0 µmol/L exogenous selenium was added (T3). Na<sup>+</sup>/K<sup>+</sup> value continued to increase with the increase in exogenous selenium concentration. At addition of 4.0 µmol/L exogenous selenium (T5), Na<sup>+</sup>/K<sup>+</sup> value was not significantly different from that of (T1) treated with 0.5 mg/L Hg alone. Hg stress significantly increased Na<sup>+</sup>/K<sup>+</sup> values in the root and Na<sup>+</sup>/K<sup>+</sup> value of roots decreased significantly after selenium supplementation, but

differences of T3, T4 and T5 treatments were insignificant.

**Discussion**

In this experiment, chlorophyll content was significantly decreased under mercury stress, which might be due to oxidative damage of accumulated reactive oxygen species caused by mercury stress, as it accelerated degradation of these pigments and severely inhibited the growth. Selenium could reduce damage to plants due to environmental stress like drought and heavy metals (Feng *et al.*, 2013) promote plant growth and development (Kaur and Nayyar, 2015). Under mercury stress, accumulation of reactive oxygen species in plants is one of the main mechanisms leading to mercury damage. MDA is considered as an oxidation product of fatty acids such as linseed oil and linoleic acid in membrane lipid, which could be used as a sensitive indicator of oxidative damage to cell (Hawrylak-Nowak, 2009). This study showed that activities of the four enzymes treated with 0.5 mg/L Hg alone were significantly increased and O<sup>2-</sup> and MDA contents were also significantly increased. After exogenous selenium was added, activities of the four enzymes significantly increased while O<sup>2-</sup> and MDA contents in rice decreased significantly. It has been reported that MDA content in the leaves has a good correlation with O<sup>2-</sup> (Li *et al.*, 2016), indicating that selenium has a good protective and repair effect on plant cell membrane. The reason may be that selenium can reduce plant absorption of mercury, thereby reducing the damage caused by mercury stress on plants (Zhang *et al.*, 2012). Protective enzymes like SOD, CAT and POD have in vivo synergistic effect on the plant, which remove excess reactive oxygen species in adversity stress, maintain metabolic balance of reactive oxygen species, so that plants tolerate, resist mercury damage to a certain extent (Hossain *et al.*, 2010). Under stress conditions, selenium has been demonstrated as an antioxidant for mung bean crops (Feng *et al.*, 2013), which supports our experiment results. In this study, it was found that use of exogenous selenium could significantly increase activity of SOD, POD, APX and CAT significantly reduce the levels of MDA and O<sup>2-</sup> then eliminate ROS and reduce oxidative damage of ROS to the cells, thus effectively alleviating oxidative damage of Hg stress to rice seedling leaves. It has been demonstrated that GPX (glutathione peroxidase) is a typical selenium-regulated oxidase in animals, which can effectively remove reactive oxygen species from animals and that selenium could significantly increase GPX gene expression and enzyme activity. In the plant, glutathione peroxidase (NnGPX) gene was overexpressed in the lotus under mercury stress and NnGPX had an important effect on plant resistance to mercury stress. NnGPX transgenic rice has stronger resistance to mercury stress (Diao *et al.*, 2014). Hence, whether selenium in the plant could regulate

NnGPX gene expression to enhance the plant's ability to resist mercury stress remains to be further studied.

This study showed that 0.5 mg/L Hg stress promoted accumulation of proline and soluble sugar in the leaves and roots of rice seedlings. The plant had an adaptive regulatory mechanism. After 0~2.0  $\mu\text{mol/L}$  exogenous selenium was added to the culture medium, soluble sugar content was significantly increased. Hawrylak-Nowak (2009) also reported that selenium could increase ability of plants to resist mercury stress. 5  $\mu\text{M}$  selenium could significantly increase proline content in leaves of cucumber seedlings under mercury stress. Selenium protected membrane lipid against peroxidation, mainly because low concentration of selenium had antioxidant capacity (Hawrylak-Nowak, 2009; Faiz *et al.*, 2015). This indicated that alleviation effect of selenium on oxidative damage induced by Hg stress in rice seedlings was related to accumulation of proline and soluble sugar. The addition of selenium further enhanced the ability of plants to resist mercury stress. Under Hg stress, plants synthesize small molecules of organic matter to enhance their osmotic adjustment and improve water conditions (Kaur *et al.*, 2014), which in turn affected plant adaptability to stress. This was the result of antioxidant capacity of proline. Proline could be used as a penetrating agent to physically quench reactive oxygen species (Sahar *et al.*, 2011).

This study showed that  $\text{Na}^+$  content in root and leaf of rice seedling increased while  $\text{K}^+$  content decreased under Hg stress.  $\text{Na}^+/\text{K}^+$  value in root and leaf decreased significantly after selenium supplementation and transport of Na to the shoots was reduced, which might be because selenium supplementation reduced sodium ion transport gene. Kaur reported that selenium application could significantly reduce  $\text{Na}^+$  uptake by mung bean leaves and anthers under mercury stress. Whether selenium reduced  $\text{Na}^+$  transport gene has not yet been confirmed (Kaur and Nayyar, 2015). In addition, under environmental stress conditions a higher amount of methylglyoxal MG would be accumulated in the plant. Methylglyoxal was a very strong cytotoxic metabolite, which could affect a variety of physiological processes and reduce glutathione of plant (Yadav *et al.*, 2008). However, MG could be detoxified by the plant via glyoxalase system. The enzyme system contains two enzymes, namely glyoxalase I and II. The two enzymes was responsible for conversion of MG to lactic acid, thereby protecting the plants from abiotic stress (Yadav *et al.*, 2008). It has been reported that excessive expression of glyoxalase (GlyI) and glyoxalase (GlyII) genes in tobacco and rice could significantly increase tolerance of crops to mercury stress (Singla *et al.*, 2008). The experiment demonstrated that activity of glyoxalase I and II were significantly decreased in rice seedlings under Hg stress (Fig. 3a and b). This indicates that glyoxalase in the rice was not fully effective in detoxification of methylglyoxal. Nevertheless, GlyI and Gly II activity was significantly increased after addition of exogenous selenium, indicating

that selenium could increase GlyI and Gly II activity and thus enhanced tolerance of rice to mercury stress.

## Conclusion

Use of exogenous selenium has a dose effect on tolerance of rice seedlings to mercury. The 0~2.0  $\mu\text{mol/L}$  exogenous selenium could promote growth of rice seedling and synthesis of chlorophyll, improved tolerance of rice to mercury stress, mainly because addition of appropriate selenium could significantly increase activity of SOD, POD, APX, CAT, GlyI and Gly II, effectively removed reactive oxygen species, reduced peroxidation of membrane, its ability to protect  $\text{K}^+$  and discharge  $\text{Na}^+$ , thus alleviated damage of mercury stress to plant and improved plant tolerance to mercury. Therefore further effort should be made to help explain the complicated mechanisms of exogenous selenium alleviates mercury toxicity in rice.

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