PHYSIOLOGICAL AND MOLECULAR BASIS OF THE EFFECTS OF EXOGENOUS SELENIUM APPLICATION ON WHEAT SEEDLING PERFORMANCE UNDER DROUGHT STRESS

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Abstract. Drought stress is a severe problem for wheat production. The purpose of the present experiment was to study the effects of the exogenous sodium selenite application at different concentrations on wheat seedlings performance under drought stress. Two wheat varieties, '*Shunmai-1718*' and '*Jintai-102*' were used as materials in this study and four selenium concentration levels were prepared from distilled water (CK), and 20 mg/L (Se1), 40 mg/L (Se2) and 60 mg/L (Se3) of sodium selenite. 7 days after the sowing, the sodium selenite solutions were foliarly applied to wheat seedlings. 9 days after the sowing, 20% PEG-6000 was used to simulate drought conditions for 7 days. The results showed that exogenous selenium significantly increased the plant height, root length, root number, fresh weight and dry weight of wheat seedlings under drought stress. Foliar application of sodium selenite under drought stress also increased antioxidant enzyme activity and the osmoprotectant contents while reducing the content of MDA and O_2^-. Furthermore, the application of sodium selenite under drought stress up-regulated the levels of transcriptional expression in some genes related to antioxidative enzymes and osmo-protectants such as Plant peroxidase, Class Ⅲ peroxidase, Glutathione S-transferase and Catalase immune-responsive.
Keyword: *wheat, selenite, drought resistance, gene expression, antioxidant*

Introduction

China is a big wheat (*Triticum aestivum* L.) planting country reaching 10.5% of the global planting area, but around 70% of it is distributed over arid and semiarid regions (Xuan et al., 2017). Many studies had showed that drought stress could severely affect the growth and development of crops (Clauw et al., 2015; Hatmi et al., 2015). The study of Hao et al. (2015) indicated that the problems of wheat yield reduction and poor grain quality caused by drought always exist in the process of wheat cultivation. Thus, effective ways to relieve the drought stress are required to achieve the goal of ensuring the stability of wheat productivity and exogenous plant growth regulators might come in handy.

Selenium (Se) is a necessary chemical element for human body. It exists in nature in two forms: inorganic Se and organic Se. Selenium has the functions of anti-cancer, anti-oxidation and improving human immunity (Broghamer et al., 1976; Scott et al., 1998). Coronary heart disease, hypertension, Kashin-Beck disease and other diseases are all related to selenium deficiency, and low selenium environment is one of the main factors for the occurrence of these diseases (Rayman, 2012). Although selenium is not an essential element in plants, it has a significant effect on the physiological characteristics of plants. A number of studies showed that Se application could enhance the crops

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performance. For example, He et al. (2019) demonstrated that foliar application of sodium selenate at low concentration could enhance the antioxidant enzymes activities and increase the chlorophyll content of rice. The study of Duan et al. (2019) revealed that exogenous Se application at heading stage could delay the senescence of rice leaves at grain filling stage. Some early studies have shown selenium can improve the drought resistance of wheat or alleviate the damage caused by drought stress (Yao et al., 2009a,b). However, the physiological and molecular basis for effects of exogenous selenium on wheat seedlings performance under drought stress remained largely unexplored.

Hence, present study was conducted with four concentration of exogenous selenite in order to explore the effect of foliar application of sodium selenite on wheat seedling under drought stress and the related physiological and molecular basis.

Materials and Methods

Plant materials and experimental details

Present experiment was conducted in the College of Agriculture with two wheat cultivars, *Yangmai9023* and *Zhengmai20*, which are widely planted in Central China. Before sowing, the selected seeds were soaked with 0.1% $HgCl_2$ for 15 minutes for sterilization, and then washed by distilled water, shade dried, the seeds were sowed into culture dish (9 cm in diameter and 50 seeds for each dish). 7 days after the sowing, the sodium selenite solutions (0 (CK), 10 (Se1), 20 (Se2), 30 (Se3) mg L^{-1} and 40 (Se4) mg L^{-1}) were foliar applied to wheat seedlings. 9 days after the sowing, 20% PEG-6000 was used to simulate drought conditions for 7 days.

Seedling quality

After 7 days of cultivation under drought stress, 15 wheat seedlings with the same growth status were selected in each dish, and their plant height, root length, root number, fresh weight and dry weight were measured.

Estimation of superoxide anion (O_2^-), malondialdehyde (MDA) and anti-oxidant responses

The MDA content and activities of peroxidase (POD), superoxide (SOD) and catalase (CAT) were detected according to the methods of Kong et al. (2017). After MDA reacted with thiobarbituric acid, the absorbance was read at the 532, 600 and 450 nm. The MDA content in the reaction solution was calculated as: MDA content (μmol/L) = 6.45 (OD532 −OD600) − 0.56OD450, and finally expressed as μmol/g FW. POD (EC 1.11.1.7) activity was estimated after the reaction in the solution including enzyme extract (50 μl), 1 ml of 0.3% H_2O_2, 0.95 ml of 0.2% guaiacol, and 1 ml of 50 $mM \cdot l^{-1}$ sodium phosphate buffer (SPB, pH 7.0). One POD unit of enzyme activity was expressed as the absorbance increase by 0.01 (U/g FW) due to guaiacol oxidation. SOD (EC 1.15.1.1) activity was measured by using nitro blue tetrazolium (NBT). In brief, 0.05 ml of an enzyme extract was added into the reaction mixture which contained 1.75 ml of SPB (pH 7.8), 0.3 ml of 130 mM methionine buffer, 0.3 ml of 750 $\mu mol \cdot L^{-1}$ NBT buffer, 0.3 ml of 100 $\mu mol \cdot L^{-1}$ ethylene diamine tetraacetic acid (EDTA)-2Na buffer and 0.3 ml of 20 $\mu mol \cdot L^{-1}$ lactoflavin. After the reaction, the absorbance was recorded at 560 nm. One unit of SOD activity was equal to the volume of the extract needed to cause 50% inhibition of the color reaction. CAT (EC 1.11.1.6) activity was estimated by adding an aliquot of enzyme

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extract (50 µl) to the reaction solution containing 1 ml of 0.3% H_2O_2 and 1.95 ml of SPB and then the absorbance was read at 240 nm. One CAT unit of enzyme activity was defined as the absorbance decrease by 0.01 (U/g FW). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was estimated by using "APX determination kit" purchased from Nanjing Jiancheng Bioengineering Institute, China (Ashraf et al., 2017). The estimation of superoxide anion (O_2^-) was according to the method of Shah et al. (2001) and the content of O_2^- was expressed as nmol g^{-1} FW.

Estimation of soluble protein, proline, reduced glutathione (GSH) and ascorbic acid (AsA)

The contents of soluble protein and proline were detected according to the methods of Li et al. (2016). In brief, the reaction mixture of 2-ml proline and 4 ml of 1.25% ninhydrin in glacial acetic acid was bathed at 100°C for 30 min, and then the absorbance of the reaction mixture was recorded at 508 nm. Protein concentration was measured based on the standard curve of bovine serum albumin after reaction with Coomassie Brilliant Blue G250 Reagent. The measurement of GSH was described by Ashraf et al. (2018) and the instructions were strictly followed and the absorbance was read at 420 and 412 nm. The determination of AsA was according to Yu et al. (2017). About 0.1 g of fresh flag leaves or endosperms were homogenized in 1 mL 6% trichloroacetic acid (TCA) solution in an ice bath, and the homogenate was centrifuged at 12 000 rpm and 4°C for10 min and the supernatant was used for AsA analysis.

Real-time quantitative RT-PCR

Fresh leaves (0.03 g) were collected for total RNA extraction. Total RNA was extracted using HiPure Plant RNA Mini Kit (Magen, Guangzhou, China). The quality and quantity of RNA was assessed by Nanodrop 2000. The Hiscript II QRT SuperMix for qPCR (+gDNA wiper) (Vazyme, Nanjing, China) was used to synthesize cDNA from 500 ng of total RNA. The following mixtures were prepared in qPCR tubes: 4.4 µl cDNA, 0.2 µl each for forward and reverse primers, 5 µl 2*chamQ SYBR qPCR Master MiX and 0.2 µl ROX reference Dye 1, ddH2O to 20 µl (Vazyme, Nanjing, China). Real-time quantitative RTPCR (qRT-PCR) was conducted in CFX96 real-time PCR System (Bio-Rad, Hercules, CA, USA). Each RNA sample was performed in triplicate. A negative control without cDNA template was always included. Primers used for qRT-PCR were listed in *Table 1*. All primers were designed using the software tool Primer 5.

Table 1. *Primer sequences of genes encoding enzymes involved in 2-AP synthesis in rice grains*

Gene name	Primer sequences
Plant peroxidase	F 5'-TGATTCGTCCATCGTCTCG-3' R 5'-CGTGTAGCATTGCCGCTTA-3'
*Class*Ⅲ *peroxidase*	F 5'-TTTGCCTCCGACTTCGTG-3' R 5'-TGCAGTTGCGCCTAATCT-3'
Glutathione S-transferase	F 5'-GCATCATCATTCCCTTCATC-3' R 5'-GCATCATCATTCCCTTCATC-3'
Catalase immune-responsive	F 5′-GTCTCAACGTGAAGCCAAGC-3′ R 5′-GCACAGTAGGTAATCGACCACA-3′

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Statistical analysis

Data were analyzed using statistical software 'Statistix 8.1' (Analytical Software, Tallahassee, FL, USA) while differences amongst means were separated by using least significant difference (LSD) test at 5% probability level. Graphical representation was conducted via Sigma Plot 14.0 (Systat Software Inc., California, USA).

Result

Seedling quality

Analysis of variance showed that foliar application of sodium selenite treatments significantly affected the wheat seedling quality (plant height, root length, root number, fresh weight and dry weight) under drought stress (*Table 2*). Comparing CK, Se1, Se2, Se3 and Se4 treatment all significantly increased plant height of rice seedling for *Zhengmai9023* and *Yangmai20*. The higher root lengths were recorded in Se1, Se2, Se3 and Se4 than CK for both cultivars. Similar trends were also observed in fresh weight and dry weight.

Table 2. *The effect of exogenous sodium selenite on wheat seedling quality under drought stress*

Cultivar Treatment	Plant height (cm)	Root length (cm)	Root number	Fresh weight (g)	Dry weight (g)
Zhengmai9023					
CK	16.37±0.20b	10.10±0.15b	5.13±0.24b	2.05±0.03b	0.23±0.00b
Se1	17.52±0.25a	11.02±0.13a	5.40±0.13ab	2.15±0.02a	0.28±0.00a
Se2	17.45±0.27a	11.12±0.16a	5.40±0.13ab	2.14±0.02a	0.28±0.01a
Se3	17.48±0.25a	10.87±0.15a	5.47±0.13ab	2.16±0.02	0.27±0.01a
Se4	17.56±0.25a	11.18±0.14a	5.67±0.13a	2.11±0.02a	0.27±0.00a
Yangmai20					
CK	15.71±0.20b	8.53±.0.23b	4.93±0.13b	1.90±0.02b	0.21±0.00b
Se1	17.54±0.29a	10.48±0.24a	5.53±0.13a	2.07±0.02a	0.25±0.01a
Se2	17.70±0.22a	10.22±0.22a	5.47±0.13a	2.06±0.02a	0.24±0.00a
Se3	17.80±0.25a	10.29±0.29a	5.33±0.13a	2.06±0.02a	0.24±0.00a
Se4	17.28±0.22a	10.59±0.25a	5.40±0.13a	2.08±0.02a	0.25±0.01a
Analysis of variance					
Cultivar (C)	ns	**	ns	**	**
Treatment (T)	**	**	**	**	**
C × T	ns	ns	ns	*	ns

Values ± SE sharing a common letter within a column don't differ significantly at ($P \leq 0.05$) according to least significant difference (LSD) test. The same as below

MDA and O_2^- content

Analysis of variance showed that sodium selenite treatments significantly influenced the contents of MDA and O2- in wheat seedling under drought stress (*Figure 1*). For *Zhengmai9023* CK, Se1, Se2, Se3 and Se4 treatments significantly decreased the O_2^- content by 7.47, 6.68, 12.06 and 16.82% while 16.53, 16.67, 20.51 and 22.97% lower MDA contents were recorded in Se1, Se2, Se3 and Se4 than in CK. For *Yangmai20* CK, Se1, Se2, Se3 and Se4 treatments significantly decreased the O_2^- content by 12.33, 16.04, 20.75 and 27.22% while 14.74, 24.30, 26.82 and 35.56% lower MDA contents were recorded in Se1, Se2, Se3 and Se4 than in CK.

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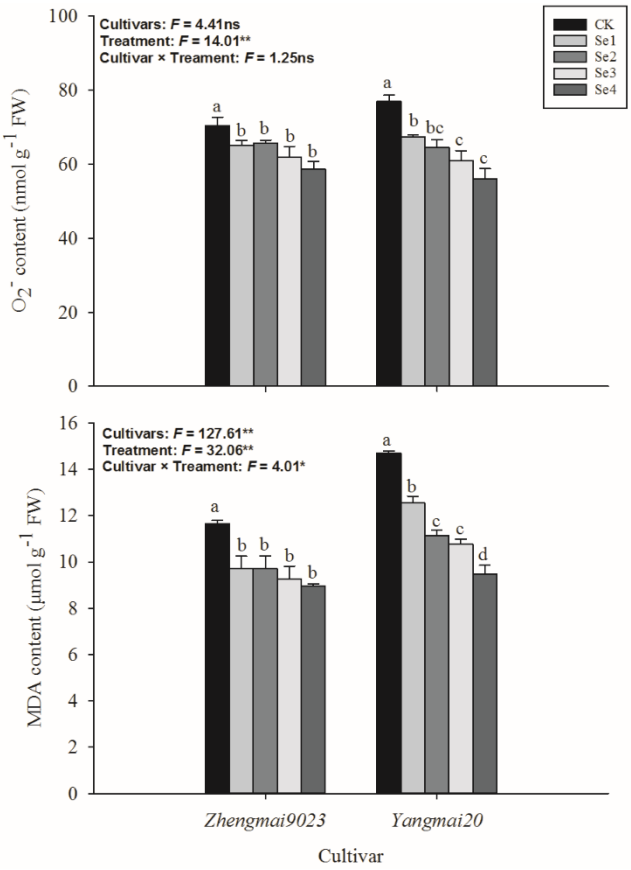

Figure 1. *Effect of exogenous sodium selenite on MDA and O_2^- content of wheat seedling under drought stress (Bars within the same cultivar followed by a different letter are significantly different at P < 0.05 probability level)*

Anti-oxidant enzymes activity

As shown in *Figure 2*, foliar application of sodium selenite significantly increased the anti-oxidant enzymes activities of wheat seedling under drought stress. Higher POD activities were recorded in Se1, Se2, Se3 and Se4 treatments than in CK in both cultivars and similar trends were also recorded in SOD activities. For CAT activities, Se1, Se2, Se3 and Se4 all significantly increased CAT activities compared to CK in *Yangmai20* whilst only Se3 and Se4 remarkably increased CAT activities in *Zhengmai9023*. For APX activities, comparing CK, Se2, Se3 and Se4 significantly increased APX activities were observed in Zheng9023 while for Yangmai20, higher APX activities were recorded in Se1, Se2, Se3 and Se4 treatments than in CK.

Osmo-protectants

As shown in analysis of variance, exogenous selenium induced dynamics in soluble protein, proline, GSH and AsA accumulation under drought stress (*Figure 3*). For *Zhengmai9023* CK, Se2, Se3 and Se4 treatments increased the soluble protein, proline, GSH and AsA contents by 10.22-19.93, 6.54-10.98, 13.40-24.92 and 12.32-22.82%, respectively. For *Yangmai20* CK, Se2, Se3 and Se4 treatments increased the soluble protein, proline, GSH and AsA contents by 23.70-25.16, 10.89-26.95, 23.41-23.94 and 21.28-31.15%, respectively.

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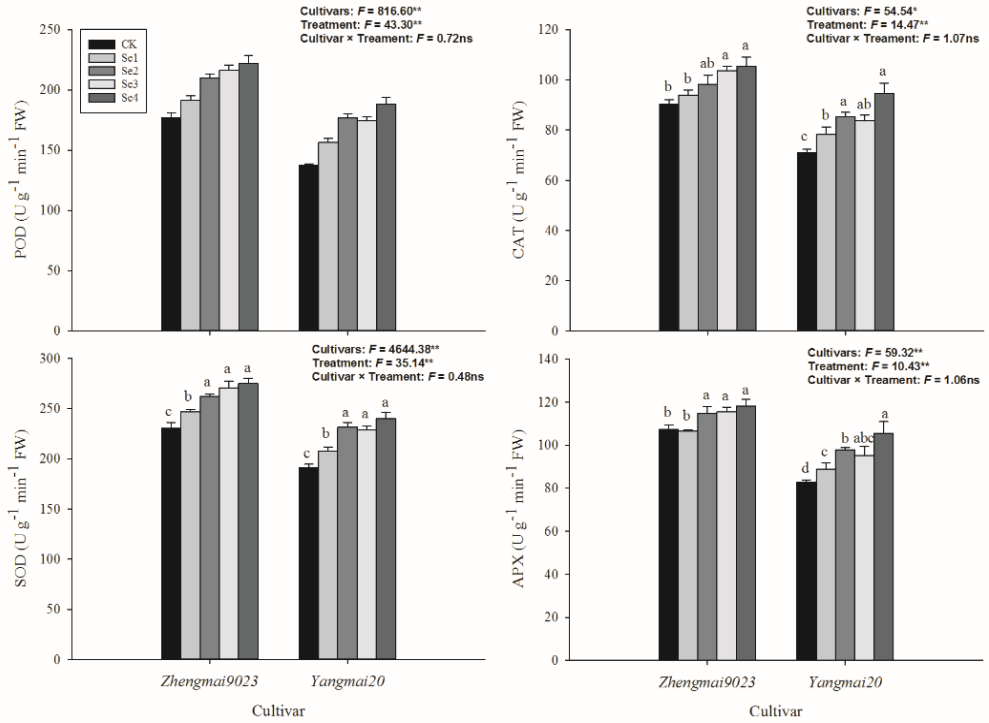

Figure 2. *Effect of exogenous sodium selenite on anti-oxidant enzymes activities of wheat seedling under drought stress (Bars within the same cultivar followed by a different letter are significantly different at P < 0.05 probability level)*

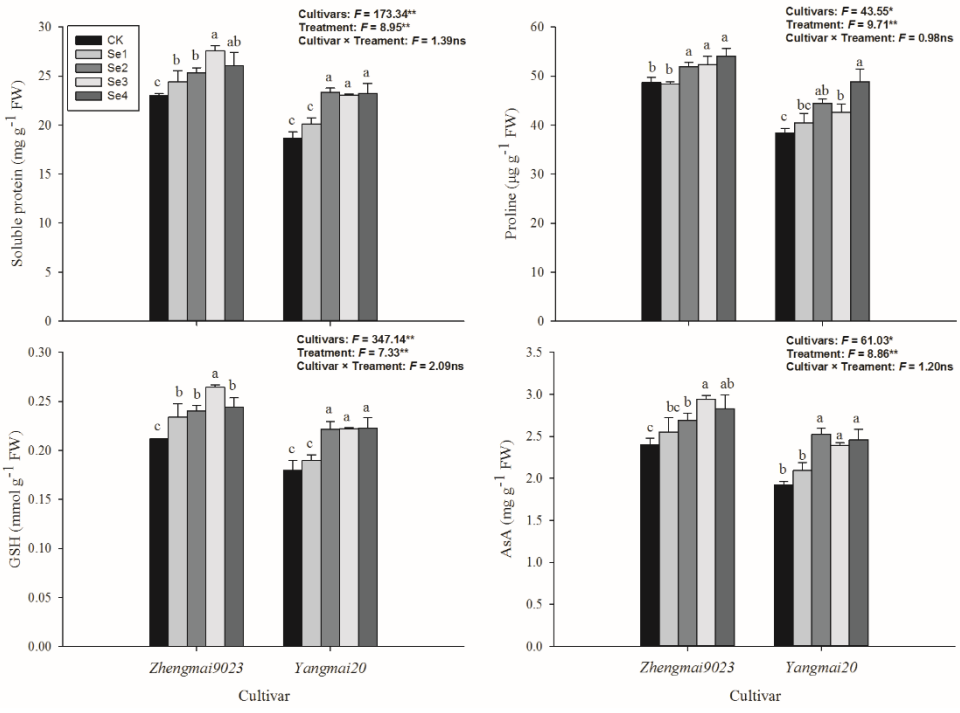

Figure 3. *Effect of exogenous sodium selenite on contents of soluble protein, proline, GSH and AsA in wheat seedling under drought stress (Bars within the same cultivar followed by a different letter are significantly different at P < 0.05 probability level*

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Real-time PCR analyses

As depicted in *Figure 4*, compared to CK, higher levels of *Plant peroxidase*, *Class Ⅲ peroxidase*, *Glutathione S-transferase* and *Catalase immune-responsive* transcripts were found in Se2, Se3 and Se4 treatments for *Zhengmai9023* and *Yangmai20*.

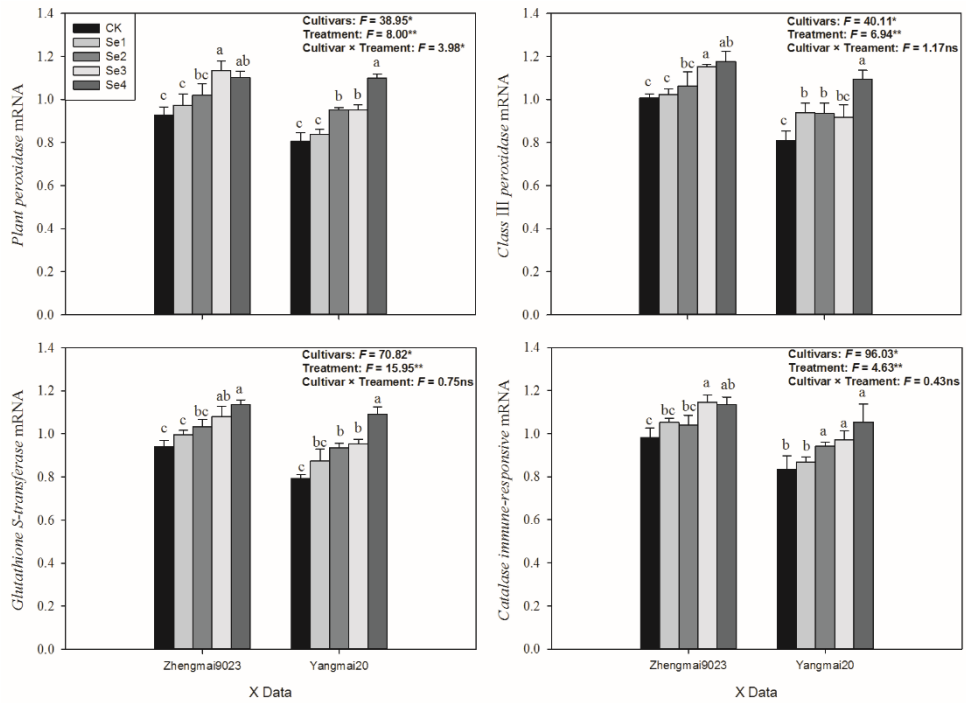

Figure 4. *Analysis of transcript levels of Plant peroxidase, Class Ⅲ peroxidase, Glutathione S-transferase and Catalase immune-responsive in wheat seedling under drought stress (Bars within the same cultivar followed by a different letter are significantly different at $P < 0.05$ probability level)*

Discussion

Water is the main limiting factor for wheat growth and yield formation (Li et al., 2015). In recent years, due to climate change, frequent drought weather is one of the main natural disasters affecting wheat production in China (Wang et al., 2014). The tillering and root growth of wheat were mainly carried out at seedling stage and hence, the growth and development of seedling significantly affected the formation of effective panicle and grain number per panicle which are the yield components of wheat. Previous study revealed that drought stress in wheat seedling stage will seriously affect tillering and spikelet differentiation, and ultimately lead to yield reduction (Leilah and Al-Khateeb, 2005). Present study revealed the alleviative effect of Se application on wheat seedling to drought stress in two wheat cultivars. Foliar applications of Se improved the wheat seedling quality under drought stress in terms of plant height, root length, fresh weight and dry weight. This result agreed with the study of Yao et al. (2009b) who indicated that treatments with selenium was able to promote the biomass accumulation of wheat seedlings under drought stress.

When the plant is under drought stress, it will affect the metabolism of reactive oxygen species (ROS) in the plant, leading to the increase of the content of reactive oxygen

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species in the plant (Kong et al., 2017). A large number of ROS can peroxidise the membrane lipids in plants to produce MDA which can damage the membrane structure of plant cells (Ashraf et al., 2017). Normally, plant itself has an antioxidant enzyme system to reduce the damage of cell membrane lipids in arid environment (Ashraf et al., 2018). Present study observed that foliar application of sodium selenite could regulated the anti-oxidative enzymatic activities in terms of SOD, POD, CAT and APX while reducing the oxidative damage by lowering the MDA and O_2^- production. The increase of O_2^- in plants will lead to the oxidation of polyunsaturated fatty acids, which will lead to the excessive accumulation of MDA. The anti-oxidative enzymes have significant roles in osmo-regulations and often help in maintaining cellular structures and functions under stress condition (Luo et al., 2018). For example, SOD dismutases superoxide radical whereas POD and CAT involved in scavenging H_2O_2 (Pan et al., 2013). In our study, the activities of SOD, POD, CAT and APX in wheat seedlings of two cultivars were significantly increased after applying exogenous selenium (*Figure 2*), and the contents of O_2^- and MDA were decreased (*Figure 1*) under PEG simulated drought stress. On the other hand, Se treatments also induced regulation in proline, soluble protein, GSH and AsA accumulation. Proteins and proline have significant roles in osmo-regulations and often help in maintaining cellular structures and functions while anti-oxidants help to quench ROS (Luo et al., 2018). Previous studies also indicated that both GSH and AsA are the most abundant non-enzymatic anti-oxidants in plants and also provide protection in plant cells from ROS-generated free radicals and serve as redox buffer (Ashraf et al., 2017). The regulations in anti-oxidative enzymatic activities and non-enzymatic antioxidants due to Se applications under drought stress may be attributed to higher levels of transcriptional expression of *Plant peroxidase*, *Class Ⅲ peroxidase*, *Glutathione S-transferase* and *Catalase immune-responsive* genes. Our results indicated that the foliar application of Se in the appropriate concentration could increase the activities of antioxidant enzymes in wheat seedlings, enhance the ability of scavenging active oxygen free radicals and reduce the degree of membrane lipid peroxidation to alleviate the adverse effects of drought stress on crops.

As far as sodium selenite concentration was concerned, the highest activities of antioxidative enzyme and the highest content of osmo-protectants were all recorded in Se2, Se3 and Se4 for both cultivars while the lowest MDA and O_2^- was also recorded in Se2, Se3 and Se4 treatments for both cultivars. Thus, the optimized selenium amendment for wheat seedlings under drought stress might be the foliar application of 20-40 mg L^{-1} sodium selenite.

Conclusion

Foliar application of sodium selenite under drought stress can promote root growth of wheat seedlings, maintain water content and biomass of plant tissues, increase activities of antioxidant enzymes and the contents of osmo-protectants while reducing the content of MDA and O_2^-. Furthermore, the application of sodium selenite under drought stress up-regulated the levels of transcriptional expression in some genes related to anti-oxidative enzyme and osmo-protectants such as *Plant peroxidase*, *Class Ⅲ peroxidase*, *Glutathione S-transferase* and *Catalase immune-responsive*. More field experiments should be conducted in the future.

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