IMPROVMENT OF GROWTH AND QUALITY AND REGULATION OF THE ANTIOXIDANT SYSTEM AND LIPID PEROXIDATION IN CHINESE CABBAGE (*Brassica pekinensis* (Lour.) Rupr.) BY EXOGENOUS SODIUM SELENITE

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(Received 15th Dec 2019; accepted 26th Mar 2020)

Abstract. Selenium (Se) is a necessary mineral nutrient for humans. In order to study the effects of exogenous sodium selenite on Chinese cabbage, an important food in people's daily life, the present study was conducted with four applications with different concentrations of sodium selenite, 0 (CK), 50 (Se1), 150 (Se2), 250 (Se3) mg L⁻¹. The results showed that the concentrations of Se, Fe and Zn in Chinese cabbage increased due to the Se application while the fresh weight and dry weight were affected beneficially by the treatment of Se just like chlorophyll contents. Foliar application of sodium selenite also remarkably increased the Vc, proline, protein and sugar contents. Furthermore, the foliar application of Se remarkably enhanced antioxidant enzyme activities of POD, SOD, CAT, APX and GR. In conclusion, exogenous sodium selenite would improve the growth and quality and enhance antioxidant resistance of Chinese cabbage.

Keywords: chlorophyll, vegetable, selenium, crop quality, antioxidant enzymes

Introduction

Selenium (Se) is one of the essential trace elements for human and animal growth. 72% of the Chinese counties (cities) are selenium deficient or low selenium areas, and two-thirds of the population suffer from selenium malnutrition or selenium deficiency (Tan et al., 2002; Xia et al., 2005). Previous research studies have proved that proper selenium supplementation is an effective measure to enhance human health, prevent diseases and prolong life (Rayman, 2000; Yang et al., 2017). Plants are the main source of selenium for humans and animals, thus the production of selenium - rich plants is an effective way to supplement human selenium.

As an important vegetable for the life of Chinese, Chinese cabbage (*Brassica pekinensis* (Lour.) Rupr.) is an indispensable delicious vegetable on the dining table of urban and rural residents in autumn and winter. Chinese cabbage not only possesses high contents of dry substances, vitamin C (Vc), protein and soluble sugar, but also has low acidity and crude fiber (Zhang et al., 2002; Su et al., 2018). Chinese cabbage also had a long history of cultivation and a large cultivated area in China (De Medici et al., 2019; Mi et al., 2019). Some previous studies have shown the beneficial effect of exogenous Se on Chinese cabbage performances. For example, the study of Wu et al. (2018) revealed that foliar application of Se selectively induced glutathione reductase (GR) and dehydroascorbic acid reductase (DHAR) in Chinese cabbage tissues and mitigated the cadmium toxicity. Dai et al. (2019) also used a year field experiment to find an interaction effect between soil application of Se and Zinc (Zn) on Chinese cabbage quality and antioxidant system through a pot experiment. However, the foliar field application of Se on Chinese cabbage was rarely reported the utilization rate of

microelements would be much higher in foliar application than in soil application (Farooq et al., 2018).

Therefore, in order to improve the scientific database on factors affecting the quality and yield of Chinese cabbage and enhance the application of Se in Chinese cabbage production, present field experiment was conducted in Shandong province, China, in 2019 with two hypotheses, that are (1) foliar application of selenite would improve the growth and development of Chinese cabbage and (2) foliar application of selenite would promote the quality of Chinese cabbage.

Materials and Methods

Experimental details

The field experiment was conducted in Heze city of Shandong province, China. The experimental soil is sandy loam soil and the pH value was 6.7, and with 25.99 g kg⁻¹ total nitrogen, 2.44 g kg⁻¹ available phosphorus and 16.90 mg kg⁻¹ available potassium. Four concentrations of sodium selenite were applied as follows: overhead sprinkling with 0, 50, 150, and 250 mg·L⁻¹ and these treatments were known as CK, Se1, Se2, Se3, respectively. The treatments were arranged in a randomized complete block design (RCBD) in triplicate. One day before harvest, three representative plants of each treatment were collected for the bio-chemical indexes including nutritional components, antioxidative enzymes, chlorophyll, malondialdehyde, Se, Fe and Zn. At harvest, ten random Chinese cabbage plants were collected from each treatment to measure the fresh weight and dry weight.

Estimation of antioxidative enzymes, malondialdehyde and chlorophyll

The activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD) and the malondialdehyde (MDA) were measured according to the methods described by Luo et al. (2018). The chlorophyll a, chlorophyll b and carotenoid contents were estimated by the methods of He et al. (2019). The activities of ascorbate peroxidase (APX) and glutathione reductase (GR) were determinate according to Dai et al. (2015).

Estimation of nutritional components of Chinese Cabbage

The protein and proline contents were determinate by the methods of Li et al. (2016) using Coomassie brilliant blue G-250 and ninhydrin colorimetry, respectively. The content of vitamin C (Vc) was estimated through titration with 2,6-dichlorophenolindophenol and the sugar content was measured by anthrone method according to Ma et al. (2014).

Determination of Se, Fe, Zn contents

The plant samples were ground into powder form and digested (0.1 g) with 1.5 ml of concentrated HNO₃ and 0.5 ml 30% H₂O₂ in a digestive stove at 180°C for 1.5 h. Total Se, Fe and Zn content in samples were determined by using atomic absorption spectrometer (Z2300, HITACHI, Japan)-HFS (HFS-3, HITACHI, Japan).

Statistical analysis

Data were analyzed on Statistix 8.1 (Analytical Software, Tallahassee, FL, USA) while differences among 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).

Results

Biomass

Foliar application of sodium selenite had impacts on fresh weight and dry weight of Chinese cabbage (*Figure 1*). Compared with CK, Se1 treatment significantly increased the fresh weight and dry weight by 36.26 and 47.70%, respectively. However, there was no remarkable difference observed among CK, Se2 and Se3 in both fresh weight and dry weight of Chinese cabbage.

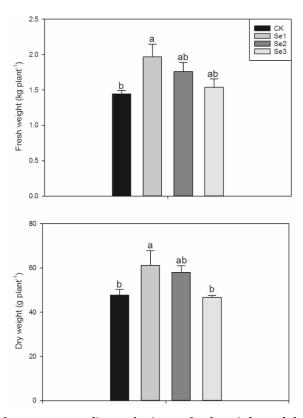


Figure 1. Effects of exogenous sodium selenite on fresh weight and dry weight in Chinese cabbage. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $(P \le 0.05)$ according to least significant difference (LSD) test

Antioxidative enzymes and lipid peroxidation

Foliar application of sodium selenite regulated the activities of POD, SOD, CAT, GR, APX and contents of MDA in Chinese cabbage (*Figure 2*). Compared to CK, Se1, Se2 and Se3 treatments significantly increased POD activities by 103.36%, 56.83% and 16.63%, respectively. For SOD activity, 31.86% and 12.67% higher activities were

recorded in Se1 and Se2 treatments than in CK whiles there was no remarkable difference between CK and Se3. Compared to CK, Se1, Se2 and Se treatments significantly increased CAT activities by 104.32%, 51.57% and 13.10%, respectively. For APX activities, 190.78%, 101.59% and 39.15% higher activities were recorded in Se1, Se2 and Se3 treatments compared with CK, respectively. Compared to CK, Se1, Se2 and Se3 treatments significantly increased GR activities by 120.71%, 63.71% and 20.95%, respectively. For the contents of MDA, Se1, Se2 and Se3 treatments significantly increased MDA contents compared with CK whilst there was no remarkable difference among Se1, Se2 and Se3 in MDA contents.

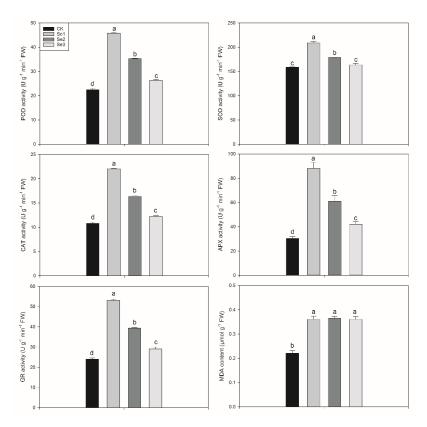


Figure 2. Effects of exogenous sodium selenite on activities of POD, SOD, CAT, GR, APX and contents of MDA in Chinese cabbage. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $(P \le 0.05)$ according to least significant difference (LSD) test

Chlorophyll content

As shown in *Figure 3*, foliar application of sodium selenite significantly affected the contents of chlorophyll a, chlorophyll b and carotenoids in Chinese cabbage. Compared with CK, Se1, Se2 and Se3 treatments all significantly increased the chlorophyll a content while the highest content was recorded in Se1 treatment. Similar trends were also found in both chlorophyll b and carotenoids contents.

Nutritional components

Foliar application of sodium selenite regulated the contents of proline, protein, Vc and sugar in Chinese cabbage (*Figure 4*). Compared to CK, Se1 and Se2 significantly

increased proline content by 40.16% and 26.18%, respectively. For Vc content, 27.07%, 17.56% and 10.46% higher contents were recorded in Se1, Se2 and Se3 treatments than in CK, respectively. Compared to CK, Se1, Se2 and Se treatments significantly increased protein content by 48.43%, 31.08% and 18.12%, respectively. For sugar content, 65.97%, 45.00% and 25.44% higher values were recorded in Se1, Se2 and Se3 treatments compared with CK.

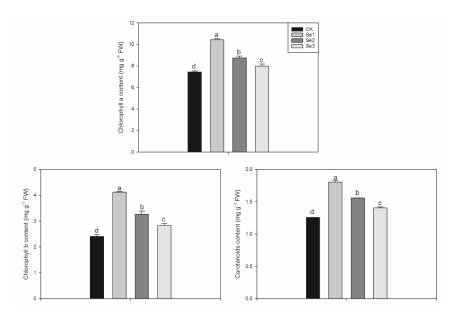


Figure 3. Effects of exogenous sodium selenite on contents of chlorophyll a, chlorophyll b and carotenoids in Chinese cabbage. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $(P \le 0.05)$ according to least significant difference (LSD) test

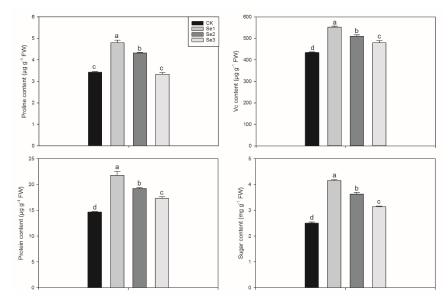


Figure 4. Effects of exogenous sodium selenite on contents of proline, protein, Vc and sugar in Chinese cabbage. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $(P \le 0.05)$ according to least significant difference (LSD) test

Se, Fe and Zn content

As shown in *Figure 5*, foliar application of sodium selenite has some impacts on Se, Fe and Zn concentrations in Chinese cabbage. The Se content increased with the increment of Se applied concentrations and the highest content was recorded in Se3 treatment. However, the Fe content showed a different trend compared to Se. The highest content of Fe was recorded in Se1 treatment while the trend was recorded as: Se1 > Se2 > Se3 > CK. Similar trend was also observed in Zn content.

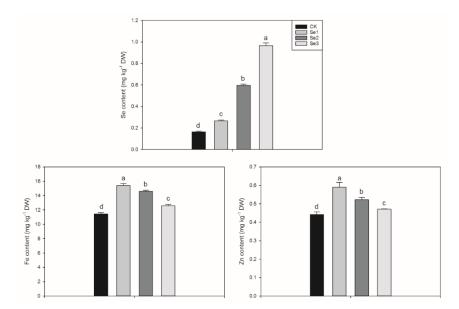


Figure 5. Effects of exogenous sodium selenite on Se, Fe and Zn content in Chinese cabbage. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $(P \le 0.05)$ according to least significant difference (LSD) test

Analysis of variance

As shown in *Table 1*, foliar application of Se had significant effects on activities of POD, SOD, CAT, APX, GR as well as MDA content. The contents of chlorophyll a, chlorophyll b and carotenoid were significantly affected by Se application. In addition, proline, Vc, protein and sugar contents and contents of Se, Fe and Zn were significantly affected by Se application. However, foliar application of Se had no significant effect on fresh weight and dry weight of Chinese cabbage.

Table 1. Variance analysis about the effects of Se on Chinese cabbage performances

Parameter	F values	Parameter	F values
Fresh weight	2.61ns	Chlorophyll b	58.45**
Dry weight	3.04ns	Carotenoid	306.39**
POD	817.81**	Proline	66.92**
SOD	144.74**	Vc	120.95**
CAT	802.02**	Protein	41.59**
APX	35.53**	Sugar	199.71**
GR	465.57**	Se content	455.59**
MDA	37.32**	Fe content	76.01**
Chlorophyll a	78.15**	Zn content	20.63**

F value and significance level (**P < 0.01, *P < 0.05 and ns P \geq 0.05)

Discussion

Present study made the first attempt about the foliar application of Se in Chinese cabbage production and revealed the beneficial effects of exogenous Se on biomass, photosynthetic pigments, quality and antioxidant system of Chinese cabbage. The results supported both hypothesizes that foliar application of sodium selenite improves growth and development as well as quality of Chinese cabbage. At the most fundamental level, foliar application of sodium selenite increased the Se content in Chinese cabbage and the Se content increased with the increment in the applied concentrations. On the other hand, the foliar application of Se significantly increased both Zn and Fe contents and the highest Zn content and Fe content were both recorded in Se1 treatment. This result indicated that exogenous Se would enhance the absorption of Chinese cabbage to Zn and Fe which was consistent with the studies of Dai et al. (2019) and Fang et al. (2008).

As expected, foliar application of sodium selenite (Se1 treatment) produced the Chinese cabbage with higher fresh weight and dry weight. The biomass was enhanced probably due to the increments in photosynthetic pigment contents including chlorophyll a, chlorophyll b and carotenoids. Our results agreed with the study of Lai et al. (2019) who demonstrated that foliar application of Se promoted the yield of fragrant rice by increasing the chlorophyll content and enhancing photosynthesis. The investigation of Wu et al. (2000) also showed that exogenous Se applied with low concentration would promote the electron transfer rate of chloroplast in paddy rice.

Besides yield, the quality of Chinese cabbage is a determinant factor in economic returns for farmers. The quality of Chinese cabbage is influenced by parameters including protein, sugar, Vc and amino acid such as proline. Present study revealed that foliar application of sodium selenite would affect the contents of those nutritional components. There were increases in the contents of proline, protein, Vc and sugar. From the results, it can be concluded that foliar application of sodium selenite clearly increased Chinese cabbage yields and improved quality.

Interestingly, we observed that exogenous selenite application showed some antioxidant properties in Chinese cabbage according to the activities of antioxidant enzymes such as POD, SOD, CAT, APX and GR. Our study coincides with the research studies of Golubkina et al. (2019) and Zimmermann and Kohrle (2002) who indicated that element Se plays an important role in immune-modulating and antioxidant properties in both mammals and plants, by defending the organism from different kinds of oxidative stress. In our study, the activities of POD, SOD, CAT, APX and GR increased due to the foliar application of sodium selenite. This result was consistent with the study of Dai et al. (2019) who also found antioxidative enzyme activities and lipid peroxidation were positive or beneficial with the application of Se.

Present study not only improved the scientific database about the effects of Se on crops, but also enhanced the application of Se in Chinese cabbage production. However, more studies are required at physiological and molecular level to reveal the mechanism about how Se affects Chinese cabbage.

Conclusion

Foliar application of sodium selenite not only significantly improved growth (Fresh weight, dry weight) by increasing the contents of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoid), but also promoted Chinese cabbage nutrient quality including Vc, proline, protein, sugar and microelements such as Se, Fe and Zn.

Furthermore, the foliar application of Se remarkably enhanced the antioxidant system of Chinese cabbage in terms of POD, SOD, CAT, APX and GR activities. In order to reveal the mechanism about how Se affects Chinese cabbage, more studies should be conducted at physiological and molecular level

Acknowledgements. This study was supported by Shandong province key research and development project (2019GNC106121).

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