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Effects of selenium on activity of glutathione peroxidase and expression of selenium metabolism-related genes in *Brassica*

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**ABSTRACT**

Inorganic selenium (Se) is absorbed and enriched by plants and converted into a stable and nutritionally important organic form, which subsequently when consumed by humans or animals results in increased Se tissue levels. *Brassica* is one of the most potent Se-enriched plants. The aim of this study was to compare differences in Se enrichment between two predominant *Brassica* plants namely *Brassica rapa* Linn L. and *Brassica tumida* Tsenet Lee L. on Se metabolic parameters. Plants exposed to soil Se levels (0, 0.5, 1, 2.5 or 5 mg/kg) were examined on the activity of glutathione peroxidase (GSH-Px), Se levels and expression of Se metabolism related genes using soil pots. Data showed that activities of GSH-Px in leaf and root of the two *Brassica* species were significantly increased in the presence of Se at 2.5 mg/kg. Se concentrations of leaf, stem and root in *B. tumida* Tsenet Lee L. and *B. rapa* Linn L. rose from 0.31 to 21.84-fold (leaf), 1.15 to 15.16-fold (stem) and 2.11 to 15.26-fold (root) in the presence of metal in a concentration-dependent manner. The highest expression levels of adenosine triphosphate (ATP), ATP sulfurylase (APS), selenocysteine methyltransferase (SMT), serine acetyltransferase (SAT), cysteine desulfurase (CysD) and \textit{S}-adenosyl-L-Met:\textit{S}-methyltransferase (MMT) in leaf of *B. rapa* Linn L. were found at 1 mg/kg Se. The highest expression levels of ATP, APS, SMT, SAT, CysD and MMT in leaf of *B. tumida* Tsenet Lee L. were observed at 2.5 mg/kg Se. The Se concentrations in leaf, stem and root of *B. rapa* Linn L. were higher than in *B. tumida* Tsenet Lee L. under the same soil Se level conditions. At the same Se level, differences in the expression of Se-related genes were observed between these two *Brassica* species. Our observations may be used to optimize the utilization of *Brassica* as a nutritional source of Se by growing this plant under certain soil conditions.

**CONTACT**

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1. Introduction

Selenium (Se), an essential trace element in humans known as ‘protective agent of life’, plays an important role in anti-aging, enhanced immunity and anti-cancer (Navarro-Alarcón and López-Martínez 2000; Fairweather-Tait et al. 2011; Rayman 2012; Ebadi and Hisoriev 2017a, 2017b, 2018). Soil Se content varies from 0.006 to 9.13 mg/kg⁻¹, with an average of 0.29 mg/kg⁻¹ in China (Fordyce 2013). There are 22 provinces, and approximately 72% land area in China contains different degrees of Se deficiency, such as purple soil in Sichuan Province (Zhao et al. 2017). Organic Se is the predominant form of this element in plants, accounting for more than 80% of the total amounts of Se (Zhang et al. 2014; Ebadi, Hisoriev, and Aliev 2017; Shahid et al. 2018). Inorganic Se was found to be adsorbed and enriched by the plant with subsequent transformation into a stable and nutritionally important organic form (Hartikainen 2005; Zhang et al. 2014). Several investigators demonstrated that ingesting Se-containing plants is the safest and most effective manner to simulate Se supplementation in humans, which then serves to improve Se nutritional levels in both humans or animals in areas where Se deficiency occurs (Abdulah et al. 2005; Olena et al. 2005; Fordyce 2013; Zhou, et al. 2017).

Se concentrations in plants were found to be related to the activity of glutathione peroxidase (GSH-Px) (Zhao et al. 2017). GSH-Px activity increased in presence of elevated exogenous Se concentrations (Zhang et al. 2014). Plants use sulphate transporters and metabolic pathways to take up inorganic Se and assimilate this element into a series of Se-containing metabolites including selenocysteine (SeCys), selenomethionine (SeMet) and Se homocysteine in the presence of sulphate assimilative enzyme (Özgür, Turgut-Kara, and Şule 2012; Longchamp et al. 2015). ATP sulfurylase (APS) is considered a key enzyme for selenate reduction and Se accumulation (Pickering et al. 2003; Zhang et al. 2014; Longchamp et al. 2015). Overexpression of APS in plants promotes the rate of this process and increases accumulation of Se in plants (Pickering et al. 2003; Zhao et al. 2017). The simultaneous overexpression of both APS and selenocysteine methyltransferase (SMT) in Brassica juncea was found to elevate the Se levels 4–9-fold (Sors and Ellis 2005; Zhao et al. 2017). Özgür, Turgut-Kara, and Şule (2012) reported that cysteine desulfurase (CysD), S-adenosyl-l-Met:L-Met S-methyltransferase (MMT) and serine acetyltransferase (SAT) were the predominant genes involved in plant metabolism and Se accumulation. The simultaneous expression of SAT and SMT increases levels of O-acetylserine (OAS), which promotes the expression of sulfur transporters, and enhances uptake and enrichment of Se in plants (Özgür, Turgut-Kara, and Şule 2012).

Brassica belongs to the Cruciferae family and demonstrated the strongest enrichment capacity for Se, followed by liliaceae and legume, and the lowest is grain crops (Sors and Ellis 2005). Brassica, containing 38 species, was the earliest classified plant in Cruciferae family, with the highest economic value and widest applications. At present, there are many reports on Se-rich crops globally (Ávila et al. 2014; Zhao et al. 2017). However, differences in Se enrichment among various Brassica species are not well known. Thus, the aim of this study was to compare Brassica tumida Tsenet Lee L. with Brassica rapa Linn L. on the effects of different Se growing concentrations on growth, GSH-Px activity, elemental absorption and Se-related gene expression.
2. Materials and methods

2.1. Plant material, soil and Se treatments

Two *Brassica* (*B. rapa* Linn L. and *B. tumida* Tsenet Lee L.) were used in the present study. The tested soil samples were collected from Jiulongpo District, Chongqing. The contents of organic matter and total nitrogen were 9.08 and 0.64 g/kg. The available amounts of N, P and K were 72.77, 54.81 and 216.7 mg/kg, the cation exchange capacity was 30.3 cmol/kg, the pH was 6.7 and the total Se and available Se in soil were 0.42 and 0.029 mg/kg, respectively.

Pot experiment was conducted with 5 Se levels control (0), 0.5, 1, 2.5 and 5 mg/kg from 4 November 2014 and 25 February 2015 in the greenhouse of College of Resources and Environmental Sciences at Southwest University, China. Five kilograms of air-dried soil was passed through a 40-mesh screen and then treated with exogenous Se (sodium selenite). After uniform mixing, the Se-treated soil was placed into plastic pots with a diameter of 25 cm and a height of 17 cm for an equilibration period of 2–3 weeks. Three seedlings of *B. rapa* Linn L. and *B. tumida* Tsenet Lee L. were planted in each pot. The base fertilizer included P (NH₄H₂PO₄), K (KCl) and N (NH₄H₂PO₄ and urea) at the concentrations of 100, 150 and 180 mg/kg, respectively. The soil moisture was measured using a soil moisture tachometer before watering. The mean moisture content in the soil was calculated over three watering sessions to establish the amount of water required to maintain maximal water-holding capacity of the soil at up to 60% (Xia et al. 2011). The experiment was performed in triplicate and arranged randomly. The fresh edible part of *B. rapa* Linn L. and *B. tumida* Tsenet Lee L. was subjected to snap freezing with liquid nitrogen and kept in the freezer at −80°C for RNA extraction. The plants were harvested on 25 February 2015, maintained at 105°C for 15 min for denaturing the enzymes and then oven-dried at 60°C until there was no further change in the weight of the sample.

2.2. Analysis of Se concentrations in soil and plants

The concentration of extractable Se in soil was determined via KH₂PO₄ (0.016 mol/L) extraction [10 g soil (dry weight) to 30 mL KH₂PO₄ (w/v)] followed by atomic fluorescence spectrometry analysis (Shahid et al. 2018). Dried plant samples were ground and digested at 170°C using 10 mL of acid mixture (8 mL of ultrapure nitric acid and 2 mL of perchloric acid) (Kyodan, Tetsuya, and Munehim 1988). The concentration of Se in the solution was analyzed using hydride generation atomic fluorescence spectrometry (PF6.3; Beijing Purkinje General Instrument Co. Ltd., China).

2.3. Detection of gene expression

2.3.1. Extraction and detection of total RNA

RNA was extracted from edible part of the sample stored in the refrigerator at −80°C. The specific procedure followed the operation manual for RNA extraction kit purchased from Tiangen Biotechnology Company (Beijing, China) (Zhao et al. 2017).
2.3.2. RNA purification and reverse transcription
Reverse transcription of RNA was performed using TaKaRa’s PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Zhao et al. 2017). The remaining DNA was treated first with DNase I, and the treatment time was extended from 2 min on manual to 20 min for complete removal of heavy contaminated genomic total DNA from RNA, followed by reverse transcription into cDNA. The resulting cDNA was stored at −20°C in refrigerator for standby application (Zhao et al. 2017).

2.3.3. Primer design and synthesis
The RT-PCR-specific primers of six Se metabolism-related genes (APR, APS, SMT, MMT, CysD and SAT) were designed by BLAST and multiple alignments (Vector NTI Advance 11.51) of Brassica crop gene family members and the 25S rRNA primers of reference genes were synthesized by Nanjing Jinsirui Science & Technology Biology Corporation (Nanjing, Chin) (Zhao et al. 2017).

2.3.4. PCR amplification of cDNA
The obtained cDNA was specifically amplified with ABI-9700 PCR instrument. The PCR product was tested by 1.0% agarose gel electrophoresis. A clear band of predicted target size (about 250 bp) means successful reverse transcription (Zhao et al. 2017).

2.3.5. Real-time quantitative PCR
The resulting cDNA was diluted 30-fold with ddH2O, and transcriptional expression level of the target gene was detected by real-time quantitative PCR (qRT-PCR) using the FastStart Essential DNA Green Master kit in Basel, Germany (Zhao et al. 2017). The test was performed using fluorescence quantitative PCR instrument’s CFX96™ Real-Time System. Data were analyzed on Bio-Rad CFX Manager 3.0 software. The operation was conducted according to the instruction manual and, with 25 s as internal reference gene, was repeated three times (Zhao et al. 2017).

2.4. Statistical analysis
Three-way analysis of univariate ANOVA and correlation analysis were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). The variables analyzed separately were Se concentration and correlations between Se levels and Se concentrations of Brassica in Brassica. The level of significant was set at $p < 0.05$ (Xu, Kachenko, and Singh 2010).

3. Results
3.1. Activity of glutathione peroxidase
GSH-Px activity in the leaf of B. tumida Tsenet Lee L. initially rose and then declined with increase of soil Se levels, reaching the highest enzymic activity when elemental level was 0.5 mg/kg, which is 1.86-fold higher than control (Figure 1). With the elevation of soil Se, GSH-Px activity of B. rapa Linn L. increased
gradually 1.08- to 3.41-fold higher than control. Leaf GSH-Px activity in *B. rapa* Linn L. was higher than in *B. tumida* Tsenet Lee L. With the rise of soil Se levels, GSH-Px activity in root of *B. tumida* Tsenet Lee L. and *B. rapa* Linn L. increased gradually, 10.3–42.7% (*B. tumida* Tsenet Lee L.) and 7.8–61.2% (*B. rapa* Linn L.), respectively, greater than control. Root GSH-Px activity in *B. tumida* Tsenet Lee L. was higher than in *B. rapa* Linn L.

### 3.2. Concentration of Se in leaf, stem and root

Se concentrations in leaf, stem and root of the two *Brassica* was elevated with increase in soil Se. Positive significant correlation was found in Se concentrations in leaf, stem and root of the two *Brassica* with soil elemental levels (Figure 2).
Compared to control, exogenous Se enhanced elemental concentrations of root, stem and leaf in *B. rapa* Linn L. and *B. tumida* Tsenet Lee L. in roots by 2.11- to 15.26-fold and 1.51- to 26.16-fold; in stem 1.33- to 14.25-fold and 1.15- to 15.16-fold and in leaf 0.31- to 8.25-fold and 1.31- to 21.84-fold, respectively. Se concentration of *Brassica* was in order of root > leaf > stem in the presence of Se. At the same level of element, Se concentration of leaf, stem and root in *B. rapa* Linn L. was greater than in *B. tumida* Tsenet Lee L. (except for root Se at 5 mg/kg Se).

### 3.3. Correlations of selenium levels in soil and selenium concentrations of *Brassica*

As shown in Table 1, significant correlation was noted between Se concentrations in root, stem and leaf of *B. rapa* Linn L. and *B. tumida* Tsenet Lee L. Significant positive correlation was also observed between Se concentrations in root, stem and leaf of the vegetables and soil elemental concentrations.

### 3.4. Detection of Se metabolism gene expression

Expression of six Se metabolism-related genes (family) in *B. rapa* Linn L. was consistent with bimodal curve characteristics correlated increase of soil Se levels (Figure 3).
The expression of six Se metabolism-related genes (family) rose at 1 mg/kg Se, then declined at 2.5 mg/kg Se but increased again at 5 mg/kg Se. Similarly, change trend was observed in members of the gene family, but significant difference was found in the expression level of different member genes in APR, APS and SAT families, and the highest expression levels were detected in APR1, APS4 and SAT2 at the same Se level.

Figure 4 illustrates that the expression of SAT, APR and CysD in *B. tumida Tsenet Lee L.* exhibited unimodal pattern with rise of soil Se, which did not change significantly in the range of 0.5–1 mg/kg Se, subsequently increased significantly at 2.5 mg/kg Se but fell at 5 mg/kg Se. The expression of APS and MMT in *B. tumida Tsenet* Lee L. decreased gradually in the range of 0.5–1 mg/kg Se, increased significantly at 2.5 mg/kg Se but declined again at 5 mg/kg Se with elevation in soil Se. SMT expression displayed large and small bimodal pattern with increase in soil Se level, which rose at 0.5 mg/kg Se, decreased at 1 mg/kg Se but increased again to the highest level at 2.5 mg/kg Se. A decline occurred at 5 mg/kg Se.

### 4. Discussion

GSH-Px is one of the main members of intracellular enzyme protection system, whose action mechanism contributes to antioxidant effect of Se (Li et al. 2018). In agreement with Zhao et al. (2017), Se concentration in plants was confirmed to be related to activity of GSH-Px. In the present study, exogenous Se increased GSH-Px activity in leaf and root of *Brassica*, which is consistent with the earlier report by Li et al. (2003).

Se concentrations in leaf, stem and root of the two *Brassica* were elevated with rise in Se levels in soil displaying a positive significant correlation. Similarly, Munierlamy et al. (2007) noted that the concentrations of Se in maize, lettuce, radish and ryegrass were higher than those found in crop plants growing in non-seleniferous soils. After Se application, elemental concentration in *B. rapa* Linn L. and *B. tumida Tsenet* Lee L. was in the order of root > leaf > stem. Tian, Chen, and Xiong (2005) reported a similar finding with different Se concentrations where the Se content in different parts of ryegrass was in order of roots > leaves > stems. Significant differences in Se concentration were found between plant species and varieties (Fordyce 2013; Li et al. 2015; Zhao et al. 2017; Shahid et al. 2018). At the same level of Se, elemental concentration of leaf, stem and root in *B. rapa* Linn L. was higher than in *B. tumida Tsenet* L. Lee L.
Lee L. (except for root Se concentration at 5 mg/kg Se level). This observation is in agreement with the earlier report by Fordyce (2013).

APS, APR, CysD, MMT, SAT and SMT are known to be the predominant genes for plant metabolism and Se accumulation (Valdez Barillas, Quinn, and Pilon-Smits 2011; Longchamp et al. 2015). In the present study, the expression level of six Se

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**Figure 3.** Real-time PCR analysis of Se metabolic related genes in leaf of *Brassica rapa* Linn L.
metabolism-related genes (family) induced by exogenous Se in the two *Brassica* demonstrated significant differences between various species and element levels. Selenite may be actively absorbed into plants through high affinity with ATP. In the present study, the expression levels of APS and APR in *B. rapa* Linn L. were markedly higher than *B. tumida* Tsenet Lee L., which may account for the findings that Se concentrations were present in leaf of *B. rapa* Linn L. in greater quantities than in *B. tumida* Tsenet Lee L.
SAT and CysD are two important enzymes involved in synthesis and regulation of cysteine, which combine to form cysteine synthase (Özgür, Turgut-Kara, and Şule 2012). In our study, SAT and CysD of leaf in Brassica demonstrated similar responsiveness under the same Se treatment. While the expression of SAT and CysD in B. rapa Linn L. was highest at 0.5 mg/kg Se in B. tumida Tsenet Lee L. expression

Figure 4. Real-time PCR analysis of Se metabolic related genes in leaf of Brassica tumida Tsenet Lee L.
highest was at 2.5 mg/kg Se. Data thus indicate the expression level of Se metabolism-related genes in *Brassica* varied in different subspecies with *B. rapa* Linn L. being more sensitive.

SMT is one of the key enzymes involved in Se metabolism in plants, which blocks adverse effects attributed to excessive concentrations of Se on plants (Araie, Suzuki, and Shiraiwa 2008). Overexpression of SMT in non-hyperaccumulator *B. juncea* led
to significant increases in Se accumulation and tolerance (LeDuc et al. 2004). In this study, the application of Se increased expression level of SMT gene in both Brassica subspecies compared to control. The increased tolerance and accumulation abilities of these two Brassica subspecies to Se may be associated with that elevated Se concentration noted in plants.

Methionine methyltransferase (MMT) plays a key role in the production of volatile compounds in plants. SeMet and Met generated selenomethionine (SeMM) and thiomethyl methionine (SMM) by the action of betaine-homocysteine methyltransferase and methionine methyltransferase (MMT). Araie, Suzuki, and Shiraiwa (2008) found that in non-enriched Se plants, the compounds SeMet, SeMM and SMM in plants produce adverse effects on the plants. In this study, the expressions of SMT and MMT in B. rapa Linn L. were higher than those in B. tumida Tsenet Lee L., indicating that B. rapa Linn L. may be more tolerant to Se-mediated stress. Furthermore, at the same Se level, different expressions of Se-related genes were observed in B. rapa Linn L. and B. tumida Tsenet Lee L., indicating the responsiveness of Brassica varies between different subspecies.

5. Conclusions

Exogenous Se increased GSH-Px activity in leaf and root of B. tumida Tsenet Lee L. and B. rapa Linn L. Se concentrations in root, stem and leaf of the two Brassica subspecies were significantly increased by exogenous Se. There was a significant positive correlation between Se concentration in root, stem and leaf and exogenous Se levels. Se concentrations in root, stem and leaf of B. rapa Linn L. were higher than in B. tumida Tsenet Lee L. under the same soil Se concentration conditions. The expression level of six Se-related genes (family) induced by exogenous Se in the two Brassica varied significantly between different species and Se levels. Data thus indicate that although Brassica is one species various subspecies respond uniquely to environmental Se exposure, which is crucial in terms of required nutritional needs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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