



**Full Length Article**

## Differences in Selenium Uptake, Distribution and Expression of Selenium Metabolism Genes in Tomatoes

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### Abstract

Effects of different levels of selenium (0, 5 and 10 mg·kg<sup>-1</sup>) on selenium uptake, distribution, and the expression levels of Se metabolism genes were compared with three tomato (*Solanum lycopersicum* L.) varieties (KT30, Defumm-8 and Luobeiqi). The results showed that Se inhibited the expression levels of APS reductase in varieties of KT30 and Luobeiqi; however, there was no significant difference in variety Defumm-8. Selenium also promoted expression levels of ATP sulfurylase of the tomato varieties. The expression levels of cysteine desulfurase of variety KT30 and variety Defumm-8 first decreased and then increased with the increasing Se concentration; however, expression levels of Se were lower than the control, and expression levels of Se in leaves of variety Luobeiqi increased. The Se concentration markedly decreased the expression levels of S-adenosyl-l-Met:l-Met S-methyltransferase in the three varieties, except variety Defumm-8 with Se at 5 mg·kg<sup>-1</sup>. Furthermore, the Se concentration markedly decreased expression levels of serine acetyltransferase of variety KT30 and variety Defumm-8, but expression levels of leaves of variety Luobeiqi increased. Expression levels of serine acetyltransferase in variety Luobeiqi increased with the increasing Se concentration but decreased in KT30 and Defumm-8. Expression levels of SeCys methyltransferase (SMT) in Luobeiqi and KT30 were reduced by Se, while promoted in Defumm-8. The Se treatment promoted expression levels of APS in three tomato varieties. © 2017 Friends Science Publishers

**Keywords:** Tomato varieties; Selenium uptake; Distribution; Selenium metabolism genes

### Introduction

Selenium (Se), a vital trace element for humans, is important for preventing disease, improving health, and preventing aging and has been considered a life-protecting agent. Selenium deficiency can cause a series of symptoms, such as cardiovascular disease, Kaschin–Beck disease and Keshan disease. The Se content of the soil is typically low, and there are more than 40 countries and regions worldwide where people experience Se deficiency. In China, about 72% county (city) are Se deficiency areas, of which nearly 1/3 are severe Se deficiency areas. Improved living standards have resulted in more attention being given to health, and people have begun taking Se supplements. Selenium supplements can be manufactured from inorganic chemicals, such as sodium selenite, but the absorption rate in the human body is low, with the risk of side effects (Wen, 2006). Researches have shown that consumption of plant products containing a high Se content, is the most efficient and safe way of Se supplementation (Finley and Davis, 2001; Chen *et al.*, 2008).

The contents of selenium in plants varied greatly, because of plant species, planting environment, and the way of cultivation, etc. There is a great difference in the content of selenium in species, and there is a significant difference

in the contents of selenium in different genotypes (Zhang *et al.*, 2006). Glutathione peroxidase (GSH-Px) was the first enzyme reported in the literature as a selenium containing enzyme, which has been found out to have four categories kinds of GSH-Px family members (Miao *et al.*, 2005). GSH-Px may be one of family member of the plant, which may or may be different vary in different developmental stages and in different stress conditions. The mRNA levels of GSH-Px increased steadily under different environmental stresses e.g., pathogen infection, high salt and heavy metals, etc. (Avsian-Kretchmer and Lev-Yadun, 2004) Therefore, GSH-Px may play a vital part in the process of oxidative signal transduction in plants (Freeman *et al.*, 2006, 2010).

In recent studies, APS play modulates the reduction of selenite and Se assimilation because of a strong similarity between sulfur and Se; it has also been illustrated that the limit of Se assimilation is selenate reduction (Du *et al.*, 2007). Overexpressed APS in transgenic plants promote the rate of this process and increases accumulation and tolerance of Se (Sors *et al.*, 2005). Many studies have proven that cysteine desulfurase (*CysD*), S-adenosyl-l-Met:l-Met S-methyltransferase (MMT) (Berken *et al.*, 2002), serine acetyltransferase (SAT) (Van Hoewyk *et al.*, 2008), and SeCys methyltransferase (SMT) were also the

main genes for plant metabolism and Se accumulation (Berken *et al.*, 2002). Apart from possessing Cys desulfurase activity, NifS-like proteins can also be characterized as SeCys lyases able to cleave selenocysteine to form alanine and elemental selenium (Mihara *et al.*, 1997). The synthesis of Se-Met is involved in possibly by homo-Cys S-methyltransferase or Met synthase. Regarding to the sulfur assimilation, Terry *et al.* (2000) reported that Se-Met is methylated to Se-methyl Se-Met (SeMM) by the Sadenosyl-l-Met:Met-S- methyltransferase.

Selenocysteine methyltransferase (SMT), a central enzyme in Se hyperaccumulators, which can methylate SeCys and inhibit toxicity by preventing SeCys from being misincorporated into proteins (Neuhierl and Böck, 1996). Therefore, selenate can be converted into methylselenocysteine and g-glutamylmethylselenocysteine rapidly by the hyperaccumulator *Astragalus bisulcatus* (Dunnill and Fowden, 1967; Zhang *et al.*, 2006). The gene encoding SMT not only has been cloned from *A. bisulcatus* (Neuhierl and Böck, 1996) but overexpressed in *Arabidopsis thaliana* and *Brassica juncea*; The strengthen Se tolerance and accumulation were brought about by this when plants were given selenite ( $\text{SeO}_3^{2-}$ ) instead of selenate (Sors *et al.*, 2005). In hyperaccumulators, accumulation of total Se was evidently higher than that was discovered in the SMT transgenic plants, which indicates that SMT enzyme is seemingly related that have a synergistic interactions of Se hyperaccumulators. Ellis *et al.* (2004) reported that it cannot be proved that the capacity differences in enzyme are related to reduction or assimilation S, which is significant for Se hyperaccumulated *A. bisulcatus*.

Sors *et al.* (2005) also proposed transgenic *A. thaliana* overexpressing APR (APS reductase) and ATP sulfurylase played significant roles in a greater selenate reduction of total Se, however the overexpressing in SAT (serine acetyl transferase) led to a tiny reduction in organic selenium. Generally, total Se accumulation in shoots was relatively low in the transgenic plants overexpressing ATPS, SAT and PaAPR (*P. aeruginosa* APR). The plant root growth was affected by selenate treatment within ATPS and SAT overexpressors, besides, less in the PaAPR transgenic plants. Thereby, it draws a conclusion that ATPS and APR enzymes are vital correlated with selenate reduction in plants (Sors *et al.*, 2005). In this research, the effect of various Se concentrations on growth and on the Se uptake and accumulation in three tomato varieties were determined. To explore differences in Se accumulations in these tomato varieties, the genes connected with Se metabolism and absorption in tomato and the expression levels during growth were also examined.

## Materials and Methods

### Plant Material, Soil and Se Treatments

Soil was collected from the Jiulongpo district in Chongqing

municipality, China. The soil pH was 6.03, organic matter content was  $37.35 \text{ g}\cdot\text{kg}^{-1}$ , total nitrogen (TN) content was  $2.62 \text{ g}\cdot\text{kg}^{-1}$ , available nitrogen content in soil was  $105 \text{ mg}\cdot\text{kg}^{-1}$ , available phosphorous content  $13.1 \text{ mg}\cdot\text{kg}^{-1}$ , available potassium content was  $100.9 \text{ mg}\cdot\text{kg}^{-1}$ , cation exchange capacity (CEC) was  $0.192 \text{ mol}\cdot\text{kg}^{-1}$ , total Se content was  $0.26 \text{ mg}\cdot\text{kg}^{-1}$ , and available Se was  $0.017 \text{ mg}\cdot\text{kg}^{-1}$ . Seedlings of three tomato varieties (KT30, Defumm-8 and Luobeiqi) were purchased from Chongqing Academy of Agricultural Sciences.

Three levels s of Se (0, 5 and  $10 \text{ mg}\cdot\text{kg}^{-1}$ ) prepared from  $\text{Na}_2\text{SeO}_3$  were set up for the pot experiment. Moreover, air-dried soil (5 kg) was sieved using a 40-mesh sieve, and homogeneously mixed with the  $\text{Na}_2\text{SeO}_3$  solution, put in a plastic pot (diameter, 25 cm; height, 17 cm), all treatments were performed in triplicate and arranged at random. The tomato seedlings were transplanted at three or four leaf stage on March 2, 2013. Nitrogen was applied basically in  $180 \text{ mg}\cdot\text{L}^{-1}$  and in three installments: 40% for basal and 30% each for seedling and flowering stages, P fertilizer ( $\text{NH}_4\text{H}_2\text{PO}_4$ ) and K (KCl) were applied in 100 and  $150 \text{ mg}\cdot\text{L}^{-1}$  respectively in the basic fertilizer, productivity was recorded since the first tomato fruit was born. The process of the greenhouse experiment was took three months, and the production was recorded (Xie *et al.*, 2014).

### Analysis of Soil Physicochemical Properties

The soil pH was measured in 1:5 (soil:water). Extractable P and cation exchange capacity (CEC) were measured according to Rayment and Higginson (1992), while the soil organic matter was determined with the method of McCleod (1975). The concentration of extractable Se was determined via  $\text{KH}_2\text{PO}_4$  ( $0.016 \text{ mol}\cdot\text{L}^{-1}$ ) extraction [10 g soil (dry weight) to 30 mL of  $\text{KH}_2\text{PO}_4$  (w/v)] followed by atomic fluorescence spectrometry analysis (Zhang *et al.*, 2014).

### Analysis of Se Concentrations in the Plants

Dried plant samples were ground and digested at  $170^\circ\text{C}$  using 10 mL (8 mL of ultrapure nitric acid and 2 mL of perchloric acid) of acid mixture (Kyodan *et al.*, 1988). The acid mixture was heated until white smoke appeared; further, 10 mL of  $6 \text{ mol}\cdot\text{L}^{-1}$  hydrochloric acid was added, and the mixture was heated until the white smoke reappeared. The residual sample was then diluted in 25 mL of ultrapure water. The concentration of Se in the solution was analyzed using hydride generation atomic fluorescence spectrometry (PF6.3, Beijing Purkinje General Instrument Co. Ltd., China).

### Detection of Gene Expression

**Total RNA extraction:** Total RNA was extracted from the plant material with RNA extraction kit purchased from Huashun Biotechnology Company. RLP lysate: RLA and Plant-PLUS were mixed at 4:1 (V/V), adding 2% of the

total volume of 2-ME. Fresh samples (100 mg) kept at  $-80^{\circ}\text{C}$  were powdered under liquid nitrogen. The samples were added to 1.5 mL centrifuge tubes pre-filled with 600  $\mu\text{L}$  RLP lysates and vibrated for 2 min for mixing the samples before the liquid nitrogen completely evaporated. Let sit for five minutes, then subjected to reverse oscillation for 30 s after adding 200  $\mu\text{L}$  chloroform, followed by 3 min at room temperature. Centrifuged for 5 min at 12 000 rpm, the supernatant was transferred to another 1.5 mL centrifuge tube of and mixed with 250  $\mu\text{L}$  75% ethanol after through mixing and then put them to the RNA adsorption column. Then centrifuged for 1 min at 12 000 rpm, the liquid was discarded in the collection tube, and the adsorption column was put back in the original collection tube. Then 500  $\mu\text{L}$  RP (protein-free solution) was added, placed for 1 min and centrifuged for 30 s at 12 000 rpm. Next, the liquid was discarded in the collection tube and the adsorption column was put back in the original collection tube. 500  $\mu\text{L}$  liquid of W3 (75% ethanol) was added, placed for 1 min, and centrifuged for 15 s at 12 000 rpm; further, the supernatant was discarded in the collection tube, the centrifuge tube was washed with 1 mL 75% ethanol and centrifuged for 1 min at 12,000 rpm. After that the adsorption column was put into another clean 1.5 mL centrifuge tube. Finally, 50  $\mu\text{L}$  of DEPC water was added to the center of the membrane adsorption and air dried for 2 min, then centrifuged for 1 min at 13 000 rpm. Lastly, the eluent contained total RNA in the samples. Two tubes were taken for electrophoresis and spectrophotometer detection after packing, and the rest were quickly preserved in refrigerator at  $-80^{\circ}\text{C}$ .

**Purification of total RNA:** RNase-free DNase I (Code No. D2215) (TARALA) was used for removing trace amounts of DNA in the RNA sampl. 20–50  $\mu\text{g}$  total RNA, 5  $\mu\text{L}$  in  $10\times$  DNase I buffer, 2  $\mu\text{L}$  DNase I (RNase-free, 5 U/ $\mu\text{L}$ ), and 0.5  $\mu\text{L}$  RNase inhibitor (40 U/ $\mu\text{L}$ ) were added in microcentrifugal tubes, and then added DEPC-treated water to a total volume of 50  $\mu\text{L}$ . The solutions were reacted for 30 min at  $37^{\circ}\text{C}$ . 50  $\mu\text{L}$  DEPC-treated water was added to make a total volume of 100  $\mu\text{L}$ . The same volume (100  $\mu\text{L}$ ) of phenol/chloroform/isoamyl alcohol (25:24:1) was added and mixed thoroughly. The solutions were centrifuged for 5 min at 12 000 rpm, and the upper supernatant was removed into another microcentrifugal tube. The same volume (100  $\mu\text{L}$ ) of chloroform/isoamyl alcohol ( $v:v=24:1$ ) was added and mixed thoroughly. The solutions were allowed to stand at room temperature and centrifuged for 5 min at 12 000 rpm; the upper supernatant was transferred to another microcentrifugal tube. The 1/10 volume (10  $\mu\text{L}$ ) of 3 M NaOAc (pH 5.2) and 250  $\mu\text{L}$  precooling ethanol were added and placed for 30–60 min at  $-20^{\circ}\text{C}$ . The solutions were centrifuged for 10 min at 12 000 rpm at  $4^{\circ}\text{C}$ ; the supernatant was abandoned and the precipitate was recovered. The precipitation was washed with  $2-4^{\circ}\text{C}$  70% ethanol and centrifuged for 10 min at 12 000 rpm at  $4^{\circ}\text{C}$  and the supernatant was then discarded and the precipitate was air or blow dried. The precipitate was then dissolved with a

proper amount of RNase-free  $\text{H}_2\text{O}$ , providing purified RNA. **RNA detection:** RNA (1  $\mu\text{L}$ ) was taken, and the OD values were set up at 260 nm and 280 nm by Nanodrop 2 000 UV-vis spectrophotometer (Thermo Scientific, USA). Concentration calculation: For single-stranded RNA, the RNA concentration was  $40\ \mu\text{g}\cdot\text{mL}^{-1}$  when  $\text{OD}_{260} = 1.0$ . The RNA concentration is calculated according to the method. Purity analysis: The ideal  $\text{OD}_{260/280}$  of RNA samples should be 1.7–2.0. Further, 1% agarose gel and  $1\times$  TAE electrophoretic buffer were prepared; 5  $\mu\text{L}$  of RNA was taken, and 2  $\mu\text{L}$  bromophenol blue was added to it. This mixture was then thoroughly mixed, electrophoresed (90 V, 1–2 h), and photos were captured with E-Gel Imager.

### Synthesis of Reverse Transcription cDNA

A 20  $\mu\text{L}$  reaction mixture was put in the microcentrifuge tubes, and cooled on ice, added 2  $\mu\text{g}$  total RNA and 1  $\mu\text{L}$  Olig (dT)18 solutions, then add to 11  $\mu\text{L}$  with DEPC-treated  $\text{H}_2\text{O}$ , mixed softly and gently centrifuged for 5 s. The solutions were kept  $70^{\circ}\text{C}$  in a water bath for 5 min, quickly transferred to an ice bath for 5 min, and gently centrifuged for 5 s. 4  $\mu\text{L}$   $5\times$  Buffer, 1  $\mu\text{L}$  RNase Inhibitor (40 U/ $\mu\text{L}$ ), 1  $\mu\text{L}$  MMLV (200 U/ $\mu\text{L}$ ) and 2  $\mu\text{L}$  dNTP (10 mM) were added to the tubes. The solutions were gently shaken and centrifuged at a low speed for 5 s. The solutions were kept at  $42^{\circ}\text{C}$  water bath for 1 h. The temperature of solutions was maintained in  $70^{\circ}\text{C}$  for 15 min, following which, the reaction was terminated, and the solutions stored at  $-20^{\circ}\text{C}$ .

### Primer Design and Synthesis

Six genes associated with Se metabolism were examined. According to cDNA sequence in NCBI GenBank of tomato (*Solanum lycopersicum* L) and other solanaceae crops (Kabelka *et al.*, 2002; Barry and Pandey, 2009) designing the RT-PCR gene-specific primers by Oligo 6.0 software (Xiao *et al.*, 2015).

### PCR Amplification of cDNA

Take the acquired cDNA, specific amplified with ABI-9700 instrument, reaction system (total volume is 25  $\mu\text{L}$ ) included with 2.5  $\mu\text{L}$   $10\times$  Buffer (including  $\text{Mg}^{2+}$ ), 0.5  $\mu\text{L}$  dNTP (10 mM), 0.5  $\mu\text{L}$  forward primer (10  $\mu\text{M}$ ), 0.5  $\mu\text{L}$  reverse primer (10  $\mu\text{M}$ ), 0.3  $\mu\text{L}$  Taq DNA polymerase (5 U/ $\mu\text{L}$ ), 0.25  $\mu\text{L}$  cDNA template, and then add dd  $\text{H}_2\text{O}$  to a total volume of 25  $\mu\text{L}$ . PCR reaction program included  $94^{\circ}\text{C}$  initial denaturation for 2 min, and  $94^{\circ}\text{C}$  denaturation for 40 s, and  $52^{\circ}\text{C}$ – $60^{\circ}\text{C}$  annealing temperature (depends on the specific reaction, APR  $56^{\circ}\text{C}$ , APS  $60^{\circ}\text{C}$ , CysD  $60^{\circ}\text{C}$ , MMT  $60^{\circ}\text{C}$ , SAT  $52^{\circ}\text{C}$ , and SMT  $58^{\circ}\text{C}$ , etc.) for 1 min, and  $72^{\circ}\text{C}$  for 45 s, and 30 cycles, and then  $72^{\circ}\text{C}$  for 10 min. PCR products with detected with electrophoresis 1.2% agarose gel (including GoldView, GV), and images were taken with ultraviolet transilluminator.

### Real-time Quantitative PCR

The cDNA obtained by reverse transcription was diluted 25-fold with dd H<sub>2</sub>O, and its specificity was detected with qRT-PCR with the 25S rRNA gene as the standard (Levesque *et al.*, 1990) using SYBR Premix Ex Taq™ II (Perfect Real Time) kit provided by TaKaRa. The PCR amplification reaction system include 10 µl SYBR® Premix Ex Taq™ II (2×), 0.8 µL F-primer (10 µmol/L), 0.8 µL R-primer (10 µmol/L), 0.4 µL ROX Reference Dye II (50×), and 100 ng cDNA template, then added ddH<sub>2</sub>O up to a total volume of 20 µL. Amplification reaction conditions were 95°C pre-denatured for 30 s, 95°C denatured for 5 s, corresponding annealing temperature annealing for 30 s, extension of 30 s, 40 cycles, and then 72°C extension of 10 min. PCR products were assayed using a rapid automated capillary electrophoresis system. Each sample was repeated three times, while 25S rRNA gene was used as the standard gene.

### Statistical Analysis

Three-way analysis of univariate ANOVA and correlation analysis were performed using SPSS version 21.0 package (SPSS, 2009). The variables analyzed separately were Se concentration and Se uptake in tomato. The transfer factors (TF; proportion of Se concentration in shoot to that in roots) were also analyzed to three-way univariate ANOVA. The level of significance was 0.05 (Xu *et al.*, 2010).

## Results

### Concentration of Se in Fruit, Leaf, Stem and Root

The Se concentrations in fruit, leaf, stem and root were significantly different ( $p \leq 0.05$ ) between Se treatments and between varieties (Fig. 1). Considering the effects of Se treatments on all varieties, Se contents in plant tissues were in the order root (9.898 mg·kg<sup>-1</sup>) > leaf (2.299 mg·kg<sup>-1</sup>) > stem (1.297 mg·kg<sup>-1</sup>) > fruit (1.176 mg·kg<sup>-1</sup>). Furthermore, Se concentrations in root, stem, leaf, and fruit increased with an increase in the applied Se fertilizer. The Se concentration in the root of 10 mg·kg<sup>-1</sup> Se treatment was 2.25 times that of the 5 mg·kg<sup>-1</sup> Se treatment for variety KT30. In the stem, leaf and fruit were 3.04, 1.81, and 2.73 times that of the 5 mg·kg<sup>-1</sup> Se treatment. For variety Defumm-8, the Se content in the root was 1.64 times that of 5 mg·kg<sup>-1</sup> Se, whereas in the stem, leaf, and fruit were 3.28, 1.83 and 2.10 that of 5 mg·kg<sup>-1</sup> Se treatment. For variety Luobeiqi, the Se concentration in the root was 1.61 times that of the 5 mg·kg<sup>-1</sup> Se treatment, whereas the stem, leaf and fruit were 2.82, 2.63 and 3.04 times that of the 5 mg·kg<sup>-1</sup> Se treatment. Highest fruit Se concentration was 2.778 mg·kg<sup>-1</sup> in variety Luobeiqi at the 10 mg·kg<sup>-1</sup> Se treatment.

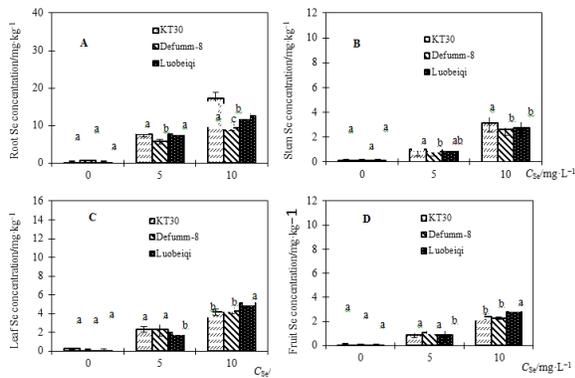
### Se Accumulation in Fruit, Leaf, Stem and Root

A marked difference was found in the Se content in the

leaves, stem, fruit, and roots between the three varieties and between the different Se concentrations (Fig. 2). The Se content in different tissues of tomato increased with the increasing Se concentration in the soil and reached a maximum at 10 mg·kg<sup>-1</sup> Se treatment. The Se accumulation (per pot) in the root was 16.19–30.92 times, for the stem was 17.03–21.69, for the leaves was 12.80–25.01, and for the fruit was 18.02–25.21 times that of the control. Moreover, the Se accumulation in the different tissues of tomato were different and was in order of stem  $\approx$  leaf > root  $\approx$  fruit ( $p \leq 0.05$ ). In this experimental condition, after treated with Se, fruit Se accumulation in variety Defumm-8 was higher than that in variety KT30 and variety Luobeiqi. On the other hand, whether treated with Se or not, total Se concentration in variety KT30 and variety Luobeiqi were higher than that in variety Defumm-8. Highest total Se concentration was 206.10 µg·pot<sup>-1</sup> in variety KT30 at 10 mg·kg<sup>-1</sup> Se treatment.

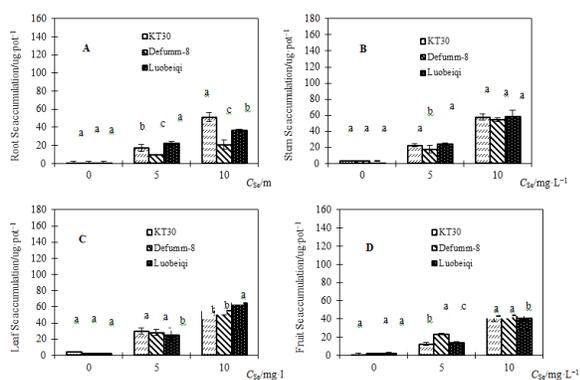
### Detection of Se Metabolism Gene Expression

Real-time PCR results of APR and six gene expression levels between the different tomato varieties and concentration are shown in Fig. 3 and Fig. 4. The expression level of APR in variety KT30 was the highest in the treatment without Se, and it markedly decreased with Se, whereas there was no marked difference of APR expression in different treatments of variety Defumm-8. There was marked difference of APR expression in different treatments of variety Luobeiqi, and it was decreased with the increasing Se concentration and reached a minimum at 10 mg·kg<sup>-1</sup>. Expression levels of ATP sulfurylase (APS) of variety KT30 first decreased and then increased and reached maximum at 10 mg·kg<sup>-1</sup> Se treatment; it was 3.67 times that of the control. The Se concentration markedly promoted expressions of APS of variety Defumm-8 and variety Luobeiqi, which increased with the increasing soil Se concentration. Moreover, expression levels of CysD of variety KT30 and variety Defumm-8 revealed the same trend with the increasing Se concentration: first decreased, then increased, and reached minimum at 5 mg·kg<sup>-1</sup> Se treatment, which decreased by 21.43% and 30.77%, respectively, compared with the control. However, Se expression levels at 10 mg kg<sup>-1</sup> Se treatments were lower than that of the control. The Se treatment promoted the expression of CysD of variety Luobeiqi, and it reached a maximum at 5 mg·kg<sup>-1</sup> Se treatment. The Se concentration markedly decreased expression levels of MMT of variety Luobeiqi and variety KT30, both increased with the increasing Se concentration; expression of SAT of variety Defumm-8 reached a maximum at 5 mg kg<sup>-1</sup> Se treatment, while reduced at 10 mg·kg<sup>-1</sup> Se treatment. Expression levels of SAT of variety KT30 and variety Defumm-8 revealed the same trend with the increasing Se concentration: first decreased and then increased; however, they were lower than the control. The expression level of SAT of variety Luobeiqi increased with the increasing Se concentration and



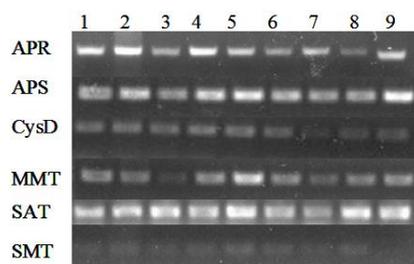
**Fig. 1:** Impacts of different Se concentrations on the Se content of root (A), stem (B), leaf (C) and fruit (D) in different tomato varieties<sup>ψ</sup>

<sup>ψ</sup>Different letters (a, b, c) indicate significant difference at  $P \leq 0.05$  among different Se levels



**Fig. 2:** Variations in Se accumulation in root (A), stem (B), leaf (C) and fruit (D) for the different tomato varieties<sup>ψ</sup>

<sup>ψ</sup>Different letters (a, b, c) indicate significant difference at  $P \leq 0.05$  among different Se levels



**Fig. 3:** Real time fluorescent quantitative PCR analysis of Se metabolism associated genes in leaf of different tomato varieties<sup>ψ</sup>

reached a maximum at  $10 \text{ mg kg}^{-1}$  Se treatment. Expression levels of SMT of variety Luobeiqi and variety KT30 were inhibited by Se treatment, whereas it was promoted in variety Defumm-8.

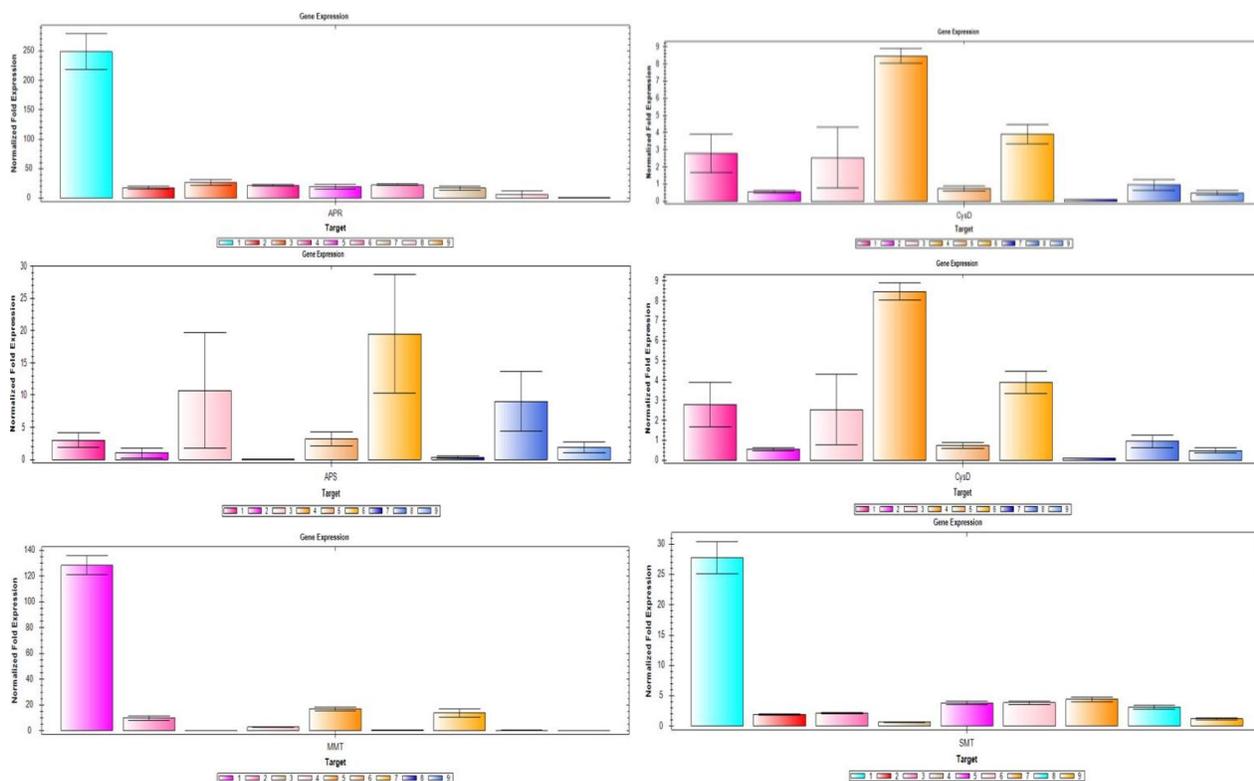
<sup>ψ</sup>Number 1, 2 and 3 indicate varieties KT30, Defumm-8 and Luobeiqi at no Se treatment; 4, 5 and 6 indicated

varieties KT30, Defumm-8 and Luobeiqi at  $5 \text{ mg kg}^{-1}$  Se treatment; 7, 8 and 9 indicated varieties KT30, Defumm-8 and Luobeiqi at  $5 \text{ mg kg}^{-1}$  Se treatment, respectively.

## Discussion

In this experiment, the Se content in roots of tomato was the highest, followed by leaf, stem and then fruit was the lowest, which was similar to the results of many scholars. For example, Shi *et al.* (1993) studied the absorption, distribution, and transformation of Se in tomato and found that the Se content in various organs in the plant in the order of root > leaf > stem > fruit > flowers. Tian *et al.* (2005) had a similar report, with different Se concentrations; the Se content in different parts of ryegrass was in order of roots > leaves > stems (Tian *et al.*, 2005). The selenium transferring from soil to plant depends upon plant species, form of Se supply, soil type, concentration and so forth (Munierlamy *et al.*, 2007). Furthermore, the content of Se in the organs is related with Se chemical form. In soil, selenite adsorbed by carbonate, amorphous iron or aluminum content mainly exists in exchangeable speciation and carbonate bound form that cannot be uptake largely by plants (Li *et al.*, 2015), whereas Se (VI) has high mobility and validity in soil which indicates that the selenate accumulation and absorption capacity of plants is stronger than selenite. There is a great difference in the plant uptake, transformation and distribution of Se (VI) and Se (IV), and Se (VI) and Se (IV) absorption and transport mechanism are relatively independent. Eustice *et al.* (1980) found that selenate was more easily transported than selenite and the organic Se selenomethionine (SMT), Terry *et al.* (2000) demonstrated the same conclusion. When selenate was absorbed, the ratio of plant Se concentration and root Se concentration was 1.4–1.72, while the ratio range was 0.6–1 when they absorbed selenomethionine (SMT); the ratio range was <0.5 when they absorbed selenite (Zayed *et al.*, 1998). The Se content in cabbage shoots was less than underground treated by Se (IV), while it was reverse for Se (VI) treatment (Fu *et al.*, 2011). There was 50% selenate absorbed by root of legume was transported to plant in 3 h, whereas for selenite, the majority remained in the roots, and only a small portion was transported to shoots (Arvy, 1993). Selenite was rarely transported to plants because it may quickly be converted to organic Se (such as SMT), which remains in the root (Zayed *et al.*, 1998). Further studies are required on synthesizing selenite in root and the transportation of synthetic substances of selenite to shoots.

The mechanism of selenium accumulation in fruits and grains has important theoretical and practical significance in improving the selenium content in fruits and grains (Zhou *et al.*, 2016). The Se content in tomato stems and leaves was greater than in roots, indicating that more root Se transported to stems and leaves, and was enriched in fruits. Zhou *et al.* (2007) found that when Se was present in abundance, it was easily transported to shoots and was



**Fig. 4:** Real-time PCR analysis of Se metabolism associated genes in tomato leaf  $\psi$

<sup>ψ</sup>Number 1,2 and 3 indicated varieties of KT30, Defumm-8 and Luobeiqi at no Se treatment; 4,5 and 6 indicated varieties of KT30, Defumm-8 and Luobeiqi at 5 mg·kg<sup>-1</sup> Se treatment; 7,8 and 9 indicated varieties of KT30, Defumm-8 and Luobeiqi at 5 mg·kg<sup>-1</sup> Se treatment, respectively

enriched in seed, while the transformation and accumulation of Se were inhibited at Se deficiency in the soil. In this experiment, similar results were obtained. This may be because exogenous Se changed the activity of the Se transporter protein, and more Se was transported to shoots when the Se level was higher.

Many studies have proven that APS, APR, *CysD*, *MMT*, *SAT* and *SMT* were also the main genes for plant metabolism and Se accumulation (Berken *et al.*, 2002; Tagmount *et al.*, 2002; Van Hoewyk *et al.*, 2008; Valdez Barillas *et al.*, 2011). In this experiment, APS expression levels increased in leaves of three tomato varieties maybe due to Se induced the expression of APS, which was conducive to metabolism and accumulation of Se for tomato. After the application of Se, APR expression in levels of three varieties of tomato did not improve, and it markedly decreased in variety KT30 and variety Luobeiqi, probably, because the increase in expression levels of APS inhibited the reduction of ASP. Expression levels of variety KT30 and variety Defumm-8 in leaves decreased, whereas they increased in variety Luobeiqi; similar results were observed for MMT and SAT. In this experiment, the Se content in the fruit of variety Luobeiqi was the highest, which may be associated with higher expression levels of *CysD*, *MMT*, and *SAT* in variety Luobeiqi. Expression levels of *SMT* in leaves of variety KT30 and variety

Luobeiqi were inhibited after Se treatment but increased in variety Defumm-8. *SMT* has been the major gene for plant metabolism and Se accumulation (Neuhierl and Bock, 2002; Nikiforova *et al.*, 2003; Ellis *et al.*, 2004). However, in this experiment, expression levels of *SMT* only increased in variety Defumm-8 after Se treatment; expression levels in variety KT30 and variety Luobeiqi decreased instead of increasing, indicating that further studies are required.

## Conclusion

The Se content of tomato fruits, roots, stems and leaves markedly increased with the increase of Se concentration in soil. The Se content in three varieties of tomato was in the order of Luobeiqi > KT30 > Defumm-8. Different expression levels of APR, APS, *CysD*, *MMT*, *SAT* and *SMT* genes were found in different tomato varieties and different concentration. The higher expression levels of *CysD*, *MMT*, and *SAT* were found in Luobeiqi variety.

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