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Transcriptome analysis of differentially expressed genes in lily leaves under selenite application

Yuying Yang[1,3], Yuqing Duan[2,3], Darong Li[1], Xiaogang Jiang[1], Hua Wang[1], Haihua Liu[1], Meide Zhang[1], Jinwen You[1]✉ & Wuxian Zhou[1]✉

Selenium (Se), an essential trace element for human health, is acquired primarily through the consumption of Se-enriched foods. Lily (*Lilium lancifolium* Thunb.) is a traditional Chinese medicinal plant that efficiently assimilates selenite through foliar uptake. This investigation elucidates the phytophysiological responses and molecular regulatory networks underlying selenite metabolism in lilies through comprehensive transcriptomic characterization. The experimental treatments consisted of graded selenite concentrations (0–8.0 mmol/L), revealing 2.0 mmol/L as the optimal concentration for enhancing biomass production and osmoprotectant accumulation. High-throughput RNA sequencing generated 59.38 Gb of clean data, yielding 76,814 functionally annotated unigenes. GO analysis revealed that the unigenes were involved mainly in cell, binding and cellular processes. Through KEGG pathway enrichment analysis, differentially expressed genes were shown to be involved mainly in translation and carbohydrate metabolism predominant pathways. Validation through RT-qPCR confirmed that pivotal enzymatic regulators including sulfite reductase, serine acetyltransferase, sterol methyltransferase, cystathionine beta-lyase, mitochondrial translation, and methionone s-methyltransferase, are important enzyme-encoding genes involved in the metabolic pathway of selenite in lily. Moderate Se exposure upregulated the expression of carbohydrate metabolism pathway genes, including *SUS*, *SPS*, and *Inv* genes, which was correlated with increased growth parameters. In contrast, supraoptimal concentrations induced a reactive oxygen species burst. Moreover, the expression levels of antioxidant genes such as superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, and glutathione reductase decreased, ultimately leading to Se toxicity in lily plants. These results delineate the regulatory network of Se accumulation and biosynthesis in lily, which helps to elucidate the physiological and molecular mechanisms of lily growth under Se accumulation.

Keywords Selenium, Lily, Selenite, RNA-sequencing, Transcriptome

As an essential component of 25 functional proteins, selenium (Se) is an important microelement for human and animal survival[1,2]. The antioxidant capacity of glutathione peroxidase (GSH-Px) with Se is 200-fold greater than that with vitamin E[3]. Se protects the human immune system and plays an important role in resistance to cancer, cardiovascular disease, diabetes and other diseases[4]. However, Se deficiency can lead to Keshan disease[5,6]. In recent years, the problem caused by Se deficiency has become increasingly severe worldwide[7,8]. Previous studies have shown that Chinese residents generally lack Se, with an average daily Se intake of only 43.3 μg, which is far below the World Health Organization's recommended value of 60 μg/d[9]. Owing to the inability of the human body to synthesize Se on its own, Se can only be obtained from the external environment[10]. At present, there are many Se-enriched health care products on the market, but these products are not accepted by the general public due to their high prices. Improving the Se content in agricultural products through agronomic methods is easy to implement and has the advantages of low cost and good effectiveness[11,12]. Therefore, we aimed to find an agricultural product with increased Se absorption efficiency to supplement Se in the human body.

The absorption, assimilation, and metabolic pathways of Se in nature are very complex. Plants absorb and assimilate exogenous Se through their roots or leaves, converting inorganic Se into more effective and stable organic Se within the plant body[13]. Inorganic Se is mainly absorbed by plants in the form of selenate (SeO_4^{2-}

[1]Key Laboratory of Chinese Herbal Medicine Biology and Cultivation, Ministry of Agriculture and Rural Affairs, Institute of Chinese Herbal Medicine, Hubei Academy of Agricultral Science, Enshi, China. [2]Hubei Key Laboratory of Biologic Resources Protection and Utilization, Hubei Minzu University, Enshi, China. [3]Yuying Yang and Yuqing Duan have the equal contribution to the research. ✉email: 554160219@qq.com; zhouwuxian@hbaas.com

) and selenite (SeO_3^2)[14]. The main forms of organic Se are selenocystine (SeCys2), selenocysteine (SeCys), selenomethionine (SeMet), etc. Compared with inorganic Se, organic Se is safer, more effective, and easier absorb and utilize by the human body[15]. Owing to the greater bioavailability of Se in plants than in animals, plant Se to some extent determines the Se level in the food chain[16]. Selenate is more soluble in water and is the most common biologically available Se in soil environments. Plants absorb selenate faster than they absorb selenite, but the conversion efficiency of selenite in plants is greater than that of selenate. In general, selenate is first converted into selenite and then further absorbed and utilized by plants. However, 90% of selenite can be directly converted into organic Se[17]. Research has shown that the Se enrichment effect of foliar application is 8 times greater than that of soil Se application[18]. In the late stage of plant growth, the absorption and assimilation of Se by leaves occur faster than those by roots. As the root-to-shoot ratio increases, the soil adsorbs and fixes more Se[19]. Therefore, we explored the absorption efficiency of Se in plants by foliar spraying of selenite.

China has extremely rich lily germplasm resources, accounting for more than half of the total number of lilies in the world[20]. Lily is a perennial herbaceous plant composed of aerial parts and underground parts. Lily has medicinal and edible value, and the underground bulbs are used as medicinal and edible parts of lily[20]. The accumulation of carbohydrates is the most important factor affecting growth of lily bulbs[21,22]. Se combines with lily polysaccharides to form Se polysaccharides, which not only exhibit pharmacological activities themselves, such as antitumor, antioxidant, immune regulatory, and antifatigue activities, but also regulate the immune and antioxidant activities of Se[23,24]. Moreover, Se polysaccharides reduce the toxicity of inorganic Se, which improves Se absorption and utilization in the body[25,26].

At present, there are few reports on the molecular mechanisms by which Se affects the vegetative growth of plants[27,28]. The lack of understanding of the physiological functions and mechanisms of Se in plants hinders the utilization of Se-enriched plant resources and the development process of Se-enriched agricultural products. Here, we used six concentrations of selenite to treat lily and compared the growth and nutrient accumulation of lily under the different concentrations of selenite. This study used RNA-seq technology to perform transcriptome analysis of selenite-treated lily. Therefore, this study explored the physiological and molecular mechanisms underlying the effects of selenite on the vegetative growth of lily through comprehensive analysis of physiological indicators and transcriptomics, providing a theoretical basis and technical support for the breeding of Se-rich lily.

Materials and methods

Plant materials and treatments

Lily seedlings were provided by the Institute of Chinese Herbal Medicine, Hubei Academy of Agricultural Science, Enshi city, Hubei Province. The experiment was conducted at an altitude of 1507 m (30°11′12″ N, 109°46′32″ E) at an experimental base. Uniform two-year-old lily seedlings were selected for this experiment. Foliar applications consisted of aqueous sodium selenite solutions (0, 0.5, 1.0, 2.0, 4.0, and 8.0 mmol/L) administered as 250 mL sprays biweekly for eight weeks. Mature leaves (positions 10–20 from the apex) were harvested for transcriptome analysis and various physiological measurements, with triplicate samples rapidly frozen in liquid nitrogen.

Chlorophyll content detection

Leaf chlorophyll was quantified through 12-h ethanol (95%) extraction of 0.1 g of tissue[28]. A spectrophotometer was used to colorimetrically calculate the absorbance at 649 nm and 665 nm when the leaves turned white. Chlorophyll content (mg/g FW) = (20.8 × A645 + 8.04 × A663) × V/M. (A: absorbance; V: volume of alcohol; M: weight of leaves)[29].

Soluble protein detection

Lily leaves (0.3 g) were ground in phosphate buffer (50 mM, pH 7.8), which contained 0.7% $NaH_2PO_4{\cdot}2H_2O$ and 1.64% $Na_2HPO_4{\cdot}12H_2O$ at pH 7.8. The supernatant was collected after centrifugation at 5000 × g at 4 °C for 20 min, and analyzed via a Coomassie brilliant blue G-250 assay.Measure the absorbance at 595 nm wavelength[28]. Aliquots of 10 µL, 20 µL, 40 µL, 60 µL, 80 µL, and 100 µL of the 1 mg/mL γ-globulin solution were separately diluted to a final volume of 100 µL with ddH_2O to prepare the standard curve. ddH_2O was used as the reagent blank. In the standard assay, the A595 for 100 µg of γ-globulin is approximately 0.4. Based on the absorbance of the test samples, the protein concentration was calculated from the standard curve.

Soluble sugar detection

Lily leaves (0.3 g) were boiled in 10 mL of 80% ethanol solution for 30 min, and the soluble sugar content was measured using anthrone reagent. 0.5 mL sample and 2 mL anthrone reagent mixed at 4 °C, cooled in a boiling water for 10 min. Measure the absorbance at 620 nm and calculate the content according to the standard curve[28].

Measurements of SOD, POD, CAT, and H_2O_2

Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)activities were measured using spectrophotometry kits (Suzhou Ke Ming Biotechnology Co., Ltd.; the catalog numbers are SOD-2-W, POD-2-Y, and CAT-2-W respectively) following the manufacturer's instructions and using a UV–vis spectrophotometer. The SOD assay reaction mixture (50 mM PBS, 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA, and an appropriate enzyme solution) was incubated under natural light at 25 ℃ for 15 min, with the control kept in the dark. Measure the absorbance at 560 nm and determine the enzyme amount that inhibits NBT reduction by 50% as one activity unit (U). POD assay reaction system (50 mM PBS,0.2% H_2O_2, 0.1% guaiacol, 0.1 mL enzyme solution). Measure the absorbance change within 1 min at 470 nm. CAT assay reaction system (50 mM PBS, 15 mM H_2O_2, 0.1 mL enzyme solution). Measure the absorbance change within 1 min at 240 nm. SOD, POD,

and CAT activities are expressed as U/min/g FW on a fresh weight basis. The H_2O_2 content was quantified with corresponding test kits (Keming, Suzhou, China; the catalog number is H2O2-2-Y) using spectrophotometry. Extract the tissue with acetone and centrifuge to obtain the supernatant. The reaction system consists of 1 mL of supernatant, 0.1 mL of 20% Ti $(SO_4)_2$, and 0.2 mL of NH_4OH. After centrifugation, the precipitate is dissolved in 2 M H_4SO_4. Measure the absorbance at 405 nm and calculate the content using the standard curve method[30,31].

Transcriptomic sequencing and analysis

Total RNA was isolated from lily leaves treated with 0, 2.0, or 4.0 mmol/L selenite using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). Libraries were prepared with a NEBNext® Ultra™ Illumina® RNA Library Preparation Kit (NEB, Ipswich, MA, USA). A NovaSeq 6000 sequencer was used to sequence paired-end RNA-seq libraries. The assembled transcripts were searched against the NCBI protein nonredundant (NR), Clusters of Orthologous Groups of proteins (COG), Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) databases. DESeq2 was used to identify DEGs ($|\log_2 FC| \geq 1$, FDR ≤ 0.05), with functional enrichment analyzed via clusterProfiler[32–36].

Quantitative real-time polymerase chain reaction (RT-qPCR)

Total RNA extracted with RNAplant plus Reagent (Tiangen, China) was reverse transcribed using the iCycler iQ5 detection system (Bio-Rad, USA) used to conduct RT-qPCR using the SYBR Green method[37]. All primers used in this study are presented in Supplemental Table 1.

Data presentation and statistical analysis

Triplicate biological replicates were analyzed in DPS (Digital Processing Systems, Hicksville, NY, USA)[38]. We considered a P value < 0.05 as significant and a P value < 0.01 as very significant.

Results

Effects of selenate on lily growth

To investigate the effects of selenite on the growth and development of lilies, lily seedlings were treated with 0, 0.5, 1.0, 2.0, 4.0, or 8.0 mmol/L selenite. As shown in Fig. 1A, the optimal concentration for promoting lily growth was 2.0 mmol/L selenite. Compared with those of the control group, the biomass, height, number of bulbils, and chlorophyll content of the lilies significantly increased. The biomass of lily seedlings gradually increased under the 0.5 mmol/L and 1.0 mmol/L selenite treatments. The 4.0 mmol/L and 8.0 mmol/L selenite treatments obviously inhibited the growth of lily, and the plants presented symptoms of wilting, leaf chlorosis, and senescence (Fig. 1 A–E). The above results showed that a low concentration (0–2.0 mmol/L) of selenite promoted the vegetative growth of lily, but concentrations above 2.0 mmol/L inhibited growth and even caused poisoning symptoms in the plants. The optimal concentration for promoting the nutritional growth of lily is 2.0 mmol/L selenite.

Effects of selenite on osmotic regulation in lily leaves

To investigate the effects of selenite on osmotic substances in lily leaves, we measured the soluble sugar and soluble protein contents. The results revealed that the soluble sugar and soluble protein contents in the 0–2.0 mmol/L selenite treatment groups were significantly greater than those in the control group (Fig. 2). In particular, in the 2.0 mmol/L selenite treatment group, the soluble sugar and soluble protein contents reached their peak values, which were, increasing by 1.52, 1.43, and 1.15 times greater than those in the control group, respectively (Fig. 2). Under the treatments of 4.0 and 8.0 mmol/L selenite, the soluble sugar and soluble protein contents significantly decreased. In addition, the ratio of soluble sugar and soluble protein content in the aboveground to underground

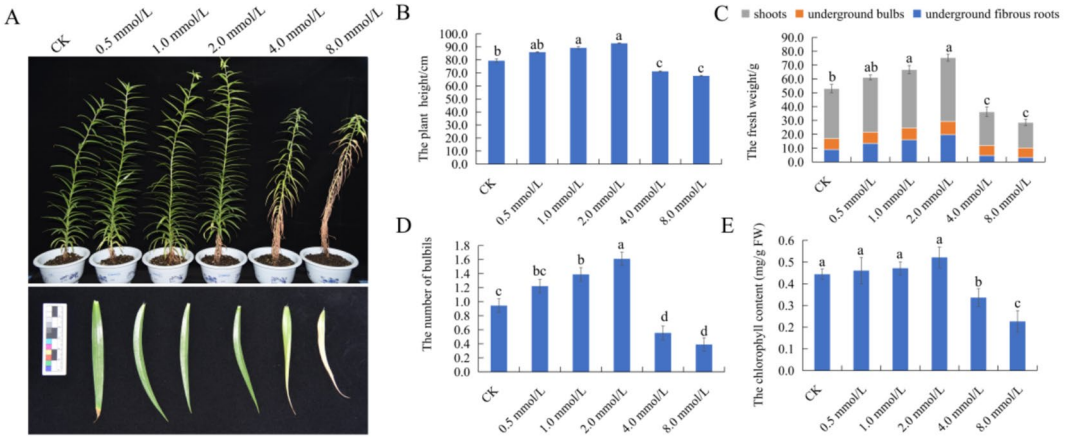

Fig. 1. The phenotype (**A**), plant height (**B**), fresh weight (**C**), number of bulbils (**D**), and chlorophyll content of lily under selenite treatments. Error bars represent standard deviations and letters significant values at $P < 0.05$.

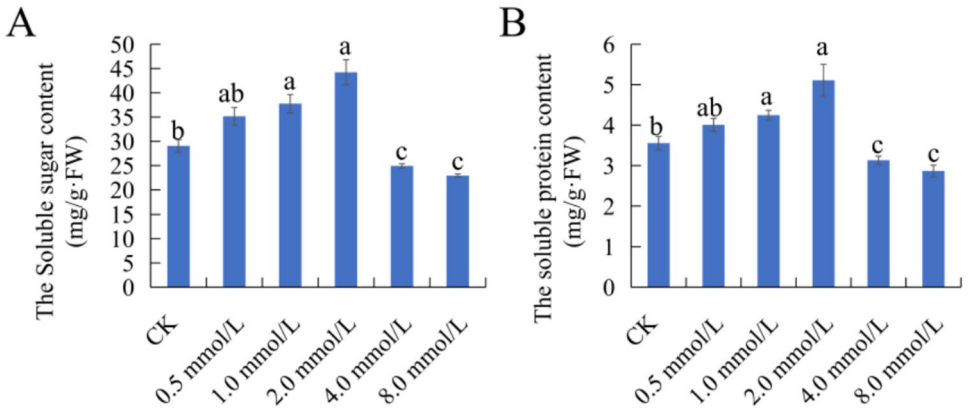

Fig. 2. The soluble sugar content (**A**) and soluble protein content (**B**) of lily under selenite treatments. Error bars represent standard deviations and letters significant values at $P < 0.05$.

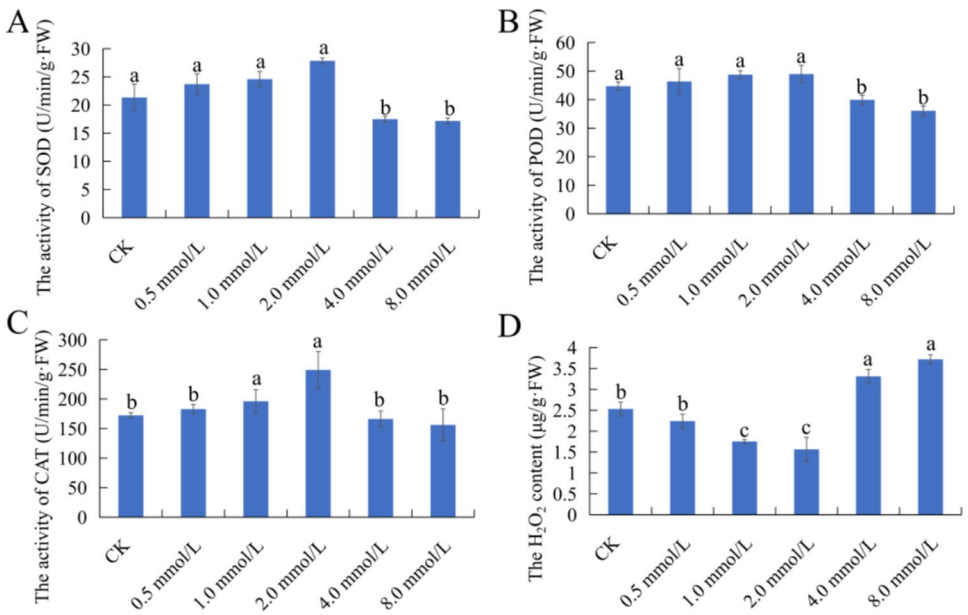

Fig. 3. The activity of SOD (**A**), POD (**B**), and CAT (**C**), and the H_2O_2 content (**D**) of lily under selenite treatments. Error bars represent standard deviations and letters significant values at $P < 0.05$.

parts also peaked in the 2.0 mmol/L selenite treatment group. In general, the treatment with 2.0 mmol/L selenite significantly improved the accumulation of nutrients in lily leaves (Fig. S1).

Effects of selenite on oxidative damage in lily leaves

When cellular metabolic activity is increased, SOD, POD, and CAT activities increase, whereas the H_2O_2 content decreases. Therefore, we used SOD, POD, CAT, and H_2O_2 as indicators to measure the oxidative damage to lily leaves under selenite treatment. Compared with the control group, the 0–2.0 mmol/L selenite treatments significantly increased the activities of SOD, POD, and CAT in lily leaves, whereas the H_2O_2 content exhibited the opposite trend (Fig. 3A–D). Under the 4.0 and 8.0 mmol/L selenite treatments, the activities of SOD and POD were significantly lower than those in control, while the H_2O_2 content was significantly greater than that in the control (Fig. 3A–D). These results showed that at concentrations exceeding 2.0 mmol/L of selenite, the higher the concentration was, the greater the degree of oxidative damage to lily leaves indicating dose-dependent oxidative damage. The above results indicated that 2.0 mmol/L selenite not only promoted the vegetative growth of lily but also did not cause oxidative damage to lily leaves. The cultivation and consumption of lily grown in a 2.0 mmol/L selenite solution was explored as a strategy for health improvement.

Transcriptome sequencing and annotation

To further reveal the physiological and molecular mechanisms of the lily response to selenite, samples from the 0, 2.0, and 4.0 mmol/L selenite treatment groups were selected as materials for transcriptome analysis. A total of

59.38 Gb of clean data were generated, with the Q30 content exceeding 91.06% and the GC content exceeding 47.59% in each library, indicating that the transcriptomic data were of high quality and could be used for further DEG analysis. A total of 76,814 unigenes were obtained, with an average length of 786.11 bp and an N50 of 1231 bp. A total of 76,814 unigenes were annotated from the six databases, including 37,271 from NR, 30,916 from GO, 17,790 from KEGG, 28,698 from Swiss-Prot, 34,373 from eggNOG, and 26,947 from Pfam (Fig. 4A). In total, 62/238 genes were upregulated/downregulated under the 2.0 mmol/L selenite treatment, and 436/656 DEGs were upregulated/downregulated under the 4.0 mmol/L selenite treatment (Fig. 4B, C).

GO enrichment analysis of differentially expressed genes

On the basis of the GO terms, a total of 30,788 unigenes could be classified into 3 gene ontology (GO) terms, including biological process, molecular function and cellular component. There were 9 biological process, 5 molecular function and 6 cellular process subcategories. In the biological process category, the most abundant terms were "cellular process," "metabolic process," and "biological regulation". In the molecular function category, "binding," "catalytic activity," and "transporter activity" were the most highly represented terms. Among the cellular component process category, the most abundant terms were "cell part", "membrane part", and "organelle" (Fig. 4D).

KEGG pathway enrichment analysis of DEGs

Enrichment analysis was conducted on the KEGG pathways in five categories: metabolism, genetic information processing, environmental information processing, cellular processes, and organic systems[39,40]. The results revealed that all the DEGs were successfully assigned to 19 KEGG pathways. KEGG pathway enrichment analysis revealed that the DEGs were significantly enriched in translation; carbohydrate metabolism; folding, sorting and degradation; amino acid metabolism; and energy metabolism (Fig. 4E). The expression analysis of key genes involved in the selenite absorption pathway in lily is worth exploring. By integrating GO and KEGG enrichment analysis, we focused on the carbohydrate metabolism process in cells. The most differentially expressed genes are involved in the metabolic process, carbohydrate, and carbohydrate metabolism pathways. Key metabolic genes in the sucrose and starch metabolic pathways may also have been enriched.

RT-qPCR analysis

To verify the accuracy of the RNA-seq data, we selected 6 highly expressed genes for RT-qPCR analysis, namely, *POL*, *OPN4*, *RPS*, *EF1G*, *FAR*, and *TAKT*. The *GAPL* gene was used as an internal reference gene (Fig. 5). The results revealed that the expression levels of these genes were highly consistent with the RNA-seq results, indicating that the RNA-seq results are reliable (Fig. S2).

The absorption and metabolic pathways of selenite in lily leaves

At present, the mechanism by which selenite is absorbed by plants is not clear. Studies have shown that plants actively absorb selenite through phosphorus transport proteins[41,42]. The metabolism of selenite in plants is relatively clear. Selenite is converted into organic Se, including SeCys and SeMet, which plants can absorb, including SeCys and SeMet. In order to verify the selenite metabolism pathway in lily leaves, the expression levels of important enzyme-encoding genes which including *sulfite reductase (SiR), serine acetyltransferase (SAT), sterol methyltransferase (SMT), cystathionine beta-lyase (CBL), mitochondrial translation (MTR), and methionone s-methyltransferase (MMT)* in the pathway were detected in lily leaves sprayed with 2 mmol/L and

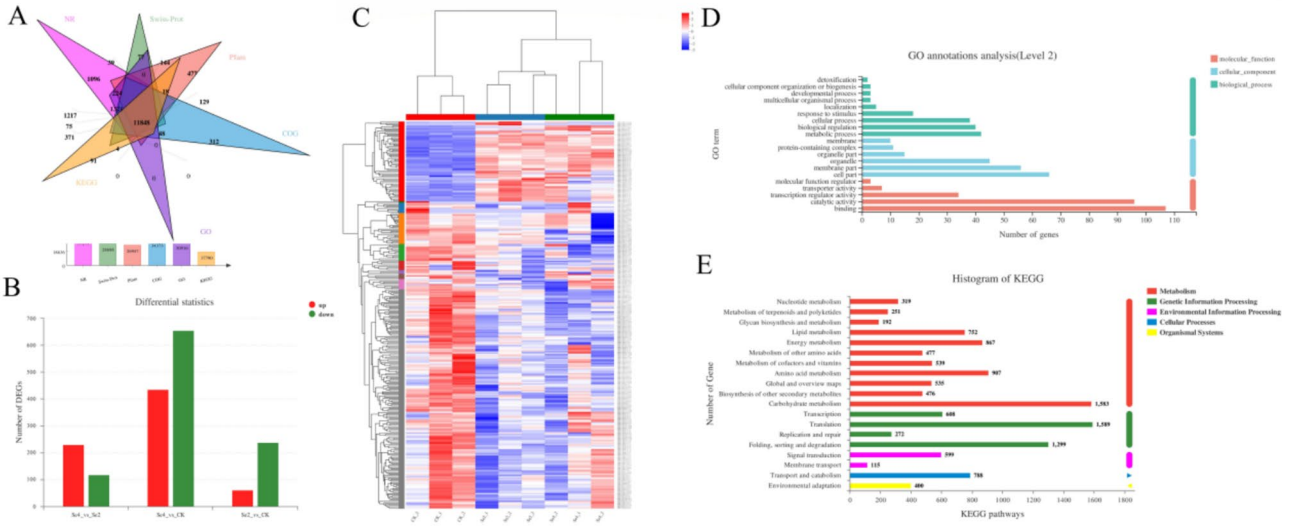

Fig. 4. Transcriptome analysis of lily under 0, 2.0 mmol/L, and 4.0 mmol/L Se treatments. (**A**) Unigenes obtained from six databases. (**B**) Statistics of the DEGs. (**C**) Hierarchical clustering analysis of DEGs. (**D**) Statistical analysis of the enriched GO terms. (**E**) KEGG pathway enrichment of the DEGs.

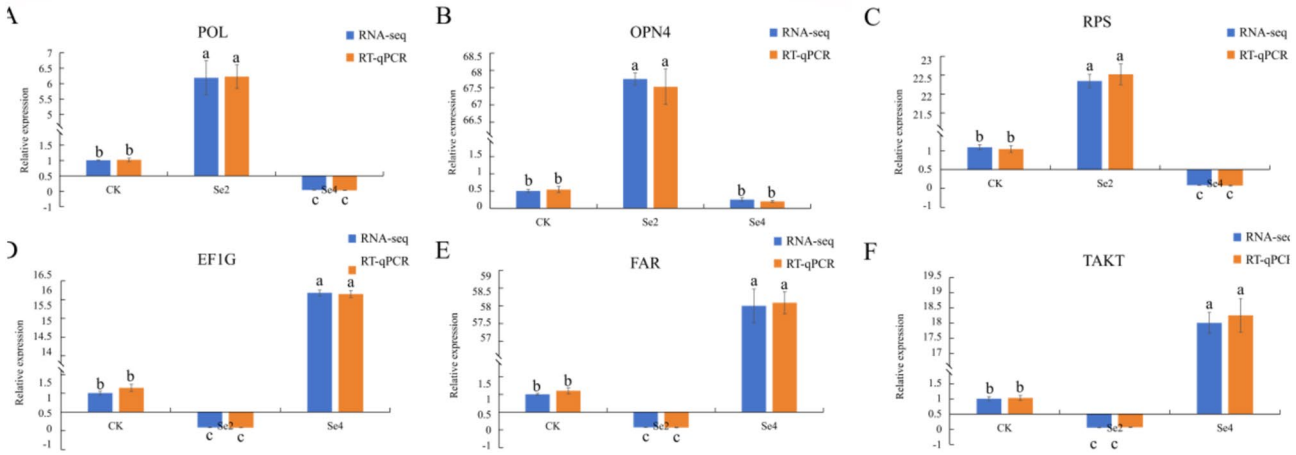

Fig. 5. RNA-seq and RT-qPCR analysis of 6 significantly expressed genes, namely, (**A**) *POL*, (**B**) *OPN4*, (**C**) *RPS*, (**D**) *EF1G*, (**E**) *FAR*, and (**F**) *TAKT*. GAPL was used as internal control for qRT-PCR. Three technical replicates were performed. Error bars represent standard deviations and letters significant values at $P < 0.05$.

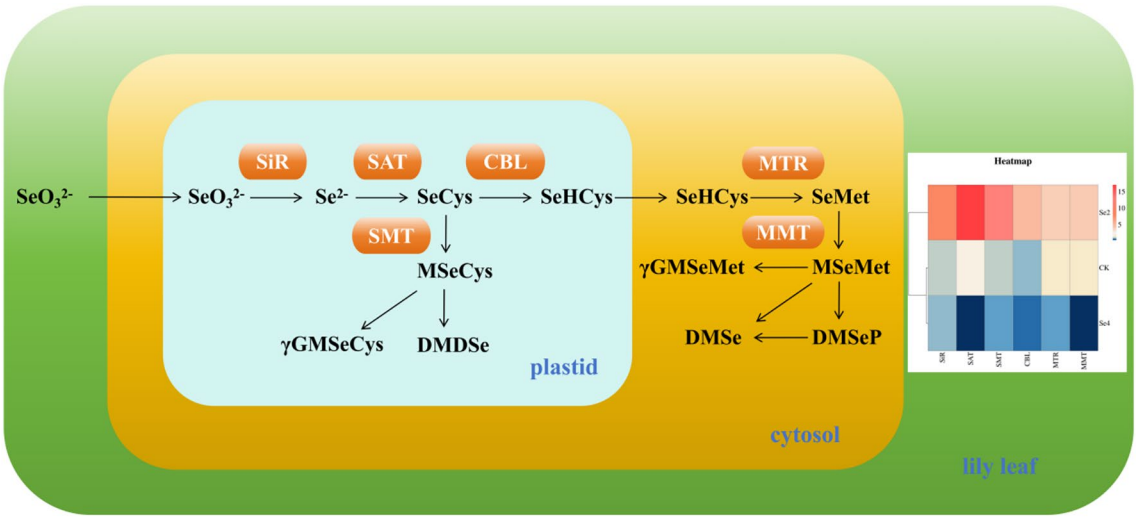

Fig. 6. Metabolic pathways of selenite in lily leaves.

4 mmol/L selenite. When lily leaves were treated with 2 mmol/L selenite, the expression levels of the six genes mentioned above significantly increased, indicating that these six genes are involved in the selenite metabolism pathway of lilies (Fig. 6). Interestingly, the expression levels of the *SiR* and *SAT* genes were high when SeO_3^{2-} was converted to SeCys, whereas the expression levels of the *CBL* and *MTR* genes were relatively low when SeO_3^{2-} was converted to SeMet. This result suggests that the process by which lily leaves absorb selenite may involve the plastid, which metabolizes SeO_3^{2-} through the conversion of γ-glutamylcysteine (γ-GMSeCys) and dimethyl diselenide (DMDSe). Moreover, the expression levels of six genes were generally reduced when lily leaves were treated with 4 mmol/L selenite, indicating that these six genes involved in the metabolism of SeO_3^{2-} were damaged by high concentrations of selenite (Fig. 6).

The physiological response of lily to selenite and the potential metabolic pathways of selenite

According to the results of the GO enrichment analysis and KEGG pathway enrichment analysis, the most differentially expressed genes are involved in the metabolic process, carbohydrate, and carbohydrate metabolism pathways. We identified the three most important genes in the sucrose and starch metabolism pathways, namely, *SUS*, *SPS*, and *Inv*.

The results revealed that when lily leaves were treated with 2 mmol/L selenite, the expression levels of these three genes were significantly increased, indicating that the conversion of low concentrations of Se into carbon sources promoted the growth and development of lilies (Fig. 7).

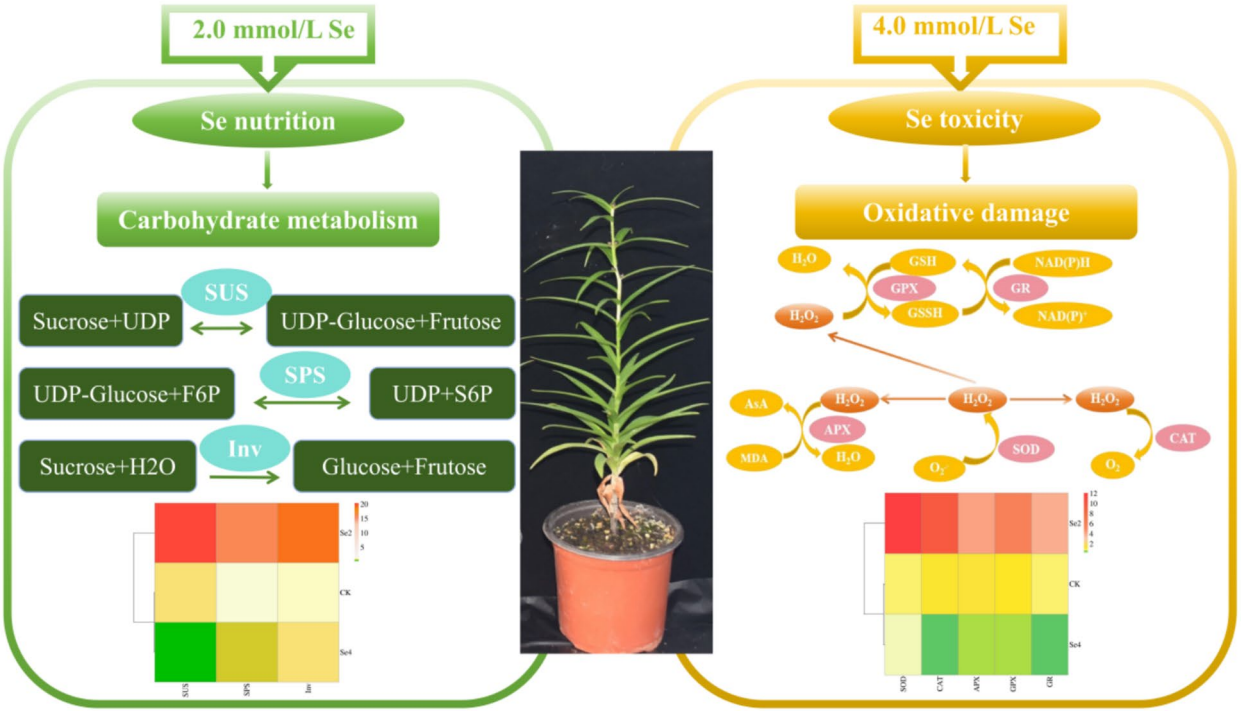

Fig. 7. Moderate Se promotes the growth and development of lily, while high concentration Se leading to toxic effect on lily.

As shown in Figs. 1, 3, and 6, when lily leaves were treated with 4 mmol/L selenite, their growth and development were impaired, their Se antioxidant capacity was weakened, and a large amount of reactive oxygen species (ROS) accumulated. The ability of genes encoding enzymes involved in the selenite metabolism pathway to metabolize SeO_3^{2-} was also impaired. Previous studies have shown that moderate Se can increase GSH-Px activity; eliminate excess free radicals; participate in energy metabolism; and promote plant root growth, development, and vitality[43]. High concentrations of Se cause the accumulation of large amounts of ROS in crops, leading to oxidative stress and subsequent toxicity[44,45]. Therefore, we measured the expression of the major antioxidant system member genes in organisms, including *SOD*, *CAT*, ascorbate peroxidase (*APX*), glutathione peroxidase (*GPX*), and glutathione reductase (*GR*). Low concentrations of selenite promoted the expression of these five genes, whereas high concentrations of selenite reduced their expression levels (Fig. 7). These results indicate that low concentrations of Se prevent plant peroxidation by scavenging excess free radicals, whereas high concentrations of Se increase the production of free radicals and promote peroxidation.

Discussion

An appropriate concentration of Se promotes the growth of lily

Treating lily leaves with 2 mmol/L selenite resulted in increased growth, development, and nutrient accumulation (Figs. 1, 2 and 7). The research results of the past year have found that Se stimulated the growth of *L. lancifolium* at low level (2.0 mmol/L) but showed an inhibitory effect at high levels (≥ 4.0 mmol/L) which is consistent with our research findings[31]. In Jiang's study, the significantly upregulated *SUS*, *bgl B*, *BAM*, and *SGA1* genes were involved in soluble sugar accumulation under Se treatment. In our study, the three most important genes in the sucrose and starch metabolism pathways are *SUS*, *SPS*, and *Inv*. Previous studies have shown that treating rice with 0.5–5 mg/kg selenite increases rice yield, proving that the experimental results in the present study have a scientific basis[46]. Moderate Se treatment also promoted photosynthesis and the synthesis of amino acids and proteins in rice[47–49]. Low-concentration selenite treatment (2.2–4.4 mg/kg) promoted tobacco growth and significantly increased plant height and dry matter weight[50]. A 9 μM selenate treatment significantly increased the dry weights of potato roots and aboveground parts[51]. The application of 0.5–1.0 mg/kg SeCys to soil significantly increased the dry weight of Chinese cabbage roots and leaves[52]. Se concentrations of 10 mg/L and 20 mg/L Nanometre also affect the dry weights of strawberry roots and aboveground parts[53]. Numerous studies have shown that different forms and concentrations of Se affect the growth and development of plants, although Se is not an essential nutrient for plants.

High concentrations of Se cause toxicity to lily

Se is an important element involved in the oxidative activity of glutathione peroxidase and in plant antioxidant defense processes[54]. A study revealed that a moderate concentration of Se stimulated the growth and antioxidant capacity of *Salvia miltiorrhiza*[55]. The toxicity caused by high concentrations of Se in crops is due to the production of toxic peroxides as oxidants[56]. When high concentrations of Se cause oxidative stress, a large amount of ROS

accumulate in the crops[44,45]. SOD, POD, and CAT enzymes protect cell membranes from oxidation and damage by removing peroxides and free radicals, thereby maintaining the integrity of the cell membrane structure and function. Research has shown that high concentrations of selenate increase the activities of SOD, POD, and CAT in rice, disrupting photosynthesis in rice leaves[57]. In this study, the activities of SOD, POD, and CAT increased but then decreased with increasing selenite concentration. However, the content of H_2O_2 was exactly opposite to the above results (Fig. 3). As a result, the photosynthetic system is disrupted, and the content of photosynthetic pigments decreases accordingly[58]. The antioxidant capacity of *Brassica napus* significantly increased when Se was added externally[59]. Similar studies have shown that Se promotes the expression of genes encoding antioxidant enzymes (AOEs), such as *SOD*, *POD*, *CAT*, *GR*, and *GST-1*, in *B. napus* while reducing the toxicity of Cr[60]. The application of 0.5–1.0 mg/kg SeCys to soil significantly increased the activities of AOEs (POD, SOD, CAT, APX, and GR) in the roots and leaves of[61]. The expression levels of the *SOD*, *CAT*, *APX*, *GPX*, and *GR* genes in lily leaves were significantly downregulated under the 4 mmol/L selenite treatment (Fig. 7). Previous studies have shown that high concentrations of Se damage the electron transfer rate and mitochondrial respiration rate of plant leaf chloroplasts, causing damage to the chloroplast membrane and ultimately leading to a decrease in the chlorophyll content in the leaves[62]. In our study, high concentrations of Se reduced the chlorophyll content in lily leaves (Fig. 1E).

Absorption and metabolism of selenite in lily leaves

Early studies implied that Se, mainly as selenate and selenite, is transported in plants via sulfur (S) and phosphate (P) transporters, respectively. Selenate is generally absorbed through sulfate transporters in plants[63]. In Se-tolerant *Arabidopsis*, *Sultr1*, *Sultr2*, *Sultr3*, and *Sultr4* encode sulfate transporters[64]. In our study, the RNA-seq results revealed that 6 genes are involved in the metabolic pathway of selenite in lily leaves. Compared with the gene encoding sulfate transporters, the gene encoding phosphate transporters is expressed at a greater level during the absorption and transport of selenite[30,65]. When lily leaves were treated with 2 mmol/L selenite, the expression of the *SiR*, *SAT*, *SMT*, *CBL*, *MTR*, and *MMT* genes was upregulated. Sulfite reductase expressed in *Arabidopsis* plastids is responsible for the reduction of selenite[66,67]. The expression level of the *SAT* gene increased significantly, which converts Se^{2-} into SeCys. However, the expression levels of MTR and MMT were lower than those of SAT and SMT. Selenite is converted to SeCys in plastids, then to SeMet in the cytosol, and finally is absorbed by plants in the form of organic selenium[68,69]. We speculate that γ-glutamylcysteine (γ-GMSeCys) and dimethyl diselenide (DMDSe) absorb and metabolize SeO_3^{2-} and that this process also occurs in plastids. Our results provide valuable information on the molecular regulation of selenite in lily.

Conclusion

This study investigated the effects of different concentrations of selenite on the vegetative growth of lily. The results revealed that the 2.0 mmol/L Se treatment significantly promoted the vegetative growth of lily, whereas the 4.0 and 8.0 mmol/L Se treatments had the opposite effect. Transcriptome analysis revealed that *SiR*, *SAT*, *SMT*, *CBL*, *MTR*, and *MMT* are important enzyme-encoding genes involved in the metabolic pathway of selenite in lily. Moreover, moderate Se treatment promoted the expression of *SUS*, *SPS*, and *Inv* genes involved in carbohydrate metabolism, thereby promoting the growth and development of lily. High-Se treatment generates a large amount of ROS and inhibits the expression of antioxidant genes such as *SOD*, *CAT*, *APX*, *GPX*, and *GR*, resulting in oxidative stress in lily. These results elucidate the genes involved in the pathways regulating Se absorption and metabolism in lily leaves, which furthers elucidates the physiological and molecular mechanisms by which Se affects lily growth and development, laying a theoretical foundation for knowing to cultivate lily with Se supplementation.

Data availability

The data presented in the study are deposited in the NGDC Genome Sequence Archive (GSA) database, https://nbdc.cncb.ac.cn/, accession number CRA017526.

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Author contributions

Wu-Xian Zhou, Jinwen You, and Yu-Ying Yang conceived the research; Yu-Qing Duan and Da-Rong Li performed the experiments; Yu-Ying Yang, Xiao-Gang Jiang, Hua Wang, Hai-Hua Liu, and Meide Zhang analyzed most data of the experiments; Yu-Ying Yang and Wu-Xian Zhou wrote the article.

Declarations

Competing interests

The authors have no conflicts of interest.

Additional information

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Correspondence and requests for materials should be addressed to J.Y. or W.Z.