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Effects of selenium application on nutrient uptake and nutritional quality of *Codonopsis lanceolata*

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ABSTRACT

Codonopsis lanceolata is widely cultivated and consumed in China, Korea, and Japan because of its beneficial effects on health; it is an effective antioxidant and reduces the risks of diabetes, obesity, and cancer. The effects of *C. lanceolata* on human health are similar to that of selenium (Se). Hence, the aim of this study was to determine whether Se application could increase Se content in *C. lanceolata* and enhance its beneficial effects on human health. Pot experiments were conducted, and sodium selenite was applied to evaluate the effects of Se application (0, 0.5, 1.0, 1.5, and 2.0 mg Se kg^{-1} soil) on *C. lanceolata*. The biomass of *C. lanceolata* increased and was the highest when treated with 1.0 mg Se kg^{-1} soil, but after this level, there was a decrease in *C. lanceolata* biomass. Compared with the corresponding organs of the control group, Selenium accumulation in stems, leaves and fruits significantly increased with Se levels increasing, the highest values appeared at 1.0 mg Se kg^{-1} soil level for roots and fruits, and at 1.5 mg Se kg^{-1} soil level for stems and leaves, respectively. Selenium application increased N, P, and K accumulation, and altered the distribution of N and K, but not of P. Compared to the control, at 1.0 mg Se kg^{-1} soil level, the contents of polysaccharide, total flavonoid, total saponin increased significantly, thereby enhancing the health benefit of *C. lanceolata*; the contents of protein, total amino acid and essential amino acid increased significantly, thereby enhancing the nutritional quality of *C. lanceolata*; the contents of Se and Zn increased concurrently, suggesting the suitability of simultaneous biofortification with Zn and Se. Furthermore, the highest concentration of Se in *C. lanceolata* root (5.93 μg g^{-1} dry weight) was considered to be safe for human consumption. Approximately 12 g (dry weight) of *C. lanceolata* root treated with 1.0 mg Se kg^{-1} soil is sufficient to fulfill the daily human requirement of Se. In conclusion, *C. lanceolata* is a good candidate for Se biofortification, and 1.0 mg Se kg^{-1} soil is the optimum.

1. Introduction

Selenium (Se) is an essential micronutrient required by humans, and the recommended dietary intake by the World Health Organization is 40–200 μg per day (Levander 1990). Low Se intake is associated with considerable health disorders, including higher risk of cancer, heart disease, and weakened immune system (Rayman, 2009). Conversely, an adequate dietary Se intake confers a variety of health benefits. Se dietary intake varies globally between populations living in different geographic regions, which is strictly related to the Se content in the soil. Approximately 72% of the land in China is Se-deficiency and Se consumption by a Chinese adult is 26–32 μg per day, which is much less than the recommended dietary allowance (50–250 μg per day) recommended dietary intake by Chinese Nutrition Association. In this context, there is an eagerly need to increase the organic Se concentration in food products as a sustainable complement in fighting Se deficiency. Several strategies have been tried. As low dietary intake of Se could be attributed to the consumption of plant-derived foods with inherently low Se concentration (Rayman, 2012), moreover, the major source of Se in most human diets is provided by plants. Therefore, Se-enriched plant foodstuff was proposed as an effective and sustainable approach to increase Se dietary intake. It is imperative to find strategies to increase Se content in plant-derived foods. Accordingly, selenium biofortification of food crops has been practiced in some Se-deficient regions by adding Se. Considerable studies have demonstrated that Se content in crops could be increased with Se application, such as *Oryza sativa* (Carey et al., 2012), *Camellia sinensis* (Hu et al., 2002), *Daucus carota* (Kápolna et al., 2009), and *Raphanus sativus* (Du et al., 2009). It is noteworthy that Se application can increase soluble sugar (Zhao et al., 2010), protein (Hu et al., 2002), and amino acid contents (Xia et al., 2012), thereby improving their nutritional quality.

Studies on Se-biofortification mainly used selenite and selenate as Se resource because they are the two main inorganic Se forms available in soil. There are mainly two ways to carry out Se-biofortification: soil

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application and foliar application or the combination of both. Soil application of Se is widely recommended as an approach to produce Se-enriched food. As a result, lots of Se-enriched agricultural products appeared such as *Oryza sativa* (Carey et al., 2012), *Camellia sinensis* (Molan et al., 2009); Compared with the soil application of Se, foliar application of Se can minimizes the impact of soil chemistry and microbiology on Se uptake and accumulation. Foliar application of Se is extensively applied in Se-biofortification of *Triticum aestivum* (Nawaz et al., 2015), *Cicer arietinum* (Poblaciones et al., 2014), *Daucus carota* (Kápolna et al., 2009) and so on. Up to date, studies on Se biofortification have mainly focused on crops and vegetables, only a few studies have been conducted on medicinal plants. Medicinal plants are used as nutraceuticals because they contain a wide spectrum of nutritional and bioactive compounds. Dong et al. (2013) reported that chlorogenic acid and carotenoid contents in *Lycium chinense* significantly increased upon Se application, enhancing its health benefits. Similar results were also observed in *Fagopyrum tataricum* Gaertn. (Tian and Wang, 2008) and *Chrysanthemum morifolium* Ramat (Chu et al., 2014).

Codonopsis lanceolata (belong to Campanulaceae) is widely cultivated and consumed in China, Korea, and Japan. It is rich in bioactive compounds such as saponins, flavonoids and polysaccharides, and possess many important physiological functions (Chang et al., 1986; Han and Cho, 1997). For example, it behaves as highly effective antioxidant and reduces the risk of diabetes, obesity, and cancer (Han and Cho, 1997) Furthermore, *C. lanceolata* is a traditional folk medicine in Korea, Japan and China, it is used for the treatment of hypertension and lung inflammatory diseases, such as asthma, tonsillitis, and pharyngitis. Noteworthy, Selenium acts as an antioxidant and plays a protective role in human immune system, which has the same effects as that of *C. lanceolata*. Moreover, Malagoli et al. (2015) showed that Se biofortification led to an increase in the Se content and the synthesis of secondary metabolites. Enhanced Se content reinforced antioxidant defense and immune function in *Chrysanthemum morifolium* Ramat (Chu et al., 2014) and *Lycium chinense* (Dong et al., 2013). Rodrigo et al. (2014) proved that the suitability of Se-enriched barley grain as a raw material could produce Se-enriched beer. Zhu et al. (2009) demonstrated that Se-enriched plants may have potential as fortified food with enhanced nutritional quality. All implied that enhanced Se content in *C. lanceolata* could enhanced its health benefits. It is therefore reasonable to infer that Se-enriched *C. lanceolata* can process Se-enriched products, thereby enhancing its market competitiveness. However, to date, studies have not been conducted to test this hypothesis. Therefore, there is an urgent need to determine whether Se application can enhance Se content and health benefits of *C. lanceolata*.

The Se content in plants is positively correlated with Se content in soils (Lukshman et al., 2015). Hence, we hypothesized that *C. lanceolata* could be used to test Se biofortification. In addition, we aimed to determine whether Se application could improve Se content in *C. lanceolata*. To this end, pot experiments were conducted with different levels of Se treatment, and the nutrient uptake and changes in nutritional quality and health benefits of *C. lanceolata* were assessed. The results could provide crucial information regarding Se-enriched *C. lanceolata* products.

2. Materials and methods

2.1. Experimental design

The pot experiment was conducted in the experimental plot of Shandong Agricultural University in Shandong Province, China, from April to October 2013. The experiment station is located at 117° 06′E (longitude) and 36° 20′N (latitude), with a relative elevation of approximately 174.4 m. The experimental area experiences a temperate monsoon climate with an annual mean temperature of 13.4 °C and annual rainfall of 688.3 mm, which mainly occurred during the summer.

Prior to planting, soil samples were randomly collected from the soil used in the pot experiment site (the location is117° 06′E65, longitude and 36° 20′N, latitude) and analyzed for physiochemical characteristics in accordance with the methods described by Bao (2000). The values of pH, available nitrogen (N), sodium bicarbonate extractable phosphorus (P), and ammonium acetate extractable potassium (K) were 7.16, 0.86 g kg^{-1}, 22.02 mg kg^{-1}, and 39.23 mg kg^{-1}, respectively. The total soil Se content determined according to the method described by Liu and Chen (2008) was 0.163 mg kg^{-1}.

Se levels (sodium selenite [Na_2SeO_3], Analytical reagent) of 0, 0.5, 1.0, 1.5, and 2.0 mg Se kg^{-1} soil were denoted as CK, $T_{0.5}$, $T_{1.0}$, $T_{1.5}$, and $T_{2.0}$, respectively. Pots were filled with 10 kg of air-dried soil. A uniform fertilizer dosage of 100 mg kg^{-1} N, 50 mg kg^{-1} P_2O_5, and 100 mg kg^{-1} K_2O in the forms of urea with 46% N content, superphosphate with 16% P_2O_5 content, and potassium sulfate with 50% K_2O content, respectively, was used as basal fertilizer and applied before transplanting. Two fecund roots of *C. lanceolata* with similar weight and vigor were planted in each pot on April 20, 2013. The designed dosages of sodium selenite were dissolved in 1000 mL of distilled water and carefully poured into each pot and the control group irrigated 1000 mL distilled water. Each treatment was replicated thrice and five pots were used for each replicate. Thus, each treatment consisted of 30 plants. The pots were arranged in a randomized complete block design. All *C. lanceolata* plants were grown under the same conditions and treated similarly throughout the experiment. The final harvest was conducted on October 20, 2013.

2.2. Sampling

At the harvest stage (October 20, 2013), *C. lanceolata* plants were uprooted from each pot, washed carefully with tap water, then with deionized water thrice. The plants were divided into roots, stems, leaves, and fruits. These plant parts were oven dried at 75 °C to obtain a constant weight and the corresponding dry weights were recorded. The oven-dried samples were ground and sieved through 2-mm sieve. These samples were stored for further analysis.

2.3. N, P, K and protein contents

Dried samples (500 mg) were weighed and digested with H_2SO_4-H_2O_2 (Bao, 2000). The digested samples were used to determine N, P, and K contents using Kjeldahl method, molybdenum blue method, and flame spectrometry, respectively. N, P, and K accumulation was calculated from N, P, and K content in different organs multiplied by the corresponding organ's biomass. The protein content was determined by multiplying the total N concentration by 6.25. Three replicates of each sample were considered to ensure the reliability of the determinations.

2.4. Micronutrient content

Calcium (Ca), iron (Fe), zinc (Zn), magnesium (Mg), and Se contents were determined using inductively coupled plasma-emission spectrometer after digestion (Liu and Chen, 2008). Dried root sample (500 mg) was digested with a mixture of HNO_3 and $HClO_4$ in the ratio of 3:1 (v/v). After filtration, the supernatant was adjusted to 50 mL with deionized water and was analyzed by an inductively coupled plasma-optical emission spectrometer (AAS, TAS-990, Beijing Purkinje General Instrument Co., Ltd., China). The accuracy of elemental analysis was verified by the use of standard reference material (purchased from GSV-1, Center for Standard Reference of China). The standard reference material was analyzed along with the samples and the analysis was considered to be accurate if the value obtained for the reference material was similar to the certified value.

Selenium accumulation was calculated from Se concentration in different organs multiplied by the corresponding organ's biomass.