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The biological effects of strontium (^{88}Sr) on Chinese cabbage

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Abstract: Steady-state strontium (^{88}Sr) plays an important role in human health. Applying a proper amount of ^{88}Sr to vegetables can improve their nutritional value. To investigate the biological effects of ^{88}S on vegetables, three-leaf Chinese cabbage (*Brassica rapa* L.) seedlings were provided with a nutrient solution containing 0, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10 mmol/L $SrCl_2$ by the hydroponic culture. The results showed that $SrCl_2$ at low concentrations (0.2 and 0.5 mmol/L) promoted the growth of Chinese cabbage, while $SrCl_2$ at high concentrations (2.0–10.0 mmol/L) significantly inhibited the growth. $SrCl_2$ at high concentrations did not decrease the chlorophyll content and protein content in Chinese cabbage leaves, nor did it affect the photosynthetic capacity of leaves. The main reason that $SrCl_2$ at high concentrations inhibited the growth of Chinese cabbage was that strontium affected the absorption of calcium. $SrCl_2$ at the concentration of 0.2 and 0.5 mmol/L could significantly increase leaf protein, chlorophyll, and water content and promote the growth of Chinese cabbage. The supplement of $SrCl_2$ at these two concentrations may be beneficial to the growth and yield of Chinese cabbage.

Keywords: vegetable; strontium fertiliser; hyperaccumulator; strontium toxicity; plant metabolism

Strontium (Sr) is the 15th most abundant element on Earth's crust, widely distributing in various environments. There are four stable isotopes of strontium that occur naturally. Their atomic weight and relative abundance are ^{88}Sr (82.58%), ^{87}Sr (7.0%), ^{86}Sr (9.86%) and ^{84}Sr (0.56%), respectively (Sasmaz and Sasmaz 2009). Radiostrontium, ^{89}Sr ($T_{1/2}$ = 50.52 days), and ^{90}Sr ($T_{1/2}$ = 28.9 years) are artificial radionuclides, which are formed during nuclear reactor operations and nuclear explosions. The average strontium concentration in soil is approximately 240 ppm, and most of it exists as insoluble compounds. Natural water sources such as rivers contain a small amount of strontium (about 70 μg/L) (Seifert 2004). Nevertheless, Sr^{2+} is one of the five main cations in seawater, with an average concentration of as high as 8.0 mg/L (Sun et al. 2005). Both strontium and calcium belong to group II in the periodic classification, so they have similar chemical characteristics. For plants, strontium is not an essential element for growth and reproduction, while it is easily absorbed into plants from the soil. Therefore, strontium is a natural constituent of botanical food. Strontium is an essential trace element for the human and has many health effects on the human body. For example, low-dose strontium (^{88}Sr) stimulates osteogenesis (Aimaiti et al. 2017).

The dietary strontium intake for humans is about 2.0 mg Sr/day/person. Most food products are poor in strontium. The mean quantity of strontium in plants (fresh weight) is about 4 ppm. Meat products, potatoes, fruit, and dairy products contain the lower contents of strontium (< 1 ppm), whereas root vegetables, seafood, and cereals contain higher

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values (1–4 ppm) (Höllriegl and München 2011). Due to different dietary habits, some people may have insufficient strontium intake. Mineral water containing below 5 mg/L strontium is beneficial to human health without adverse effects, which is a common method of supplementing strontium. Adding strontium fertiliser to vegetables to increase their strontium contents is also a feasible way. At present, botanical studies on strontium are mainly focused on "the phytotoxicity and phytoremediation of strontium (including radiostrontium)" (Li et al. 2006, Sasmaz and Sasmaz 2009, Chen et al. 2012) and "the uptake and distribution of strontium" (von Firck et al. 2002, Tsukada et al. 2005, Hoseini et al. 2012, Wang et al. 2017, Gupta et al. 2018). The promotion effects of low-concentration stable strontium on plant growth have been shown in *Solanum laciniatum* Aiton, *Platymonas subcordiformis* G.S.West, maize, oilseed rape and soy plants (Kartosentono et al. 2001, Li et al. 2006, Moyen and Roblin 2010, 2013, Chen et al. 2012, Sowa et al. 2014). However, these studies all ignore this phenomenon. Chinese cabbage (*Brassica rapa* L.) is one of the most productive vegetables in East Asia. Hence, it was used as a model plant to study the biological effects of ^{88}Sr on vegetables in this study.

MATERIAL AND METHODS

Plant materials and growth conditions. The seeds of a Chinese cabbage cultivar (*Brassica rapa* L. ssp. *pekinensis*. Brand name: Qingmaye, Tianjin Seed Co., LTD) were germinated in sand moistened with Arnon's revised Hoagland solution (pH 5.7, containing 4 mmol/L Ca^{2+}). All seedlings grown in a greenhouse under conditions of day/night temperature at 25 °C/15 °C, 16 h/8 h light/dark photoperiod, and the maximum illumination 1 000 μmol/m^2/s. When the seedlings had 2–3 leaves, every five uniform seedlings were transplanted into a ceramic pot (diameter: 12 cm; depth: 16 cm), which contained 3 L Hoagland solution without (control) or with 0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 mmol/L strontium chloride ($^{88}SrCl_2$), respectively. The treatment solutions were renewed every 3 days to ensure a stable $SrCl_2$ concentration. All Chinese cabbage seedlings were harvested for the determination of the physiological and biochemical parameters after a 30-days exposure to $SrCl_2$.

Fresh weight, leaf water content, and maximum leaf area. The fresh weight (FW) of the individual plant was determined gravimetrically on 30 days after exposure to $SrCl_2$ treatments. The dry weight (DW) of leaves was obtained from the corresponding fresh weight of the leaves, which were placed in an oven at 75 °C for 72 h. The leaf water content was defined as the ratio of FW/DW. The leaf area measurement was performed using an LI-3000C portable area meter (Li-Cor Biosciences, USA).

Protein content and chlorophyll content. The total soluble protein content in leaves was determined by Coomassie brilliant blue staining, using bovine albumin for calibration. Chlorophyll was extracted from fully expanded leaves with 80% acetone and determined at two wavelengths (663.2 nm and 646.8 nm), according to Porra (2002).

Photosynthetic gas exchange. Gas exchange analysis was carried out using a TARGAS-1 portable photosynthetic system (PP Systems, USA). The leaf net photosynthetic rate (P_n) and stomatal conductance (g_s) were determined at a CO_2 concentration of 380 μmol/mol, 40% relative humidity, and a saturation light intensity of 800 μmol/m^2/s.

Ca^{2+} content and Sr^{2+} content. For the determination of the total Ca^{2+} and Sr^{2+} content, the fresh leaves were first washed with deionised water and then dried in an oven at 75 °C for 72 h. Subsequently, the dried samples were accurately weighed (0.5 g) and homogenised. The aliquots of dry samples were afterward digested with HNO_3:$HClO_4$ (4:1, *v*/*v*) for 6 h in Teflon tubes. After the digested samples were evaporated to dryness, the residues were dissolved with deionised water. The quantification of Ca^{2+} and Sr^{2+} was performed by an Agilent 7700 ICP-MS (Agilent Technologies Inc., USA).

Expression of the data. Data were expressed as mean ± standard deviation (SD) with the number of replicates. ANOVA and Duncan's *LSR* (least significant range) test were used to analyse the differences among the treatments. Means were separated at a significant level ($P < 0.05$) by different letters.

RESULTS AND DISCUSSION

Plant growth. At present, strontium is regarded as a harmful pollutant for plants in almost all related studies. For example, in the presence of 3 mmol/L $Sr(NO_3)_2$ for 2–4 days, the radicle elongation increment of maize was significantly reduced (Seregin and Kozhevnikova 2004, Kozhevnikova et al. 2009). Some results also show that trace amounts of radioactive strontium and stable strontium can slightly promote plant growth, but no analysis has been per-

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formed in these studies (Kartosentono et al. 2001, von Firck et al. 2002, Seregin and Kozhevnikova 2004). Moyen and Roblin (2010) first clearly pointed out that low-concentration strontium (0.1–1.0 mmol/L) can stimulate the growth of maize. This promotion effect was observed after the maize seedlings were strontium-treated for 7–14 days.

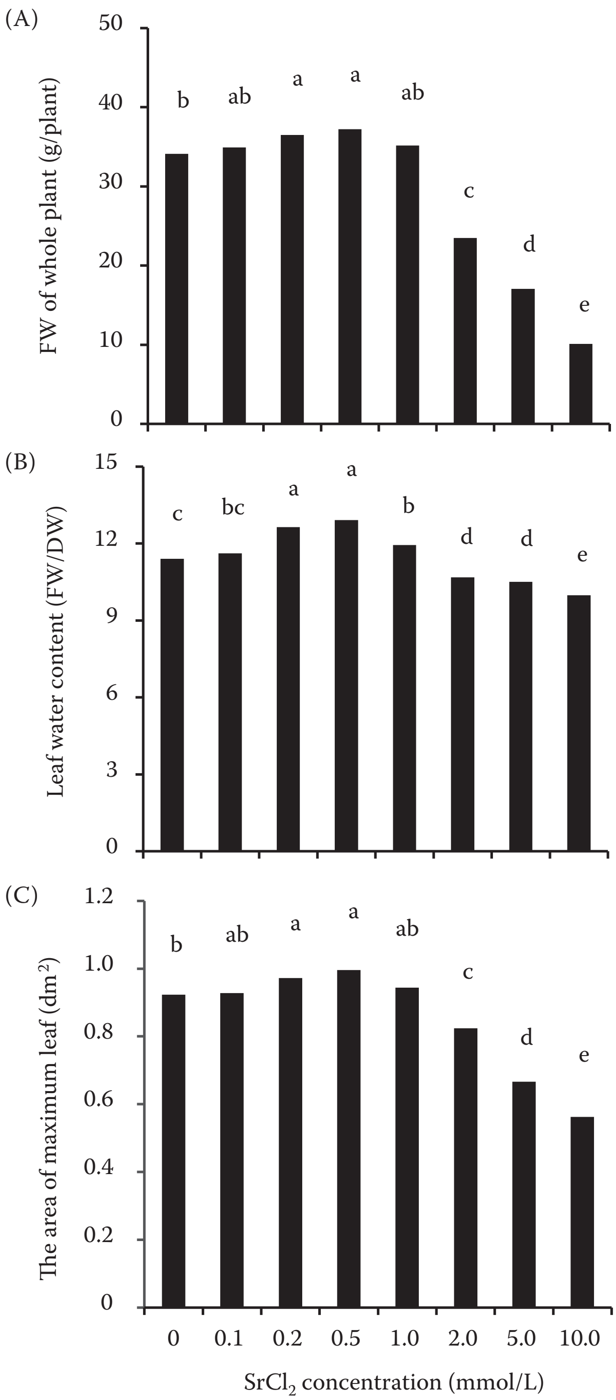

Figure 1. (A) The fresh weight (FW) of whole plant, (B) leaf water content, and (C) maximum leaf area of Chinese cabbage seedlings after incubation in 0.1–10.0 mmol/L $SrCl_2$ for 30 days. The data are means ± standard deviation (n = 10). DW – dry weight

Our result showed that strontium at low concentrations (0.1–1.0 mmol/L) did not inhibit the growth of Chinese cabbage seedlings (Figure 1A) after Sr-treatment for 30 days. On the contrary, 0.2 and 0.5 mmol/L $SrCl_2$ slightly promoted their growth ($P < 0.05$), by which the fresh weight of the whole plant was increased by 7.0% and 9.1% compared with the control seedlings, respectively. However, Sr^{2+} at high concentrations (2.0–10.0 mmol/L) significantly inhibited the growth of Chinese cabbage seedlings. After a 30-days exposure to 2.0, 5.0, and 10.0 mmol/L $SrCl_2$, the fresh weight of Chinese cabbage seedlings dropped to 68.8, 50.0, and 29.7% of the control group, respectively. The effect of the strontium treatment on the maximum leaf area was similar to the fresh weight of the whole plant (Figure 1C). Sr^{2+} at high concentrations affected both cell division and elongation, leading to inhibition of leaf extension (Kozhevnikova et al. 2009). However, Sr^{2+} at concentrations of 2.0–10.0 mmol/L did not inhibit the growth of maize seedlings (Moyen and Roblin 2010, 2013), indicating that maize has a better strontium-tolerance mechanism. The change of the leaf water content also showed the same trend with the growth of Chinese cabbage seedlings (Figure 1B). This phenomenon has only been reported in maize plants (Moyen and Roblin 2010). The higher the water contents in plant cells, the stronger the metabolic activity. Strontium at low concentrations may promote plant metabolism.

Metabolic activity. The protein content is often used to reflect the metabolic status of plant cells. The strontium treatment induced a continuous increase in leaf protein content with the increase of the $SrCl_2$ concentration in the nutrient medium (Figure 2A). Moreover, the chlorophyll contents of leaves treated with 0.1–0.5 mmol/L $SrCl_2$ were all significantly higher than that of the control group (Figure 2B). The promotion of chlorophyll synthesis by low concentration strontium did not attract much attention in previous studies (Li et al. 2006, Chen et al. 2012, Zheng et al. 2016). The results in Figure 2 suggested that 0.1–10.0 mmol/L $SrCl_2$ had no destructive effect on leaf cells of Chinese cabbage seedlings. Although the protein content and chlorophyll content of the leaves increased by $SrCl_2$ at the concentration of 0.2–1.0 mmol/L, their photosynthetic capacity and stomatal conductance did

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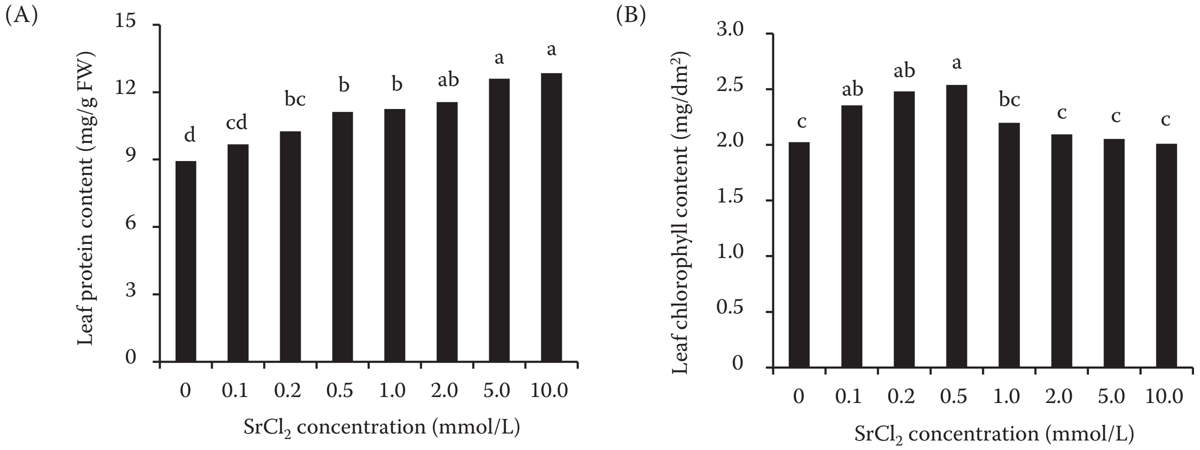

Figure 2. (A) The leaf protein content and (B) chlorophyll content of Chinese cabbage seedlings after incubation in 0.1–10.0 mmol/L $SrCl_2$ for 30 days. The data are means ± standard deviation (*n* = 5). FW – fresh weight

not change significantly (Figure 3). Chen et al. (2012) found that the photosynthetic activities of oilseed rape seedlings increased after exposure to 10 mmol/L $SrCl_2$ for 7 days. However, with the extension of the treatment time to 14–21 days, the 10 mmol/L $SrCl_2$ treatment decreased the chlorophyll content and photosynthetic rate. The longer the strontium treatment time, the higher the concentration of strontium accumulated in the leaves (Moyen and Roblin 2010, Chen et al. 2012). Sr^{2+} at high concentrations could decrease the Mg content in the plant tissues, which is a component of chlorophyll (Moyen and Roblin 2010). Hence, the inhibitory effect of strontium on leaves might be related to the strontium accumulation concentration in leaves.

The relationship between calcium and strontium. Because Sr shares chemical analogy with essential plant macronutrient Ca, plants can easily uptake Sr^{2+} via Ca^{2+} channels in the roots, despite the fact that Sr is not an essential element for plant metabolism (Arne et al. 2015). The absorbed Sr^{2+} is primarily transferred to and accumulated in the leaves in most plants (Gupta et al. 2018). The maize plant, as an exception, accumulated Sr^{2+} mainly in the roots (Moyen and Roblin 2010, 2013). After exposure to 10 mmol/L Sr^{2+} for 21 days, the Sr^{2+} contents in leaves of oilseed rape seedlings are as high as 29 mg/g DW (Chen et al. 2012). The results in Figure 4 showed that the strontium content in Chinese cabbage leaves increased rapidly with the increase of the strontium treatment concentration, which was 29.87 mg/g after exposure to 10 mmol/L Sr^{2+} for 30 days. Rice, maize, sorghum, and soy are less capable of accumulating strontium than oilseed rape and Chinese cabbage (Tsukada et al. 2005, Moyen and Roblin 2013, Sowa et al. 2014, Wang et al. 2017).

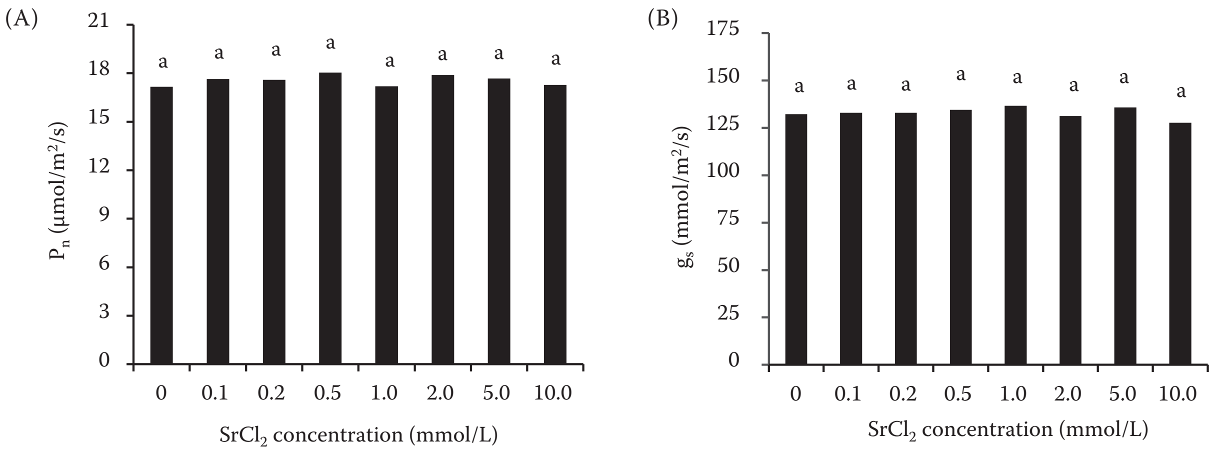

Figure 3. (A) Photosynthetic rate (P_n) and (B) stomatal conductance (g_s) of Chinese cabbage leave exposed to 0.1–10.0 mmol/L $SrCl_2$ for 30 days. The data are means ± standard deviation (*n* = 5)

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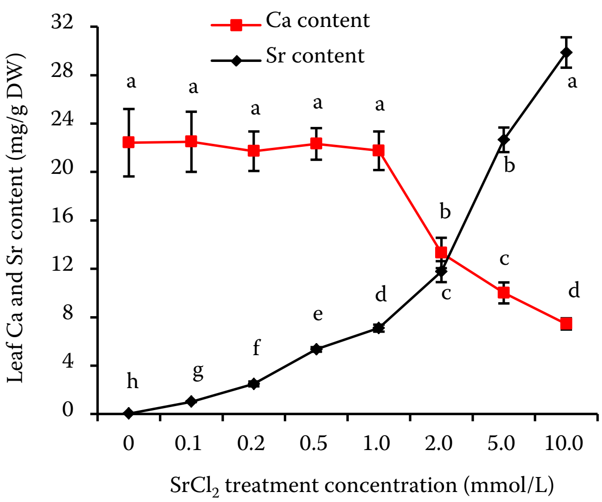

Figure 4. The Ca^{2+} and Sr^{2+} content in the leaves of Chinese cabbage seedlings exposed to 0.1–10.0 mmol/L $SrCl_2$ for 30 days. The data are means ± standard deviation (*n* = 5). DW – dry weight

These results indicate that Chinese cabbage is a strontium-hyperaccumulators.

There is a competitive inhibition between strontium absorption and calcium absorption. In the presence of calcium, the strontium absorption of plants is significantly reduced (Kozhevnikova et al. 2009, Moyen and Roblin 2010). Conversely, strontium also inhibits calcium absorption. Under the same concentration conditions, the absorption of strontium by maize plants was greater than that of calcium (Moyen and Roblin 2010, 2013). Compared with the control group, 0.1–1.0 mmol/L strontium-treatment did not affect the calcium content in Chinese cabbage leaves. However, 2.0, 5.0, and 10.0 mmol/L strontium-treatment significantly reduced the calcium content in the leaves, which decreased to 59.4, 44.7, and 33.2% of the control group, respectively. Calcium-deficiency affects leaf bud development and young leaf extension (Hepler 2005). Therefore, it is speculated that the reduction of the calcium content under high strontium conditions (2.0–10.0 mmol/L) is the main reason for the growth inhibition of Chinese cabbage. In addition, a large amount of strontium in the cells would interact with some functional groups of proteins and interfere with their functions. Unlike other heavy metals, strontium is less toxic to plants and can even replace some functions of calcium (Kozhevnikova et al. 2009). For example, strontium can reconstitute oxygen evolution in the oxygen-evolving complex of photosystem II (Boussac et al. 2004).

The low-promoting and high-inhibiting effects of strontium on plants are common phenomena. Supplementing plants with 0.2–0.5 mmol/L strontium chloride can both promote the growth of vegetables and increase their nutritional value. The food with excessive strontium content is harmful to human health, and Chinese cabbage has a strong ability to accumulate strontium. Thus, we recommend strontium fertiliser at a low concentration (0.2 mmol/L) to be applied at the seedling stage of Chinese cabbage to avoid the accumulation of excessive strontium in the leaf head (the edible part). In addition, Chinese cabbage, as a strontium-hyperaccumulators, could be used for phytoremediation of strontium pollution.

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