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ARTICLE

The Addition of Calcium and Strontium Improves Salt Tolerance of Chinese Cabbage at the Germination Stage

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ABSTRACT: Strontium has similar chemical properties to calcium and has recently been recognized as a non-essential beneficial element for plants. In order to compare the effects of strontium and calcium on improving salt tolerance of Chinese cabbage during the germination stage, 2, 4, and 8 mmol/L of $SrCl_2$, $CaCl_2$ or an equimolar mixture of both were added separately to a 150 mmol/L NaCl solution. The results showed that Ca-Sr addition significantly increased seed viability, seed vigor, seed germination rate and seed germination uniformity of Chinese cabbage compared with the salt-control group. The differences in germination percentage (GP) and germination energy (GE) among the Ca-addition and Sr-addition groups were not significant, and the differences in coefficient of rate of germination (CRG), index of rate of germination (IRG) and coefficient of variation of the germination time (CV_T) were relatively small, but clear differences were observed in germination index (GI), vigor index (VI) and coefficient of uniformity of germination (CUG). The results of GI and VI indicated that the higher the concentration of Ca-addition or Sr-addition, the more significant the enhancement of seed vigor. Under saline stress (150 mmol/L NaCl), the Ca-Sr co-addition outperformed Sr-treatment alone, and Ca-addition achieved the highest seed vigor at equivalent concentrations. Furthermore, all Ca-Sr treatments significantly enhanced the uniformity of Chinese cabbage sprouts exposed to 150 mM NaCl, with the best uniformity improved by the addition of 2 and 4 mmol/L $SrCl_2$. Ca-Sr treatments increased the salt tolerance of Chinese cabbage sprouts during the germination stage mainly because the Ca^{2+} and Sr^{2+} significantly enhanced plasma membrane stability and reduced oxidative stress (as indicated by decreased contents of malondialdehyde and $O_2^{\cdot-}$ contents) in sprouts. The decrease of soluble sugar and proline content caused by Ca-Sr addition implies that elevated levels of these osmolytes were not the primary contributors to improved seed germinability in Chinese cabbage. These findings demonstrate that Sr is a beneficial element for enhancing salt tolerance in plants, laying a theoretical foundation for the development and application of strontium in agriculture.

KEYWORDS: Chinese cabbage; seed germinability; germination indices; Ca-Sr addition; salt stress; membrane stability; oxidative stress

1 Introduction

More than 800 million hectares of land are affected by salinity and alkalinity worldwide [1]. Cultivating salt-tolerant crops is an important strategy for the utilization of saline-alkali wasteland. Although substantial progress has been made in breeding salt-tolerant grain crops, their economic returns remain low in high salinity soils. This is primarily due to the high demand for freshwater and input costs [2,3]. Salt-tolerant vegetables with high economic yield and profitability offer a more viable approach to developing saline-alkali land. Many salt-tolerant vegetables have been successfully cultivated and adopted. For example, tomatoes not only grow well under saline conditions, but may also exhibit enhanced quality attributes [4]. Seed germination is the initial stage of agricultural production, yet it is also the most salt-sensitive phase in the crop life cycle [5]. Enhancing salt tolerance during seed germination is therefore critical for successful vegetable cultivation in saline-alkali soils.

Our previous results showed that the seeds of the salt-tolerant Chinese cabbage variety, Juhong 65, had a low germination percentage and could not survive under 300–400 mmol/L NaCl, whereas its seedlings could survive for up to two months under the same salinity level [6]. Halophyte seeds are also sensitive to salt stress during germination and typically initiate germination only after soil salinity is reduced by rainfall [7]. Therefore, vegetable cultivation in saline-alkali soil commonly employs seedling transplantation or direct sowing following freshwater irrigation. To further improve seed germination and survival rate under saline conditions, various physiological interventions have been explored. Soil inoculation with *Streptomyces avidinii*, *Bacillus proteolyticus* and *Bacillus safensis* has been shown to significantly improve the germination rate of mung beans, peppers, cucumbers and common bean under saline conditions [8,9]. Microbial seed coatings using beneficial bacteria also exhibit a comparable promoting effect [10]. The application of exogenous silicon has also been found to effectively improve the germination ability of seeds under saline conditions [11]. A growing number of studies have focused on the use of plant growth regulators, such as gibberellin, brassinolide, ethylene, kinetin, melatonin, and salicylic acid to improve seed germinability [12–16]. In recent years, modern nanomaterials (such as carbon nanoparticles and nano-selenium) and physical methods (such as constant-frequency and variable-frequency ultrasonic treatments) have also been employed to improve seed germinability under saline conditions [17–19]. Since high salinity disrupts ionic balance and impairs nutrient uptake, supplementation with organic or mineral nutrients in the saline medium can also enhance seed germinability [20,21]. For example, the exogenous application of amino acids, including Gly, Cys, Ser and Met, can significantly alleviate the inhibition of salt stress on seed germination of *Arabidopsis thaliana* [22,23]. Soaking seeds with glucose and sucrose under salt stress significantly enhances antioxidant capacity, thereby improving seed germination and early seedling growth [23]. The exogenous addition of phosphorus, potassium, calcium, magnesium and other mineral nutrients is particularly practical in saline-alkali agriculture, and demonstrates a pronounced effect on promoting seed germination and seedling growth [24–27]. Among mineral nutrients, calcium is most commonly reported to enhance seed germinability under salt stress [27–35]. Ca-addition can also improve plant salt tolerance throughout all developmental stages [36,37]. Strontium and calcium belong to the same group of elements in the periodic table and exhibit similar chemical properties. Both strontium and calcium are essential nutrients for humans and animals. Recently, strontium has been identified as a new non-essential beneficial element for plants [38,39].

To explore the beneficial role of strontium in plant salt tolerance, this study compared the effects of Ca and Sr supplementation on seed germinability of Chinese cabbage (a widely cultivated vegetable in East Asia) under saline conditions, which would lay a solid foundation for further agricultural applications of strontium.

2 Materials and Methods

2.1 Plant Material and Experimental Design

The seeds of Chinese cabbage (*Brassica rapa* L. ssp. *Pekinensis*. Cultivar name: Qingmaye) were purchased from Tianjin Kerun Seed Co., Ltd. (Tianjin, China). The seed germination experiment included a blank-control group (B-CK), a salt-control group (S-CK) and a calcium-strontium addition group (Ca-Sr addition). The B-CK group received half-strength Hoagland nutrient solution without $Ca(NO_3)_2$ (hereinafter referred to as "nutrient solution"). The S-CK group was treated with 150 mmol/L NaCl dissolved in the nutrient solution (hereinafter referred to as "salt solution"). In the Ca-Sr addition groups, $CaCl_2$ or $SrCl_2$ was added to the salt solution to the final concentration of 2, 4 or 8 mmol/L (labeled as "2Ca", "4Ca", "8Ca" and "2Sr", "4Sr", "8Sr", respectively). In Ca-Sr mixed addition group, equimolar concentrations of $CaCl_2$ and $SrCl_2$ were added to the salt solution to the total concentration of 2, 4 or 8 mmol/L (labeled as "1Ca + 1Sr", "2Ca + 2Sr", "4Ca + 4Sr", respectively). Five glass Petri dishes (15 cm in diameter, 2 cm in height) were prepared for each treatment group with 2 layers of filter paper on the bottom. The corresponding treatment solution (30 mL) was poured into each Petri dish to moisten the filter paper, and the solution depth was about 1 mm. One hundred uniform, viable seeds were evenly distributed in each Petri dish, which was then covered to minimize evaporation. The Petri dishes were incubated in an artificial climate chamber at 22°C with a light intensity of 50 μmol/m^2/s and a 16 h/8 h light/dark photoperiod. The solution in Petri dishes was renewed daily to maintain constant solute concentrations. The number of newly germinated seeds in each Petri dish was recorded every 24 h. Germination was defined as radicle protrusion of at least 2 mm through the seed coat. The germination experiment was ended on the 6th day when no additional germination was observed. Germination indices of Chinese cabbage seeds were calculated based on the recorded data. After the germination experiment, all sprouts from each Petri dish were collected to measure total fresh weight and calculate the average fresh weight of each sprout (g FW/plant). The sprouts were washed twice with distilled water and blotted dry with absorbent paper. The prepared sprouts were used directly or frozen with liquid nitrogen for subsequent physiological analyses.

2.2 Calculation of Seed Germination Indices

Germination indices were calculated using the following formulas [40].

$$\text{Germination percentage (GP)} = N_6/N_T$$

$$\text{Germination energy (GE)} = N_2/N_T \times 100\%$$

$$\text{Germination index (GI)} = \sum_{i=1}^{k} \frac{n_i}{d_i}\ (\text{seed/d})$$

$$\text{Vigor index (VI)} = (FW/plant) \times \text{GI}\ (\text{g} \cdot \text{seed/plant/d})$$

$$\text{Coefficient of rate of germination (CRG)} = \frac{\sum_{i=1}^{k} n_i}{\sum_{i=1}^{k} d_i n_i} \times 100\%\ (1/\text{d}) \quad (1)$$

$$\text{Average germination days } (\bar{d}) = 1/\text{CRG (d)}$$

$$\text{Index of rate of germination (IRG)} = \frac{\sum_{i=1}^{k} N_i}{k}\ (\text{seed/d})$$

$$\text{Coefficient of uniformity of germination (CUG)} = \frac{\sum_{i=1}^{k} n_i - 1}{\sum_{i=1}^{k} n_i (d_i - \bar{d})^2}\ (1/\text{d}^2)$$

$$\text{Coefficient of variation of the germination time } (CV_T) = S_d/\bar{d}$$

The symbols in the formulas are described as follows: N_2: Total number of germinated seeds on the 2nd day; N_6: Total number of germinated seeds on the 6th day; N_T: Total number of seeds in each Petri dish (100 seeds); n_i: The number of newly germinated seeds on the *i*th day; d_i: Corresponding germination days (*i*th day); k: Total germination days (6 days); FW: The average fresh weight per sprout (g); N_i: Total number of germinated seeds on the *i*th day.

2.3 Determination of Plasma Membrane Permeability

A total of 0.2 g fresh sprouts, washed with distilled water, were placed in a test tube (containing 20 mL distilled water). The tubes were vacuum-infiltrated for 15 min and subsequently kept at room temperature (25°C) for 30 min. The original conductivity (OC) of the supernatant was measured with a conductivity meter. The test tube was subsequently immersed in boiling water for 15 min. The total conductivity (TC) was measured after the tube was cooled to 25°C. Plasma membrane permeability was calculated as OC/TC × 100%.

2.4 Determination of Malondialdehyde and Superoxide Anion ($O_2^{\cdot -}$)

Lipid peroxidation was quantified by measuring the malondialdehyde (MDA) content. MDA was extracted from 1 g frozen sprouts of Chinese cabbage by grinding with 10 mL 10% trichloroacetic acid. The MDA content was determined following the method of Hodges et al. [41]. Superoxide anion radical ($O_2^{\cdot -}$) was extracted from 0.5 g fresh sprouts by grinding with 5 mL 50 mM phosphate buffer (K_2HPO_4-KH_2PO_4, pH7.8). The $O_2^{\cdot -}$ concentration was determined by monitoring the formation of nitrite, the product of hydroxylamine reaction with $O_2^{\cdot -}$ [42].

2.5 Determination of Soluble Sugar and Proline

Anthrone reagent was added to the MDA extract to develop color, and the absorbance was determined at 620 nm. The soluble sugar concentration was calculated using a standard curve of glucose.

Proline was extracted from 0.5 g frozen sprouts by grinding with 5 mL 3% sulfosalicylic acid. Proline concentration was determined using acid-ninhydrin binding methods [43].

2.6 Statistical Analysis

A randomized block design was used in this research. All values were expressed as mean ± standard deviation (SD) based on five replicates. One-way ANOVA and Duncan's LSR test were used to assess the differences among treatments. Means were considered significantly different at $p < 0.05$, as indicated by different lowercase letters.

3 Results

3.1 Ca-Sr Addition Improves the Seed Viability of Chinese Cabbage under Saline Conditions

Seed viability refers to the potential of seeds to germinate, or the vitality of embryos, which is commonly expressed as germination percentage (GP). In agriculture, a GP value above 85% is typically required for qualified vegetable seeds. Under non-saline condition (B-CK), the GP of Chinese cabbage seeds reached 98.2%, indicating high-quality seed vigor (Fig. 1). The GP values of Chinese cabbage seeds under 50 and 100 mmol/L NaCl treatments showed no significant difference compared with that of the B-CK group. Under the condition of 150 mmol/L NaCl, the germination rate decreased to 56.8%, which falls below agricultural production standards. Under the condition of 200 mmol/L NaCl, the GP value further dropped to 18.6%, rendering the seeds agriculturally unviable. Therefore, 150 mmol/L NaCl was selected as the salt stress level for the salt-control group (S-CK) and subsequent treatments of Ca-Sr addition.

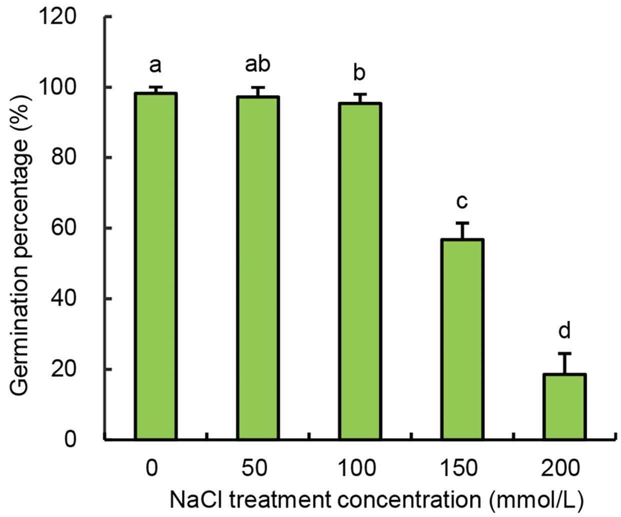
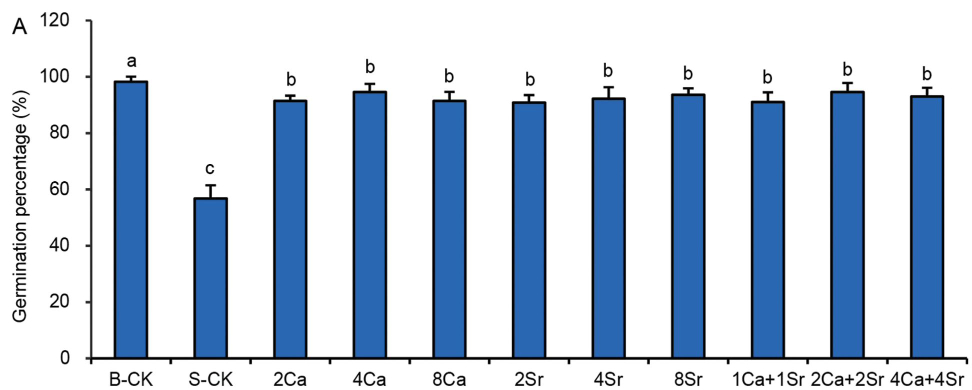


Figure 1: Effects of different NaCl concentrations on the germination percentage of Chinese cabbage seeds. Means were separated at a significant level ($p < 0.05$) by different lowercase letters

Under the condition of 150 mmol/L NaCl, the GP values of Chinese cabbage seeds increased to over 90% following Ca-addition, Sr-addition or the Ca-Sr mixed addition (Fig. 2A). Therefore, Sr-addition exhibited a comparable promoting effect on seed germination to Ca-addition under saline conditions. The germination energy (GE) exhibited a similar trend (Fig. 2B). The difference between GP and GE in the Ca-Sr addition groups was only 1.6%–3.4%, indicating rapid and synchronized germination, with most seeds germinating within 2 days. In contrast, the difference between GP and GE of the S-CK group was 9.8%, indicating slower and more dispersed germination. However, GP and GE were not sensitive enough to distinguish the differences among different Ca-Sr treatments in their promotive effects on germination.



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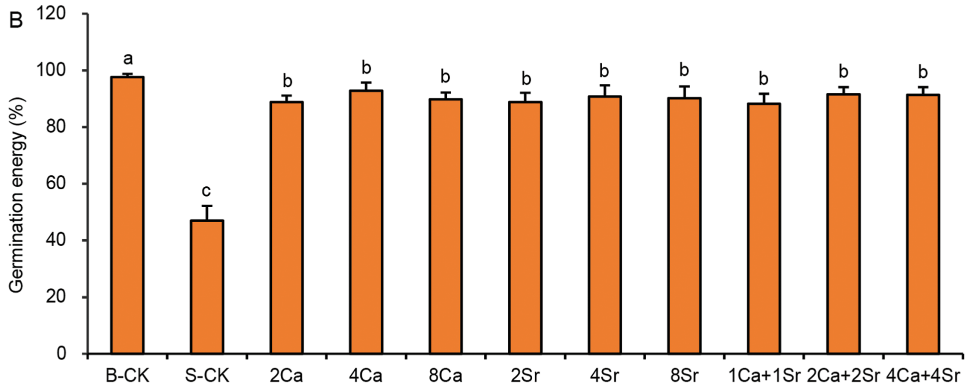
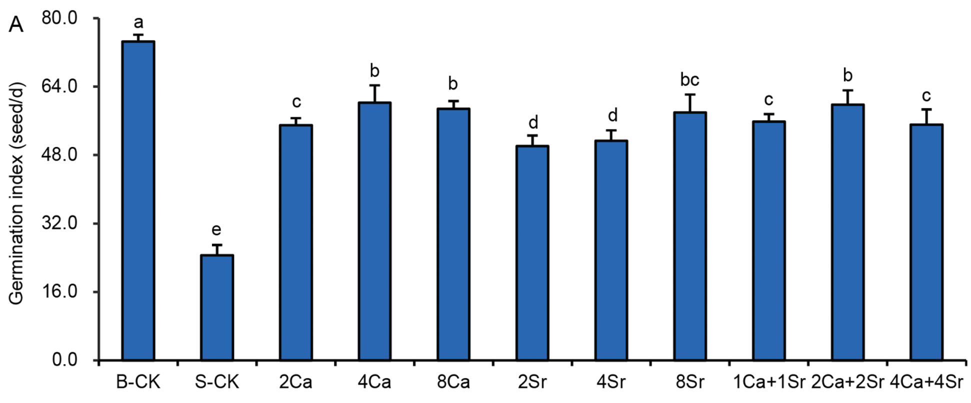


Figure 2: Effects of calcium-strontium addition on germination percentage (**A**) and germination energy (**B**) of Chinese cabbage seeds under 150 mmol/L NaCl. The B-CK group received half-strength Hoagland nutrient solution without $Ca(NO_3)_2$. The S-CK group was treated with 150 mmol/L NaCl dissolved in the nutrient solution. In the Ca-Sr addition groups, $CaCl_2$ or $SrCl_2$ was added to the salt solution to the final concentration of 2, 4 or 8 mmol/L, labeled as "2Ca", "4Ca", "8Ca" and "2Sr", "4Sr", "8Sr", respectively. In Ca-Sr mixed addition group, equimolar concentrations of $CaCl_2$ and $SrCl_2$ were added to the salt solution to the total concentration of 2, 4 or 8 mmol/L, labeled as "1Ca + 1Sr", "2Ca + 2Sr", "4Ca + 4Sr", respectively. Means were separated at a significant level ($p < 0.05$) by different lowercase letters

3.2 Ca-Sr Addition Enhances Seed Vigor of Chinese Cabbage under Saline Condition

Seed vigor is defined as the capacity of seeds to germinate rapidly and uniformly, and to produce robust seedlings, reflecting their growth potential and adaptability under various environmental conditions. Germination index (GI) is one of the most commonly used indicators of seed vigor. As shown in Fig. 3A, the GI of Chinese cabbage seeds was 74.5 seeds/d under the B-CK condition, indicating strong seed vigor. Under the 150 mmol/L NaCl condition, the GI rapidly decreased to 24.6 seeds/d, approximately one-third of that of the B-CK group. All Ca-Sr treatments resulted in a GI more than doubled that of the S-CK group. The promoting effects of 2Sr and 4Sr addition on GI were slightly lower than those of the other Ca-Sr addition treatments, yielding GI values of 50.1 and 51.33 seeds/d, respectively.



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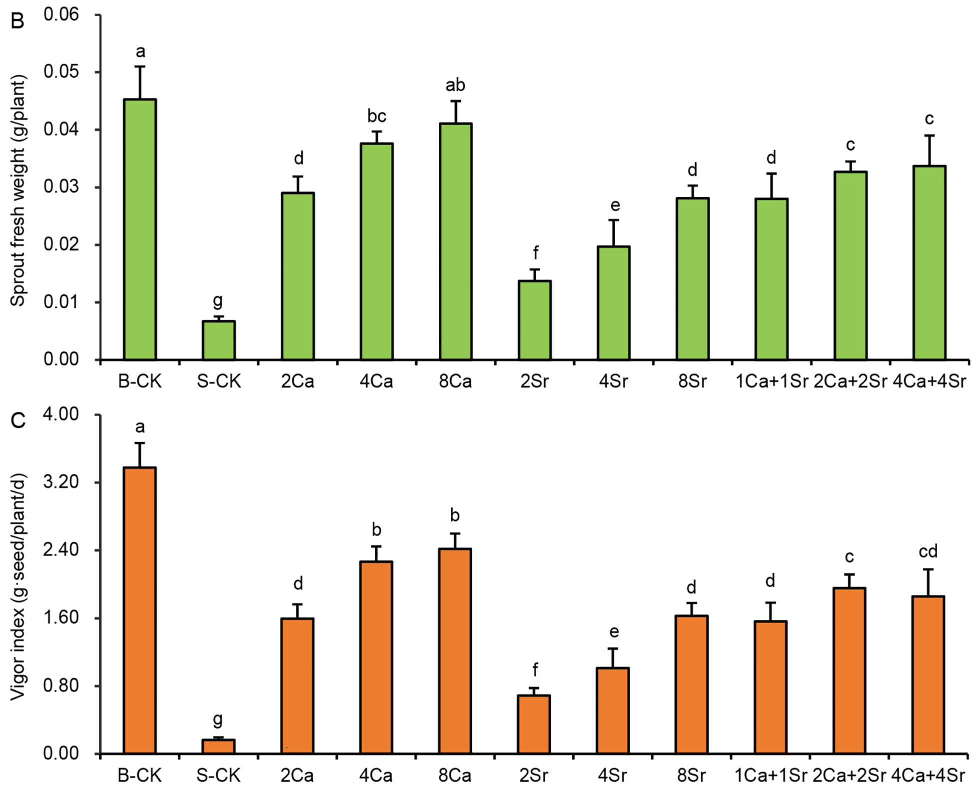

Figure 3: Effects of calcium-strontium addition on germination index (**A**), sprout fresh weight (**B**) and vigor index (**C**) of Chinese cabbage seeds under 150 mmol/L NaCl. The B-CK group received half-strength Hoagland nutrient solution without $Ca(NO_3)_2$. The S-CK group was treated with 150 mmol/L NaCl dissolved in the nutrient solution. In the Ca-Sr addition groups, $CaCl_2$ or $SrCl_2$ was added to the salt solution to the final concentration of 2, 4 or 8 mmol/L, labeled as "2Ca", "4Ca", "8Ca" and "2Sr", "4Sr", "8Sr", respectively. In Ca-Sr mixed addition group, equimolar concentrations of $CaCl_2$ and $SrCl_2$ were added to the salt solution to the total concentration of 2, 4 or 8 mmol/L, labeled as "1Ca + 1Sr", "2Ca + 2Sr", "4Ca + 4Sr", respectively. Means were separated at a significant level ($p < 0.05$) by different lowercase letters

Another key index of seed vigor is the vigor index (VI), calculated by multiplying GI by the fresh weight per sprout. Fresh weight measurement on the 6th day of germination showed increasing Ca concentration resulted in larger Chinese cabbage sprouts (Fig. 3B). The fresh weight of individual sprout treated with 2, 4 and 8 mmol/L $CaCl_2$ was 4.33, 5.61 and 6.13 times as much as that of the S-CK group, respectively. Ca-addition significantly improved the growth of Chinese cabbage sprouts under saline condition. Sr-addition exhibited a similar effect on the sprouts, although its growth promoting effects were consistently lower than those of $CaCl_2$ at equivalent concentrations. The fresh weight of sprouts treated with 2, 4 and 8 mmol/L $SrCl_2$ was 2.04, 2.94 and 4.19 times as much as that of the S-CK group, respectively. The growth promoting effect of Ca-Sr mixed addition fell in between the effects of Ca and Sr treatment alone at the same concentrations. Among all Ca-Sr treatments, 4–8 mmol/L $CaCl_2$ had the best growth promoting effect under 150 mmol/L NaCl. Consistent with GI trends, VI is more clearly differentiated among Ca-Sr treatments (Fig. 3C), primarily due to the increase in sprout fresh weight. GI and VI were more sensitive indicators for evaluating seed germinability of Chinese cabbage than GP and GE under saline conditions.

3.3 *Ca-Sr Addition Increases Seed Germination Rate of Chinese Cabbage under Saline Condition*

Seed germinability can also be assessed by the germination rate. The coefficient of rate of germination (CRG), the reciprocal of the average germination time ($\overline{d}$), is commonly used to evaluate seed germination rate. Under non-saline condition (B-CK), the CRG of Chinese cabbage seeds was 0.672 (1/d), and the average germination time was about 1.5 d (Fig. 4A). Under 150 mmol/L NaCl (S-CK), CRG decreased significantly to 0.387 (1/d), and the average germination time was extended to about 2.6 days. Following Ca-Sr addition in 150 mmol/L NaCl, the CRG of Chinese cabbage seeds increased to more than 0.500, and the average germination time was shortened to less than 2 days. However, the variation of CRG among Ca-Sr treatments was relatively low, with a maximum variation of less than 15%. In general, CRG values followed the trend: Ca-addition > Ca-Sr mixture > Sr-addition at equivalent concentrations. For both Ca and Sr applied individually, the germination rate of Chinese cabbage seeds increased with the increase of addition concentrations. Germination rate can also be quantified by the index of germination rate (IRG). The results of IRG also showed that Ca-Sr treatments significantly accelerated the germination of Chinese cabbage seeds under saline condition (Fig. 4B). However, CRG and IRG were not as sensitive as GI and VI. Variation in CRG and IRG among different Ca-Sr treatments was minimal.

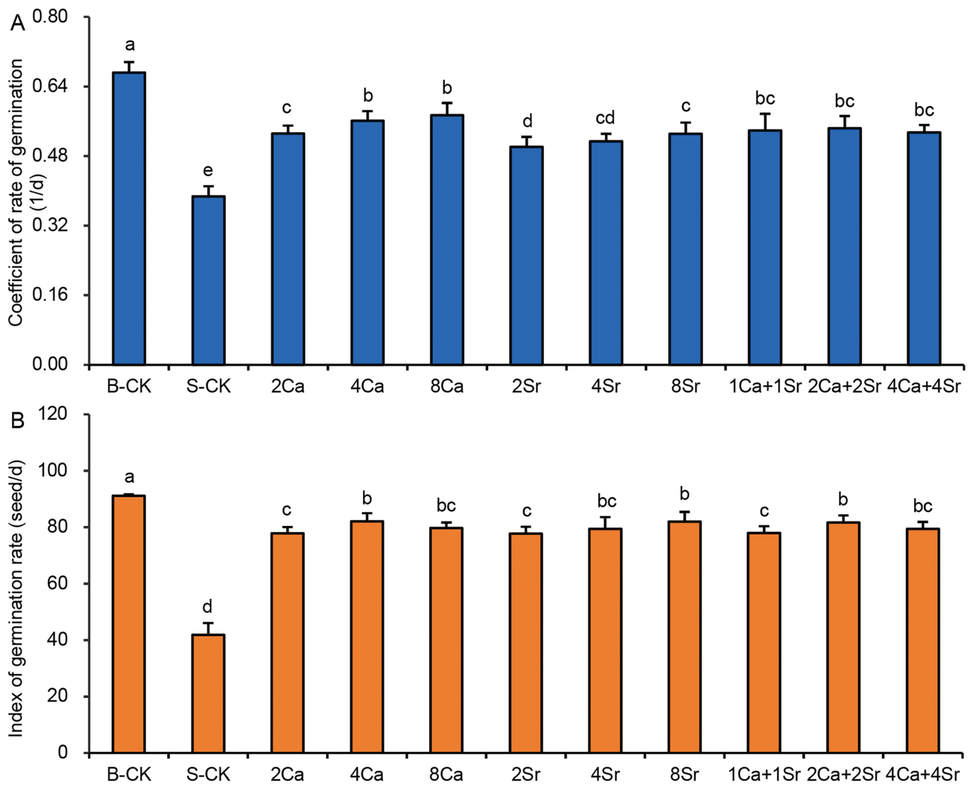

Figure 4: Effects of calcium-strontium addition on coefficient of rate of germination (**A**) and index of rate of germination (**B**) of Chinese cabbage seeds under 150 mmol/L NaCl. The B-CK group received half-strength Hoagland nutrient solution without $Ca(NO_3)_2$. The S-CK group was treated with 150 mmol/L NaCl dissolved in the nutrient solution. In the Ca-Sr addition groups, $CaCl_2$ or $SrCl_2$ was added to the salt solution to the final concentration of 2, 4 or 8 mmol/L, labeled as "2Ca", "4Ca", "8Ca" and "2Sr", "4Sr", "8Sr", respectively. In Ca-Sr mixed addition group, equimolar concentrations of $CaCl_2$ and $SrCl_2$ were added to the salt solution to the total concentration of 2, 4 or 8 mmol/L, labeled as "1Ca + 1Sr", "2Ca + 2Sr", "4Ca + 4Sr", respectively. Means were separated at a significant level ($p < 0.05$) by different lowercase letters

3.4 Ca-Sr Addition Improves Seed Germination Uniformity of Chinese Cabbage under Saline Condition

The uniformity of germination is an important indicator in vegetable cultivation, which can be quantified by the coefficient of uniformity of germination (CUG) and the coefficient of variation of germination time (d_i) (CV_T). A higher CUG indicates greater germination uniformity, whereas a lower CV_T value reflects more synchronized germination. The CUG results showed that all Ca-Sr treatments enhanced the germination uniformity of Chinese cabbage seeds, among which, 2Sr, 4Sr and 8Ca addition groups exhibited the same germination uniformity as the B-CK group (Fig. 5A). CUG was more sensitive than CV_T in reflecting treatment differences. The CUG values in all Ca-Sr treatments were more than double that of the S-CK group, with clear differences observed under different treatment concentrations. Although the CV_T value of all Ca-Sr addition groups was significantly lower than that of the S-CK group, CV_T did not show a significant difference between the S-CK group and the B-CK group (Fig. 5B). Therefore, CUG is recommended as a reliable indicator for evaluating seed germination uniformity under saline conditions.

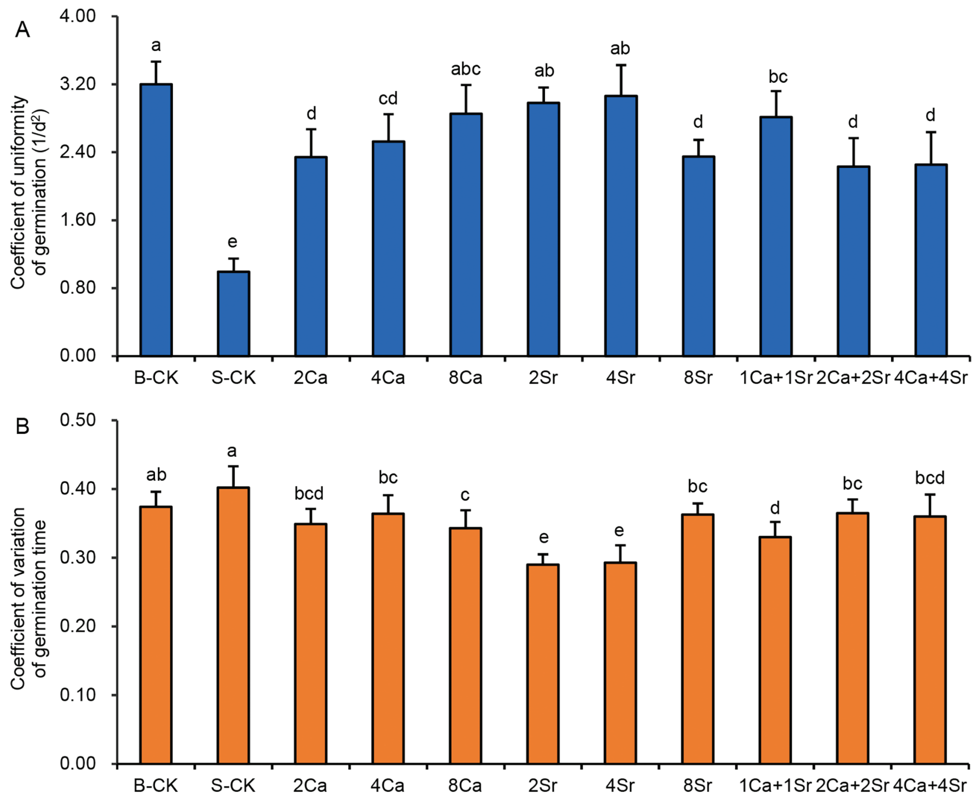

Figure 5: Effects of calcium-strontium addition on coefficient of uniformity of germination (**A**) and coefficient of variation of the germination time (**B**) of Chinese cabbage seeds under 150 mmol/L NaCl. The B-CK group received half-strength Hoagland nutrient solution without $Ca(NO_3)_2$. The S-CK group was treated with 150 mmol/L NaCl dissolved in the nutrient solution. In the Ca-Sr addition groups, $CaCl_2$ or $SrCl_2$ was added to the salt solution to the final concentration of 2, 4 or 8 mmol/L, labeled as "2Ca", "4Ca", "8Ca" and "2Sr", "4Sr", "8Sr", respectively. In Ca-Sr mixed addition group, equimolar concentrations of $CaCl_2$ and $SrCl_2$ were added to the salt solution to the total concentration of 2, 4 or 8 mmol/L, labeled as "1Ca + 1Sr", "2Ca + 2Sr", "4Ca + 4Sr", respectively. Means were separated at a significant level ($p < 0.05$) by different lowercase letters

3.5 Ca-Sr Addition Enhances Plasma Membrane Stability of Chinese Cabbage Sprouts under Saline Condition

Among all treatments, 8Ca-addition treatment showed the greatest effect in promoting seed vigor under saline conditions. Therefore, the addition treatments of 8Ca, 8Sr, and "4Ca + 4Sr" were selected to investigate the physiological mechanisms by which Ca-Sr addition enhanced salt tolerance of Chinese cabbage sprouts during the germination stage. As shown in Fig. 6A, plasma membrane permeability of Chinese cabbage sprouts increased significantly under 150 mmol/L NaCl, reaching 3.51 times the level of the B-CK group. Following treatments with 8Ca, 8Sr, and 4Ca + 4Sr, the plasma membrane permeability of Chinese cabbage sprouts significantly decreased to 1.15, 1.89, and 1.25 times that of the B-CK group, respectively. At equivalent concentrations, Sr-addition was less effective than Ca-addition or the Ca-Sr combination in stabilizing membrane integrity.

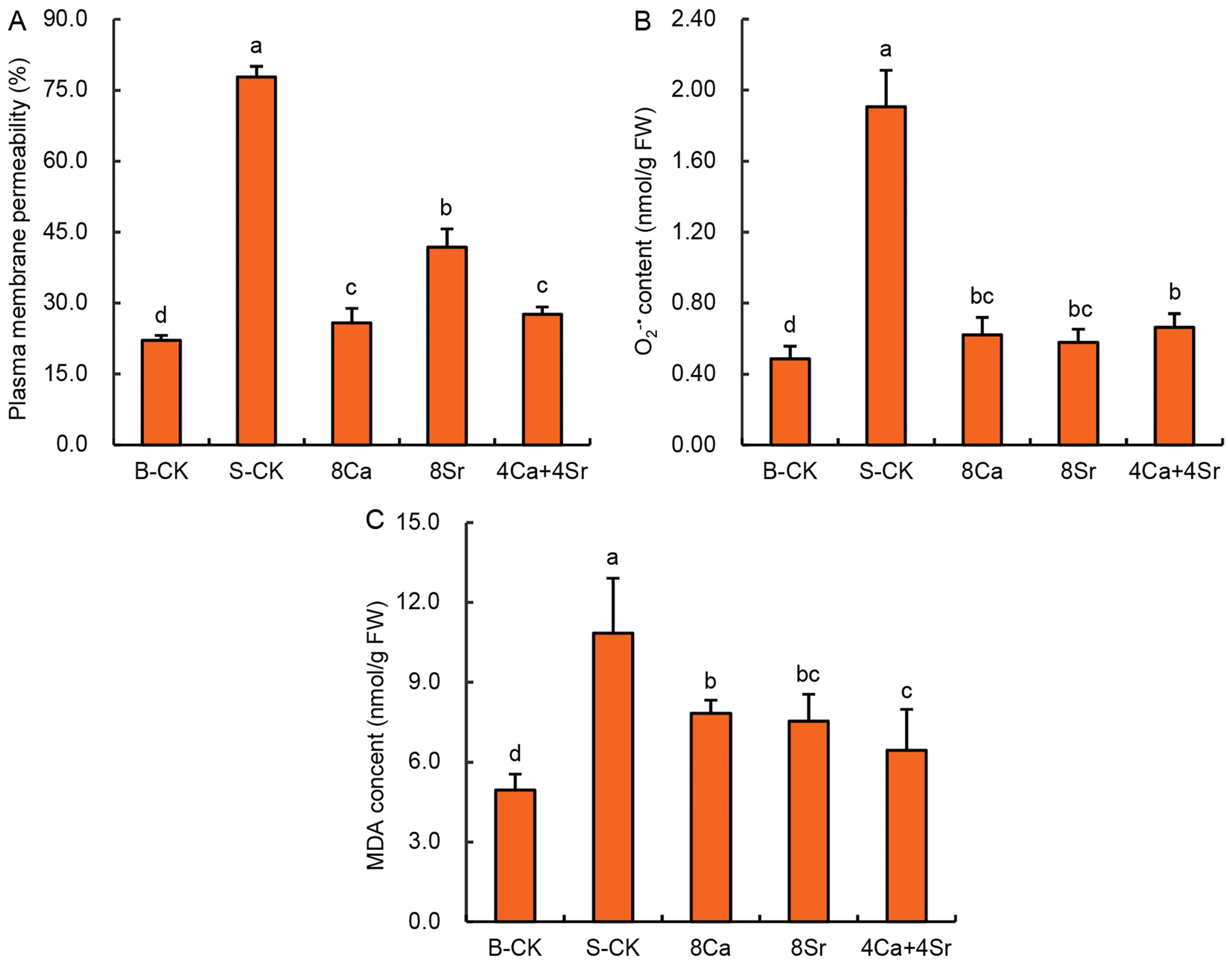

Figure 6: Effects of calcium-strontium addition on plasma membrane permeability (**A**), $O_2^{\cdot -}$ content (**B**) and malondialdehyde (MDA) content (**C**) of Chinese cabbage sprouts under 150 mmol/L NaCl. Means were separated at a significant level ($p < 0.05$) by different lowercase letters

3.6 *Ca-Sr Addition Alleviates Oxidative Stress in Chinese Cabbage Sprouts under Saline Condition*

Salt stress often induces secondary oxidative stress, leading to elevated production of reactive oxygen species within cells. As shown in Fig. 6B, the content of $O_2^{\cdot -}$ in Chinese cabbage sprouts treated with 150 mmol/L NaCl was 3.92 times that of the B-CK group. The addition of 8Ca, 8Sr, and 4Ca + 4Sr in 150 mmol/L NaCl significantly reduced the accumulation of $O_2^{\cdot -}$ in the sprouts, which were 1.28, 1.15, and 1.37 times that of the B-CK group, respectively (Fig. 6B). Malondialdehyde (MDA), a product of membrane lipid peroxidation, also confirmed that Ca-Sr addition significantly reduced oxidative damage in Chinese cabbage sprouts treated with 150 mmol/L NaCl (Fig. 6C). The MDA content in sprouts treated with 150 mmol/L NaCl was 2.19 times that of the B-CK group. Following the addition of 8Ca, 8Sr, and 4Ca + 4Sr in 150 mmol/L NaCl, the MDA contents in the sprout decreased to 1.58, 1.52, and 1.30 times that of the B-CK group, respectively. The above results indicate that Ca-Sr treatments effectively alleviated the oxidative damage of membrane lipids in the sprouts under salt stress.

3.7 *Ca-Sr Addition Decreases the Accumulation of Organic Osmotytes Regulation Substances in Chinese Cabbage Sprouts*

Under salt stress, plants generally accumulate low-molecular-weight organic osmolytes, such as proline and soluble sugars to maintain cellular osmotic balance. As shown in Fig. 7A, the proline content in Chinese cabbage sprouts treated with 150 mmol/L NaCl increased dramatically, reaching 57.8 times that of the B-CK group. With the addition of 8Ca, 8Sr, and 4Ca + 4Sr in 150 mmol/L NaCl, the proline content in the sprouts decreased to 19.6, 44.1, and 33.3 times that of the B-CK group, respectively. The increase multiple in the content of soluble sugars in the sprouts was relatively small after exposure to 150 mmol/L NaCl, increasing to only 2.33 times that of the B-CK control group. Following the addition of 8Ca, 8Sr, and 4Ca + 4Sr in 150 mmol/L NaCl, the soluble sugar contents in the sprouts decreased to 1.40, 1.60, and 1.19 times that of B-CK group, respectively (Fig. 7B). These results indicate that Ca-Sr-enhanced salt tolerance in sprouts was not associated with elevated proline or soluble sugar accumulation.

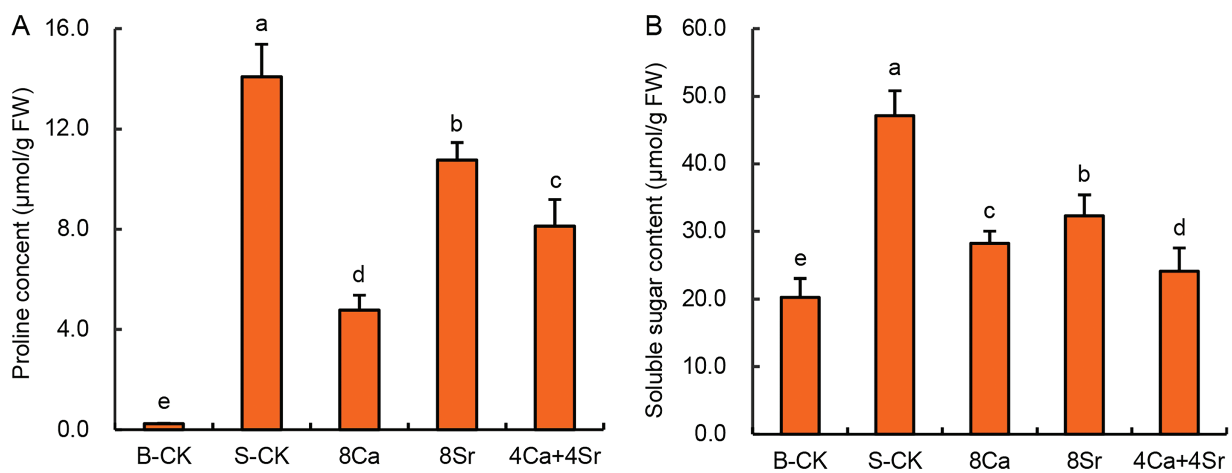

Figure 7: Effects of calcium-strontium addition on soluble sugar content (**A**) and proline content (**B**) of Chinese cabbage sprouts under 150 mmol/L NaCl. Means were separated at a significant level ($p < 0.05$) by different lowercase letters

4 Discussion

The germination stage is the most critical and sensitive period in the life cycle of crops under environmental stress conditions [44]. For example, although cv. Qingmaye is a relatively salt-tolerant Chinese cabbage cultivar, its seed germinability was significantly inhibited under 150 mmol/L NaCl (Fig. 1). There are several key reasons why the germination period is particularly sensitive to salt stress. Firstly, salt-resistant genes are not fully expressed during the germination stage [45], or salinity may suppress the expression of both germination and salt-tolerant genes [32,46]. Secondly, salt-resistant anatomical structures are not yet developed, leaving cells vulnerable to salt-induced damage [47]. Thirdly, sprouts have limited osmotic regulation and water absorption capacity [24]. Fourth, salt stress disrupts nutrient uptake and leads to ionic imbalances in sprouting tissues [20,48]. Fifth, photosynthesis of the sprouts is at a low level. So sprouts mainly rely on stored reserves, which are limited and rapidly depleted under stress. Therefore, main physiological measures to improve salt tolerance during the germination stage include the exogenous application of organic osmotic regulatory substances, plant hormones, mineral nutrients or biostimulants [49]. Among these measures, the addition of exogenous Ca is the most widely used. Sr and Ca are both group IIA elements, and Sr exhibits chemical properties very similar to Ca. It is hypothesized that the addition of Sr may also enhance seed germinability under salt stress, similar to calcium.

This study demonstrated that Ca-addition, Sr-addition, and Ca-Sr mixed addition all significantly improved seed germinability of Chinese cabbage under saline condition (Fig. 2). According to the VI value, seed vigor increased with rising concentrations of Ca and Sr under salt stress. Since the difference between the 4Ca group and the 8Ca group was not statistically significant, 4–8 mmol/L $CaCl_2$ was identified as the optimal concentration in this study. In previous studies involving other plants species, the optimal calcium addition concentration ranges from 5–10 mmol/L $CaCl_2$, which aligns well with the findings of our study [28,29,34,35,50]. In some studies, $CaCl_2$ was applied as a seed priming agent to improve seed germinability prior to salt exposure. In these cases, the seeds were soaked in $CaCl_2$ solution for 12 h [30,32,46]. This priming method requires a higher concentration of $CaCl_2$ (6–15 g/L, or 54–135 mmol/L). The promoting effect of Ca^{2+} on seed germination under saline conditions is universal for plants. However, most studies have focused on the effects of calcium on GP, GE, GI and VI, while relatively few studies have examined its effects on germination rate and germination uniformity [32]. In this study, seed viability, seed vigor, seed germination rate and seed germination uniformity of salt-treated Chinese cabbage seeds were systematically compared among Ca-addition and Sr-addition treatments. VI was the most sensitive index among these germination indices. The VI results showed that calcium was more effective than strontium at equivalent concentrations (Fig. 3). From the CRG results, the germination rates of Ca-addition groups were also slightly higher than those of Sr-addition groups at the same concentration, while IRG did not differ significantly (Fig. 4). Based on CUG and CV_T, the 2 and 4 mmol/L $SrCl_2$ treatments showed greater improvement in germination uniformity than the corresponding $CaCl_2$ treatments (Fig. 5). The overall effects of Sr-addition and Ca-addition on salt-tolerance of Chinese cabbage were comparable during the germination stage, indicating that strontium is beneficial to seed germination under salt stress. These findings provide new evidence supporting strontium as a beneficial element for plants.

Several mechanisms explain how calcium enhances seed germinability of Chinese cabbage under saline conditions. Calcium integrates the cell wall in the form of calcium pectin, which can slow salt absorption and stabilize cellular membranes [51]. Strontium had a similar effect to calcium in enhancing the stability of plasma membrane of Chinese cabbage sprout under saline conditions (Fig. 6A). As a signal substance, calcium can directly activate the metabolic activity of seeds (such as increasing the activity of amylase and upregulating the expression of glucose metabolism genes), thereby improving seed vigor and germination rate [32]. Calcium signal is also involved in regulating the expression of salt-resistant genes. $CaCl_2$-addition has been shown to upregulate the expression of *NHX2*, *NHX4*, *SOS1*, *AKT1*, *AKT2*, *HKT1*, *HAK1* and *KUP* genes [34,46], enhancing K^+ uptake and decreasing the Na^+/K^+ ratio to maintain ion homeostasis under salt stress. Our results showed that Ca-Sr addition significantly reduced the content of $O_2^{\cdot -}$ and the level of membrane lipid peroxidation (MDA content) under 150 mmol/L NaCl (Fig. 6B,C). The effect of calcium addition on alleviating the oxidative damage caused by salt stress is well documented [30,46]. Nevertheless, we propose that the alleviation of oxidative damage by Ca-addition is mainly due to the decreased production of reactive oxygen species. Kamran et al. found that Ca-addition increased the contents of soluble sugar and proline in the sprouts of Chinese cabbage, thereby enhancing osmotic protection ability of cells [31]. Similarly, nitric oxide (NO) improved the germination of wheat seeds exposed to salinity via modulating sugar and proline metabolism [52]. In contrast, we found that Ca-Sr addition significantly reduced soluble sugar and proline contents in Chinese cabbage sprouts under 150 mmol/L NaCl (Fig. 7). A marked increase in proline contents may be a consequence of salt stress rather than a cause [53], because exogenous application of proline did not improve salt tolerance of *Kochia Scoparia* during the germination stage [54]. The mechanism by which strontium enhances salt tolerance during germination may resemble that of calcium, but further investigation is needed to confirm this hypothesis.

5 Conclusion

Under 150 mmol/L NaCl, the addition of 2–8 mmol/L $CaCl_2$ and $SrCl_2$ significantly enhanced seed viability, seed vigor, seed germination rate and seed germination uniformity of Chinese cabbage, mainly because calcium and strontium could significantly enhance the stability of biomembrane under salt stress. When comparing the effects of Ca-addition and Sr-addition, GI, VI and CUG were the more sensitive indices, followed by CRG, IRG, and CV_T. GP and GE did not significantly differ among the various Ca-Sr addition treatments. Therefore, GI, VI and CUG are more informative for evaluating seed germinability under salt stress. At equal concentrations, calcium was more effective than strontium in enhancing seed vigor, while strontium outperformed calcium in improving germination uniformity. These results provide strong evidence that strontium is a beneficial element for enhancing salt tolerance. The application of calcium and strontium represents a feasible strategy to promote seed germination and sprout growth in saline–alkaline soils.

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