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ORIGINAL ARTICLE

Silicon can improve seed germination and ameliorate oxidative damage of bud seedlings in cucumber under salt stress

Tianyun Gou[1] · Xinhang Chen[1] · Rong Han[1] · Jiaqi Liu[1] · Yongxing Zhu[2] · Haijun Gong[1]

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Abstract

It has been extensively demonstrated that silicon has beneficial effects on plant growth and development under salt stress; whereas less attention has been paid to its effect on seed germination, and the underlying mechanism is also unknown. Here, we investigated the effect of silicon on seed germination and oxidative damage of bud seedlings in cucumber under salt stress. The results showed that, 0.3 mM silicon could increase seed germination percentage, germination index and seedling vigor index under 200 mM NaCl. Twelve hours after germination initiation, the expression of *CYP707A1*, which encodes ABA 8′-hydroxylase, was increased by added silicon under salt stress; while the expressions of *GA20ox*, *GA3ox* and *GA2ox*, which encode genes involved in gibberellin metabolism, were not changed in seeds. Thirty-six hours after germination initiation, added silicon markedly inhibited the expressions of ABA biosynthesis genes (*NCED1* and *NCED2*) and gibberellin catabolism gene *GA2ox*. The α-amylase activity was higher in silicon-applied seeds than the control under salt stress. Compared with salt stress alone, added silicon improved the growth and plasma membrane integrity of bud seedlings, while decreasing reactive oxygen species accumulation and lipid peroxidation. Added silicon decreased the activities of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase, and the concentrations of protein and proline in radicles of bud seedlings under salt stress, implying a stress alleviation. These results suggest that silicon might decrease ABA level, maintain high gibberellin level and increase α-amylase activity, therefore improving cucumber seed germination under salt stress. The alleviation of oxidative damage by added silicon contributed to the improvement of bud seedling growth under salt stress.

Keywords Cucumber · Oxidative damage · Salt stress · Seed germination · Silicon

Introduction

Globally, about 800 million hectares of arable land is adversely affected by soil salinity (Azeem et al. 2015). In China, nearly 100 million hectares of land is salinized (Luo et al. 2017). Moreover, secondary soil salinization is increasing due to over utilization of chemical fertilizers, watering with salty water, and unreasonable crop rotation. Soil salinization affects seed germination, growth and development of crops, and breeding salt-tolerant crop cultivars is a sustainable approach to deal with the soil salinity. However, this still remains challenging due to variable climate conditions. On the other hand, the use of exogenous substances is an optional approach to increase crop tolerance to salt stress (Li et al. 2015).

Silicon (Si) is among the most abundant elements in soil (Coskun et al. 2019). Although silicon is not essential for most plants, yet it possesses many positive effects on the growth and development of plants (Frew et al. 2018). Silicon's beneficial effect is more pronounced under adverse environmental conditions, including various abiotic and biotic stresses (Etesami and Jeong 2018). The beneficial role of silicon on tolerance to salt stress has been extensively demonstrated in different plants, including both silicon-accumulating plants such as rice (Gong et al. 2006), cucumber (Zhu et al. 2016) and sorghum (Yin et al. 2016), and low silicon-accumulating plants like tomato (Romero-Aranda et al. 2006) and soybean (Lee et al. 2010). The positive role of silicon on salt

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✉ Haijun Gong
gongnavy@163.com

[1] College of Horticulture, Northwest A&F University, Yangling 712100, Shaanxi, China

[2] College of Horticulture and Gardening, Yangtze University, Jingzhou 434025, Hubei, China

tolerance suggests its application in improving salt tolerance of crops in production. Extensive studies have been conducted to clarify the mechanisms of silicon-mediated plant salt tolerance. The proposed mechanisms mainly include decreasing salt ions uptake and/or their transport to the shoot (Shi et al. 2013; Ali et al. 2016), inhibiting water loss via transpiration (Matoh et al. 1986), improving root water uptake (Zhu et al. 2015), and enhancing antioxidant defense (Li et al. 2015). Coskun et al. (2019) proposed a "apoplastic obstruction hypothesis" to explain the mechanism of silicon-mediated tolerance to different environmental stresses including salt stress. However, this hypothesis can not explain the mechanism of silicon's action very well in some cases, such as silicon-mediated increases in both shoot Na^+ accumulation in maize and its salt tolerance (Bosnic et al. 2018). The studies so far suggest that silicon may be involved in plant physiological processes under salt stress, and the role and mechanism of silicon-mediated tolerance to salt stress are not adequately understood and remain to be further explored.

Silicon can not only improve plant growth, but also has a beneficial effect on seed germination in some plants in normal conditions. For example, Toledo et al. (2011) reported that silicon addition increased the germination percentage and vigor in seeds of white oat. Torabi et al. (2012) found that silicon addition improved the germination of borage seeds. Under salt stress, studies on silicon's effect on seed germination are very limited. In squash, Siddiqui et al. (2014) found that addition of nano-SiO_2 improved the germination percentage and bud seedling vigor under 120 mM NaCl. Azeem et al. (2015) observed that in wheat, silicon priming increased the germination percentage and improved the growth of seedlings under salt stress. In common bean, Alsaeedi et al. (2017) also observed a beneficial role of engineered silica nanoparticles on the seed germination in saline conditions. However, the mechanism of silicon-mediated enhancement of seed germination is still unclear. Plant hormones can regulate seed germination. Abscisic acid (ABA) and gibberellin acid (GA) are the two major plant hormones that control seed dormancy and germination (Zhang et al. 2014). However, information is lacking about the possible regulation of silicon on their levels or metabolism.

Cucumber (*Cucumis sativus* L.) is a popular vegetable with large cultivation areas and consumption globally, and it is very sensitive to soil salinity (Huang et al. 2009). In China, cucumber is a vegetable crop with the largest cultivation area (Wu and Han 2017), including cultivation in open field and protected facilities. Soil salinization has been threatening cucumber production. On one hand, it decreases seed germination of cucumber and affects seedling quality; on the other hand, it causes a reduction in cucumber quality and yield (Huang et al. 2009; Zhang et al. 2017a). Moreover, in some regions, salty and polluted water is used for irrigation (Wei and Xu 2005), which further worsens soil salinization.

In this work, we examined silicon's effect on seed germination, expressions of genes involved in ABA and gibberellin (GA) metabolism, and oxidative damage in bud seedlings of cucumber under salt stress. The study may provide a basis for further exploration of the deep mechanism for silicon-mediated improvement in seed germination in saline conditions. Our study suggests an application of silicon fertilizer in cucumber production in salinized soils and regions irrigated with salty water.

Materials and methods

Plant material and germination conditions

The seeds of cucumber (*C. sativus* L. 'Jinyou 1') were sterilized at 55 °C for 15 min (using water bath) and then transferred to Petri dishes lined with two layers of filter paper moistened with the following solutions: (1) CK, distilled water; (2) Na, NaCl solution; (3) NaSi, NaCl plus silicon (as sodium silicate). For concentration screening, 50–250 mM NaCl and 0.1–0.5 mM Si were applied, as indicated in Fig. 1; while for the other experiments, 200 mM NaCl and 0.3 mM Si were used. The solution pH was adjusted to 5.8 with diluted H_2SO_4. Every treatment was replicated three times.

Germination characteristics

Seeds were considered being germinated with a minimum radicle length of 2 mm (Alsaeedi et al. 2018). The number of germinated seeds was counted at the time points as indicated in Fig. 1. Germination percentages (GP), germination index (GI) and seedling vigor index (SVI) were calculated according to Biju et al. (2017) using the following formulae:

$$\mathrm{GP}\ (\%) = n/N \times 100,$$

where n and N are the numbers of germinated seeds and total tested seeds, respectively.

$$\mathrm{GI} = \sum (Gt/Dt),$$

where Gt is the number of germinated at t day, and Dt is the corresponding day of germination.

$$\mathrm{SVI} = \mathrm{GP} \times \text{mean of radicle length}.$$

Expression of genes related to abscisic acid (ABA) and gibberellin (GA) synthesis and catabolism

After germination initiation for 12 h and 36 h, the seeds were collected, frozen in liquid N_2 immediately and then stored at

− 80 °C until analysis. The total RNA of seeds was extracted using Trizol Reagent (Omega, Norcross, USA) according to the manufacturer's instruction. For qPCR analysis, the first-strand cDNA was synthesized using HiScript® Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, Nanjing, China) according to the manufacturer's protocol, including a step for digestion of genomic DNA. The qPCR analysis was performed on a CFX 96 Real-Time PCR system (Bio-Rad) using ChamQ™ SYBR® qPCR Master Mix (Vazyme, Nanjing, China) with gene-specific primers (Table 1). The specific primers of reference genes [*actin* and *elongation factor 1* (*EF1*)] and genes related to ABA and GA synthesis and catabolism were designed using Oligo 7.0 and listed in Table 1. The primers were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). Relative expression of the interested genes to reference genes was calculated using the $2^{-\Delta C_t}$ method. Each treatment included three biological replicates.

Table 1 Gene-specific primers used for real-time PCR

Gene	Accession no.	Primer sequence
NCED1	EU391614	F: CACGCTGTCAGCATCAAT CATGG
		R: GAAGGCGTGATACGCACG TGG
NCED2	HQ008768	F: AAGACGACGGCCACATCT TAGCC
		R: GACGTAAATTCACACTCATCC CGC
CYP707A1	HQ008767	F: TCGGAGTTCTGTTTGCGGCT
		R: TGGTAAAGGGCATAGTTCGT
GA20ox	NM_001305727	F: ATCCGTTCCTTATGTTGCTG
		R: CCTCATTATTGATTCATTGTCC
GA2ox	NM_001282132	F: CCGACGAACTTATCGCTGC
		R: ATTCTTTTGCTGCCGTATCC
GA3ox	XM_008462834	F: ATTCCCTCTTCTCCCTTCCT
		R: ACGCAACCCACATCAGCC
Actin	XM_004135888	F: TTCTGGTGATGGTGTGAGTC
		R: GGCAGTGGTGGTGAACATG
EF1	EF446145	F: ACTTTATCAAGAACATGA TTAC
		R: TTCCTTCACAATTTCATCG

Determination of α-amylase activity

The α-amylase activity was determined according to Białecka and Kępczyński (2010) with modifications. Cucumber seeds were homogenized in 15 mM phosphate buffer, pH 7.0 (g/mL, 1:9). The homogenate was centrifuged at 12,000 × *g* for 15 min. The clear supernatant was heated at 70 ℃ for 15 min and α-amylase activity was assayed by adding 0.5 mL of extract to 1 mL of 1.0% soluble starch. After 15 min of incubation at 40 °C, the reaction was stopped by adding 2 mL of 0.4 M NaOH, and the maltose concentration was determined by DNS method.

Bud seedling growth and oxidative damage analysis

After 90 h of germination, the radicle length was recorded. The radicle was collected for analysis of membrane integrity, lipid peroxidation, reactive oxygen species levels, antioxidant enzyme activities, and level of proline.

Measurement of relative electrolyte leakage and malondialdehyde content in radicles

The relative electrolyte leakage of radicles was determined by following the method of Tuna et al. (2007) with an electrical conductivity meter (DDS-307A, Shanghai).

The malondialdehyde content in radicles was assayed by thiobarbituric acid reaction following the method of Shi et al. (2016) with modification. Radicles were homogenized in 5% (w/v) trichloroacetic acid. The homogenates were centrifuged at 5000 × *g* for 15 min at 4 °C and the supernatants were kept for analysis. The extract was mixed with an equal volume of 0.5% (w/v) of thiobarbituric acid prepared in 5% (w/v) trichloroacetic acid, and then heated in boiling water for 20 min. The reactions were stopped by placing the reaction tubes in an ice bath. The reaction mixture was centrifuged at 7888 × *g* for 10 min, and the supernatant absorbance was read at 450 nm, 532 nm and 600 nm. The malondialdehyde concentration was calculated as follows: malondialdehyde concentration (μM) = $6.452 (OD_{532} - OD_{600}) - 0.559 \times OD_{450}$.

Histochemical staining analysis

The loss of plasma membrane integrity of radicles was detected using the Evans blue staining method (Yamamoto et al. 2001). The radicles were incubated in 0.025% (w/v) Evans blue (in 100 μM $CaCl_2$, pH 5.6) for 10 min, and then washed with 100 μM $CaCl_2$ (pH 5.6). The radicles were observed under a stereoscopic microscope (MZ10F, Leica, Germany) and photographed.

Histochemical detection of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) was done according to Fukao et al. (2011). Fresh radicles immediately placed in a 0.25 mg/mL NBT solution in 10 mM potassium phosphate buffer (pH 7.8) for 0.5 h in the dark. To detect H_2O_2, fresh radicles were soaked with 1 mg/mL DAB in 50 mM Tris–acetate (pH 5.0) for 0.5 h. The membrane lipid peroxidation of radicles

was monitored with the Schiff's reagent staining method (Pompella et al. 1987).

Soluble protein content and antioxidant enzyme activity analysis

The radicles were homogenized in ice-cold 100 mM Na-phosphate buffer (pH 6.8), after which the extract was centrifuged at 12,000×*g* for 20 min at 4 °C. The supernatant was used to determine the content of soluble protein and activities of antioxidant enzymes. The protein content was assayed according to Bradford (1976). The activities of antioxidant enzymes were determined according to Gong et al. (2005).

SOD activity was assayed using the nitroblue tetrazolium method. The reaction mixture (2 mL) contained 50 mM Na-phosphate buffer (pH 7.8), 12.8 mM methionine, 2.25 mM NBT, 30 µM EDTA-Na_2, 60 µM riboflavin and enzyme extract. The reaction mixture was illuminated for 2.5 min at a light intensity of 52 µmol m^{-2} s^{-1}, after which the absorbance was measured at 560 nm. One unit of SOD was defined as that being contained in the volume of extract which caused a half inhibition of NBT reduction.

POD activity was measured by monitoring the oxidation rate of guaiacol. The reaction was initiated by adding enzyme extract to 1.8 mL of Na-phosphate buffer (0.2 M, pH 6.0) containing 10 mM H_2O_2 and 10 mM guaiacol. The color development rate was determined by recording the absorbance of reaction solution at 470 nm ($E = 26.6$ mM^{-1} cm^{-1}).

CAT activity was assayed by recording the decomposition rate of H_2O_2 at 240 nm ($E = 39.4$ mM^{-1} cm^{-1}). The reaction mixture contained 0.15 M Na-phosphate buffer (pH 7.0), 10 mM H_2O_2 and enzyme extract.

APX activity was determined by measuring the oxidation rate of H_2O_2-dependent ascorbate at 285 nm ($E = 2.8$ mM^{-1} cm^{-1}). The reaction mixture contained 50 mM Na-phosphate (pH 7.8), 0.1 mM EDTA-Na_2, 0.5 mM ascorbate, 1 mM H_2O_2 and enzyme extract.

Measurement of proline level

Proline in radicles was extracted in 3% (w/v) sulfosalicylic acid in boiling water, and the concentration was measured using the ninhydrin regent according to Bates et al. (1973). Finely ground radicles (0.15 g) were homogenized in 2 mL of 3% sulfosalicylic acid. The homogenate was centrifuged at 12,000×*g* for 10 min, and the supernatant was used to determine proline concentration. 2 mL of supernatant was mixed with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin regent (2.5 g ninhydrin, 60 mL glacial acetic acid, 40 mL of 6 M H_3PO_4), and the mixture was incubated in boiling water for 30 min. The reaction was terminated by placing the test tubes in an ice bath. After addition of 5 mL toluene, the tubes were vortexed for 15 s. The absorbance of toluene phase was read at 520 nm, and the proline concentration was calculated using a standard curve obtained from L-proline (Bates et al. 1973).

Statistical analysis

Data were subjected to one-way ANOVA with Statistical Package for Social Sciences (SPSS) software for Windows (version 22.0; SPSS, Inc., Chicago, IL, USA). Where *F* tests were significant ($P < 0.05$), the mean values were separated by Duncan's multiple range test.

Results

Germination responses

Mild salt stress at 50 mM NaCl did not affect the germination percentage of cucumber seeds (Fig. 1a). Although 100 mM NaCl delayed the germination, after 42 h, the germination percentage did not show a difference between the control and salt stress at this salt level (Fig. 1a). At 150 mM or higher concentrations of NaCl, the germination percentage was significantly decreased. At 42 h, the germination percentage was 57.6%, 32.6% and 15.6% under 150, 200 and 250 mM NaCl, respectively (Fig. 1a). In the following experiments investigating the effect of silicon on seed germination and bud seedling growth, 200 mM NaCl was used.

Under 200 mM NaCl stress, the effect of silicon on seed germination was investigated. Addition of 0.1 and 0.3 mM of silicon could improve the seed germination under salt stress, and the positive effect was more obvious at 0.3 mM; however, silicon addition at a higher concentration of 0.5 mM did not demonstrate any positive effect (Fig. 1b). Therefore, 0.3 mM of silicon was used in the following experiments. To confirm the positive effect of silicon under salt stress, the effect of added Na_2SO_4 on seed germination was also investigated under salt stress. The result showed that Na_2SO_4 addition had no effect on the germination percentage (Fig. 1c). In addition, addition of silicon did not affect the seed germination of cucumber in normal conditions (Fig. 1d).

Under salt stress (200 mM NaCl), the germination index and seedling vigor index were dramatically decreased; whereas in the presence of added silicon (0.3 mM), both parameters were significantly increased by two- and three-fold, respectively (Fig. 2a, b).

Expression of genes in ABA and GA biosynthesis and catabolism in seeds

9-*cis*-Epoxycarotenoid dioxygenase (NCED) is a key enzyme in ABA synthesis (Zhang et al. 2014). In this

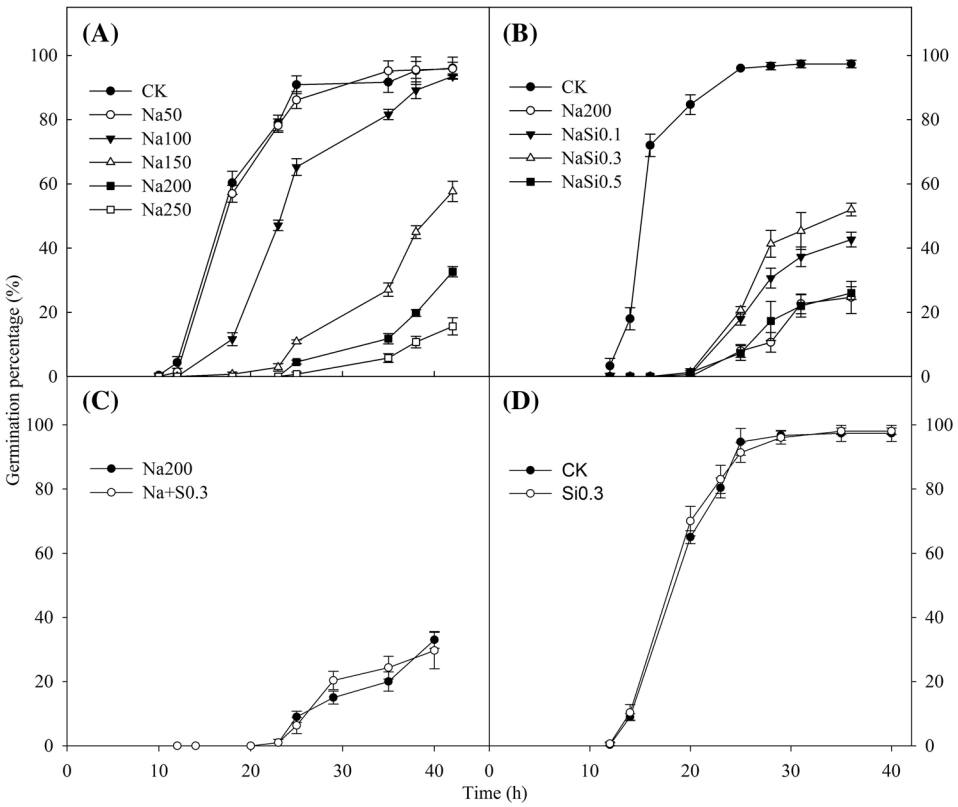

Fig. 1 Effect of salt stress, silicon or sulphur on germination percentage of cucumber seeds. **a** Effect of salt stress (50–250 mM NaCl) on seed germination percentage; **b** effect of Si supplement on germination percentage under salt stress (200 mM NaCl); **c** germination percentage in the absence or presence of added sulphur (0.3 mM) under salt stress (200 mM NaCl); **d** germination percentage in the absence or presence of added silicon (0.3 mM) in control conditions. Data are the mean ± SD ($n = 3$). *CK* control, *Si* silicon, *Na* salt (NaCl) stress, *NaSi* salt stress plus silicon, *S* sodium sulfate

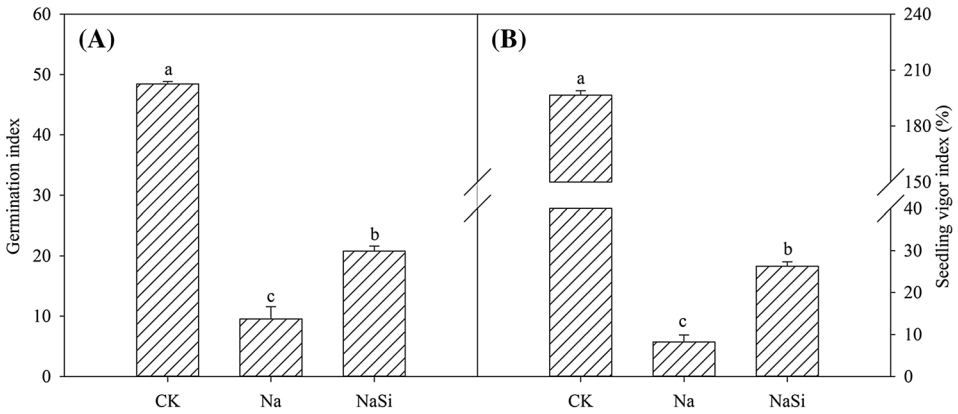

Fig. 2 Effect of silicon on seed germination index and seedling vigor index of cucumber under salt stress. **a** Germination index; **b** seedling vigor index. In the calculation of GI and SVI, the data of 24 h (1 day) and 36 h (1.5 days) were used. Data are the mean + SD ($n = 3$). Bars with different letters are significantly different between the treatments at $P < 0.05$

study, at 12 h, salt stress increased the expression of *CsNCED1* significantly, and there was no difference between salt stress alone and salt stress plus silicon treatment (Fig. 3a); while the expression of *CsNCED2* was decreased under salt stress, with the decrease being less in 'NaSi' than 'Na' treatment (Fig. 3b). At 36 h, the expressions of *CsNCED1* and *CsNCED2* were dramatically increased under salt stress alone, and these were unchanged in 'NaSi' treatment compared with 'CK' (Fig. 3a, b). Compared with that in the control, the expression of *CsCYP707A1*—a gene related to ABA catabolism, was decreased in 'Na' treatment, but it was increased in 'NaSi' at 12 h; whereas at 36 h, the gene expression was increased in 'Na' treatment and it was not changed in 'NaSi' treatment compared to the control (Fig. 3c).

GA20ox and *GA3ox* are genes of GA biosynthesis, whereas *GA2ox* is a GA catabolism gene (Zhang et al. 2014; Vishal and Kumar 2018). In this study, at 12 h, the expressions of *GA20ox* and *GA2ox* were basically not changed under salt stress regardless of silicon addition; while the expression of *GA3ox* was increased in 'Na' and 'NaSi' treatment (Fig. 3e). At 36 h, compared with the control, *GA20ox* expression was increased under salt stress, but there was no obvious difference (less than twice) between 'Na' and 'NaSi' treatment (Fig. 3d). *GA3ox* expression was not changed under salt stress alone, but it

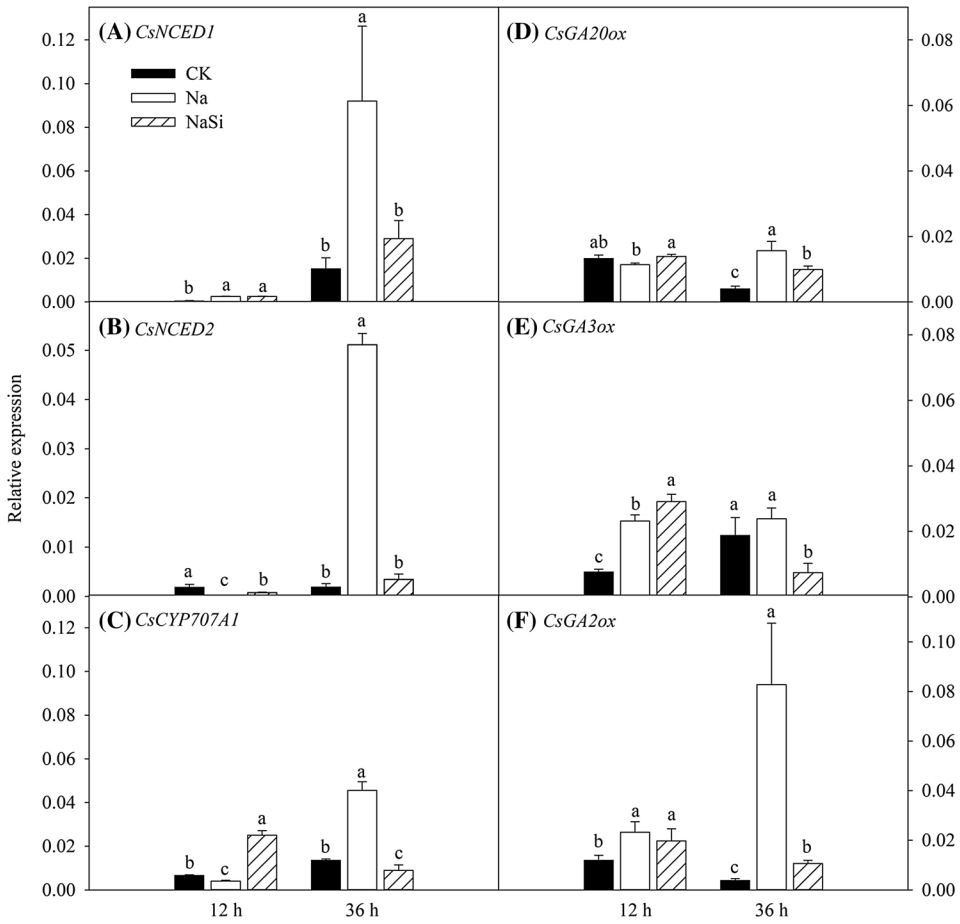

Fig. 3 Expression of genes related to ABA and GA synthesis and catabolism in cucumber seeds under salt stress in the absence or presence of added silicon. **a**, **b** ABA synthesis gene NCEDs; **c** ABA catabolism gene *CYP707A1*; **d**, **e** GA synthesis genes *GA20ox* and *GA3ox*; **f** GA catabolism gene *GA2ox*. Data are the mean + SD ($n = 3$). Bars with different letters are significantly different between the treatments at $P < 0.05$

was decreased in 'NaSi' (Fig. 3e). The *GA2ox* expression was significantly increased under salt stress, especially in the absence of added silicon (Fig. 3f).

Activity of α-amylase

The activity of α-amylase was decreased under salt stress alone (Fig. 4). Under salt stress, addition of silicon significantly increased the α-amylase activity (Fig. 4).

Growth, plasma membrane integrity and relative electrolyte leakage of bud seedlings

The radicle length was markedly decreased under salt stress; whereas it was obviously increased by silicon addition (Fig. 5a). The relative electrolyte leakage was markedly increased under salt stress, and it was significantly decreased by addition of silicon under salt stress (Fig. 5b). The loss of plasma membrane integrity was shown by Evans blue staining, and the result showed that salt stress reduced the plasma membrane integrity, which was improved by added silicon (Fig. 5c).

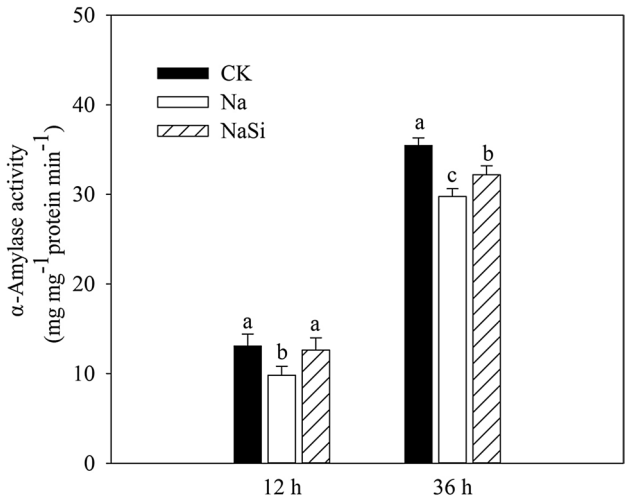

Fig. 4 Effect of silicon on the activity of α-amylase in cucumber seeds under salt stress. Data are the mean + SD ($n = 3$). Bars with different letters are significantly different between the treatments at $P < 0.05$

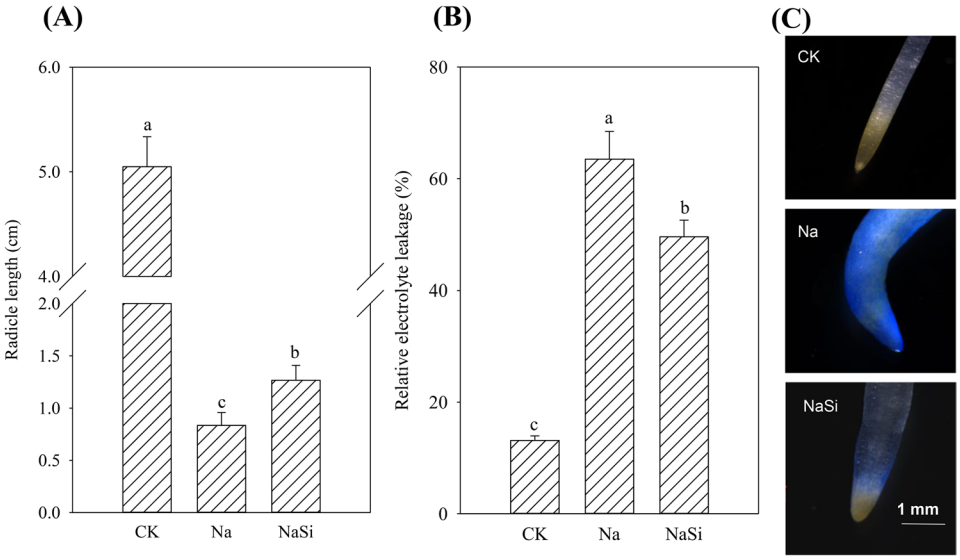

Fig. 5 Effect of silicon on the length, relative electrolyte leakage and plasma membrane integrity of radicles under salt stress. **a** Radicle length; **b** radicle electrolyte leakage; **c** plasma membrane integrity shown by histochemical staining. Data are the mean + SD for radicle length and electrolyte leakage ($n = 3$). Bars with different letters are significantly different between the treatments at $P < 0.05$

Lipid peroxidation and reactive oxygen species levels in radicles

Under salt stress, the levels of H_2O_2 and O_2^- were increased, while in the presence of silicon addition, the levels were obviously lower than those of salt stress alone (Fig. 6a, b). Schiff's reagent staining was used to check lipid peroxidation of radicles. Salt stress induced obvious lipid peroxidation in the radicle; while in the presence of added silicon, the lipid peroxidation was obviously decreased (Fig. 6c). Malondialdehyde level can be used to assess lipid peroxidation in tissues (Li et al. 2015). In this study, salt stress caused an increase of malondialdehyde concentration in radicles (Fig. 6d). In the presence of supplemented silicon, the malondialdehyde level was not increased under salt stress (Fig. 6d), indicating silicon-mediated alleviation of lipid peroxidation in the present stress conditions.

Antioxidant enzymes' activities in radicles

Under salt stress, the activities of antioxidant enzymes including SOD, CAT, POD and APX were all significantly

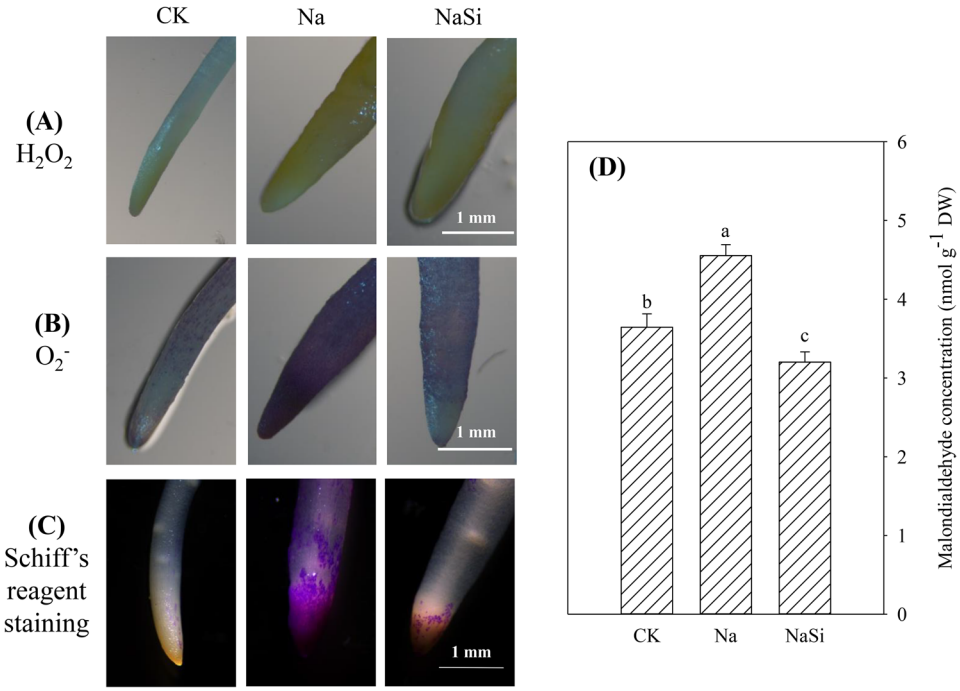

Fig. 6 Effect of silicon on reactive oxygen species levels and lipid peroxidation in radicles under salt stress. **a** Hydrogen peroxide level; **b** superoxide anion level; **c** Schiff's reagent staining showing lipid peroxidation of radicles; **d** malondialdehyde content. Representative photos are shown for the histochemical staining. Data are mean + SD for malondialdehyde content ($n = 3$). Different letters above bars indicate significant differences between the treatments at $P < 0.05$

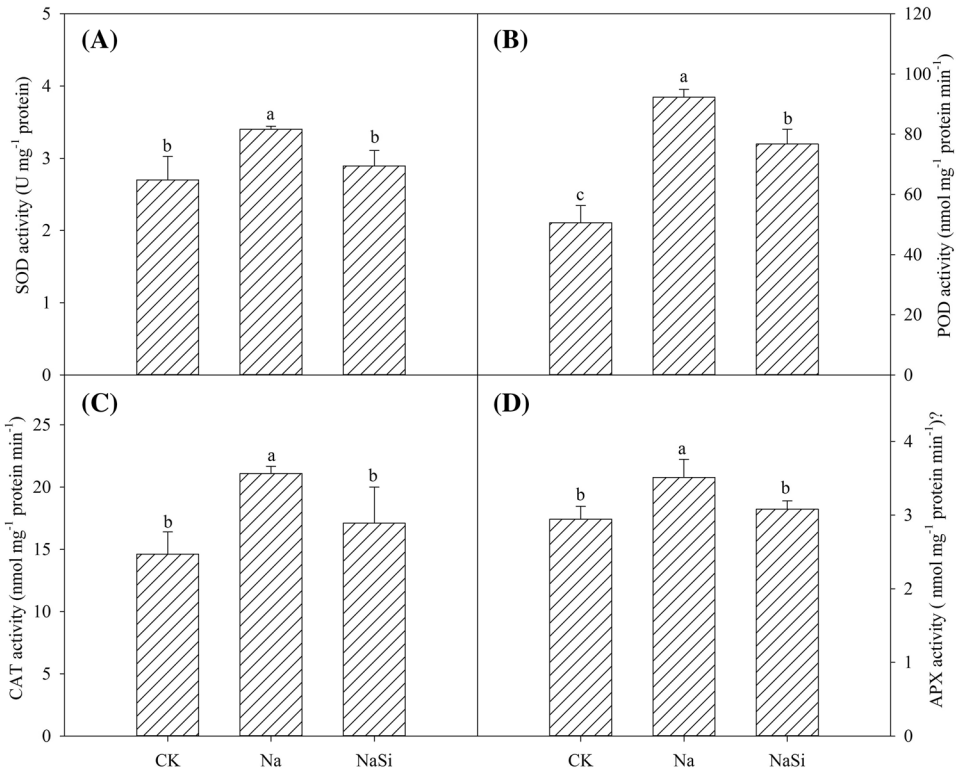

Fig. 7 Effect of silicon on activities of antioxidant enzymes in radicles under salt stress. **a** SOD activity; **b** POD activity; **c** CAT activity; **d** APX activity. Data are the mean + SD ($n = 3$). Different letters above bars indicate significant differences between the treatments at $P < 0.05$. *SOD* superoxide dismutase, *POD* peroxidase, *CAT* catalase, *APX* ascorbate peroxidase

increased in radicles (Fig. 7a–d). In the presence of silicon addition, compared with the control, the activities of SOD, CAT and APX were unchanged under salt stress (Fig. 7a, c, d). Added silicon inhibited the increase of POD activity under salt stress (Fig. 7b).

Concentrations of protein and proline in radicles

The levels of protein and proline in radicles were markedly increased under salt stress, and the increases were less in the presence of silicon addition (Fig. 8a, b).

Discussion

The beneficial role of silicon on plant salt tolerance has been intensively investigated. Seed germination and seedling emergence are crucial stages in the cycle of plant life (Biju et al. 2017), and these stages are extremely sensitive to adverse environmental factors, such as soil salinity. However, investigations on the effect of silicon under salt stress during the early period of seedling establishment are very limited (Siddiqui et al. 2014; Azeem et al. 2015; Alsaeedi et al. 2017, 2018; Zhang et al. 2017b). Moreover, the mechanism for silicon-mediated enhancement of seed germination is unclear. Cucumber is very sensitive to soil

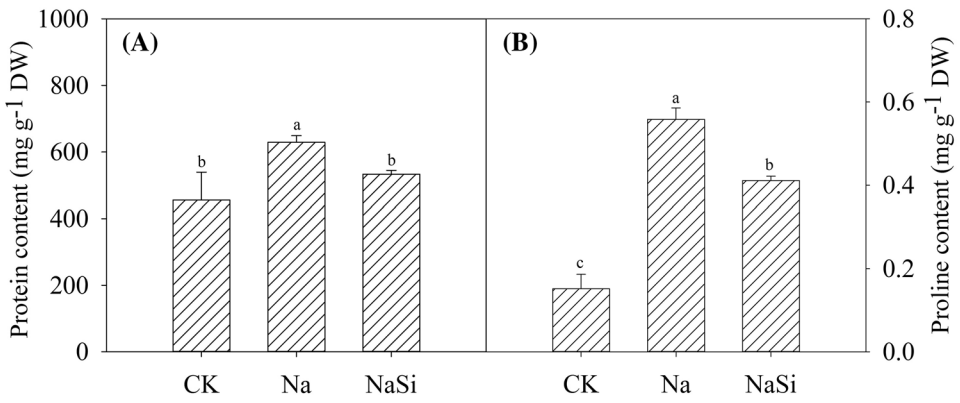

Fig. 8 Effect of silicon on protein and proline concentrations in radicles under salt stress. Data are the mean + SD ($n = 3$). Different letters above bars indicate significant differences between the treatments at $P < 0.05$

salinity (Huang et al. 2009). The increase of soil salinization and irrigation with salty and polluted water not only cause poor cucumber seedling establishment, but also affect subsequent growth, development and yield. In this study, the results demonstrated that inclusion of silicon could improve the seed germination characteristics of cucumber under salt stress, as the germination percentage (Fig. 1b), germination index (Fig. 2a) and seedling vigor index (Fig. 2b) were all increased. Besides, the radicle length was also increased in silicon-added treatment under salt stress (Fig. 5a). The positive effect of silicon on seed germination and growth of bud seedling suggests a potential application of silicon in cucumber production in salinized soils and areas watered with salty and polluted water.

In the present work, added silicon did not affect the seed germination of cucumber in normal conditions (Fig. 1d). Similar phenomenon was also reported in tomato (Shi et al. 2014) and lentil (Biju et al. 2017). However, these results are inconsistent with those observed in borage (Torabi et al. 2012), white oat (Toledo et al. 2011) or common bean (Alsaeedi et al. 2017), where beneficial roles of silicon on the seed germination were demonstrated. These studies suggest that, in normal conditions, silicon's effect on seed germination may be genotype-dependent.

ABA and GA are the two major plant hormones that control seed dormancy and germination (Zhang et al. 2014). ABA has been demonstrated to promote seed dormancy and delay seed germination, whereas GA can enhance seed germination (Tuan et al. 2018). ABA level in seeds is controlled by the balance of its biosynthesis and catabolism. In ABA biosynthesis, the rate-liming step is oxidative cleavage of 9-*cis*-neoxanthin and violaxanthin, which is catalyzed by 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Tuan et al. 2018). ABA 8′-hydroxylase, which is encoded by *CYP707A*, is mainly responsible for catalyzing ABA catabolism (Zhang et al. 2014). In plants, there are a lot of different GAs, but only a few of them are biologically active (Zhang et al. 2014). The concentration of active GAs in seeds is regulated by its biosynthesis and inactivation. The main enzymes involved in GA biosynthesis are GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox), and the enzyme involved in GA inactivation is GA 2-oxidase (GA2ox) (Tuan et al. 2018). Up to now, information is still lacking about silicon's effect on the level or metabolism of plant hormones in germinating seeds, regardless in normal or stress conditions. Given the important roles of ABA and GA in regulating seed germination, we investigated the expressions of genes involved in their biosynthesis and catabolism/inactivation (Fig. 3). At 12 h, the expression of *CsNCED1* was increased under salt stress, and it was not affected by added silicon (Fig. 3a, b). The expression of *CYP707A1* at this time point was much higher in 'NaSi' than 'Na' treatment (Fig. 3c). On the other hand, the expressions of *GA20ox*, *GA3ox* and *GA2ox* at 12 h did not show obvious difference between 'Na' and 'NaSi' treatment (Fig. 3d–f). These results suggest that, at 12 h, silicon may have enhanced ABA catabolism under salt stress, but it did not affect GA metabolism. At 36 h, the expressions of *CsNCED1* and *CsNCED2* were dramatically increased under salt stress, but they were unchanged in the presence of supplemented silicon (Fig. 3a, b). Although the expression of *CYP707A1* was also increased under salt stress alone at 36 h, the fold of change was less than that of *CsNCED1* or *CsNCED2*, suggesting that salt stress promoted ABA synthesis, which was inhibited by added silicon. Silicon addition might also inhibit GA catabolism, as shown by the much lower expression of *GA2ox* in 'NaSi' than 'Na', although the expression of *GA3ox* was also lower in 'NaSi' than 'Na' (Fig. 3e, f). Overall, our results suggest that silicon may decrease the level of ABA while maintain higher level of GA, therefore improving the germination of cucumber seeds under salt stress.

Starch degradation is necessary for seed germination and it provides energy for germinating seeds. The degradation of starch is catalyzed by hydrolytic enzymes, such as α-amylase (Biju et al. 2017). In this work, the α-amylase activity was markedly decreased under salt stress alone; while in the presence of supplemented silicon, it was not changed (12 h) or decreased less (36 h) (Fig. 4). These results are in accordance with those observed by Biju et al. (2017) in drought-sensitive or moderately drought-tolerant lentil genotypes under drought. However, Biju et al. (2017) reported that silicon addition decreased the α-amylase activity in drought-tolerant lentil genotypes under drought stress, suggesting genotype-dependent effect of silicon. In this work, silicon-mediated increase in α-amylase activity may have facilitated seed germination under salt stress (Fig. 1b).

In this work, we observed that silicon addition not only enhanced seed germination of cucumber (Fig. 1b), but also improved the growth of bud seedlings under salt stress, as shown by the increased radicle length (Fig. 5a). The improvement in bud seedling growth under salt stress corresponded with the decrease of its relative electrolyte leakage and maintenance of higher membrane integrity (Fig. 5b, c). Environmental stresses cause accumulation of reactive oxygen species (H_2O_2, O_2^-, etc.), which induces oxidative damage to functional macromolecules such as membrane lipid, protein and nucleic acid (Biju et al. 2017). To scavenge reactive oxygen species, plants have evolved an antioxidant defense system (Shi et al. 2016). There have been a good amount of papers reporting silicon's effect on oxidative damage and antioxidant defense in plants under salt stress (Zhu and Gong 2014). For example, Siddiqui et al. (2014) reported that the activities of SOD, CAT, POD, APX and glutathione reductase (GR) in squash leaves were all increased under salt stress, and they were further increased by added silicon. Zhang et al.

(2017b) observed that the activities of SOD, CAT, POD, APX and GR were unchanged in licorice seedlings under salt stress, and they were not changed by added silicon either, except the activity of APX, which was increased. Zhang et al. (2018) reported that the SOD, CAT and POD activities in licorice leaves were unchanged or decreased under salt stress, depending on the stress intensity; and the activities in silicon-applied plants were maintained higher than those without added silicon. Farhangi-Abriz and Torabian (2018) found that the activities of SOD, CAT, POD and APX in both roots and leaves were all increased under salt stress, and suitable concentrations of silicon further increased the activities. These studies all showed that silicon addition could decrease the concentration of malondialdehyde—a lipid peroxidation product (Shi et al. 2016). These studies suggest the complexity of changes in antioxidant enzymes activities. Despite this, the alleviative effect of silicon on plant oxidative damage has been frequently observed under salt stress. However, the majority of the studies were performed at seedling stage and later stages, while little information is available at earlier stage. Here, we investigated silicon's effect on oxidative damage and antioxidant defense at bud seedling stage of cucumber. The salt-stress-induced increase in H_2O_2 and O_2^- levels in radicles could partly be inhibited by added silicon (Fig. 6a, b). Accordingly, lipid peroxidation was inhibited in silicon treatment under salt stress, as shown by the lighter Schiff's reagent staining and the decrease of malondialdehyde concentration (Fig. 6c, d). The SOD, CAT, POD and APX activities in radicles were all significantly increased under salt stress alone, while they were not changed or less increased in the presence of added silicon (Fig. 7a–d). The increase of these antioxidant enzymes activities under salt stress might contribute to reactive oxygen species scavenging; while inhibition of the enzyme activities by added silicon suggests less demand for the scavenging of reactive oxygen species, the level of which was lower in silicon-added treatment (Fig. 6a, b). In a word, our results indicate a positive effect of silicon in alleviating oxidative damage of salt stressed bud seedlings, which may have contributed to the improvement of growth.

Accumulation of compatible solutes is an adaptation strategy of plants under stress conditions, and it can decrease tissue osmotic potential and therefore facilitates water uptake. Proline is an important compatible organic solute (Zhang et al. 2017b). Proteins can also play a role in osmotic adjustment under salt stress (Ashraf and Harris 2004). In this study, the proline and protein concentrations in radicles were increased under salt stress, whereas silicon addition partly or fully inhibited the increase (Fig. 8). In comparison to the control, the increase of proline and protein levels in radicles may have contributed to the adaptation of bud seedlings to the salt stress. The lower proline and protein levels in silicon-added bud seedlings under salt stress may suggest an alleviation of stress injury.

Conclusion

Silicon addition could improve the germination of cucumber seeds under salt stress. Silicon-mediated improvement in seed germination may be attributed to the decrease of ABA level, maintenance of high gibberellin level, and increase of α-amylase activity in the seeds under salt stress. Silicon addition improved the growth and membrane integrity of bud seedlings, while decreased reactive oxygen species accumulation and lipid peroxidation, implying that silicon alleviated the oxidative damage of salt-stressed bud seedlings.

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