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EFFECT OF NANO-SILICON APPLICATION ON SALT TOLERANCE OF PEPPER (*CAPSICUM ANNUUM* L.)

Sebnem Kusvuran[1]✉, Bekir Cengil[1], Ferhat Mutlu[2]

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Abstract

The effectiveness of nano-silicon (nSi) in increasing salt tolerance in pepper was investigated. Commercial varieties of Mızrak were used as material. To stress the plants, 4 salt doses, namely 0 (control), 50, 100, and 150 mM NaCl, were used, and 0, 0.5, 1, 2, and 3 mM doses of nSi were sprayed on the plants every seven days. Malondialdehyde (MDA), chlorophyll *a* and *b*, total carotenoid, and enzymatic activity were examined. NaCl treatment led to decreases in photosynthetic pigment contents (chlorophyll *a* and *b*, total carotenoid). NSi applications significantly increased superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase activity. However, MDA content was significantly decreased. The results support foliar application of nSi to increase the defense system of pepper, which enables it to tolerate the negative effects induced by salinity.

Key words: catalase, MDA, oxidative stress, salinity, superoxide dismutase

Introduction. Salinity, among the most important limiting factors affecting plant growth and development, and thus yield, occurs in different forms [1]. Salt stress affects plants negatively by inhibiting germination, growth and development, flowering, and fruit set. High sodium concentrations in saline soil limit water uptake and nutrient absorption. Water deficiency and nutritional imbalance

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highlight primary stresses, including osmotic stress and ionic stress [2]. Salt stress results in an excessive accumulation of reactive oxygen species (ROS), which may result in lipid peroxidation, protein oxidation, enzyme inactivation, DNA damage, and/or interactions with other essential plant cell components. High concentrations of salt may induce stomatal closure, reducing the availability of carbon dioxide in the leaves and inhibiting carbon fixation. This results in chloroplasts being exposed to high levels of excitation energy, which increases the production of ROS including hydrogen peroxide (H_2O_2), superoxide anion, hydroxyl radical, and singlet oxygen [3].

Nanoparticles have a wide range of uses in agriculture, including as plant growth and development promoters, herbicides, nano-pesticides, and nanofertilizers. The small use of nanofertilizers contributes to fertilizer savings and helps reduce the increased soil toxicity caused by the accumulation of chemical fertilizers and pesticides. In addition, they are effective in providing tolerance to stress conditions by stimulating different mechanisms at the physiological and biochemical level under abiotic or biotic stress conditions [4]. Si (silicon) is generally considered a “valuable element” or “structural element” useful for plant growth, physiological/metabolic pathways, cell structure, and the alleviation of a wide variety of abiotic or biotic environmental stresses. Many studies have confirmed its role in different plant species in increasing growth, development, and yield under abiotic stress conditions such as salinity, heavy metal stress, and drought [5, 6]. Silicon nanoparticles positively affect leaf relative water content and water use efficiency by increasing turgor pressure. Si particles are also effective in increasing tolerance to abiotic stress conditions because they increase antioxidant enzyme activity and are effective in ion regulation, for example [5].

As a result of salinity, which is one of the most important results of global climate change, it is vital to ensure agricultural sustainability by considering different applications in response to the negative effects in yield and yield parameters in many agricultural products. In this direction, the current study aimed to determine the effects of nano-Si (nSi) on pepper growth and development against salt stress.

Material and methods. The Mızrak pepper variety was used as the material. The seeds were sown in vials containing a mixture of peat:perlite at a ratio of 2:1. At 55 days after sowing, the seedlings were transplanted into 12 L plastic pots (peat:perlite), with 3 plants in each pot. Applications of salt stress were initiated with four doses of NaCl (0, 50, 100, or 150 mM NaCl) eighteen days after the plants (when the plants reach the 4–5 leaf stage) were transplanted. Different doses of nSi applications (0, 0.5, 1, 2, or 3 mM) were initiated together with the stress application and were sprayed on the leaves every seven days.

Sixteen days after salt application when stress effects were clearly visible, plants were evaluated using photosynthetic pigments [7], and biochemical parameters such as lipid peroxide content (malondialdehyde, MDA) [8]; ascorbate peroxidase (APX), catalase (CAT), glutathione reductase, and superoxide dismutase (SOD) antioxidative enzyme activities. SOD was assayed according to KARANLIK [9], by monitoring the superoxide radical-induced nitroblue tetrazolium (NBT) reduction at 560 nm. CAT activity was measured based on the decomposition rate of H_2O_2 at 240 nm. The APX activity was determined by measuring the consumption of ascorbate by following the absorbance at 290 nm. Glutathione reductase (GR) activity was determined by measuring the enzyme-dependent oxidation of the NADPH by following the absorbance at 340 nm [10].

The study was carried out according to the randomized plot design with 3 replications. The JMP (version 8.0) program was used in the statistical analysis of the data. Differences between means were grouped according to the Tukey test ($p \leq 0.05$).

Results and discussion. ***Photosynthetic pigments.*** Treatment of pepper plants with 50, 100, and 150 mM NaCl reduced the content of the photosynthetic pigments Chl *a*, Chl *b*, and total carotenoids by 7–32%, 12–47%, and 18–33%, respectively (Table 1). Application of nSi slightly increased the content of photosynthetic pigments in control plants without salt application, especially at 1 mM nSi. nSi application along with salt application significantly limited the decrease in photosynthetic pigments due to NaCl. Accordingly, a decrease of 3–29% in Chl *a*, 5–56% in Chl *b* and 8–61% in total carotenoid contents was prevented. When 150 mM NaCl application was examined, the highest Chl *a*, Chl *b* and total carotenoid values were determined as 0.711 and 1.921 mg g^{-1} FW, 0.819 and 0.896 mg g^{-1} FW, 0.914 and 1.110 mg g^{-1} FW in 0.5 and 1 mM nSi applications, respectively. When the nSi applications were compared, 0.5 and 1 mM in particular greatly increased the levels of photosynthetic pigments in leaves. The improvement with these applications was 9–61% on average. Salt stress causes the disintegration of chloroplast, instability of pigment protein complexes, the destruction of chlorophyll, and changes in the amount and composition of carotenoids. The inhibition of chlorophyll biosynthesis, acceleration of its degradation, and oxidative damage caused by salinity are considered the causes of decreased chlorophyll content. Studies have shown that salt stress causes significant reductions in plant growth in cowpeas, beans, broad beans, and soybeans, but Si applications greatly improve the growth and development of plants by increasing total photosynthetic pigment content, photosynthetic velocity, chlorophyll content, stomatal conductivity, transpiration, and intercellular carbon dioxide concentration [11]. HAGHIGHI and PESSARAKLI [12] and AVESTAN et al. [11] emphasized that nSi applications significantly limited losses in chlorophyll content under salt stress conditions.

Malondialdehyde content. MDA content was increased under stress conditions (41–248%); the highest MDA content (248% increase) was 14.74 µmol g^{-1}FW, observed with 150 mM NaCl application (Table 1). In nSi applications, the change was at the level of 20–169%, and the increase in MDA content was limited by an average of 17%. Among nSi applications, an improvement of 12–33%

Table 1

Effects of nano-Si (nSi) application on morphological parameters of pepper under saline condition (S: Salt; MDA: µmol g^{-1} F.W., Chl *a*, *b*, total carotenoids: mg g^{-1} F.W.; SOD: U $min^{-1}mg^{-1}$ F.W.; CAT, GR, APX: µmol min^{-1} mg^{-1} F.W.)

S (mM)	nSi (mM)	MDA	Chl *a*	Chl *b*	Total carotenoids
0	0	4.78 ± 0.88^{jk}	$2.266\pm0.11^{a-d}$	$1.187\pm0.19^{a-c}$	$1.039\pm0.07^{b-d}$
	0.5	3.91 ± 0.91^{kl}	2.375 ± 0.05^{ab}	1.307 ± 0.14^{a}	1.168 ± 0.10^{a}
	1	3.29 ± 0.05^{l}	2.504 ± 0.19^{a}	1.345 ± 0.20^{a}	1.179 ± 0.08^{a}
	2	3.73 ± 0.31^{kl}	$2.285\pm0.07^{a-d}$	1.251 ± 0.10^{ab}	1.068 ± 0.04^{ab}
	3	3.54 ± 0.49^{kl}	$2.345\pm0.05^{a-c}$	$1.234\pm0.03^{a-c}$	1.127 ± 0.02^{ab}
50	0	6.72 ± 0.30^{hi}	$2.096\pm0.12^{c-e}$	$1.043\pm0.16^{b-e}$	$0.844\pm0.06^{f-h}$
	0.5	$5.90\pm0.38^{h-j}$	$2.296\pm0.25^{a-d}$	$1.205\pm0.19^{a-c}$	1.043 ± 0.13^{bc}
	1	5.75 ± 0.46^{ij}	2.422 ± 0.04^{ab}	1.255 ± 0.07^{ab}	1.102 ± 0.03^{ab}
	2	6.18 ± 0.41^{hi}	$2.253\pm0.08^{a-d}$	$1.141\pm0.03^{a-c}$	$0.919\pm0.02^{e-g}$
	3	6.37 ± 0.62^{hi}	$2.354\pm0.29^{a-c}$	$1.159\pm0.07^{a-c}$	$0.928\pm0.02^{c-f}$
100	0	10.59 ± 0.24^{de}	$1.672\pm0.09^{g-i}$	$0.753\pm0.08^{f-h}$	0.759 ± 0.08^{hi}
	0.5	8.44 ± 0.33^{g}	$2.072\pm0.09^{d-f}$	$1.059\pm0.06^{b-d}$	$0.904\pm0.04^{e-g}$
	1	7.05 ± 1.17^{h}	$2.171\pm0.19^{b-e}$	$1.181\pm0.32^{a-c}$	$1.018\pm0.14^{b-e}$
	2	9.01 ± 0.58^{fg}	$1.814\pm0.03^{f-h}$	$0.900\pm0.06^{d-f}$	$0.859\pm0.03^{f-h}$
	3	8.74 ± 0.56^{g}	$1.732\pm0.11^{g-i}$	$1.003\pm0.04^{c-e}$	0.890 ± 0.04^{fg}
150	0	14.74 ± 0.97^{a}	1.534 ± 0.06^{ii}	0.628 ± 0.07^{h}	0.689 ± 0.05^{i}
	0.5	11.69 ± 1.95^{cd}	$1.711\pm0.32^{g-i}$	$0.819\pm0.009^{e-f}$	$0.914\pm0.03^{d-f}$
	1	10.13 ± 0.34^{ef}	$1.921\pm0.06^{e-g}$	$0.896\pm0.10^{d-g}$	1.110 ± 0.01^{ab}
	2	12.96 ± 0.88^{b}	1.621 ± 0.36^{hi}	0.658 ± 0.12^{gh}	0.746 ± 0.06^{hi}
	3	12.87 ± 0.96^{bc}	$1.659\pm0.06^{g-i}$	$0.689\pm0.15^{f-h}$	$0.792\pm0.08^{g-i}$

*Each value represents the mean of four replicates. For each parameter of each different letters are significantly different at $p \leq 0.05$ according to Tukey test.

was achieved with 0.5 and 1 mM nano-Si applications. The most important mechanism in the formation of tissue damage due to free radicals resulting from stress is the peroxidation of lipids in the cell membrane. Lipid peroxidation causes the destruction of membrane integrity and increased permeability of the cell to electrolytes [3]. ISMAIL et al. [6] reported that a decrease in MDA content occurred with nSi applications in their study on peas.

Antioxidative enzyme activities. Antioxidative enzyme activity (SOD, CAT, APX, and GR) levels were evaluated in control, S, and S + nSi treatments (Table 2). Antioxidative enzyme activity increased due to the increase in salt level. When salt levels without nSi application were examined, the lowest SOD, CAT, APX, and GR enzyme activities were determined at 50 mM salt concentration and an increase of 263%, 98%, 59%, and 51%, respectively, occurred compared to control plants. However, SOD, CAT, APX and GR activities were found to be highest at 150 mM NaCl level, and an increase of 478%, 184%, 212% and 154%, respectively, was determined at this dose. As is evident in Table 2, nSi treatments had a serious effect on antioxidative enzyme activities of pepper under salt stress. In nSi applications, enzyme activities increased by 3–64% (SOD), 15–97% (CAT), 33–94% (APX), and 6–57% (GR) compared with salt-stressed plants not treated with nSi. In this study conducted on pepper, the highest SOD and CAT activities were 342.95 U $\min^{-1}$$mg^{-1}$ F.W. and 1119.4 µmol $\min^{-1}$ mg^{-1} F.W., respectively, in 1 mM nSi application; APX and GR activities were determined at 20.33 µmol $\min^{-1}$ mg^{-1} F.W. and 9.08 µmol $\min^{-1}$ mg^{-1} F.W. in 0.5 mM nSi application. In the 0.5 and 1 mM nSi applications, the increase in antioxidative enzyme activity was higher than in other applications, at 51–478%. Normal cellular metabolism or stressful environmental conditions such as drought, heavy metals, herbicides, nutrient deficiency, radiation, or salinity generate ROS in plant cells [13]. Enzymatic antioxidant defense systems include APX, CAT, DHAR, GR, MDHAR, POX, and SOD; non-enzymatic antioxidant defense systems include ascorbate, carotenoids, glutathione, glycine betaine, phenolic compounds, polyamines, proline, and sucrose. Antioxidative enzyme activities, such as those of SOD, CAT, GR, and APX, increased in response to the application of nSi in a salt-stressed medium, as has been reported elsewhere. Si alleviates salt stress with maintenance of cell form by increasing antioxidative enzyme activity and enhancing plant hydration status, thereby increasing plasma membrane permeability [14]. The present findings support those of SIDDIQUI et al. [15], who demonstrated that nano-SiO_2 stimulated the activity of antioxidant enzymes. The use of nano-silicon enhances antioxidant activity and therefore protects against salt stress [16].

Salt stress is one of the main abiotic stress factors affecting plant production and productivity. In this study, pepper seedlings treated with NaCl and nSi showed significant changes in growth and physiological characteristics. When the seedlings were exposed to salt stress, photosynthetic pigment content decreased, while antioxidative enzyme (SOD, CAT, APX, and GR) activity and MDA content increased. However, nSi significantly reduced the adverse effects of salinity by attenuating cellular oxidative damage. In this study, the responses of pepper plants to different doses of salt stress and the effectiveness of different doses of nSi applications against the negative effects of salt were examined. Thus, the aim was to determine the effective concentrations at different stress levels. As a matter of fact, studies conducted on different species such as pea [6], strawberry [11], tomato [12] and lemongrass [17] have stated that nSi applications increase tolerance under abiotic stress conditions such as salt stress. The obtained results support the effectiveness of nSi induced protection in pepper against salt stress (NaCl 50, 100 and 150 mM). Similar results were reported by TANTAWY et al. [18] It has also been stated by and reported that Si application can reduce salt stress damages in sweet pepper plants and nano-silicon application is more effective and

Table 2

Effects of nano-Si (nSi) application on morphological parameters of pepper under saline condition (S: Salt; MDA: µmol g^{-1} F.W., Chl *a*, *b*, total carotenoids: mg g^{-1} F.W.: SOD: U min^{-1}mg^{-1} F.W.; CAT, GR, APX: µmol min^{-1} mg^{-1} F.W.)

S (mM)	nSi (mM)	SOD	CAT	APX	GR
0	0	38.09±2.67^{l}	237.32±6.63^{l}	3.83±0.35^{k}	2.43±0.62^{i}
	0.5	41.98±2.09^{l}	261.51±9.05^{l}	4.13±0.43^{k}	2.53±0.23^{i}
	1	43.89±3.24^{l}	273.43±10.16^{l}	4.07±0.17^{k}	2.39±0.34^{i}
	2	41.65±1.38^{l}	259.50±18.62^{l}	4.02±0.43^{k}	2.22±0.16^{i}
	3	39.40±0.73^{l}	245.44±14.58^{l}	4.08±0.14^{k}	2.37±0.15^{i}
50	0	138.28±9.69^{k}	471.43±13.04^{k}	6.11±0.14^{j}	3.67±0.45^{h}
	0.5	167.67±7.04^{hi}	571.46±17.64^{ij}	11.89±0.92^{f}	4.52±0.41^{gh}
	1	159.32±6.92^{ij}	573.28±16.17^{ij}	10.31±1.15^{gh}	4.28±0.84^{gh}
	2	146.10±4.10^{jk}	521.00±18.49^{jk}	10.57±0.61^{g}	3.98±0.28^{h}
	3	143.01±2.67^{jk}	501.89±18.66^{k}	8.15±0.66^{i}	3.91±0.32^{h}
100	0	182.47±2.78^{gh}	582.01±15.78^{hi}	9.35±0.21^{h}	5.20±0.32^{fg}
	0.5	289.96±7.19^{c}	915.39±15.85^{d}	17.66±0.49^{d}	8.19±0.57^{ab}
	1	300.46±9.45^{c}	990.56±21.33^{c}	17.87±0.96^{cd}	7.73±0.71^{bc}
	2	193.71±3.91^{fg}	712.45±17.36^{f}	16.18±0.93^{e}	6.49±0.73^{de}
	3	178.902±8.92^{gh}	668.48±16.30^{fg}	12.46±1.01^{f}	5.67±0.33^{ef}
150	0	220.22±5.43^{e}	676.07±17.37^{fg}	11.98±0.27^{f}	6.19±0.38^{d-f}
	0.5	320.78±2.85^{b}	10556.25±20.60^{b}	20.33±0.55^{a}	9.08±0.58a
	1	342.95±2.03^{a}	1119.48±29.80^{a}	19.21±0.97^{ab}	8.86±0.32^{a}
	2	240.80±8.00^{d}	841.63±18.84^{e}	18.93±0.65^{bc}	7.69±0.87^{bc}
	3	211.98±4.19^{ef}	635.27±15.90^{gh}	15.97±0.83^{e}	6.75±0.34^{cd}

[*]Each value represents the mean of four replicates. For each parameter of each different letters are significantly different at $p \leq 0.05$ according to Tukey test.

efficient than normal silicon application. Although similar results were obtained in our study, it was concluded that especially 2 and 3 mM nSi applications may have negative effects, therefore 0.5–1 mM nSi applications may be more effective. In light of all the data, 0.5 and 1 mM nSi doses were determined to be the most effective doses at all stress levels. Nano-Si application under salt conditions was revealed to be advantageous for the metabolic processes of pepper. Therefore, applying nSi to pepper plants in salt circumstances is a useful technique for increasing their tolerance.

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[1] *Cankiri Karatekin University, Food and Agriculture Vocational School,*
18100, Cankiri, Turkey
e-mails: `skusvuran@gmail.com`, `bekircengil@gmail.com`

[2] *Cankiri Karatekin University, Graduate School of Natural and Applied Sciences,*
Department of Agriculture and Life Sciences, Cankiri, Turkey
e-mail: `mutlu_ferhat@yahoo.com`