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Nano-silica modulates salt stress response in lettuce by enhancing growth, antioxidant activity, and mineral uptake

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Abstract: Salt stress is a significant abiotic factor that limits crop growth and yield. Nano-fertilisers, effective even in small quantities, have gained prominence for their ability to enhance plant growth and stress tolerance. This study investigated the effects of silica nanoparticles (SiNPs) at different concentrations (0, 100, 200, and 400 mg/L solution) under varying saline water application levels (0.6, 1.2, 2.4, and 3.6 dS/m) on growth parameters, antioxidant enzyme activity, and nutrient uptake in lettuce. The greenhouse experiment followed a randomised complete block design with three replications. Results demonstrated that SiNPs effectively increased head diameter and plant height by approximately 8% and 14%, respectively, compared to the control. Similarly, dry matter content improved by 22% with SiNP-400. While salinity stress significantly increased electrolyte leakage and lipid peroxidation (as indicated by malondialdehyde (MDA) content), SiNPs reduced MDA levels by 21%, indicating lower oxidative damage. Soil-plant analysis development (SPAD) values improved by 6%, and leaf relative water content increased by 4% with the application of SiNPs. Enzyme activity analysis revealed that salinity stress enhanced superoxide dismutase (SOD) and catalase (CAT) activities, but SiNP-400 reduced SOD and CAT levels by 23% and 50%, respectively, suggesting a decrease in oxidative stress. Furthermore, SiNPs enhanced nutrient uptake, significantly increasing the contents of Mg, Fe, and Zn while reducing Na accumulation. The highest Mg, Zn, and K concentrations were recorded under the SiNP-400 treatment. These findings highlight the potential of silica nanoparticles in mitigating the effects of salt stress and improving plant resilience, highlighting their role in sustainable agriculture.

Keywords: abitic stress; *Lactuca sativa* L.; plant nutrients; stress condition; vegetable

Lettuce (*Lactuca sativa* L.) is one of the most widely consumed leafy vegetables and holds significant economic value, particularly in the restaurant and food processing sectors. Lettuce ranks among the world's most popular vegetables, yet its nutritional significance remains underappreciated (Kim et al. 2016). Its popularity stems from its role as a staple ingredient in ready-to-eat meals, especially among younger generations worldwide. However, like other vegetable crops, lettuce production faces substantial challenges due to salinity stress, particularly in protected cultivation systems (Gruda et al. 2024). Salinity is a critical abiotic stressor that limits agricultural sustainability and crop productivity. Climate change models predict an exacerbation of soil salinity due to rising global temperatures, further threatening vegetable production (Zaman et al. 2018).

To address these challenges, researchers have increasingly focused on advanced fertilisation strategies that enhance plant resilience under stress conditions. In this context, nanotechnology has emerged as a promising tool in modern agriculture (Abdel-Aziz et al. 2016, Kim et al. 2018, Usman et al. 2020, Kilic et al. 2025). Nanofertilisers offer a sustainable alternative to conventional fertilisers by enhancing nutrient-use efficiency and reducing losses, thereby contributing to improved plant growth under abiotic stress conditions (Chhipa 2017, Raliya et al. 2017, Cakmakci et al. 2022b). Among these, silica nanoparticles (SiNPs) have garnered attention for their potential role in mitigating the adverse effects of environmental stressors, particularly in arid and semi-arid regions (Aqaei et al. 2020, Mathur and Roy 2020).

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Silicon-based nanomaterials have been demonstrated to enhance plant growth and development under various abiotic stress conditions, including salinity, drought, and heavy metal toxicity. Their role in mitigating salinity stress is attributed to enhanced physiological and biochemical responses. For example, studies on tomato have demonstrated that SiO_2/TiO_2 nanocomposites significantly increased biomass and stress tolerance (Rolón-Cárdenas and Rodríguez-González 2025). Additionally, silicon nanoparticles improve antioxidant defence systems, enhance nutrient uptake, and reduce oxidative damage. However, while SiNPs have shown potential benefits in several crops, their effects on lettuce under saline conditions remain underexplored.

Recent studies have shown that the exogenous application of nanoparticles and osmolytes can enhance plant tolerance to salinity. Abd-Elzaher et al. (2024) reported that the application of proline, silicon, and zinc nanoparticles in wheat significantly improved growth and yield under salt stress by increasing chlorophyll content, potassium uptake, and the K^+/Na^+ ratio while reducing sodium accumulation. Similarly, Liang et al. (2024) demonstrated that SiO_2-NPs enhanced stress tolerance in cotton seedlings subjected to combined salt and low-temperature stress by improving the K^+/Na^+ balance and increasing the activities of antioxidant enzymes. This highlights the potential of SiNPs in mitigating the adverse effects of salinity by maintaining ionic homeostasis and reinforcing antioxidant defence mechanisms.

Studies suggest that nanomaterials, including silicon dioxide nanoparticles (NPs), can mitigate salt-induced damage. El-Kinany et al. (2025) found that foliar spraying with SiO_2-NPs improved root development, plant growth, and flowering traits in carnations under saline irrigation while enhancing enzymatic defence mechanisms. These findings support the notion that SiNPs can be practical tools in enhancing plant resilience to salinity stress.

Nanoparticles can also influence photosynthesis by modifying chlorophyll fluorescence and leaf reflectance. Kalisz et al. (2023) reported that metal and metal oxide nanoparticles affected the photosynthetic apparatus of lettuce, with their impact varying depending on the type of nanoparticle and concentration. Notably, silicon-based nanoparticles exhibited positive effects on fluorescence parameters, suggesting their potential benefits in stress mitigation.

Beyond physiological and biochemical improvements, silicon and silica nanoparticles also contribute to soil quality and nutrient uptake. Their application has been linked to increased absorption of essential elements such as calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), and zinc (Zn) (Alsaeedi et al. 2019). While the precise mechanisms underlying their effects remain to be fully elucidated, studies suggest that nano-silicon (SiNPs) reduces reactive oxygen species (ROS) accumulation and lipid peroxidation by enhancing silica uptake (Mathur and Roy 2020). Additionally, silica application has been shown to enhance the efficiency of nitrogen fertilisers, leading to improved photosynthesis, increased chlorophyll content, and enhanced crop quality (Mattson and Leatherwood 2015, Kah et al. 2018). Given the superior performance of nanofertilisers compared to conventional fertilisers, the utilisation of SiNPs is expected to play a crucial role in sustainable agricultural production (El-Naggar et al. 2020).

Despite the increasing body of research on the role of silicon in stress mitigation, limited studies have specifically investigated the effects of SiNPs on lettuce growth and physiological responses under saline conditions. Previous studies have focused on conventional silicon fertilisers or SiNP applications in other crops, leaving a knowledge gap regarding their potential in lettuce cultivation. This study aims to address this gap by evaluating the effectiveness of SiNPs in enhancing lettuce's resilience to salinity stress through improved nutrient uptake, antioxidant activity, and physiological performance. The main objectives of this study are: (1) to assess the potential of SiNPs as a novel approach for sustainable lettuce production under saline conditions, and (2) to evaluate the physiological and yield responses of lettuce to SiNP applications under salinity stress. It is hypothesised that SiNP treatment will mitigate the effects of salt stress by enhancing ionic balance (increasing the K^+/Na^+ ratio), boosting antioxidant defence mechanisms, improving water relations, and promoting overall plant growth. This study offers new insights into the role of SiNPs in stress mitigation and proposes a novel strategy for enhancing lettuce production in saline environments.

MATERIAL AND METHODS

Material and experimental design. The experiment was conducted in the greenhouse of the Department of Horticulture at Van Yuzuncu Yil University. A standard lettuce cultivar (*Lactuca sativa* var. *crispa* L.) cv. Kislik Kivircik (Bursa Seed)

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was selected as the plant material. The lettuce seedlings were initially grown in plastic vials containing a mixture of peat and perlite (2:1, *v*/*v*). After 24 days, the seedlings were transplanted into 4-L pots filled with 4-mm sieved soil and kept under greenhouse conditions for 55 days. The soils were collected from an agricultural area located in Gevaş, near Van Province, Türkiye, from the A and B layers at 0–30 cm depth after removing the O layer. According to the FAO classification, the soils used in the experiment belong to the calcic cambisol class. The texture of the surface soil layer (0–30 cm) in the study area is sandy clayey loam, considering sand (47.8%), clay (37.3%) particle and silt (14.9%). Some chemical and hydraulic properties of the soil are pH 7.83, organic C 0.94%, $CaCO_3$ 9.2%, total N 0.081%, 15.7 mg P/kg, 244.0 mg K/kg, field capacity 31.1%, and permanent wilting point 17.6%.

Silica nanoparticles with a particle size of 44 nm and a purity of 99% were obtained from Nanocar Nano Technology, a commercial firm (Ankara, Türkiye). Throughout the growth period, the seedlings were irrigated with two nutrient solutions: solution A (10.3% N, 7.5% K, 8.6% Ca, 0.3% Fe) and solution B (containing, 2.1% N, 6.4% P, 11.6% K, 1.6% Mg, 0.01% Zn, 0.003% Cu, 0.1% Mn, 0.003% B, 0.004% Mo). These nutrient solutions were applied twice (100 mL, 0.5% mixed solutions) throughout the growth period.

Inside the greenhouse, temperature and relative humidity were monitored using an automatic weather station (HOBO, Camphell Scientific INC.Massachusetts, USA). The mean daily temperature was 22 ± 5 °C, and the relative humidity was 55 ± 10% (Figure 1). The lettuce seedlings were assigned to a randomised experimental design with three replicates, resulting in 48 pots.

Silica nanoparticle and salinity treatments. The experiment involved four salinity treatments using NaCl (Sw0.6 – 0.60 dS/m, control tap water, Sw1.2 – 1.2 dS/m, Sw2.4 – 2.4 dS/m, Sw3.6 – 3.6 dS/m) and four different concentrations of silica nanoparticles (SiNP-0 – 0 mg/L, control; SiNP-100 – 100 mg/L; SiNP-200 – 200 mg/L; SiNP-400 – 400 mg/L). Throughout the growing season, the SiNPs were applied three times *via* soil drench, with each pot receiving 150 mL (total 450 mL) of the respective SiNPs solution. The amount of irrigation water to be applied to each pot was calculated based on the volume of moisture content in the pots using a portable moisture meter (HH2 Moisture Meter, WET Sensor, Delta-T Devices, Cambridge, UK). In a preliminary study, the moisture meter was calibrated using the soil type employed in the experiment, and the specific volumetric moisture content of the medium at pot (field) capacity was determined. Irrigation was applied at the soil moisture level to reach the pot capacity. In Sw2.4 and Sw3.6 treatments where the salt content was high, the amount of salt was divided into the 1st and 2nd irrigations to prevent sudden salt stress on the plant.

Yield and morphological properties measurements. Plant length and head diameter were measured using a calliper, and the number of leaves per plant was counted. Additionally, the fresh and dry weights of the plants were determined. The lettuce leaves were separated from their stems and weighed to obtain fresh weight. Subsequently, the same leaf samples were dried in an oven at 65 °C for 48 h, then weighed again to get the dry weight (DW). The dry weight ratio to fresh weight was calculated to determine the plants' dry weight.

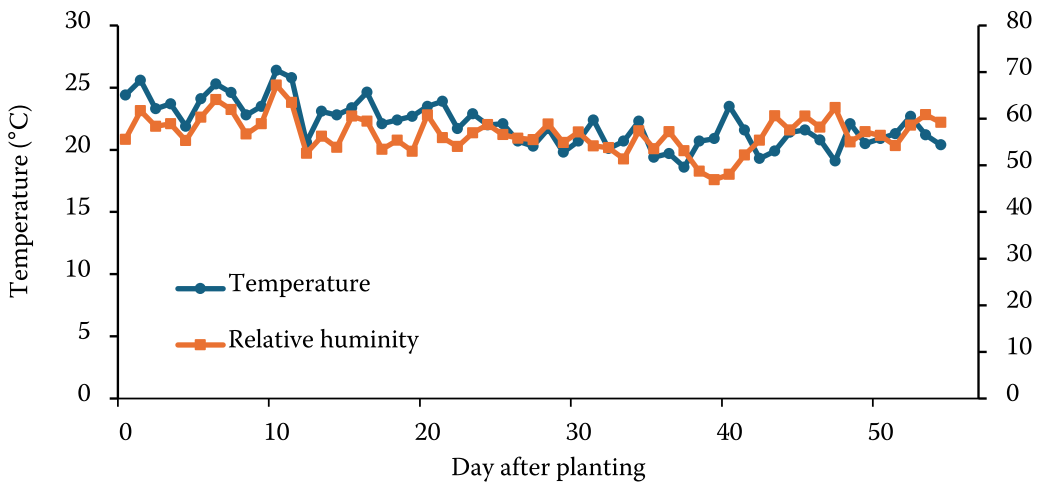

Figure 1. Greenhouse temperature and humidity values

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SPAD measurement. Soil-plant analysis development (SPAD) values were measured on four parts of the plant leaf using a chlorophyll meter (Minolta SPAD-502, Tokyo, Japan) for each replicate. The average of these measurements was recorded as the SPAD value for each experimental unit.

Antioxidant enzymatic activity. These frozen leaf samples, stored at –20 °C, were homogenised using a cold mixture containing 5 mL of 50 mmol potassium phosphate buffer and 0.1 mmol Na-EDTA (pH 7.6). The homogenate was centrifuged at 18 000 rpm for 30 min at 4 °C. The subsequent enzyme analyses were conducted at +4 °C. Catalase (CAT) activity was determined by measuring the rate of hydrogen peroxide dissociation at a wavelength of 240 nm, using the method described by Cakmak and Marschner (1992). Superoxide dismutase (SOD) activity was assessed by measuring the inhibition of nitro blue tetrazolium (NBT) at a wavelength of 560 nm, using a modified method derived from Jebara et al. (2010). The activity of SOD was determined based on the reduction of 50% of NBT as a unit. Ascorbate peroxidase (APX) activity was analysed using the method described by Nakano and Asada (1981). The absorbance value was measured at 290 nm immediately after adding the extract to determine APX activity. APX activity was defined as the amount of enzyme required to consume 1 µmol of ascorbate per minute. To assess the accumulation of malondialdehyde (MDA) as an indicator of lipid peroxidation, 0.5 g leaf sample was homogenised with 0.1% trichloroacetic acid (TCA). The resulting homogenate was then centrifuged at 15 000 rpm for 15 min. Next, 1 mL of the supernatant was mixed with 0.5% thiobarbituric acid, which had been dissolved in 2 mL of 20% trichloroacetic acid. The mixture was then incubated at 95 °C for 30 min and rapidly cooled in an ice bath. The mixture was centrifuged at 10 000 rpm for 10 min. The absorbance value of the resulting solution was measured at wavelengths of 532 nm and 600 nm. The content of MDA was calculated using the molar absorption coefficient of 155 mmol/g, as described by Heath and Packer (1968).

Electrolyte leakage. Electrolyte leakage (EL) was determined by measuring the electrolyte leakage from leaf cells, following the method described by Shi et al. (2006). Disks were taken from the leaves of each plant and immersed in 30 mL of deionised water at room temperature for 24 h. The water solution's electrical conductivity (EC) value was measured and recorded as EC1. The leaf disks were then subjected to a water bath at 95 °C for 20 min. After cooling the samples to room temperature, the EC value was measured again and recorded as EC2. The EL was calculated using the following equation:

$$EL = \left(\frac{EC1}{EC2}\right) \times 100$$

Leaf relative water content. To determine leaf relative water content (LRWC), leaf samples with a diameter of 10 mm were collected before harvest. The samples were carefully weighed using a precision scale, and the recorded weight was referred to as the leaf fresh weight (LFW). These leaf samples were placed in distilled water for 4 h. After the immersion, the leaf turgor weights (LTW) were determined by weighing the same samples. Subsequently, the samples were dried in an oven set at 65 °C for approximately 48 h to obtain the leaf dry weights (LDW). The LRWC was calculated using the following equation, as described by Ors et al. (2021):

$$LRWC(\%) = 100 \times \frac{LFW - LDW}{LTW - LDW}$$

Leaf mineral concentration. The mineral concentration analysis was performed using the dry combustion method outlined by Kacar and Inal (2010). Leaf samples, which had been dried at 65 °C for approximately 48 h, were ground using a porcelain mortar. From the ground samples, 0.5 g were selected and subjected to combustion in an oven at 550 °C. After combustion, a washing step was carried out using 10 mL of 0.5 mol H_2SO_4. The concentrations of potassium (K), calcium (Ca), sodium (Na), magnesium (Mg), iron (Fe), and zinc (Zn) were determined using atomic absorption spectrophotometry. Additionally, the concentrations of phosphorus (P), copper (Cu), and manganese (Mn) were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo Fisher, Scientific Inc., Waltham, USA).

Statistical analysis. The data were analysed using the general linear model approach in SPSS (version 23.0 software Van Yuzuncu Yıl University, Van, Türkiye), and the significance level ($P < 0.05$) between means was determined using Duncan's multiple range test (Duncan 1955).

RESULTS

Growth and physiological parameters. The study results showed that the growth and physiological parameters of lettuce were significantly influenced by the levels of NaCl applications and SiNPs treat-

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ments. Increasing levels of NaCl resulted in a decrease in head diameter, plant height, and dry matter ratio ($P < 0.01$). However, applying SiNPs positively influenced these parameters, promoting increased head diameter, plant height, and dry matter (DM) values. In terms of head diameter, the highest SiNP treatment (SiNP-400) resulted in an approximate 8% increase compared to the control treatment (SiNP-0) (Figure 2A). A similar trend was observed for plant height, where the highest SiNPs treatment (400 mg/L) led to a significant increase of approximately 14% compared to the control treatment (Figure 2B). Furthermore, the application of the highest SiNP-400 concentration significantly increased the dry matter content by approximately 22% compared to the control treatment (0 mg/L) (Figure 2C).

The results indicate that the increased doses of NaCl applications had a significant effect on SPAD (chlorophyll content) but did not have a considerable impact on the leaf relative water content of lettuce leaves (Figures 3A–B). However, the electrolyte leakage was substantially increased ($P < 0.01$) in response to NaCl application (Figure 3C), indicating an increase in membrane damage due to salinity stress. On the other hand, the application of SiNPs treatments had a positive influence on SPAD and LRWC values. The mean values showed that the highest SiNPs treatment (SiNP-400) resulted in an approximate 6% increase in SPAD value compared to the control treatment (SiNP-0). Similarly, in terms of LRWC, the highest mean value was observed in the SiNP-200 application, with a roughly 4% increase compared to the control treatment.

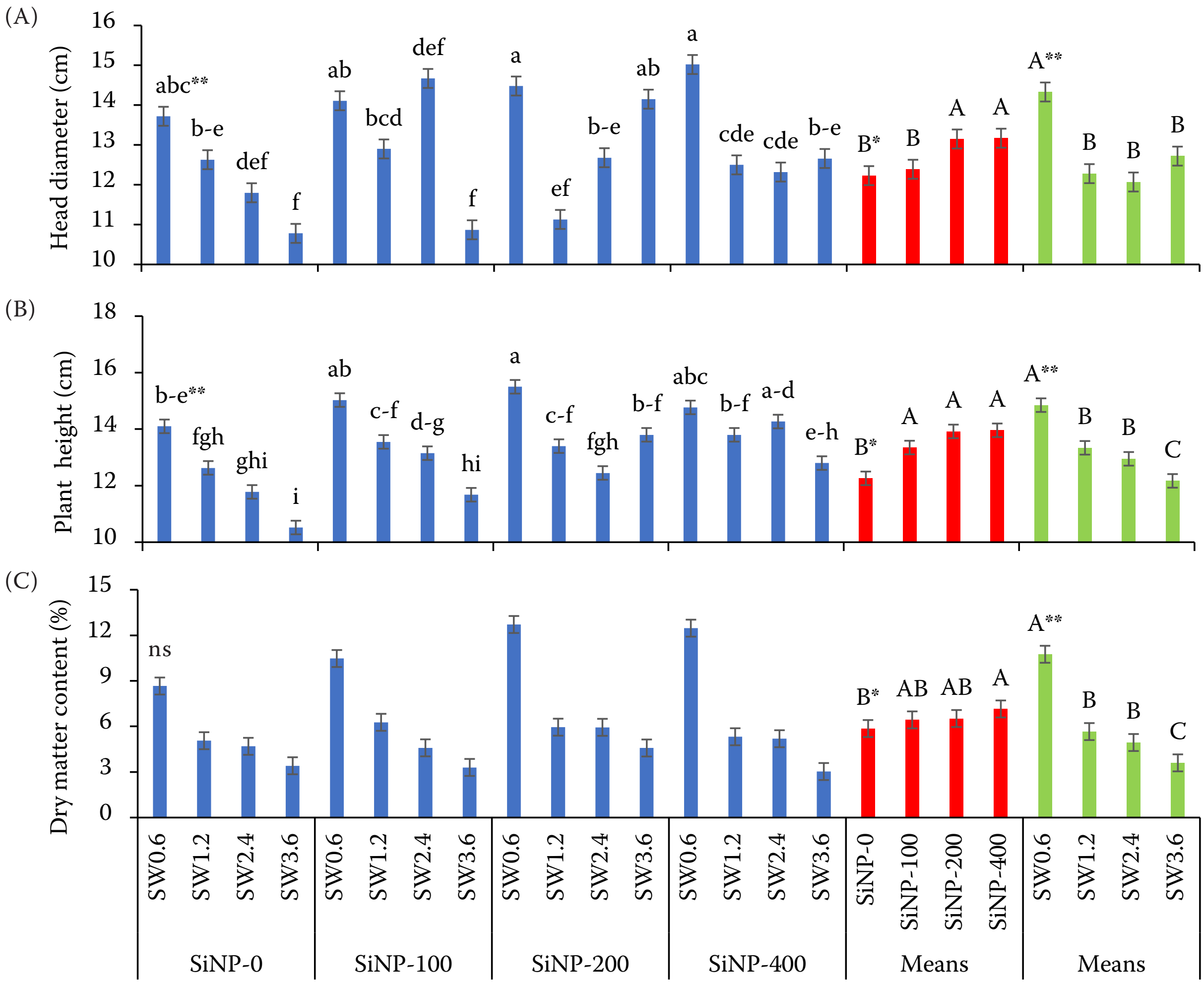

Figure 2. Effect of different doses of nano silica and saline irrigation water on (A) head diameter; (B) plant height, and (C) dry matter content. SiNP-0 – non-nano silica particles-control; SiNP-100 – 100 mg/L nano silica; SiNP-200 – 200 mg/L nano silica; SiNP-400 – 400 mg/L nano silica; Sw0.6 – saline water 0.6 dS/m (control); Sw1.2 – saline water 1.2 dS/m; Sw2.4 – saline water 2.4 dS/m; Sw3.6 – saline water 3.6 dS/m; $^{*}P < 0.05$; $^{**}P < 0.01$; ns – not significant

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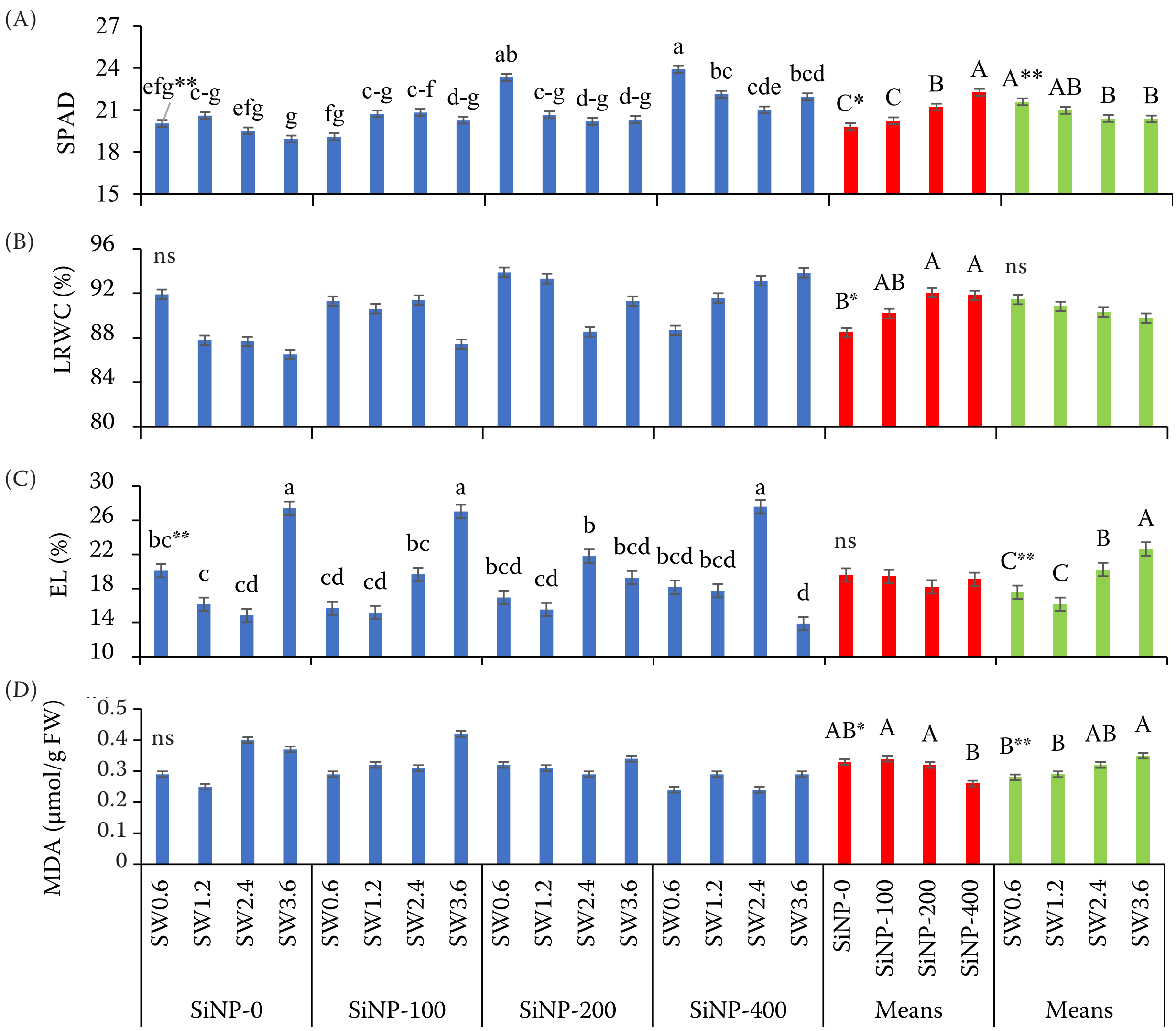

Figure 3. Effect of different doses of nano-silica and saline irrigation water on (A) soil-plant analysis development (SPAD) values; (B) leaf relative water content (LRWC); (C) electrolyte leakage (EL), and (D) malondialdehyde (MDA). SiNP-0 – non-nano silica particles-control; SiNP-100 – 100 mg/L nano silica; SiNP-200 – 200 mg/L nano silica; SiNP-400 – 400 mg/L nano silica; Sw0.6 – saline water 0.6 dS/m (control); Sw1.2 – saline water 1.2 dS/m; Sw2.4 – saline water 2.4 dS/m; Sw3.6 – saline water 3.6 dS/m; *P < 0.05; **P < 0.01; ns – not significant; FW – fresh weight

Antioxidant enzyme activity and lipid peroxidation. SOD activity increased with increasing NaCl levels, indicating an increased defence mechanism against oxidative stress. However, higher doses of SiNPs significantly reduced SOD activity (P < 0.01). The SiNP-400 treatment showed a reduction of approximately 23% in SOD activity compared to the SiNP-0 treatment (Figure 4A). Similarly, catalase activity increased progressively with increasing NaCl salinity levels, indicating an enhanced oxidative stress response in lettuce plants under salinity stress (P < 0.01). However, applying SiNPs treatments significantly decreased CAT activity, indicating a reduction in oxidative damage caused by NaCl salinity (P < 0.01). The highest SiNPs treatment (SiNP-400) resulted in approximately a 2-fold decrease in CAT activity compared to the control treatment (Figure 4B). In contrast, APX activity increased with SiNPs applications. The most significant increase in APX activity was observed at the Sw1.2 level. The SiNP-200 treatment showed the lowest APX value compared to the control treatment (SiNP-0). Still, the SiNP-200 and SiNP-400 treatments increased APX activity (Figure 4C). MDA content indicated lipid peroxidation increased with NaCl salinity applications. However, SiNPs applications decreased MDA levels, indicating a reduction in lipid peroxidation and oxidative damage. The SiNP-400 treatment resulted in a decrease

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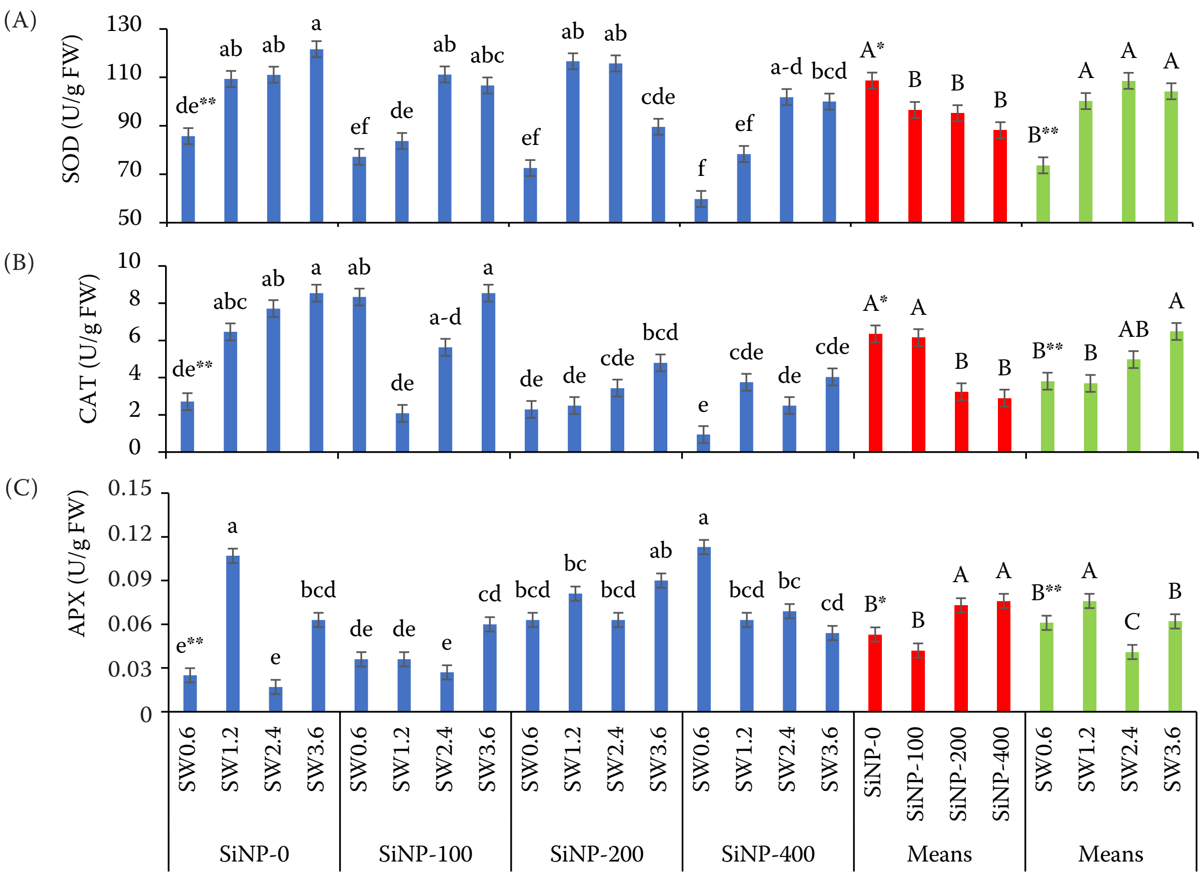

Figure 4. Effect of different doses of nano-silica and saline irrigation water on (A) superoxide dismutase (SOD); (B) catalase (CAT), and (C) ascorbate peroxidase (APX). SiNP-0 – non-nano silica particles-control; SiNP-100 – 100 mg/L nano silica; SiNP-200 – 200 mg/L nano silica; SiNP-400 – 400 mg/L nano silica; Sw0.6 – saline water 0.6 dS/m (control); Sw1.2 – saline water 1.2 dS/m; Sw2.4 – saline water 2.4 dS/m; Sw3.6 – saline water 3.6 dS/m; $^*P < 0.05$; $^{**}P < 0.01$; FW – fresh weight

of approximately 21% in MDA content compared to SiNP-0 (Figure 3D).

Mineral content in lettuce. The mineral content analysis of lettuce leaves revealed significant effects of NaCl salinity, SiNPs applications, and their interactions on the contents of K, Mg, Fe, Zn and Na (Table 1). However, no significant effects were observed on Ca contents due to the application of SiNPs. Increasing levels of NaCl salinity led to a substantial reduction in Fe content in lettuce leaves. Conversely, the contents of Mg, Zn, and Na increased with NaCl application. SiNPs applications prominently improved Mg, Fe, and Zn contents in lettuce leaves, indicating their positive impact on mineral uptake. SiNPs also alleviated the adverse effect of salt on Fe uptake, resulting in higher Fe content than control applications. Furthermore, Na contents decreased progressively with SiNPs treatments. Increasing doses of nano-silica have a beneficial effect on reducing sodium accumulation in lettuce leaves. Likewise, SiNP treatments enhanced the K uptake compared to the control (SiNP-0). The highest Mg, Zn, and K accumulation was observed at the SiNP-400 silica nanoparticle application. The control SiNP-0 application exhibited the lowest Ca, Fe, K, and Zn accumulation. The effect of NaCl salinity stress and SiNPs applications on some mineral matter contents of lettuce plants. Increasing concentrations of NaCl resulted in a decrease in K uptake in plant tissue, accompanied by a corresponding insignificant decrease in the K^+/Na^+ ratio. In addition, the effect of nanosilica applications on the reduction of the Na/K ratio was not found to be statistically significant.

DISCUSSION

The present study investigated the potential mitigating effects of silica nanoparticles on lettuce plants under saline conditions, considering the significant impact of salinity on agricultural production. Recent

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Table 1. NaCl salinity stress and silica nanoparticles (SiNPs) applications affect some mineral matter contents of lettuce plants

		Ca	K	Mg	Fe	Zn	Na (%)	K^+/Na^+
		(%)			(mg/L)			
SiNP-0	Sw0.6	1.07 ± 0.08^{ns}	5.38 ± 1.30	0.51 ± 0.03^{ns}	252.5 ± 11.50^{b**}	26.07 ± 7.97^{g**}	1.55 ± 1.16^{cd**}	2.78 ± 0.68^{ns}
	Sw1.2	1.18 ± 0.19	4.22 ± 0.58	0.52 ± 0.06	197.14 ± 12.07$^{c–f}$	25.22 ± 4.96^{g}	1.36 ± 0.11^{def}	3.13 ± 0.66
	Sw2.4	1.22 ± 0.26	4.21 ± 0.07	0.60 ± 0.12	139.38 ± 5.86^{h}	24.11 ± 4.52^{g}	1.78 ± 0.01^{bc}	2.07 ± 0.45
	Sw3.6	1.47 ± 0.00	3.08 ± 1.01	0.80 ± 0.02	104.14 ± 1.19^{i}	23.66 ± 7.07^{g}	2.81 ± 0.06^{a}	1.98 ± 1.91
SiNP-100	Sw0.6	1.24 ± 0.27	4.65 ± 0.32	0.50 ± 0.08	166.50 ± 24.02$^{f–h}$	36.47 ± 2.86^{g}	1.17 ± 0.26^{f}	3.56 ± 0.99
	Sw1.2	1.41 ± 0.12	4.52 ± 0.45	0.59 ± 0.09	179.33 ± 25.79$^{d–g}$	28.47 ± 6.84^{g}	1.58 ± 0.05^{cd}	2.73 ± 0.31
	Sw2.4	2.25 ± 1.46	4.40 ± 0.44	0.57 ± 0.03	210.41 ± 24.06^{cd}	29.16 ± 5.98^{g}	1.75 ± 0.02^{bc}	2.52 ± 0.34
	Sw3.6	1.43 ± 0.07	4.43 ± 0.15	0.65 ± 0.18	203.25 ± 20.73^{cde}	78.69 ± 1.82^{f}	1.99 ± 0.06^{b}	2.19 ± 0.04
SiNP-200	Sw0.6	1.67 ± 0.01	5.60 ± 0.02	0.55 ± 0.01	287.72 ± 0.96^{a}	81.29 ± 8.50^{ef}	1.51 ± 0.45^{cde}	3.34 ± 1.67
	Sw1.2	1.58 ± 0.29	4.58 ± 1.12	0.65 ± 0.04	251.04 ± 21.41^{b}	32.24 ± 7.19^{g}	1.63 ± 0.01^{cd}	3.14 ± 0.53
	Sw2.4	1.87 ± 0.01	4.82 ± 0.03	0.75 ± 0.02	199.43 ± 0.25$^{c–f}$	133.98 ± 18.18^{b}	1.72 ± 0.05^{bc}	2.87 ± 0.18
	Sw3.6	2.12 ± 0.05	5.36 ± 0.45	0.80 ± 0.03	160.29 ± 15.14^{gh}	97.18 ± 18.35^{de}	1.78 ± 0.10^{bc}	3.65 ± 0.98
SiNP-400	Sw0.6	1.12 ± 0.02	5.78 ± 0.50	0.58 ± 0.01	193.70 ± 2.12$^{c–g}$	110.75 ± 0.78^{c}	1.26 ± 0.05^{ef}	3.94 ± 1.28
	Sw1.2	1.49 ± 0.01	3.83 ± 0.38	0.63 ± 0.02	169.03 ± 0.53$^{e–h}$	113.28 ± 13.83^{c}	1.52 ± 0.07^{cde}	2.30 ± 0.44
	Sw2.4	1.83 ± 0.27	4.39 ± 0.99	0.72 ± 0.09	196.66 ± 4.56$^{c–f}$	114.63 ± 14.61^{c}	1.64 ± 0.04^{cd}	2.85 ± 0.35
	Sw3.6	1.99 ± 0.01	4.24 ± 0.38	0.79 ± 0.01	227.01 ± 24.63^{bc}	179.46 ± 7.63^{a}	1.80 ± 0.07^{bc}	2.31 ± 0.01
Means								
SiNP-0		1.19^{ns}	4.22^{B**}	0.59^{B**}	179.57^{C**}	24.86^{D**}	1.88^{A**}	2.49^{ns}
SiNP-100		1.62	4.50^{AB}	0.57^{B}	189.59^{C}	41.12^{C}	1.62^{B}	2.84
SiNP-200		1.78	4.67^{AB}	0.70^{A}	231.07^{A}	180.18^{B}	1.66^{B}	3.25
SiNP-400		1.63	4.98^{A}	0.69^{A}	196.61^{B}	127.87^{A}	1.55^{B}	3.03
Sw0.6		1.25^{ns}	5.30^{ns}	0.53^{C**}	221.98^{A**}	57.17^{D**}	1.38^{D**}	3.41^{ns}
Sw1.2		1.41	4.38	0.60^{B}	204.12^{B}	45.59^{C}	1.52^{C}	2.86
Sw2.4		1.78	4.31	0.65^{B}	183.88^{C}	70.15^{B}	1.72^{B}	2.56
Sw3.6		1.79	4.14	0.76^{A}	173.67^{C}	94.75^{A}	2.09^{A}	2.49
P SiNP		0.129	0.019	0.003	0.000	0.000	0.000	0.386
P Sw		0.076	0.171	0.000	0.000	0.000	0.000	0.183
P SiNP × Sw		0.719	0.314	0.414	0.000	0.000	0.000	0.758

SiNP-0 – non-nano silica particles-control; SiNP-100 – 100 mg/L nano silica; SiNP-200 – 200 mg/L nano silica; SiNP-400 – 400 mg/L nano silica; Sw0.6 – saline water 0.6 dS/m (control); Sw1.2 – saline water 1.2 dS/m; Sw2.4 – saline water 2.4 dS/m; Sw3.6 – saline water 3.6 dS/m; $^{**}P < 0.01$; ns – not significant

studies have investigated the role of nano-silicon in mitigating salinity stress in various plant species (Farhangi-Abriz and Torabian 2018, Ismail et al. 2022, Sayed et al. 2022). Excess salt in the soil disrupts water balance, induces osmotic stress, and leads to ion toxicity and nutrient imbalances, negatively affecting

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plant growth and physiological functions (Zhang et al. 2018). Consistent with previous reports indicating that salinity stress adversely impacts plant growth parameters (Abdeldym et al. 2020, Sayed et al. 2022), our findings demonstrated that NaCl-induced salt stress significantly reduced physical parameters, including head diameter, plant height, and dry weight. High salinity levels cause osmotic imbalance at the soil-root interface, impairing the plant's ability to absorb water (Shelden and Munns 2023). Consequently, fresh and dry weights declined in plants subjected to salt stress.

SiNPs application mitigated the adverse effects of NaCl-induced stress, as treated lettuce plants exhibited significantly higher growth parameters than untreated plants under saline conditions. This suggests that nano-silicon effectively enhances salt stress tolerance. The beneficial effects of SiNPs on growth parameters can be attributed to their role in enhancing water uptake and improving water-use efficiency, thereby counteracting the osmotic stress caused by salinity. Additionally, SiNPs may regulate nutrient uptake and ion homeostasis, which are typically disrupted under saline conditions. Further research is required to elucidate the precise mechanisms through which SiNPs alleviate salt stress in lettuce. The findings contribute to the growing body of literature on nanotechnology applications in agriculture, highlighting the need for further studies to optimise the use of SiNPs to mitigate salt stress.

Chlorophyll is a crucial component of photosynthesis, capturing light energy and converting it into chemical energy (Maxwell and Johnson 2000). In the present study, salinity stress did not significantly affect the SPAD value, while SiNPs application substantially increased it. Reports on the effects of salinity stress on SPAD values have been inconsistent. For instance, Sayed et al. (2022) observed a decrease in SPAD values following NaCl application. In contrast, Haghighi and Pessarakli (2013) found no significant effect of salinity on SPAD values in tomato leaves, which aligns with our study. The observed increase in SPAD values following SiNP treatment under salinity stress can be attributed to enhanced antioxidant defence mechanisms and reduced oxidative damage. Previous studies have demonstrated that SiNPs enhance antioxidant enzyme activities, thereby reducing oxidative stress in plants exposed to adverse conditions, including salinity (Farhangi-Abriz and Torabian 2018, Singh et al. 2020, Tondey et al. 2021). SiNPs contribute to chlorophyll retention by mitigating oxidative damage and enhancing photosynthetic efficiency under salt stress conditions. Further research is needed to elucidate these protective effects' underlying mechanisms fully.

The findings regarding leaf relative water content under salinity stress in this study align with those of Haghighi and Pessarakli (2013), who reported no significant reduction in LRWC due to NaCl application. However, other studies have shown that salinity stress negatively affects leaf water status, resulting in a decline in LRWC (Hanafy et al. 2008, Abdul Qados and Moftah 2015). In contrast, our study demonstrated that SiNPs significantly increased LRWC under saline conditions, consistent with the findings of Ismail et al. (2022), who reported enhanced leaf water status following SiNP treatment. The ability of SiNPs to inhibit excessive transpiration, a common consequence of NaCl-induced stress, could explain the increased LRWC (Liang 1999). This highlights the potential of SiNPs in maintaining leaf hydration and improving plant resilience to salt stress.

Salinity stress significantly affected physiological parameters, as evidenced by an increased electrolyte leakage in lettuce leaves from elevated Na^+ accumulation. High levels of Na^+ and Cl^- ions contribute to the production of reactive oxygen species, which leads to oxidative damage, membrane instability, degradation of biological macromolecules, and lipid peroxidation, ultimately resulting in cell death (Swapnil et al. 2017, Campos et al. 2019). Osmotic stress and ion imbalances further exacerbate the damage caused by salinity (Monetti et al. 2014). The present study supports the understanding that salt stress, including EL, severely impacts plant physiological integrity. However, SiNPs application mitigated some of these detrimental effects by improving LRWC and potentially reducing the damaging impact of salt stress on cellular structures. Further investigation is required to decipher the specific role of SiNPs in maintaining membrane stability and enhancing plant resilience under salinity stress. The observed increase in superoxide dismutase and catalase activities in lettuce plants under salt stress indicates the activation of plant defence mechanisms against oxidative stress. Salinity stress disrupts ion distribution within plant cells, leading to excessive ROS accumulation, which can damage macromolecules such as DNA, lipids, and proteins (Arif et al. 2020). Plants rely on enzymatic antioxidant defence systems, including SOD and CAT, to eliminate reactive oxygen species and mitigate oxidative stress-related damage (Rajput et al. 2021). Previous studies have documented the

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activation of CAT and SOD under salinity stress conditions (Gupta and Huang 2014, Cakmakci et al. 2022a). The observed increase in antioxidant enzyme activity reflects the plant's adaptive response to oxidative stress. Interestingly, ascorbate peroxidase activity exhibited differential responses to SiNPs applications. The highest APX activity was recorded under 1.2 dS/m NaCl application, while the lowest was observed under SiNP-100 treatment. However, SiNP-200 and SiNP-400 treatments increased APX activity, suggesting potential toxicity at higher SiNP concentrations. The antioxidant defence system in plants operates through a sequential process, with SOD playing a primary role in ROS detoxification, followed by the ascorbate-glutathione cycle, where enzymes such as CAT and APX contribute to the removal of hydrogen peroxide (Gupta and Huang 2014, Ismail et al. 2022). Contrary to expectations, SiNPs treatments under salt stress conditions in our study led to a significant decrease in CAT and SOD enzyme activities in lettuce leaves. This contrasts with findings by Fan et al. (2022), who reported enhanced antioxidant enzyme activities following SiNPs application. These discrepancies could be attributed to variations in plant species, SiNPs concentrations, and environmental conditions. Further research is necessary to fully elucidate the role of SiNPs in modulating antioxidant enzyme activities under salt stress, particularly in evaluating potential toxic effects at higher concentrations and their interactions with the plant's antioxidant defence mechanisms.

The decrease in Fe content and the increase in Mg and Zn contents observed in lettuce leaves with increasing salinity levels in the present study are consistent with previous findings (Chaichi et al. 2017, Tondey et al. 2021). High soil salinity can disrupt the mineral-nutrient relationships in plants by affecting nutrient availability, transport, and metabolism (Hu and Schmidhalter 2005). The negative impact of salinity on nutrient uptake is attributed to factors such as ion competition, decreased nutrient mobility, and interference with nutrient absorption mechanisms. On the other hand, SiNPs applications in the present study led to increased uptake of Ca, K, Mg, Fe, and Zn elements in lettuce leaves. This enhancement in mineral uptake can be attributed to the positive effects of SiNPs on plant growth parameters and yield. Improved mineral uptake is known to enhance plant resistance to abiotic stress factors. In particular, the increased intake of K has been associated with alleviating salt stress in plants. K^+ ions are crucial in maintaining turgor pressure and ion homeostasis in plant cells under salinity stress (Hu and Schmidhalter 2005). The results indicated that SiNPs treatments decreased Na accumulation. This suggests that high-dose SiNPs applications inhibit Na uptake in lettuce leaves under saline conditions. Under salinity stress, high Na accumulation in the root zone can inhibit K absorption by plant roots and affect numerous enzymatic activities in plant cells, where K plays a crucial role. Therefore, increasing K content and decreasing Na content (increasing the K^+/Na^+ ratio) will alleviate oxidative damage (Alsaeedi et al. 2018). This leads to improvements in cell turgor, increased CO_2 assimilation and water uptake, ultimately resulting in the maintenance of the nutritional status of plant cells. In this study, SiNP treatments increased potassium uptake while inhibiting Na uptake and improved plant growth. Maintaining a high K^+/Na^+ ratio is a characteristic of salt tolerance (Fakhrfeshan et al. 2018). The minimum value of the K^+/Na^+ ratio is approximately 1 (Maathuis and Amtmann 1999). In our study, the K^+/Na^+ ratio was higher than 1 and reached approximately 2, even at the highest NaCl concentration in the medium. With the increasing applications of nanosilica, this value approached 4.

In conclusion, this study demonstrates that salinity stress significantly impairs lettuce growth, nutrient uptake, and oxidative balance. However, silica nanoparticles effectively mitigate these adverse effects by enhancing plant resilience. SiNPs application improved growth parameters (head diameter, plant height, and dry matter content), increased chlorophyll retention (SPAD values), and optimised mineral uptake, particularly Mg, Fe, Zn, and K, while reducing Na accumulation. Notably, SiNPs alleviated oxidative stress, as evidenced by decreased lipid peroxidation (MDA) and modulation of antioxidant enzymes (SOD and CAT). The efficacy of SiNPs was concentration-dependent, with 100 mg/L showing the most favourable outcomes, while higher doses (200–400 mg/L) occasionally induced stress, highlighting the need for precise dosage optimisation. These findings highlight SiNPs as a sustainable strategy to enhance salinity tolerance in crops. However, further research is warranted to refine application protocols (timing and dosage) across diverse plant species and stress conditions. By bridging knowledge gaps in nano-enabled agriculture, SiNPs could emerge as an eco-friendly alternative to conventional fertilisers, bolstering food security in saline-affected regions.

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