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Effect of Silica Nanoparticles on Physiological, Antioxidant, and Ionic Responses of Plantago Ovata under Combined Drought and Salinity Stress

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ABSTRACT

Combined drought and salinity stress can severely impede plant growth by altering water relations, ion balance, and oxidative metabolism. This study evaluated the effect of *Plantago ovata* on coupled drought and salinity stress, as well as assessed whether silica nanoparticles may minimise the negative impacts of these conditions, this surface of silica characterized by using XRD technique. Plants put under combined stress revealed a marked drop in relative water content, growth, and chlorophyll concentration, while hydrogen peroxide and malondialdehyde increased, indicating more oxidative damage. Foliar application of silica nanoparticles helped the stressed plants retain greater growth, higher hydration status, and enhanced chlorophyll content. The treatment also decreased oxidative damage and supported the function of antioxidant enzymes. In addition, silica nanoparticles inhibited sodium buildup and encouraged potassium retention, resulting in an improved K^+/Na^+ ratio. Overall, the data imply that silica nanoparticles can improve the resistance of *P. ovata* to combined severe drought and salt stress by enhancing water status, lowering oxidative injury, and promoting ionic equilibrium.

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INTRODUCTION

Salinity and drought are two of the greatest, pervasive abiotic factors that reduce crop production, especially in semi-arid and arid environments. Among abiotic stressors, salt and drought are significant factors that limit plant growth [1, 2]. Drought primarily affects plant water content and physiology, whereas salinity affects ion transport in plants [3, 4]. Under natural conditions, both stresses usually occur together, producing greater impacts than either stress alone [5].

The simultaneous presence of both drought and salinity exerts stricter constraints on plant development compared to either one alone. The osmotic and ionic stress due to these factors

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interferes with cell equilibrium and causes ionic imbalance [6]. Nanostructured silicon materials have recently become a popular means of improving stress tolerance in plants [(7]. Salinity stress causes an excess of ions that impair cellular functions [8]. Nanomaterials derived from silicon have been shown to reduce stress-induced damage, thereby improving physiological performance [9]. When plants are subjected to drought and salinity stresses simultaneously, they experience significant physiological disturbance, leading to stunted growth and development [10]. The generation of large quantities of oxygen-reactive species is among the main effects of abiotic stressors, which causes harm to proteins, lipids, and nucleic acids [11]. The defence mechanism of antioxidants is essential in neutralizing these reactive substances [12]. Moreover, reactive oxygen forms serve as molecules that control the physiological reactions associated with stress [13]. Nevertheless, when these reactive oxygen compounds are in excess, oxidative stress ensues [14]. During periods of extreme stress, antioxidant defences may fail to maintain redox homeostasis [15].

New developments in nanotechnology have opened up avenues to enhance plants' reaction to abiotic stresses [16]. The efficacy of nanoparticles is determined by their properties that permit them to interact with living organisms and impact physiological functions [17, 18]. Silica nanoparticles have gained substantial interest because of their capacity to boost plants' stress reactivity by regulating antioxidant levels and water equilibrium [19, 20]. Additionally, silica is recognised to be a good stimulator of plant growth and resilience to stress [21].

Although there have been many investigations into nanomaterials, very little information is available on their contributions under drought and salt stresses [22]. Previous research has mostly been conducted in isolation from stress factors [23]. The presence of environmental stress factors poses a significant problem for plant life and adaptation [24]. This study evaluated whether foliar-applied SiNPs can improve growth, water relations, antioxidant metabolism, and ionic balance in *P. ovata* under the combined stresses of salinity and drought.

Consequently, the present research was designed on the hypothesis that silicon nanoparticles can improve the water status of *P. ovata* to lessen the combined impacts of salinity and drought, protect photosynthetic pigments, limit oxidative injury, and maintain a more stable ionic balance. Accordingly, this investigation aimed to evaluate the impact of silicon nanoparticles on oxidative stress markers, growth, chlorophyll content, relative water content, antioxidant responses, and Na^+ and K^+ regulation in *P. ovata* under combined drought and salinity stress. It was expected that plants treated with silicon nanoparticles would perform better under combined stress than untreated, stressed plants, with improved growth and physiological status, reduced formation of H_2O_2 and MDA, in addition to a higher K^+/Na^+ ratio.

MATERIALS AND METHODS

Plant materials and growth conditions

The seeds of *P. ovata* were bought from a certified local seed supplier in Kirkuk city, Iraq, and kept dry until further use. Before planting, for five minutes, after being surface-sterilised with sodium hypochlorite (1%), the seeds were washed with filtered water, eliminating residues of the sterilization agent. After that, the seedlings were placed in plastic containers (25 cm in diameter), with the soil mixture consisting of sand with sandy loam in a 2:1 proportion.

The research was conducted at Kirkuk University, Iraq, in a greenhouse. A steady temperature of 24–28°C and a 55–65% humidity range were maintained. The physicochemical properties of the soil were determined before the start of the experiment. The soil's pH was 7.4, its electrical conductivity was 2.1 dS m^{-1}, its organic carbon content was 1.18%, its available nitrogen was 74.6 mg kg^{-1}, its accessible phosphorus was 18.3 mg kg^{-1}, and its accessible potassium was 165.4 mg kg^{-1}. Sandy loam was the classification given to the soil texture.

The seedlings were then supplied with distilled water after germination to make sure that there was uniform establishment. Three uniform plants per pot were created by thinning seedlings. Every plant was raised under controlled conditions [25].

Nanoparticles of silicon preparation and their application

The nanoscale silicon dioxide particles (SiO_2-NPs; 20-50 nm particle size, purity >99%) were acquired from Sigma-Aldrich in St Louis, Missouri, USA as shown in Fig. 1. The product batch

information and supplier specifications were recorded at the time of purchase. According to the supplier's technical information, the particles had a nanoscale silica composition with nearly spherical morphology and aqueous dispersibility. Stock suspensions were prepared in purified water at 50, 100, and 150 mg L^{-1}. There was no Tween-20 or surfactant applied to the suspensions. The pH of the prepared suspensions was approximately 6.8-7.0 before foliar application. To reduce aggregation and obtain a more uniform suspension, each suspension was sonicated for 30 min at 40 kHz immediately before spraying. The suspensions stayed well mixed after sonication, showing no sign of settling for both prep and spraying. So, they had good short-term stability. Each morning, we thoroughly covered the leaves by spraying till runoff using a hand sprayer. We repeated the treatment three times weekly throughout the stress period. This concentration level was picked due to earlier studies that showed its helpful benefits [26, 27].

Experimental design

The experimental design was a CRD with eight treatments, each replicated four times. Each replicate was represented by one pot containing three plants; therefore, the experiment included 32 pots in total. The pot was considered the biological replicate. All pots were randomly arranged within the greenhouse and were repositioned periodically to reduce positional effects related to light and temperature. Stress treatments started 21 days after planting and continued until samples were collected for the different measurements. The different treatments used in the experiment are as follows:

(1) Control (no stress, no SiNPs)
(2) Only SiNPs (100 mg L^{-1})
(3) Stress due to drought
(4) Stress due to salinity (100 mM NaCl)
(5) Combined stress (drought and salinity)
(6) Combined stress with SiNPs (50 mg L^{-1})
(7) Combined stress with SiNPs (100 mg L^{-1})
(8) Combined stress with SiNPs (150 mg L^{-1})

The SiNP treatments were applied only under coupled salinity and drought stress, as this study's primary goal was to assess whether SiNPs could mitigate the more severe co-stress condition rather than individual stress effects. Drought-only and salinity-only treatments were included as stress references to compare the severity of every single stress with the combined stress. Therefore, drought + SiNP and salinity + SiNP treatments were

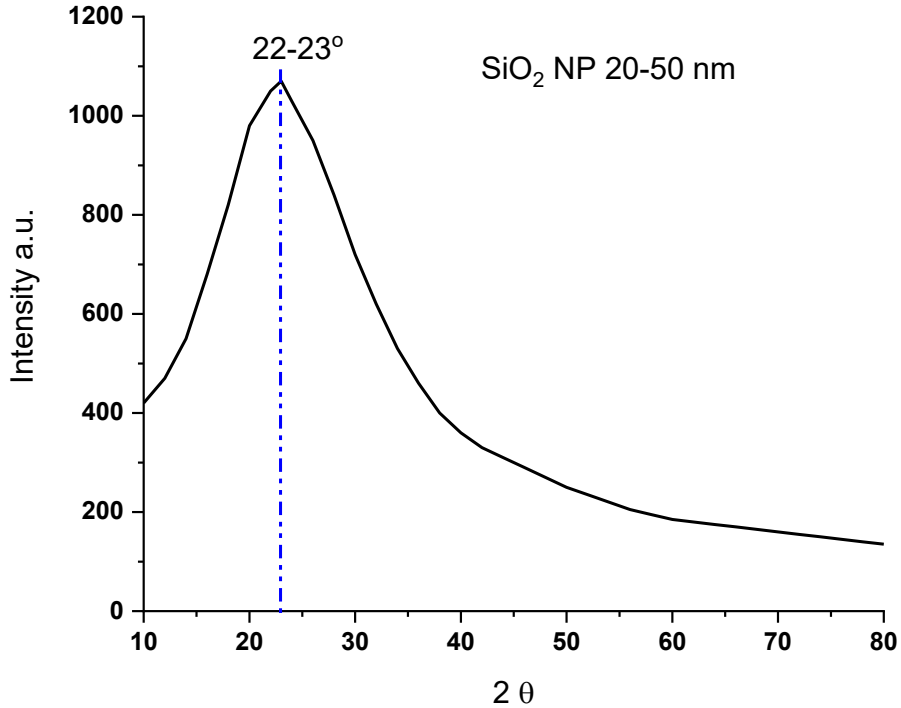

Fig. 1. XRD of Silica nanoparticles

not included in the present experimental design.

Treatments for stress

Stress treatments were initiated 21 days after planting. Reducing and keeping soil moisture at 45% of field capacity caused drought stress. Every day, the pots were weighed to check the moisture content of the soil, and the lost water was replaced gravimetrically to maintain the required moisture level throughout the stress period. Salinity stress was applied gradually by adding NaCl solution in two steps to mitigate osmotic shock. On the first day, plants received 50 mM NaCl and 100 mM NaCl on the following day. For the combined stress treatment, both reduced soil moisture and 100 mM NaCl were maintained together until sampling [28].

Growth measurements

Growth measurements were recorded at harvest, 45 days after planting. They measured success by the plants' height, length of root, number of leaves, and fresh and dried weights. The plant's height and the length of its roots were measured with a ruler, whereas leaves were counted manually. The dry mass was calculated following oven drying at 70°C, while the fresh weight was taken immediately after harvesting [29].

Relative water content (RWC)

RWC was computed from freshly collected leaves in accordance with the technique described previously [30]. FW was measured immediately upon leaf collection. The leaves were dried at 70 °C in order to calculate their drying weights (DW) following soaking them in filtered water at 25 ± 2 °C for four hours to determine the turgid weight (TW). RWC was calculated using the following formula:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Chlorophyll Determination

The amount of chlorophyll was measured spectrophotometrically following extraction with acetone (80%). Two grams of fresh leaf tissues were homogenized and filtered to obtain a clear chlorophyll extract. At 663 and 645 nm, respectively, the absorption values of chlorophyll a and b were determined, utilising a UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan). Following that, standard formulas were applied to calculate the amounts of total chlorophyll, chlorophyll a, and chlorophyll b [31]. The final chlorophyll values were reported as mg g^{-1} FW.

$$\text{Chlorophyll a} = [12.7 \times A_{663} - 2.69 \times A_{645}]$$
$$\text{Chlorophyll b} = [22.9 \times A_{645} - 4.68 \times A_{663}]$$
$$\text{Total chlorophyll content} = [20.2 \times A_{645} + 8.02 \times A_{663}]$$

Membrane Stability Index (MSI)

Ten millilitres of filtered water were added to test tubes containing 0.2 grams of freshly harvested leaf tissues to assess the membrane stability index. Next, the conductivity of electricity was determined after a 30-minute incubation period at 40 °C (C_1). For ten minutes, the specimens were heated to 100°C in boiling water to determine ultimate conductivity (C_2). The calculation of MSI was as follows according to [32]:

$$MSI\ (\%) = [1 - (C_1 / C_2)] \times 100$$

Proline measurement

The approach previously published [33] was used to calculate the proline content. Five milliliters of 3% sulfosalicylic acid were employed to standardize 0.5 grams of fresh leaf tissues. Centrifugation was performed on the mixture for ten minutes at 4 °C at 10,000 × g. Then, two milliliters each of the standard supernatant, glacial acetic acid, and ninhydrin acid reagent were mixed. After one hour of incubation at 100 °C, the combination was rapidly cooled in a bath of ice. Proline level was determined utilizing a curve of calibration after absorption was observed at 520 nm [34]. The findings were represented as µmol g^{-1} FW, and each test was run in triplicate.

Lipid peroxidation (MDA)

The TBA, or thiobarbituric acid, procedure previously reported was employed to calculate the amount of MDA [35]. Five milliliters of trichloroacetic acid (TCA) (0.1%), had been employed to standardize 0.5 grams of fresh leaves tissue. Following that, the homogeneous product was spun up for 10 minutes at 4 °C at 10,000 × g. Next, 4 milliliters of 0.5% TBA made in 20% TCA was combined with one milliliter of the supernatant. After thirty minutes of heating to 95 °C, the combination was rapidly chilled in the bath of ice. Absorption that is not specific at 600

nm was deducted from the absorbance at 532 nm. The attenuation value of 155 mM^{-1} cm^{-1} was used to compute the MDA level [36]. The outcomes were represented as (nmol g^{-1} FW), and each test was run in triplicate.

Hydrogen Peroxide (H_2O_2)

The technique previously described [37] was employed to ascertain the amount of hydrogen peroxide. Fresh leaf tissue weighing 0.5 grams was homogenized using five milliliters of cold (0.1%) trichloroacetic acid. The resulting solution was spun up for fifteen min. at 4 °C at 12,000 × g. Next, 1 milliliter of 1 M potassium iodide and half a milliliter of ten mM potassium phosphate buffer (pH level 7.0) were combined with half a milliliter of the supernatant. The absorbing capacity was calculated at 390 nm after the reaction mixture was held in dark conditions for thirty minutes at room temperature. A typical calibration curve was employed to calculate the H_2O_2 concentrations. The findings were represented as µmol g^{-1} FW, and each test was run in triplicate.

Enzymatic antioxidant assay

Spectrophotometric analysis was applied to identify the actions of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD). In 5 millilitres of 50 mM cooled phosphate buffer with a pH of 7.0, including polyvinylpyrrolidone (PVP) with 1 mM EDTA. The homogeneous material was spun at 12,000 times speed at 4°C for fifteen minutes, and then the enzyme was extracted from the clear supernatant. The activity of SOD was assessed based on its ability to prevent nitro blue tetrazolium (NBT) from being reduced photochemically.

The interaction combination includes an enzyme extract, phosphate buffer, riboflavin, NBT, methionine, and EDTA, and after being exposed to light, the absorption value was reported at 560 nm. The quantity of enzymes needed to prevent NBT degradation by 50% was represented as one unit of SOD efficiency. CAT efficiency was evaluated via tracking the breakdown of hydrogen peroxide through the reduction in absorption at 240 nm, utilising an absolute extinction of 39.4 mM^{-1} cm^{-1}. POD function was tested by detecting guaiacol oxidation at 470 nm, based on an extinction value of 26.6 mM^{-1} cm^{-1}. Protein levels in the enzyme extracts were calculated by employing the Bradford technique, using bovine serum albumin as the reference. Every enzyme test was done in triplicate, and the activities were stated as U mg^{-1} protein according to the specified method [38].

Analysis of ions

Potassium and sodium contents were evaluated using dried leaf samples. The oven was set at 70°C to dry these leaves before being milled into fine powder. The powdered substance was digested with 65% nitric acid at 80°C for 2 h till a clear extract was produced. Following chilling, purified water was used to filter and dilute the digested specimens to a predetermined ultimate volume. A flame photometer was applied to ascertain the concentrations of Na^+ and K^+ (Jenway PFP7, UK), and the results have been expressed as mg g^{-1} DW based on dry weight [39].

Statistical analysis

The findings were assessed by applying a one-way (ANOVA) to compare variances among the experimental therapies. The treatments were analysed as a single-factor fixed effect, with each treatment combination handled as a separate treatment group. Therefore, one-way (ANOVA) was utilised to calculate the total response of the quantified variables among therapies. The normality and homogeneity of variance hypotheses were assessed by the Shapiro-Wilk and Levene tests, respectively. Duncan's test of multiple ranges was applied for mean comparisons at $P < 0.05$. SPSS programmes (IBM Corp., version 26) were applied to every statistical analysis. The findings are displayed based on four biological replicas as the mean ± standard error (SE) [40].

RESULTS AND DISCUSSION

Distinct variations were noticed in how the plants looked based on their treatment. The control plants showed typical growth, with well-developed shoots and plentiful leaves. In contrast, drought, salinity, and their combined application clearly inhibited plant growth. Stressed plants had shorter branches, smaller canopies, and fewer leaves than the controls. The greatest reduction was noticed under the combined stress treatment, when plant height declined to 19.7 cm, compared with 26.3 cm under drought stress and 24.8 cm under salinity stress. SiNPs' applicability improved the appearance and growth process of plants under stress, with greater shoot development and leaf production (Fig. 2). These visual observations

were consistent with the quantitative data. While combined stress really took its toll, the treated plants clearly recovered in terms of shoot and leaf growth.

Parameters related to the growth of *P. ovata* were significantly impacted by the treatments applied (Table 1). The control plants exhibited normal growth behaviour with a plant height of 36.4 cm, whereas SiNPs treatment without the presence of any stress factor increased the plant height to 39.2 cm. Drought stress and salinity stress led to plant heights of 26.3 and 24.8 cm, respectively, where the minimum plant height was reported under combined stress conditions (19.7 cm). However, treatment with SiNPs under combined stress increased plant height, with concentrations of 50, 100, and 150 mg L^{-1} producing plant heights of 25.6, 29.8, and 32.7

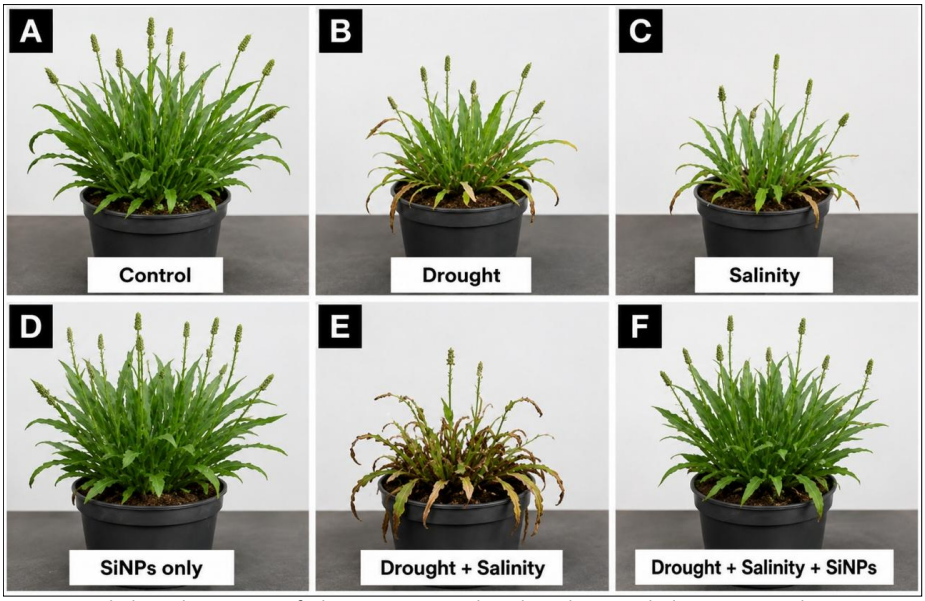

Fig. 2. Morphological responses of *Plantago ovata* to drought, salinity, and silica nanoparticle treatments. Control plants (A) showed normal growth, while drought (B) and salinity (C) reduced growth performance. Silica nanoparticle treatment improved growth under non-stress (D) and combined stress conditions (F), whereas combined drought and salinity stress (E) caused the most severe growth reduction.

Table 1. Effects of silica nanoparticle treatments on growth parameters of *Plantago ovata* under drought and salinity stress.

Treatment	Plant height (cm)	Root length (cm)	Leaves per plant	Fresh weight (g)	Dry weight (g)
Control	36.4 ± 1.2 b	18.7 ± 0.9 b	14.0 ± 0.7 b	9.82 ± 0.41 b	3.46 ± 0.18 b
SiNPs (100 mg L^{-1})	39.2 ± 1.3 a	20.5 ± 1.0 a	16.0 ± 0.8 a	10.94 ± 0.46 a	3.89 ± 0.21 a
Drought	26.3 ± 1.1 e	14.2 ± 0.7 d	10.0 ± 0.5 d	6.35 ± 0.28 d	2.14 ± 0.12 d
Salinity (100 mM NaCl)	24.8 ± 1.0 f	13.6 ± 0.6 d	9.0 ± 0.4 e	5.92 ± 0.26 e	1.97 ± 0.10 e
Drought + Salinity	19.7 ± 0.9 g	10.4 ± 0.5 e	7.0 ± 0.3 f	4.36 ± 0.21 f	1.51 ± 0.09 f
Stress + SiNPs (50 mg L^{-1})	25.6 ± 1.0 f	13.9 ± 0.6 d	10.0 ± 0.5 d	6.41 ± 0.30 d	2.16 ± 0.11 d
Stress + SiNPs (100 mg L^{-1})	29.8 ± 1.2 d	15.7 ± 0.7 c	12.0 ± 0.6 c	7.84 ± 0.34 c	2.63 ± 0.13 c
Stress + SiNPs (150 mg L^{-1})	32.7 ± 1.3 c	17.2 ± 0.8 b	13.0 ± 0.7 b	8.92 ± 0.39 b	3.08 ± 0.15 b

The value is illustrated as the mean ± SE (n = 4). Various letters inside one column, utilising the Duncan multiple range test at ($P < 0.05$), show significant variations.

cm, respectively. Based on the grouping letters in Table 1, these variances were noteworthy at P < 0.05. The improvement was most evident at 150 mg L^{-1}, which gave the highest plant height among the SiNPs-treated stressed plants. Root length, leaf count, fresh weight, and dry weight followed the same general response.

Combined drought and salinity stress dramatically lowered growth, hydration status, and metabolic stability in *P. ovata*. Stressed plants revealed notable declines in plant height, biomass accumulation, and leaf development, indicating serious perturbation in water relations and overall metabolic activity [30]. The combined effect of drought and salinity was more destructive than either stress alone, suggesting that water deficiency and sodium buildup acted together to enhance osmotic imbalance and membrane damage [36]. Under these conditions, *P. ovata* encountered more physiological pressure because reduced water supply was accompanied by increased salt toxicity. This combined stress also activated multiple defensive mechanisms, adding more complexity to the plant response at the physiological level [23].

Physiological attributes were significantly altered by stress and SiNPs application (Table 2). The relative water content decreased under stress therapy, with the lowest value observed in the combined stress treatment (54.6%). Similar results were observed in the membrane stability index and chlorophyll content. SiNPs increased the values of these parameters in response to stress therapies, with RWC rising to 79.5% at 150 mg L^{-1}.

Table 2. Effects of silica nanoparticles on physiological attributes of *Plantago ovata* under stress conditions.

Treatment	RWC (%)	Chlorophyll (mg g^{-1} FW)	Membrane stability (%)
Control	86.3 ± 1.4 b	2.41 ± 0.08 b	82.7 ± 1.6 b
SiNPs (100 mg L^{-1})	89.4 ± 1.6 a	2.58 ± 0.09 a	85.9 ± 1.8 a
Drought	65.2 ± 1.3 e	1.73 ± 0.07 d	63.8 ± 1.5 e
Salinity	62.7 ± 1.2 f	1.65 ± 0.06 d	60.9 ± 1.4 f
Drought + Salinity	54.6 ± 1.1 g	1.32 ± 0.05 e	52.4 ± 1.2 g
Stress + SiNPs (50 mg L^{-1})	66.9 ± 1.3 e	1.78 ± 0.07 d	64.3 ± 1.4 e
Stress + SiNPs (100 mg L^{-1})	72.8 ± 1.5 d	2.01 ± 0.08 c	70.7 ± 1.6 d
Stress + SiNPs (150 mg L^{-1})	79.5 ± 1.7 c	2.19 ± 0.08 bc	76.2 ± 1.7 c

The value is illustrated as the mean ± SE (n = 4). Various letters inside one column, utilising the Duncan multiple range test at (P < 0.05), show significant variations.

Table 3. Effects of silica nanoparticles on proline content in *Plantago ovata* under stress conditions.

Treatment	Proline (µmol g^{-1} FW)
Control	2.8 ± 0.2 d
SiNPs (100 mg L^{-1})	3.1 ± 0.3 d
Drought	6.9 ± 0.4 c
Salinity	7.5 ± 0.5 c
Drought + Salinity	10.8 ± 0.6 a
Combined stress + SiNPs (50 mg L^{-1})	8.6 ± 0.5 b
Combined stress + SiNPs (100 mg L^{-1})	7.2 ± 0.4 c
Combined stress + SiNPs (150 mg L^{-1})	6.5 ± 0.3 c

The value is illustrated as the mean ± SE (n = 4). Various letters inside one column, utilising the Duncan multiple range test at (P < 0.05), show significant variations.

Chlorophyll content and membrane stability had the same trend. The statistical letters in Table 2 indicate that SiNPs significantly improved these physiological traits under combined stress at $P < 0.05$, especially at the higher concentrations.

Silica nanoparticles boost plant growth under stressful conditions, shown by improved rates in growth, water content, and chlorophyll. Improved hydration status suggests that SiNPs enhanced water uptake and retention, possibly through improved root hydraulic conductance, reduced transpirational loss, and stabilisation of cellular membranes. These findings imply that SiNPs play an important role in ensuring physiological stability during stressful situations [26]. It has been previously shown that silica nanoparticles increase stress resistance due to increased water storage and structural protection [27].

Reduction in water and chlorophyll contents under stress conditions is an indication of poor water status and photosynthesis in plants [29]. However, enhancement in these factors by SiNPs demonstrates increased water absorption and retention ability and photosynthesis ability [41]. The membrane stability index results also validate the significance of SiNPs in the preservation of cell integrity in stressful situations.

Proline accumulation increased markedly under stress conditions (Table 3). Higher proline production was noted for drought- and salt-stressed plants than for control plants, with the maximum amount of proline being produced in the case of stress combination (10.8 µmol g^{-1} FW). SiNPs application progressively reduced proline accumulation under combined stress. This reduction suggests that SiNPs-treated plants were exposed to lower osmotic stress intensity than untreated plants under the combined pressures of salinity and drought. The decline in the proline buildup in the plants that were treated also shows that the stress intensity has been reduced since proline is related to osmotic stress [33].

The oxidative stress markers showed significant differences due to stress treatments (Table 4). The maximum values of MDA and H_2O_2 occurred with drought plus salinity stress treatment. SiNPs treatment reduced both of these indicators of oxidative stress, and the maximum reduction was found with a 150 mg L^{-1} concentration. Antioxidant enzymes like POD, CAT, and SOD became more active in response to stress, while less activity occurred in treated stressed plants. The grouping letters in Table 4 support notable variations between treatments at $P < 0.05$. The lower H_2O_2 and MDA values in SiNPs-treated plants indicate reduced oxidative injury, while the moderated enzyme activities suggest a lower need for stress-induced antioxidant activation.

Notably, the results demonstrated the decrease in the markers of oxidative stress after exposure to SiNPs. The reduction in hydrogen peroxide and malondialdehyde shows that there is no lipid peroxidation or generation of reactive oxygen species [11]. It has already been demonstrated that reactive oxygen species cause cell damage if uncontrolled [12]. This protective effect is consistent with enhanced antioxidant buffering and greater maintenance of redox homeostasis [34]. Whereas there was an enhancement in the activities of antioxidant enzymes due to stress conditions, the lower values seen under SiNPs treatment indicate less oxidative stress. This shows that SiNPs assist in keeping the equilibrium of redox through restraining the generation of

Table 4. Effects of silica nanoparticles on oxidative stresses and antioxidant enzyme activity indices in *Plantago ovata*.

Treatment	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	POD (U mg^{-1} protein)	MDA (nmol g^{-1} FW)	H_2O_2 (µmol g^{-1} FW)
Control	112 ± 4 b	56 ± 2 b	71 ± 3 b	2.14 ± 0.11 e	3.28 ± 0.15 e
SiNPs (100 mg L^{-1})	124 ± 5 c	63 ± 3 c	78 ± 4 c	1.92 ± 0.10 f	3.04 ± 0.14 f
Drought	146 ± 6 d	74 ± 3 d	92 ± 4 d	3.86 ± 0.17 c	5.47 ± 0.22 c
Salinity	152 ± 6 d	78 ± 3 d	97 ± 4 d	4.02 ± 0.18 c	5.84 ± 0.24 c
Drought + Salinity	169 ± 7 a	89 ± 4 a	112 ± 5 a	4.78 ± 0.21 a	6.92 ± 0.29 a
Stress + SiNPs (50 mg L^{-1})	156 ± 6 d	80 ± 3 d	98 ± 4 d	3.94 ± 0.18 c	5.61 ± 0.23 c
Stress + SiNPs (100 mg L^{-1})	143 ± 5 d	73 ± 3 d	90 ± 4 d	3.21 ± 0.15 d	4.82 ± 0.20 d
Stress + SiNPs (150 mg L^{-1})	131 ± 5 c	66 ± 3 c	83 ± 4 c	2.68 ± 0.13 e	4.11 ± 0.18 e

The value is illustrated as the mean ± SE (n = 4). Various letters inside one column, utilising the Duncan multiple range test at ($P < 0.05$), show significant variations.

reactive oxygen species that are reactive [14].

The concentrations of ions were greatly influenced by salinity and combined stress (Table 5). The concentration of Na^+ increased with stress treatment, especially with combined stress, whereas the K^+ content decreased to give a low K^+/Na^+ ratio. The application of SiNPs decreased Na^+ uptake but raised the K^+ content to improve the K^+/Na^+ ratios with increased concentrations of nanoparticles. The lowest K^+/Na^+ ratio was recorded under combined stress, while the application of SiNPs progressively improved this ratio. The highest improvement among stressed plants was recorded at 150 mg L^{-1}, and the statistical grouping letters in Table 5 confirm significant treatment differences at $P < 0.05$.

Aside from their regulatory function in redox metabolism, silica nanoparticles were also involved in the maintenance of ionic homeostasis. This was seen from the lower level of Na^+ accumulation and higher rate of potassium intake, hence the better K^+/Na^+ ratio required for enzyme activities during salt stress [42]. Ionic homeostasis is generally acknowledged as a crucial component of salt resistance in plants.

The correlation analysis indicated that all growth, physiological, biochemical, and ion characteristics were highly intercorrelated (Fig.

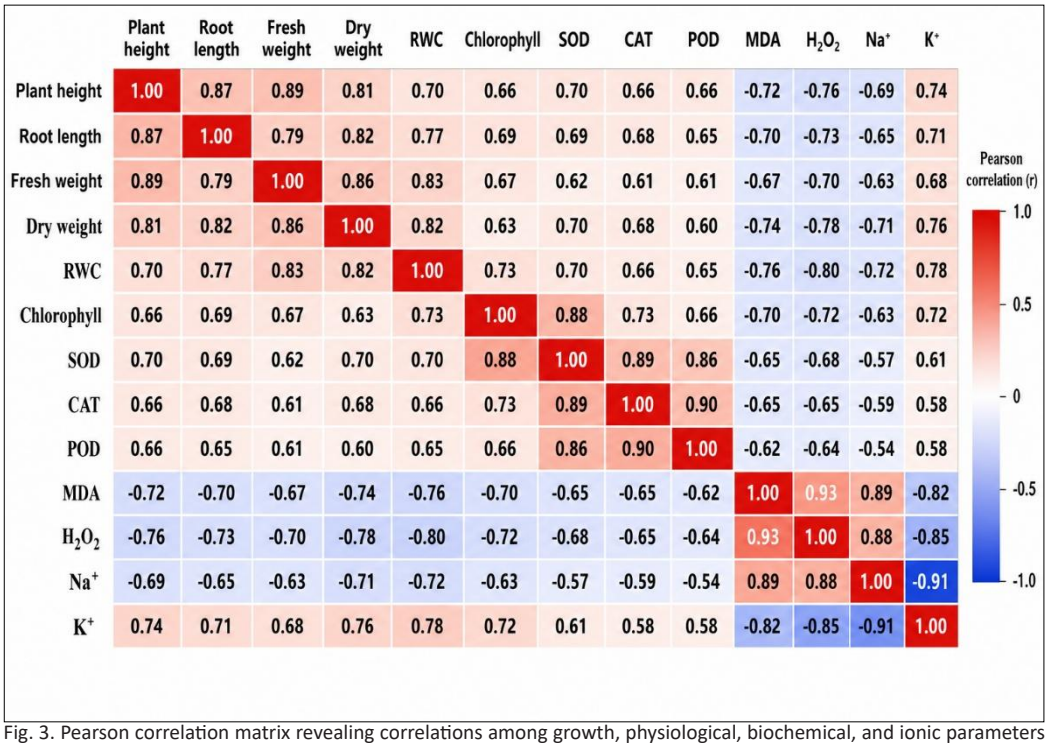

Fig. 3. Pearson correlation matrix revealing correlations among growth, physiological, biochemical, and ionic parameters in *Plantago ovata*. Red denotes positive connections, whilst blue denotes negative correlations.

Table 5. Effects of silica nanoparticles on ion accumulation and ionic balance in *Plantago ovata* under stress conditions.

Treatment	Na^+ (mg g^{-1} DW)	K^+ (mg g^{-1} DW)	K^+/Na^+ ratio
Control	1.21 ± 0.05 f	8.54 ± 0.32 b	7.06 ± 0.28 a
SiNPs (100 mg L^{-1})	1.17 ± 0.04 f	9.02 ± 0.35 a	7.71 ± 0.31 a
Drought	1.68 ± 0.07 e	7.14 ± 0.29 c	4.25 ± 0.19 c
Salinity	3.42 ± 0.14 b	6.11 ± 0.26 d	1.79 ± 0.08 e
Drought + Salinity	4.05 ± 0.18 a	5.62 ± 0.24 d	1.39 ± 0.07 f
Stress + SiNPs (50 mg L^{-1})	3.18 ± 0.13 c	6.47 ± 0.27 d	2.03 ± 0.09 e
Stress + SiNPs (100 mg L^{-1})	2.64 ± 0.11 d	7.12 ± 0.29 c	2.70 ± 0.12 d
Stress + SiNPs (150 mg L^{-1})	2.11 ± 0.09 e	7.84 ± 0.31 c	3.71 ± 0.16 c

The value is illustrated as the mean ± SE (n = 4). Various letters inside one column, utilising the Duncan multiple range test at ($P < 0.05$), show significant variations.

3). There were positive correlations between growth and physiological characteristics, including chlorophyll and relative water content. Oxidative stress indicators had a negative correlation with growth parameters. Sodium (Na^+) had a negative correlation, while potassium (K^+) had a positive correlation with growth parameters. These relationships agree with the treatment results, where better growth was associated with higher RWC, chlorophyll content, and K^+ accumulation, whereas growth reduction was linked with higher Na^+, H_2O_2, and MDA levels.

Further evidence for integration was provided by correlation and principal component analysis. The positive correlation of growth characteristics, potassium, relative water, and chlorophyll contents is suggestive of the coordination of control of the physiology of plants under stress. Negative correlation with oxidative stress markers is indicative of the damaging consequences of stress on plants' physiology.

PCA provided extra insight on the association between treatments and assessed attributes (Fig. 4). The first main component accounted for 56.8% of the variation as a whole and was largely connected with growth features, chlorophyll content, relative water content, and K^+ concentration. The second major component explained 25.6% of the variation and was related to oxidative stress markers and antioxidant enzyme activity. Together, PC1 and PC2 accounted for 82.4% of the overall variance. The PCA biplot showed a distinct difference between SiNPs-treated plants, stress-tolerant plants, and control plants, showing differential physiological and biochemical responses under the three treatments.

Overall, this suggests that silica nanoparticles improve plant resilience when facing both drought and salt stress. These stresses alter physiological, biochemical, and ionic processes. Unlike studies that investigate a single stress factor, the present research evaluates plant responses to the simultaneous occurrence of drought and salinity. Therefore, it offers a greater understanding of the

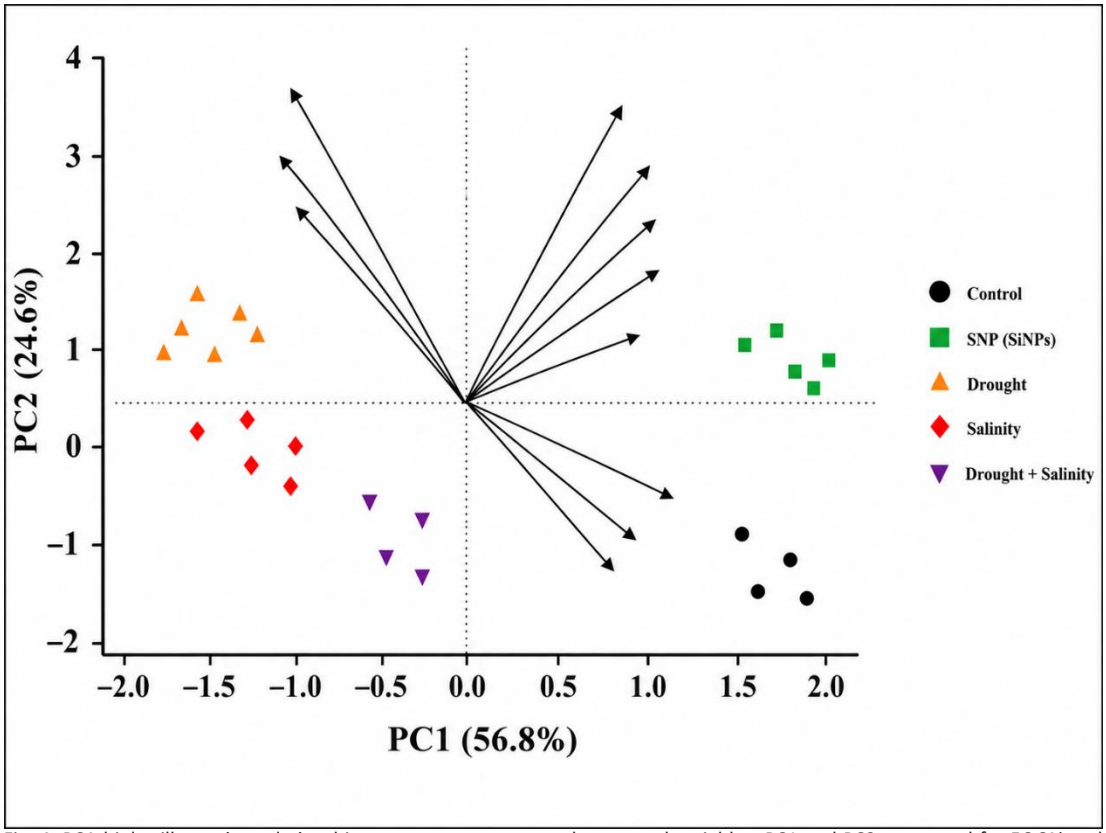

Fig. 4. PCA biplot illustrating relationships among treatments and measured variables. PC1 and PC2 accounted for 56.8% and 25.6% of the total variation, respectively.

systems involved in stress adaptation.

Study Limitations

This work gives helpful details regarding *P. ovata*'s reaction to salinity stress and drought; however, there are a few restrictions to be aware of. The experiments were conducted in a greenhouse with regulated growth substrate, humidity, and temperature, which may not adequately replicate the variety of outdoor environments. Field settings are more complex and contain various interacting elements that were not investigated in this study. Furthermore, the use of four biological replicates may limit the broader application of the data. The study also did not address the molecular mechanisms involved in SiNPs-mediated stress tolerance, such as ion transport, aquaporin function, and antioxidant-related gene expression. Therefore, further research under field settings and at the molecular level is necessary to comprehend the function of silica nanoparticles for improving the tolerance of plants to environmental challenges.

CONCLUSION

Silica nanoparticles boosted the ability of *P. ovata* to survive a combination of drought and salt stress. Treated plants maintained greater growth under adverse conditions, along with higher chlorophyll content and improved hydration status. The treatment also lowered oxidative stress, indicating lower cellular damage. The improvement in the K^+/Na^+ ratio further implies that silica nanoparticles helped control ion balance under stress. These findings indicate the possible use of nano-silica to boost crop tolerance in settings where drought and salinity occur simultaneously.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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