



RESEARCH ARTICLE



Effects of Iron Nanoparticles and Salinity on Growth and Physiology of Onion (*Allium cepa*)

Fouzia Hameed¹, Shamas Ul Rehman¹, Zainab Naseem², Atia Nazir³, Saher Nawaz^{1,4}  

¹Department of Botany, Ghazi University, Dera Ghazi Khan 32200, Pakistan

²Department of Botany, University of Agriculture, Faisalabad 38040, Pakistan

³School of Agricultural Engineering and Food Science, Shandong Research Center of Engineering & Technology for Clean Energy, Shandong University of Technology, Zibo 255000, China

⁴State Key Laboratory of Grassland Agro-ecosystems and Herbage Improvement, College of Ecology, Lanzhou University, Lanzhou 730000, China

METADATA

Paper history

Received: 30 July 2026

Revised: 24 September 2026

Accepted: 21 October 2026

Published online: 31 October 2026

Corresponding author

Saher Nawaz¹

Keywords

Photosynthesis

Oxidative stress

Malondialdehyde

Mineral nutrients

Chlorophyll

Antioxidants

Citation

Hameed F, Rehman SU, Naseem Z, Nazir A, Nawaz S (2026) Interactive effects of iron nanoparticles and salinity on growth and physiology of onion (*Allium cepa*).

Innovations in STEAM: Research & Education 4(2): 26040203.

<https://doi.org/10.63793/ISRE/0038>.

ABSTRACT

Background: Salinity is one of the most significant abiotic stresses affecting crop production, which affects plant growth, photosynthesis, oxidative balance and nutrient uptake. The application of nanotechnology, especially the iron nanoparticles (Fe-NPs) is a promising strategy for enhancing plant tolerance to saline environments.

Objective: The objective of this study was to investigate the role of spinach-mediated iron nanoparticles in alleviating salinity-induced damage in onion (*Allium cepa* L.) in terms of growth, physiological, biochemical and mineral responses.

Methodology: The pot experiment was carried out in 3 × 4 factorial completely randomized design (3 replicates) with salinity: 0, 150 and 200 mM NaCl and Fe-NP foliar application: 0, 250, 350 and 450 ppm. The growth parameters, photosynthetic pigments, oxidative stress parameters, antioxidant metabolites and mineral nutrients were measured by standard procedures.

Results: The salinity reduced growth, photosynthetic contents, and nutrient accumulation, and increased the malondialdehyde (MDA) accumulation that suggests increased oxidative damage. Foliar Fe-NPs improved growth parameters, increased chlorophyll (a & b) and carotenoids, and decreased lipid peroxidation, while increasing antioxidant compounds such as flavonoids, ascorbic acid, and phenolics. Fe-NPs also positively affected mineral nutrient balance by enhancing the Fe accumulation and minimizing ionic disturbances due to salinity. The greatest ameliorative action was observed at 450 ppm.

Conclusion: The spinach mediated iron nanoparticles significantly reduced the physiological and biochemical constraints of onion under salinity stress by enhancing photosynthetic pigments, antioxidant defense and nutrient homeostasis. Iron nanoparticles are a promising approach to enhance onion performance under salinity stress.

INTRODUCTION

Salinity is one of the major abiotic stresses affecting agricultural productivity in areas where irrigation and soil conditions lead to the buildup of soluble salts. Exposure to higher concentrations of salt for longer periods leads to reduced plant growth, photosynthesis, metabolic activity,

water uptake and ionic balance (Attiq *et al.* 2021), eventually causing a reduction in yield of crops (Raphael and Parthasarathi 2026). Salinity effects are especially evident in the disruption of the photosynthetic processes. Salt-induced water limitation and ionic imbalance may cause decreased pigment accumulation and impairment of photosynthetic machinery (Liu *et al.* 2019). Thus,



chlorophyll (a, b and total chlorophyll) and carotenoids are good markers to assess plant physiological response to salinity (Bistgani *et al.* 2019). Another consequence of salt exposure is the increased formation of reactive oxygen species (ROS). When ROS production becomes excessive, cellular membranes and other macromolecules may be damaged. One outcome is lipid peroxidation and the associated accumulation of malondialdehyde (MDA), which is widely used to assess membrane injury. Plants also rely on non-enzymatic antioxidants, including phenolics, flavonoids, and ascorbic acid, to strengthen their antioxidant protection (Kanwal *et al.* 2024). Mineral nutrition is also affected by salinity because the accumulation of Na^+ can interfere with the uptake and distribution of other essential ions, particularly K^+ . The ability to maintain ionic balance is therefore important for several processes, including enzyme activity, osmotic adjustment, stomatal functioning, and photosynthetic metabolism (Zamir *et al.* 2026). Consequently, implementation of efficient strategies to improve nutrient status while limiting salt-induced ionic disturbances may contribute to greater plant tolerance.

Onion is an economically important vegetable crop cultivated extensively in many agricultural systems (Sekara *et al.* 2017). Its productivity depends on adequate vegetative development, efficient nutrient acquisition, and efficient photosynthetic machinery. However, salinity stress can substantially impair onion growth by reducing morphological attributes, including shoot and root development, leaf number, leaf area, and fresh and dry biomass accumulation (Patil *et al.* 2026). Nanotechnology provides a potential approach for improving plant resilience to combat various stresses like salinity (Al-Khayri *et al.* 2023). Iron is an essential micronutrient involved in important metabolic and photosynthetic processes, and nanoparticle-based Fe delivery may provide an alternative means of supplying Fe to stressed plants (Sen *et al.* 2025). Plant extracts particularly attractive approach to obtain green-synthesized nanoparticles such as iron, because plant-derived compounds can participate in nanoparticle formation and may provide an alternative to conventional synthesis routes (Shahzadi *et al.* 2025). Although the beneficial effects of nanoparticles in improving salt tolerance have been reported in several crops, very limited information is available relevant to the integrated response of green-synthesized iron nanoparticles under salinity stress in onion plants, particularly considering growth performance, photosynthetic pigments, oxidative stress responses, and mineral nutrient balance. We hypothesized that foliar application of spinach-mediated iron oxide nanoparticles would partially alleviate salinity-induced damage in onion by improving growth and photosynthetic pigment accumulation, strengthening antioxidant-related responses, and improving mineral nutrient balance. Therefore, this study is directed to investigate the influence of various concentrations of spinach-mediated iron oxide nanoparticles on growth and physiological characteristics of

onion plants under saline conditions. For this purpose, influence of nanoparticles under high salt contents on photosynthetic pigments, oxidative-stress-related attributes, and changes in mineral nutrient accumulation, particularly K^+ , Na^+ , and Fe, is determined.

MATERIALS AND METHODS

Experimental site and soil preparation

The experiment was conducted during 2023–2024 in the Department of Botany, Ghazi University, Dera Ghazi Khan, Pakistan. Soil samples were collected from the upper 0–30 cm layer of an experimental site located in the Department of Botany, Dera Ghazi Khan. The soil was air-dried for approximately three days, crushed, cleared of stones and other coarse materials, then passed through a 2 mm sieve for further cleaning. After that, physicochemical properties were analysed by standard analysis protocols (Table 1).

Plant material and experimental design

The experiment was designed to estimate the influence of salinity stress and foliar-applied spinach-mediated iron nanoparticles (Fe-NPs) on onion (*Allium cepa* L.) plants. The design was completely randomized (CRD) with a factorial arrangement of treatments. In a controlled environment, plant seedlings were grown in pots. After thirty days of seedling establishment, saline stress was applied. Three concentrations of NaCl (0, 150, and 200 mM) were applied in combination with foliar application of Fe-NP (0, 250, 350, and 450 ppm) as a mitigant.

Table 1: Physicochemical properties of soil

Soil characters	Value
Texture of soil	Sandy loam
pH	7.8
EC_e	4.90 dS m^{-1}
Saturation (%)	42
Organic matter (%)	0.56
Available phosphorus (mg kg^{-1})	8.47
Available potassium (mg kg^{-1})	151

Synthesis of nanoparticles

By using Abert *et al.* (2022) method synthesis of iron-based nanoparticles was done in the laboratory of the Botany Department at Ghazi University, Dera Ghazi Khan. The iron oxide nanoparticles were extracted from spinach (*Spinacia oleracea*). For this purpose, ferric chloride hexahydrate was mixed with ferrous chloride tetrahydrate (1:2 molar ratio), then dissolved in 100 mL of water. The mixture was heated at 80 °C with constant stirring. After 10 min, spinach extract (20 mL) was added. The solution changed color from pale yellow to dark brown, indicating the formation of nanoparticles. For controlled precipitation of Fe-nanoparticles, 20 mL of sodium hydroxide was added

gradually (3 mL per min). The mixture was left to cool at ambient temperature. Then decantation was done to get the nanoparticles. To remove the impurities, thorough rinsing was done using distilled water. Then dried at room temperature for experimental use.

Data collection and analysis

Plants were uprooted approximately 15 days after Fe-NP treatment for morphological, physiological, and elemental analysis measurements. For fresh and dried analysis, harvested plants were stored carefully. For fresh analysis, plants were labeled, placed in zipper bags, and frozen at -40°C . For dry analysis, half of harvested plants were put in brown bags, then oven-dried for 1 week at 65°C .

Growth parameters

For fresh biomass determination, plants were uprooted, shoots and roots were separated and weighed immediately on a top-load weight balance. The plant shoots and roots were put in brown bags, then, for complete drying, placed in an oven at 65°C and then weighed on a top-load weight balance.

Photosynthetic pigments

Arnon (1949) and Davies (1977) methods were used to determine photosynthetic contents of onion plants by using fresh leaves. 0.1 g of sample was ground with 5 mL of acetone (80%). The solution was filtered to take readings at various wavelengths (480, 663, and 645 nm) using a spectrophotometer. For final calculations, the following formulas were used:

$$\begin{aligned}\text{Chl. } a \text{ (mg/g f. wt.)} &= [12.7(A_{663}) - 2.69(A_{645})] \times 5/1000 \times 0.1 \\ \text{Chl. } b \text{ (mg/g f. wt.)} &= [22.9(A_{645}) - 4.68(A_{663})] \times 5/1000 \times 0.1 \\ \text{Carotenoids (mg/mL)} &= [(A_{480}) + 114(A_{663}) - 0.638(A_{645})] \times V/2500 \times W\end{aligned}$$

Oxidative stress and antioxidants

Heath and Packer (1968) method was used to determine malondialdehyde in plants to measure lipid peroxidation. Fresh samples of roots and shoots (0.5 g) were ground with 5 mL trichloroacetic acid (TCA; 5%), centrifuged (15 min), and the supernatant collected. Mixed with TBA (thiobarbituric acid) and incubated for 25 min (100°C). Measurements were taken at 533 and 600 nm.

Flavonoid concentration was determined separately in leaf and root tissues using the Zhishen *et al.* (1999) method with fresh plant extract. For extraction, methanol (80%) was used. Sodium nitrate and hydroxide were added to the plant extract with aluminum chloride. The readings were noted at 510 nm using a spectrophotometer. Final readings were calculated using a standard curve. To measure the total phenolic content in roots and shoots Julkunen-Tiitto (1985) Folin-Ciocalteu colorimetric assay was used. Fresh root and

shoot samples were ground with acetone (80%). Extracted and mixed with reagent (Folin-Ciocalteu) and sodium carbonate solution. At 730 nm readings were noted using a spectrophotometer.

Fresh roots and leaves were used to determine ascorbic acid content by following the Gillespie and Ainsworth (2007) method. Samples were ground with metaphosphoric acid (5%) and centrifuged. Supernatant collected and mixed with 2,6-dichlorophenol indophenol. Using a spectrophotometer, readings were noted at 520 nm.

Elemental analysis

0.5 g of dried plants were digested with concentrated acid (HNO_3). Added distilled water for final volume of 50 mL. To estimate phosphate-P contents Yoshida *et al.* (1971) procedure was used. The digested sample was mixed with HNO_3 (2N) and reagent (molybdate-vanadate). Incubated (20 min), and values were taken at 420 nm. Kowalenko and Lowe (1973) procedure was followed to calculate nitrate-N. The digested sample was mixed with CTA (chromotropic acid). Values were taken at 430 nm. Atomic absorption was used to estimate potassium, iron, and sodium using digested samples.

Statistical analysis

The experimental factorial design was completely randomized. Salinity and nanoparticle concentrations were recorded as two factors. For analysis of data, two-way analysis of variance (ANOVA) was used to evaluate the influence of salinity concentrations on Fe nanoparticle levels in Statistix 8.1 software. Then Tukey's test was used for further analysis, where significance was at $P \leq 0.05$. Origin software was used to represent graphical pictures of results, where values were shown as mean $\pm$ standard error (SE).

RESULTS

Growth attributes

ANOVA showed that salinity and foliar-applied Fe-NP significantly affected onion growth attributes. The interaction between salinity levels and Fe-NP treatments was significant ($P < 0.05$) for shoot and root biomass parameters (Fig. 1). Salinity stress significantly reduced shoot (fresh and dry) and root (fresh and dry) weights as compared to control plants. Increasing levels of NaCl (0, 150, and 200 mM) progressively decreased biomass accumulation, and the lowest values were recorded under 200 mM NaCl without Fe-NP application. It was observed at 200 mM NaCl there was more decline in growth compared to 150 mM salinity. However, exogenous application of Fe-NP significantly enhanced

growth traits in normal and saline conditions. Compared with untreated plants, Fe-NP-treated plants showed increased shoot and root biomass, with a progressive improvement as Fe-NP concentration increased. Shoot and root (fresh and dry) weights were enhanced at 450 ppm Fe-NPs, followed by 350 and 250 ppm Fe-NPs treatment.

Photosynthetic pigments

Statistical analysis revealed that salinity stress and foliar Fe-NP application significantly affected photosynthetic pigment contents of onion plants. Salinity significantly diminished chlorophyll (a, b & total) and carotenoid contents, whereas Fe-NP application significantly improved pigment accumulation in non-stress and stress conditions (Fig. 2). The interaction between salinity and Fe-NP treatments showed significant variation among treatment combinations ($P < 0.05$). Results showed that increasing NaCl concentration from 0 to 150 and 200 mM significantly decreased chlorophyll *a* and *b* content. The maximum reduction was observed under 200 mM NaCl without Fe-NP application, whereas control plants maintained the highest chlorophyll *a* concentration. Foliar-applied Fe-NPs significantly improved chlorophyll (*a* & *b*) contents, and maximum enhancement was recorded in 450 ppm Fe-NPs followed by 350 ppm and 250 ppm treatments under normal and stress conditions.

High salt contents significantly reduced the total chlorophyll contents, whereas Fe-NP application progressively increased chlorophyll accumulation. The highest value of total chlorophyll was observed under 450 ppm Fe-NPs, followed by 350 ppm and 250 ppm treatments, demonstrating the protective effect of Fe-NPs on the photosynthetic apparatus under salt stress. Carotenoid contents also showed significant variation among salinity $\times$ Fe-NP combinations. Increasing NaCl concentration reduced carotenoid accumulation, while Fe-NP application significantly improved carotenoid content under stress conditions.

Oxidative stress and antioxidant responses

Salinity and foliar Fe-NP application significantly influenced oxidative stress and antioxidant metabolites in onion (Fig. 3a, 3b). Their interaction was also significant ($P < 0.05$), as reflected by the different Tukey grouping letters among treatment combinations.

MDA accumulation increased significantly in both leaf and root tissues under salinity and reached its highest level at 200 mM NaCl, indicating greater lipid peroxidation. Foliar Fe-NP application lowered MDA relative to untreated salt-stressed plants. In contrast, flavonoids, ascorbic acid, and total phenolics generally increased following Fe-NP application. The highest antioxidant accumulation occurred

at 450 ppm Fe-NPs, followed by 350 and 250 ppm. Thus, Fe-NP treatment was associated with lower oxidative damage and stronger antioxidant responses under saline conditions (Fig. 3a, 3b).

Elemental composition

Statistics revealed that salinity and foliar-applied Fe-NP significantly affected mineral nutrient accumulation in onion plants (Fig. 4a, 4b, 4c). The salinity $\times$ Fe-NP interaction was significant ($P < 0.05$) for all analysed elements except leaf potassium, which showed a non-significant interaction ($P > 0.05$). Increasing NaCl concentration disturbed mineral balance by decreasing essential nutrient accumulation and increasing sodium levels, particularly under 200 mM NaCl stress. Foliar Fe-NP application significantly improved nutrient status under saline conditions by enhancing iron accumulation, improving potassium retention, and reducing excessive sodium accumulation. Iron concentration in leaf and root tissues increased progressively with increasing Fe-NP levels, with the maximum accumulation recorded under 450 ppm Fe-NPs, followed by 350 and 250 ppm treatments. Similarly, Fe-NP application improved nitrate and phosphate accumulation under salt stress. Overall, Fe-NPs enhanced nutrient acquisition and helped to maintain ionic homeostasis under saline conditions.

Correlation matrix

Pearson correlation of treatment means showed tight coupling among most traits (Fig. 8). Growth traits (shoot and root fresh and dry weight) were strongly positively correlated with chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoids, nitrate, phosphate, potassium, iron and phenolics ($r = 0.84$ to 0.99 , $P < 0.001$). The same growth traits were strongly negatively correlated with leaf and root MDA ($r \approx -0.95$ to -0.98 , $P < 0.001$) and with leaf and root sodium ($r \approx -0.95$ to -0.96 , $P < 0.001$). Sodium itself was strongly positively correlated with MDA ($r \approx 0.96$ to 0.97 , $P < 0.001$). Nitrate, phosphate and potassium were strongly positively correlated with one another ($r \geq 0.99$) and negatively correlated with sodium ($r \approx -0.83$ to -0.89 , $P < 0.001$). Flavonoids and ascorbic acid were only weakly or moderately correlated with the other traits ($|r| \leq 0.57$), so they did not track the growth and nutrient response (Fig. 5).

DISCUSSION

Salinity affects crop productivity by causing osmotic imbalance, ionic toxicity, oxidative damage and disrupting normal physiological processes. The onion growth such as shoot and root biomass decreased with the increase of NaCl concentration in this study. Salinity-induced growth

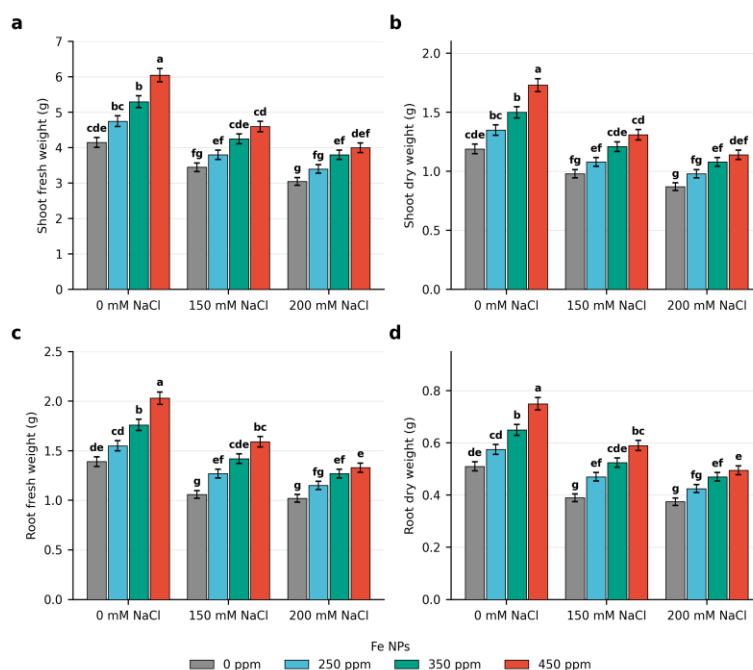


Fig. 1: Foliar application of spinach-mediated iron nanoparticles (Fe NPs) improves growth of onion plants under salinity stress. a, Shoot fresh weight. b, Shoot dry weight. c, Root fresh weight. d, Root dry weight. Plants were grown under 0, 150 or 200 mM NaCl and sprayed with Fe NPs at 0, 250, 350 or 450 ppm. Data are mean $\pm$ SE. Different letters indicate significant differences among treatments ($P \leq 0.05$).

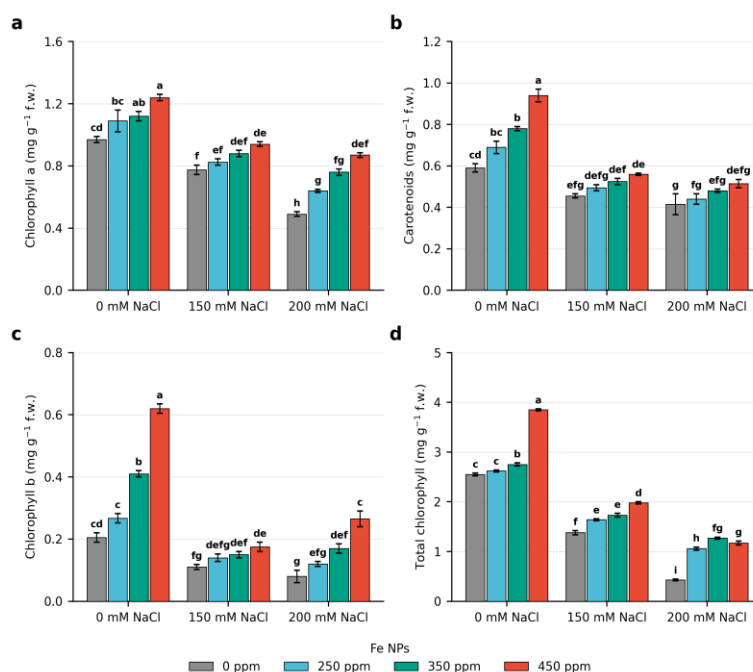


Fig. 2: Spinach-mediated iron nanoparticles (Fe NPs) restore photosynthetic pigments in onion plants under salinity stress. a, Chlorophyll a. b, Carotenoids. c, Chlorophyll b. d, Total chlorophyll content. Plants were grown under 0, 150 or 200 mM NaCl and sprayed with Fe NPs at 0, 250, 350 or 450 ppm. Pigment contents are expressed as mg g^{-1} fresh weight (FW). Data are mean $\pm$ SE. Different letters indicate significant differences among treatments ($P \leq 0.05$).

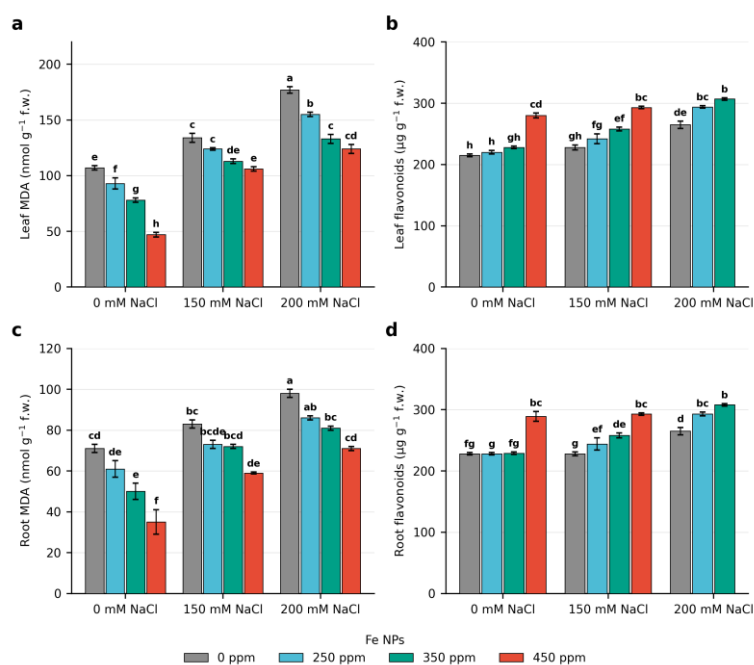


Fig. 3a: Foliar application of spinach-mediated iron nanoparticles (Fe NPs) improves growth of onion plants under salinity stress. a, Shoot fresh weight. b, Shoot dry weight. c, Root fresh weight. d, Root dry weight. Plants were grown under 0, 150 or 200 mM NaCl and sprayed with Fe NPs at 0, 250, 350 or 450 ppm. Data are mean $\pm$ SE. Different letters indicate significant differences among treatments ($P \leq 0.05$).

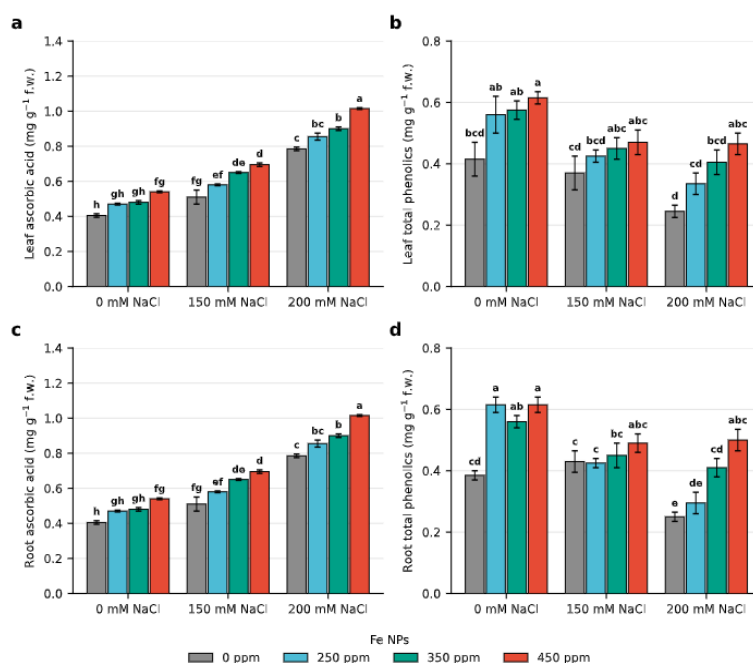


Fig. 3b: Spinach-mediated iron nanoparticles (Fe NPs) enhance non-enzymatic antioxidants in onion plants under salinity stress. a, Leaf ascorbic acid. b, Leaf total phenolics. c, Root ascorbic acid. d, Root total phenolics. Plants were grown under 0, 150 or 200 mM NaCl and sprayed with Fe NPs at 0, 250, 350 or 450 ppm. Contents are expressed as mg g⁻¹ fresh weight (f.w.). Data are mean $\pm$ SE. Different letters indicate significant differences among treatments ($P \leq 0.05$).

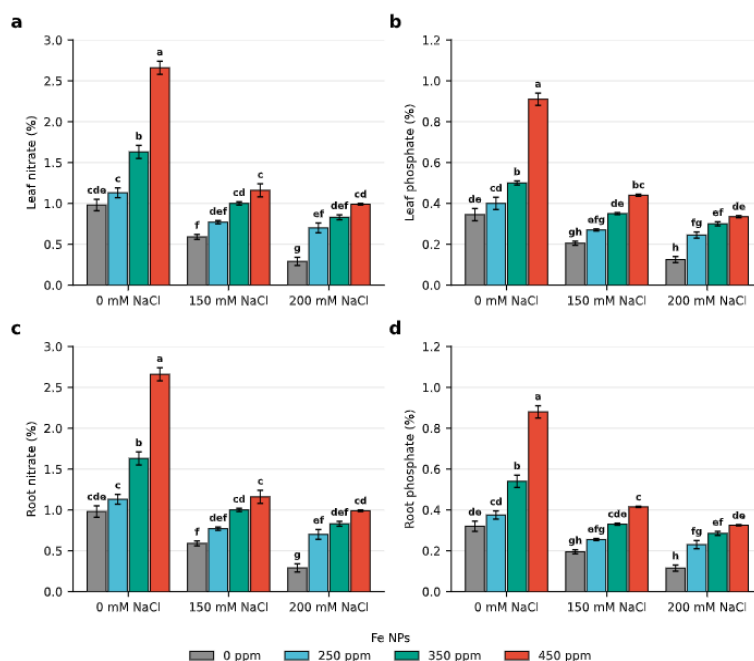


Fig. 4a: Spinach-mediated iron nanoparticles (Fe NPs) improve nitrate and phosphate accumulation in onion plants under salinity stress. a, Leaf nitrate. b, Leaf phosphate. c, Root nitrate. d, Root phosphate. Plants were grown under 0, 150 or 200 mM NaCl and sprayed with Fe NPs at 0, 250, 350 or 450 ppm. Contents are expressed as a percentage (%). Data are mean $\pm$ SE. Different letters indicate significant differences among treatments ($P \leq 0.05$).

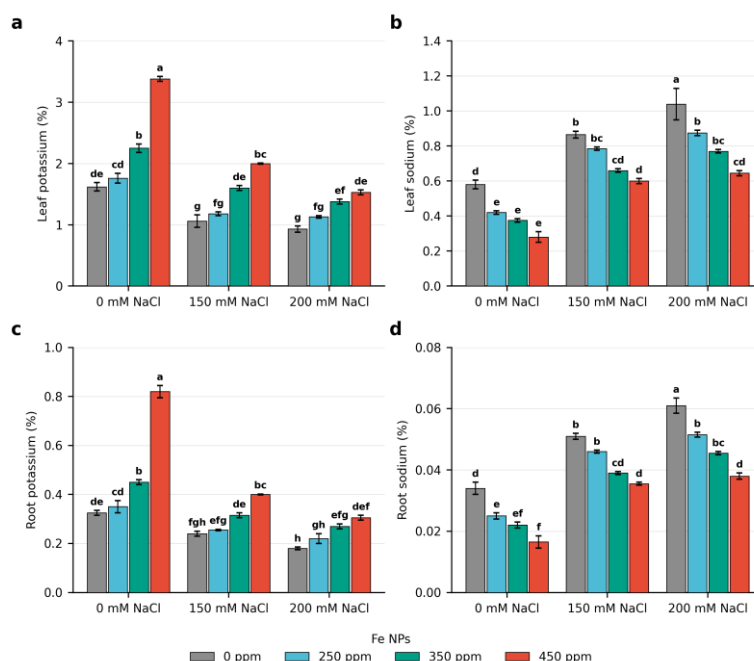


Fig. 4b: Spinach-mediated iron nanoparticles (Fe NPs) increase potassium and reduce sodium accumulation in onion plants under salinity stress. a, Leaf potassium. b, Leaf sodium. c, Root potassium. d, Root sodium. Plants were grown under 0, 150 or 200 mM NaCl and sprayed with Fe NPs at 0, 250, 350 or 450 ppm. Contents are expressed as a percentage (%). Data are mean $\pm$ SE. Different letters indicate significant differences among treatments according to Tukey's test ($P \leq 0.05$).

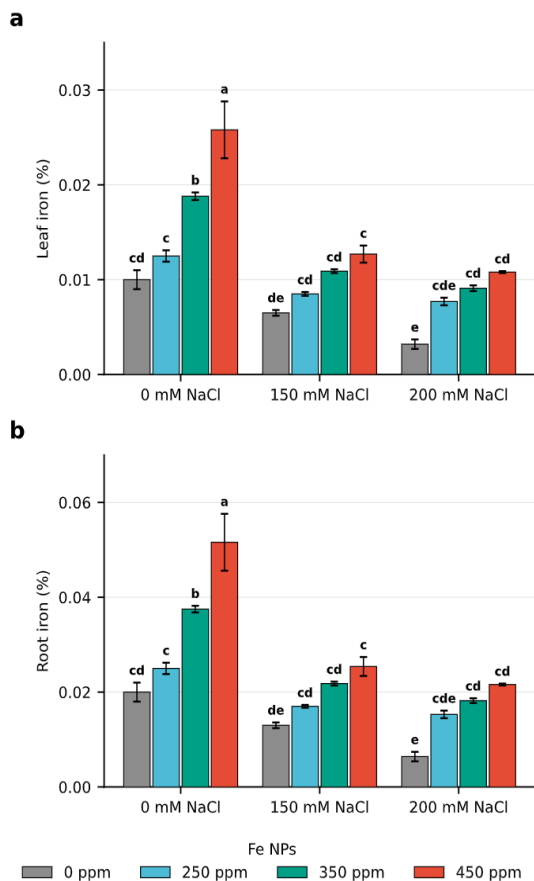


Fig. 4c: Spinach-mediated iron nanoparticles (Fe NPs) increase iron accumulation in onion plants under salinity stress. a, Leaf iron. b, Root iron. Plants were grown under 0, 150 or 200 mM NaCl and sprayed with Fe NPs at 0, 250, 350 or 450 ppm. Iron content is expressed as a percentage (%). Data are mean $\pm$ SE. Different letters indicate significant differences among treatments according to Tukey's test ($P \leq 0.05$).

inhibition can be due to cell growth limitation, reduction in water availability, decreased uptake of nutrients, and the build-up of Na^+ ions (Balasubramaniam *et al.* 2023). Ion balance of cells is also affected by high salt concentrations, and this disturbs metabolic activity leading to a decreased biomass production (Gul *et al.* 2022). Results indicated that the activity of growth inhibition increased with NaCl concentration, being more pronounced at 200 mM than at 150 mM. Foliar-applied spinach-mediated Fe-NPs significantly improved root and shoot biomass under high salt contents, demonstrating their protective role against salt-induced growth inhibition. Iron is considered an essential nutrient because of its involvement in chlorophyll biosynthesis, respiratory machinery, electron transport, and various other enzymatic activities (Rai *et al.* 2021). Improved Fe availability through nanoparticle application can enhance metabolic activity and nutrient utilization, which has been shown to enhance plant growth under stress

(Zia-ur-Rehman *et al.* 2023). The increase in surface area and bioavailability of Fe-NPs facilitates efficient nutrient delivery compared with conventional iron sources. A meta-analysis of Fe-based nanomaterials reported that Fe-NPs significantly improve plant biomass, antioxidant activity, and stress tolerance by enhancing nutrient availability and reducing oxidative damage (Tan *et al.* 2026). The application of iron-based nanoparticles improved growth and physiological characteristics of wheat plants by enhancing biomass productivity and reducing salt-induced damage (Singh *et al.* 2025).

The reductions in chlorophyll *a*, chlorophyll *b*, and carotenoids under high salinity indicate disturbance of the photosynthetic system. Under salt stress, chlorophyll level can be reduced due to limitation in its biosynthesis, increased degradation and oxidative damage to chloroplast membranes (Li *et al.* 2015). Carotenoids also play a role in photoprotection, through their ability to dissipate excess excitation energy and to scavenge ROS. They could then be expected to display less photoprotection under conditions of high salinity (Ramachandran *et al.* 2023).

Fe-NP application resulted in an increase in photosynthetic pigments, indicating the alleviating effect of Fe supply on the protection of pigment-related processes under salt stress. Iron is associated with chlorophyll biosynthesis and electron transfer via iron-containing proteins such as ferredoxin and cytochromes. Similarly, Fe-NPs were seen to enhance PSII activity and photosynthetic performance and pigment stability under saline conditions. The carotenoids increased following the treatment with Fe-NP, which is also consistent with enhanced protection from photo-oxidative damage and oxidative injury. Kreslavski *et al.* (2023) similarly reported that Fe_3O_4 nanoparticles improved photosynthesis, PSII activity, and pigment stability under saline conditions. The increase in carotenoids observed after Fe-NP treatment is also consistent with improved photoprotection and lower oxidative injury.

The rise in malondialdehyde (MDA) in onion leaves and roots showed that salinity increased oxidative stress in the present study. Higher MDA is associated with lipid peroxidation and membrane injury resulting from excessive ROS production (Nawaz *et al.* 2025). Under saline conditions, disruption of electron transport and other metabolic processes can increase ROS formation, thereby affecting membrane stability and cellular function (Hameed *et al.* 2021).

The decline in MDA after Fe-NP application indicates that oxidative injury was reduced. Iron participates in antioxidant metabolism, including as a cofactor in several antioxidant enzymes, and Fe-NPs may influence ROS-scavenging and cellular redox regulation (Kejik *et al.* 2021). In the present study, Fe-NP application also increased flavonoids, ascorbic acid, and phenolic compounds, showing a stronger non-enzymatic antioxidant response. Phenolics and flavonoids can directly scavenge ROS, while ascorbic acid contributes to regulation of the cellular redox

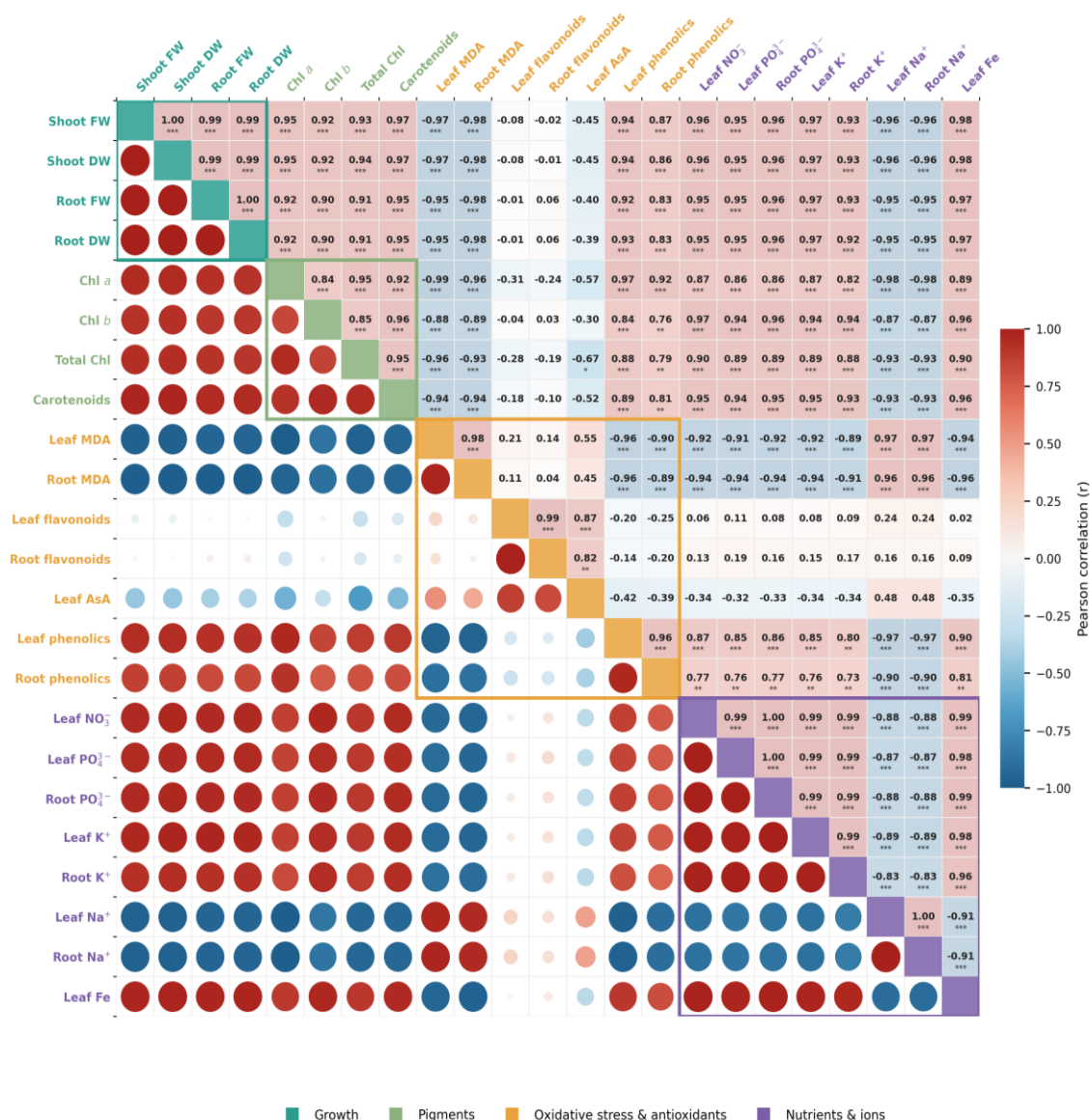


Fig. 5: Correlation among growth, pigment, antioxidant and nutrient traits of onion plants under salinity stress and Fe NP treatment. Pearson correlation matrix of 23 traits calculated from treatment means (3 NaCl levels $\times$ 4 Fe NP concentrations; $n = 12$). Lower triangle: circle size and color show the correlation coefficient (r), with red for positive and blue for negative correlation. Upper triangle: r values with significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Trait groups are color-coded: growth (teal), pigments (green), oxidative stress and antioxidants (orange), and nutrients and ions (purple). MDA, malondialdehyde; AsA, ascorbic acid; Chl, chlorophyll; FW, fresh weight; DW, dry weight.

state (Raphael and Parthasarathi 2026). Consistent with these responses, Hou *et al.* (2024) reported improved antioxidant metabolites and reduced oxidative stress following the use of Fe-based nanomaterials under stressful environments.

Salinity also altered mineral nutrient composition, including nitrate, phosphate, potassium, sodium, and iron (Nawaz *et al.* 2022). Excessive Na⁺ accumulation is a major component of salt toxicity because it competes with

essential nutrients and disrupts ionic balance. K⁺ is particularly important for salt tolerance because it regulates osmotic adjustment, enzyme activation, stomatal movement, and cellular metabolism. In the present study, salinity reduced K⁺ accumulation, while Fe-NP application improved nutrient balance under stress conditions. However, the salinity $\times$ Fe-NP interaction for leaf K⁺ was non-significant, suggesting that Fe-NP application did not significantly modify the effect of salinity on leaf K⁺

accumulation. The significant increase in Fe accumulation following Fe-NP application confirms effective uptake and utilization of foliar-applied nanoparticles. Improved stress tolerance may be mediated by enhanced Fe availability, which may lead to increased activity of antioxidant enzymes, electron transport and formation of chlorophyll (Kreslavski *et al.* 2023). Likewise, better accumulation of nitrate and phosphate following Fe-NP application is indicative of better nutrient uptake and utilization under saline conditions. An improvement in nutrient uptake and ion homeostasis with nanoparticles use has been reported as an important mechanism of salinity tolerance (Jamei *et al.* 2026). The decrease in Na^+ after Fe-NP application suggests that the plants in salinity are better controlled by the flow of Na^+ . Saline condition requires proper balance of Na^+ and K^+ intake for proper growth of plant: sodium high concentration causes disturbance in metabolic activities and causes damages in the cellular structure. Recent research shows that iron-based nanoparticles can control ion homeostasis, improve the efficacy of nutrient assimilation, and improve antioxidant defense systems in salinity (Alsamadany *et al.* 2024). Overall, it was found that use of Fe-NPs in the presence of excessive salt load significantly reduced the physico-biochemical disturbances in the onion plants. Multiple mechanisms were responsible for the positive effect of Fe-NPs such as improved biomass production, preservation of photosynthetic pigments, suppression of oxidative damage, increase of antioxidant metabolites, and regulation of balance of mineral nutrients. Of the applied treatment, 450 ppm Fe-NPs exhibited the highest ameliorative effect, suggesting that optimal application of Fe-NPs could be a favorable approach for enhancing the onion performance in saline environment (Fig. 5).

CONCLUSION

The growth, photosynthetic pigments, antioxidants, and mineral nutrient balance of onion plants were significantly reduced by salinity. The results show that foliar application of Fe-NPs also has a positive effect on alleviating the damage caused by salt stress by enhancing biomass production, maintaining photosynthetic pigments, reducing oxidative stress, and enhancing antioxidant metabolites. Fe-NPs also has the ability to regulate nutrient homeostasis by increasing Fe availability and controlling ionic balance under saline conditions. All treatments exhibited ameliorative activity with the 450 ppm Fe-NPs exhibiting the highest, followed by 350 and 250 ppm treatments. Overall, foliar Fe-NP application is a promising approach to enhance salinity stress tolerance in onion.

ACKNOWLEDGEMENTS

The authors acknowledge the Department of Botany, Ghazi University, Dera Ghazi Khan, Pakistan for providing laboratory facilities.

AUTHOR CONTRIBUTIONS

FH, SUR and SN conceived and designed the study; FH, SUR, ZN and AN performed the experiments and analyzed the data; FH wrote the first draft. SN supervised the work and administered the project; all authors reviewed, edited and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

The data will be made available on a fair request to the corresponding author.

ETHICS APPROVAL

Not applicable.

FUNDING SOURCE

The study did not receive any financial support.

REFERENCES

- Abert HO, Aduwamai HU, Adamu SS (2022) Effect of green synthesized iron oxide nanoparticles using spinach extract on Triton X-100-induced atherosclerosis in rats. *Biochemistry Research International* 2022(1): 9311227. <https://doi.org/10.1155/2022/9311227>.
- Al-Khayri JM, Rashmi R, Surya Ulhas R, Sudheer WN, Banadka A, Nagella P, Aldaej MI, Rezk AAS, Shehata WF, Almaghasla MI (2023) The role of nanoparticles in response of plants to abiotic stress at physiological, biochemical, and molecular levels. *Plants* 12(2): 292. <https://doi.org/10.3390/plants12020292>.
- Alsamadany H, Anayatullah S, Zia-ur-Rehman M, Usman M, Ameen T, Alharby HF, Alharbi BM, Abdulmajeed AM, Yong JWH, Rizwan M (2024) Residual efficiency of iron-nanoparticles and different iron sources on growth and antioxidants in maize plants under salt stress: life cycle study. *Heliyon* 10(7): e28973. <https://doi.org/10.1016/j.heliyon.2024.e28973>.
- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24(1): 1–15. <https://doi.org/10.1104/pp.24.1.1>.
- Attiq W, Tayyab Q, Ali Q (2021) Salinity stress in crops: mechanisms and mitigation techniques using PGPR and silicon. *Plant and Environment* 2(1): 36–54. <https://doi.org/10.54219/plantenviro.2021.9254>.
- Balasubramaniam T, Shen G, Esmaeili N, Zhang H (2023) Plants' response mechanisms to salinity stress. *Plants* 12(12): 2253. <https://doi.org/10.3390/plants12122253>.
- Bistgani ZE, Hashemi M, DaCosta M, Craker L, Maggi F, Morshedloo MR (2019) Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenensis* Celak. *Industrial Crops and Products* 135: 311–320. <https://doi.org/10.1016/j.indcrop.2019.04.055>.
- Davies B (1977) Carotenoids in higher plants. In: *Lipids and Lipid Polymers in Higher Plants*. Berlin, Heidelberg: Springer, pp 199–217.
- Gillespie KM, Ainsworth EA (2007) Measurement of reduced, oxidized and total ascorbate content in plants. *Nature Protocols* 2(4): 871–874. <https://doi.org/10.1038/nprot.2007.101>.

- Gul Z, Tang ZH, Arif M, Ye Z (2022) An insight into abiotic stress and influx tolerance mechanisms in plants to cope in saline environments. *Biology* 11(4): 597. <https://doi.org/10.3390/biology11040597>
- Hameed A, Ahmed MZ, Hussain T, Aziz I, Ahmad N, Gul B, Nielsen BL (2021) Effects of salinity stress on chloroplast structure and function. *Cells* 10(8): 2023. <https://doi.org/10.3390/cells10082023>
- Heath RL, Packer L (1968) Photooxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125(1): 189–198. [https://doi.org/10.1016/0003-9861\(68\)90654-1](https://doi.org/10.1016/0003-9861(68)90654-1)
- Hou D, Cui X, Liu M, Qie H, Tang Y, Xu R, Zhao P, Leng W, Luo N, Luo H, Lin A, Wei W, Yang W, Zheng T (2024) The effects of iron-based nanomaterials (Fe NMs) on plants under stressful environments: machine learning-assisted meta-analysis. *Journal of Environmental Management* 354: 120406. <https://doi.org/10.1016/j.jenvman.2024.120406>
- Jamei R, Onori O, Jamei M (2026) Nanoparticles and salinity stress: emerging insights into plant responses and stress mitigation. *Plant Stress* 20(3): 101265. <https://doi.org/10.1016/j.stress.2026.101265>
- Julkunen-Tiitto R (1985) Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry* 33: 213–217. <https://doi.org/10.1021/jf00062a013>
- Kanwal R, Maqsood MF, Shahbaz M, Naz N, Zulfiqar U, Ali MF, Jamil M, Khalid F, Ali Q, Sabir MA, Chaudhary T (2024) Exogenous ascorbic acid as a potent regulator of antioxidants, osmo-protectants, and lipid peroxidation in pea under salt stress. *BMC Plant Biology* 24(1): 247. <https://doi.org/10.1186/s12870-024-04947-3>
- Kejřík Z, Kapláněk R, Masařík M, Babula P, Matkowski A, Filipenský P, Veselá K, Gburek J, Sýkora D, Martásek P, Jakubek M (2021) Iron complexes of flavonoids-antioxidant capacity and beyond. *International Journal of Molecular Sciences* 22(2): 646. <https://doi.org/10.3390/ijms22020646>
- Kowalenko CG, Lowe LE (1973) Determination of nitrates in soil extracts. *Soil Science Society of America Journal* 37(4): 660. <https://doi.org/10.2136/sssaj1973.03615995003700040051x>
- Kreslavski VD, Shmarev AN, Ivanov AA, Zharmukhamedov SK, Strokina V, Kosobryukhov A, Yu M, Allakhverdiev SI, Shabala S (2023) Effects of iron oxide nanoparticles (Fe₃O₄) and salinity on growth, photosynthesis, antioxidant activity and distribution of mineral elements in wheat (*Triticum aestivum*). *Functional Plant Biology* 50(11): 932–940. <https://doi.org/10.1071/FP23085>
- Li J, Hu L, Zhang L, Pan X, Hu X (2015) Exogenous spermidine is enhancing tomato tolerance to salinity-alkalinity stress by regulating chloroplast antioxidant system and chlorophyll metabolism. *BMC Plant Biology* 15(1): 303. <https://doi.org/10.1186/s12870-015-0699-7>
- Liu B, Soundararajan P, Manivannan A (2019) Mechanisms of silicon-mediated amelioration of salt stress in plants. *Plants* 8(9): 307. <https://doi.org/10.3390/plants8090307>
- Nawaz S, Ahmad MSA, Nazir A, Nijabat A, Leghari SK, Gulshan AB, Hussain F, Khan MA, Awan AN, Naseem Z (2022) Gamma irradiation a potent mitigant of saline stress in maize crop. *GU Journal of Phytosciences* 2: 9–16.
- Nawaz S, Wahid A, Shahbaz M, Basra SM (2025) Evaluation of heat stress induced plant metabolites in *Fagopyrum esculentum* Moench. by exogenous application of plant growth promoters. *Energy & Environment* 36(3): 1105–1131. <https://doi.org/10.1177/0958305X231192355>
- Patil MB, Patil SS, Mahadule PA, Pawar AR, Gade KA, Arunachalam T (2026) Soil salinity impairs soil microbial activity, nutrient availability, plant nutrient uptake, and yield of onion (*Allium cepa* L.). *Frontiers in Plant Science* 17: 1860923. <https://doi.org/10.3389/fpls.2026.1860923>
- Rai S, Singh PK, Mankotia S, Swain J, Satbhai SB (2021) Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. *Plant Stress* 1: 100008. <https://doi.org/10.1016/j.stress.2021.100008>
- Ramachandran P, Pandey NK, Yadav RM, Suresh P, Kumar A, Subramanyam R (2023) Photosynthetic efficiency and transcriptome analysis of *Dunaliella salina* under hypersaline: a retrograde signaling mechanism in the chloroplast. *Frontiers in Plant Science* 14: 1192258. <https://doi.org/10.3389/fpls.2023.1192258>
- Raphael D, Parthasarathi T (2026) Halophilic plant growth-promoting bacterial consortium reshapes soil microbiota to enhance salinity tolerance, antioxidant defense, and yield in *Vigna mungo* L. *Frontiers in Microbiology* 17: 1878014. <https://doi.org/10.3389/fmicb.2026.1878014>
- Sekara A, Pokluda R, Del Vacchio L, Somma S, Caruso G (2017) Interactions among genotype, environment and agronomic practices on production and quality of storage onion (*Allium cepa* L.) – a review. *Horticultural Science* 44(1): 21–42. <https://doi.org/10.17221/92/2015-HORTSC>
- Sen B, Roy D, Narayan M, Sarma H (2025) Nanoparticle-driven stress alleviation: exploring the roles of metal and metal oxide nanoparticles in plant abiotic stress management. *Discover Plants* 2(1): 74. <https://doi.org/10.1007/s44372-025-00153-z>
- Shahzadi S, Fatima S, ul ain Q, Shafiq Z, Janjua MRSA (2025) A review on green synthesis of silver nanoparticles (SNPs) using plant extracts: a multifaceted approach in photocatalysis, environmental remediation, and biomedicine. *RSC Advances* 15(5): 3858–3903. <https://doi.org/10.1039/D4RA07519F>
- Singh A, Bol R, Lovynska V, Singh RK, Sousa JR, Ghazaryan K (2025) Application of nanoparticles for salinity stress management and biofortification in wheat: a review of dual approaches and insights. *Frontiers in Plant Science* 16: 1592866. <https://doi.org/10.3389/fpls.2025.1592866>
- Tan U, Gören HK, Canavar Ö (2026) Meta-analysis of nanoparticles in plant drought responses: functional shifts across stress intensities and nanoparticle types. *Physiologia Plantarum* 178(1): e70701. <https://doi.org/10.1111/pp1.70701>
- Yoshida S, Forno DA, Cock JH (1971) *Laboratory Manual for Physiological Studies of Rice*. Los Baños: International Rice Research Institute, p 61.
- Zamir H, Shah DA, Rauf F, Ahmad H, Khan S, Zulfiqar U, Alotaibi MS, Ergasheva O, Sotiboldiyeva D, Gururani MA, Korai SK (2026) Salinity signaling networks in wheat: crosstalk among Ca²⁺, ROS, phytohormones, and metabolic signals in salt adaptation. *Plant Signaling & Behavior* 21(1): 2700909. <https://doi.org/10.1080/15592324.2026.2700909>
- Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry* 64: 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)
- Zia-ur-Rehman M, Mfarrej MFB, Usman M, Anayatullah S, Rizwan M, Alharby HF, Zeid IMA, Alabdallah NM, Ali S (2023) Effect of iron nanoparticles and conventional sources of Fe on growth, physiology and nutrient accumulation in wheat plants grown on normal and salt-affected soils. *Journal of Hazardous Materials* 458: 131861. <https://doi.org/10.1016/j.jhazmat.2023.131861>