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ORIGINAL PAPER

Fascinating Role of Silicon dioxide Nanoparticles and Co-inoculation of Mycorrhiza and Rhizobacteria to Combat NaCl Stress: Changes in Physiological Characteristics, Uptake of Nutrient Elements, and Enhancing Photosystem II Activities in Wheat

Farnaz Ahmadi-Nouraldinvand[1] · Raouf Seyed Sharifi[1] · Seyed Ataollah Siadat[2] · Razieh Khalilzadeh[3]

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Abstract
Salinity stress is one of the global problems that limit crop production. The application of silicon dioxide nanoparticles (SiO_2-NPs) and the inoculation of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) with wheat are novel approaches to reducing the negative effects of salinity stress. Therefore, the main objective of this experiment was to investigate the role of SiO_2-NPs and the use of co-inoculation of AMF and PGPR for wheat plant tolerance under salt stress. The experiment was carried out as a factorial based on a randomized complete block design. Accordingly, a pot experiment was conducted on wheat growing under salt stress (0, 35, 70, and 105 mM NaCl), using bio-fertilizers (control, mycorrhizal fungi, *Flavobacterium* and *Pseudomonas* bacteria, co-inoculation of AMF and PGPR), and SiO_2-NPs (0, 30, and 60 mg/L) as additives. The results showed that, under 105 mM salinity stress, the co-inoculation of AMF and PGPR as well as the use of SiO_2-NPs enhanced chlorophyll a (8.81%), b (12.93%), total Chl (22.68%), carotenoid content (18.88%), membrane stability index (14.82%), plant height (13.37%), and spike length (13.9%) parameters compared to control treatment. The application of AMF and PGPR individually or in combination and the use of SiO_2-NPs improved physiological parameters such as leaf area index, chlorophyll fluorescence parameters, anthocyanin, and chlorophyll index. Salinity stress reduced the uptake of phosphorus and K^+, as well as increasing the uptake of Na^+. However, co-inoculation of AMF and PGPR as well as 60 mg/L SiO_2-NPs resulted in increased Si, P, and K^+, as well as decreased Na^+ uptake, which finally increased grain yield. Generally, this finding implies that co-inoculation of AMF and PGPR as well as the application of SiO_2-NPs could be used as additives for the improvement of wheat and the uptake of nutrients under salinity stress.

Keywords Salinity stress · Photosynthesis · Nanoparticles · Bio-fertilizer · Flavonoid

1 Introduction

One of the most important agricultural crops with the most cultivated area in dry areas is bread wheat (*Triticum aestivum* L.), which is one of the main sources of carbohydrates, proteins, fats, vitamins, minerals, and other nutrients for human consumption (Zhao et al. 2022). After corn and rice, this plant ranks third in the world and is the most important food crop for urban and rural farmers worldwide, as well as a source of straw for animal feed (Hussein et al. 2022). Therefore, there is a direct link between wheat production and the issue of food security (Jing et al. 2020). Abiotic stressors can thereby inhibit plant development and yield, including salinity, drought, and heavy metal stress. One of the most prominent abiotic stresses is soil salinity, which is

✉ Farnaz Ahmadi-Nouraldinvand
Farnaz_ahmadi@rocketmail.com

[1] Department of Plant Production and Genetics Engineering, University of Mohaghegh Ardabili, Ardabil, Iran

[2] Department of Plant Production and Genetic Engineering, Agricultural Sciences and Natural Resources University of Khuzestan, Ahvaz, Iran

[3] Department of Plant Production and Genetic Engineering, Faculty of Agriculture, Lorestan University, Khorramabad, Iran

increasingly problematic globally because it occurs in all climates and can be a threat to agricultural products such as wheat (EL Sabagh et al. 2021; Loo et al. 2022; Ahmadi Nouraldinvand et al. 2023a). Indeed, salinity inhibits plant growth by generating reactive oxygen species (ROS), disrupting nutrient hemostasis, causing ion toxicity, and creating osmotic potential in the root environment (Gebrehiwet et al. 2021). While chloride ions are thought to be poisonous to plants and cause oxidative stress and crop loss, sodium chloride is a prominent component of salty soils (Mousavi et al. 2022). Or, to put it another way, high salt concentrations subject plants to osmotic and ionic stress, which eventually has a negatively impact on photosynthesis at all stages and thus reduced crop yield (Evelin et al. 2019).

According to reports, increased Na^+ and Cl^- levels in leaf tissue can have a major impact on metabolic activities, which can be repressed and restrict photosynthesis itself. NaCl, on the other hand, causes osmotic and ionic stressors that damage the physiology and photosynthetic system, such as stomata closure, reduced leaf expansion, and decreased leaf area index (LAI) (Lu et al. 2021). In addition, researchers have reported that a decrease in potential (F_V/F_m), effective quantum yield of PSII electron transport, chlorophyll fluorescence, and LAI have also been associated with salt stress (Ali et al. 2022; Canora et al. 2022). Utilizing advantageous soil microorganisms is thus one of the sustainable strategies to increase yield under environmental stress conditions (Ahmadi-Nouraldinvand et al. 2022). Researchers have recently proposed using microorganisms as bio-fertilizers because of their great efficacy and compatibility with the ecology of ecosystems (Karimi & Noori 2022).

Among bio-fertilizers, plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) species have been used to increase salt tolerance in plants. In fact, AMF and PGPR play an important role in improving plant growth through various mechanisms (Toubali & Meddich 2023). It is known that the combined use of AMF and PGPR can improve plants' ability to respond to abiotic conditions like salinity and drought through a variety of processes, including increased potassium uptake and reduced sodium intake by the plant (Chen et al. 2022). Accordingly, it is reported that co-inoculation with PGPR and AMF through reduction of Na^+ and Cl^- absorption promotes lettuce growth under salt stress conditions compared with control (Ouhaddou et al. 2022). Several studies have shown that the application of AMF and PGPR improves plant growth and the uptake of phosphorus from the soil (Ahmadi Nouraldinvand et al. 2023b), chlorophyll fluorescence parameters, and contents of chlorophylls and carotenoid pigments (Wahid et al. 2020; Omer et al. 2022).

One way that silicon (Si) can be used to improve plants is in the form of nanoparticles. According to recent research, nanoparticles (NPs) are among the best alternatives to questionable techniques now in use for protecting plants from abiotic stressors (Moradi et al. 2022). Indeed, crop productivity has been increased by the use of nanoparticles as growth stimulators, soil-improving agents, fertilizers, insecticides, and sensors for regulating numerous agricultural parameters on the farm (Sabagh et al. 2021).Furthermore, the role played by NPs in the reduction of abiotic stress-related toxicity in plants is of enormous agricultural significance (Rajput et al. 2021). In other words, the use of silicon nanoparticles (Si-NPs) is reportedly a successful substitute for adding Si as part of conventional mineral fertilizers (Avestan et al. 2019). Indeed, Si-NPs exhibit high potential in the agriculture field and may work better in mitigating various environmental stresses through their utilization as nano-fertilizers (Rastogi et al. 2019). In agriculture, Si-NPs can be applied by spraying the leaves or directly into the roots. The Si-NPs applied by foliar application can penetrate the leaves and be transported to the various parts via the cuticle or the stomata (Wang et al. 2022). Si accumulates in the cell wall of plants in the form of SiO_2, which improves the mechanical function of the cell wall as well as functioning as a physical barrier to prevent water loss and improve plant resistance to adverse environmental conditions (Gaur et al. 2020). Si also regulates polyamine metabolism, enhances H-ATPase activity in the plasma membrane and tonoplast, promotes Na^+ excretion from the cell, and reduces Na^+ translocation and its damage to plants (Vandegeer et al. 2021).

Several studies show that the use of optimal concentrations of Si-NPs can play an important role in increasing growth and improving the quality of products. It has been reported that the use of Si-NPs can be effective in improving agriculture and producing quality products due to their ease of penetration into plant cells and their ability to affect plant growth and development through metabolism (Du et al. 2022; Shen et al. 2022). Researchers have reported that under salinity stress, the application of Si spray is more efficient than the use of Si soil application, due to its relatively low solubility in the soil (Zhu et al. 2019). Also, it has been reported that the application of Si-NPs under environmental stress conditions such as salinity stress increased the growth and yield of wheat due to the improvement of photosynthesis rate, increase of water status, chlorophyll fluorescence parameters, and reduction of transpiration (Mushtaq et al. 2019).

Regarding the effects of silicon nanoparticles and bio-fertilizers on improving plant conditions under salt stress conditions, the use of silicon nanoparticles together with bio-fertilizers is expected to be effective as an environmentally friendly approach to improving salt stress tolerance in crops such as bread wheat. So, because of the importance of bio-fertilizers and the foliar utility of silicon dioxide nanoparticles in reducing the effects of abiotic

stresses such as salinity, and due to a lack of research on the interaction of those factors on the uptake of nutrient elements and enhancement of photosystem II activities in wheat under salinity, it was investigated.

2 Material and Methods

2.1 Greenhouse Experimental Design and Treatments

The purpose of this experiment was to determine the appropriate concentration of silicon dioxide nanoparticles (SiO_2-NPs) and the application of plant growth-promoting rhizobacteria (PGPR) as well as arbuscular mycorrhizal fungi (AMF) to evaluate their effects on the enhancing photosystem II activity, uptake of nutrient elements, and improving physiological traits in wheat under salinity stress conditions. This project was carried out in 2021–2022, at the greenhouse of the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Iran. The experimental design was arranged three factors in a completely randomized block design with three replications. The experimental factors were included different salt levels (control, 35, 70, and 105 mM NaCl), foliar application of SiO_2-NPs (control, 30, and 60 mg/L) and bio-fertilizer (control, inoculation with mycorrhiza fungi, inoculation with *Flavobacterium* and *Pseudomonas* strain 18,798, co-inoculation of *Flavobacterium* and *Pseudomonas* strain 18,798 with mycorrhiza). The wheat cultivar of Mihan was used in this study was obtained from the Agriculture Research Center, Ardabil, Iran. This cultivar's pedigree is Bkt/90-Zhong 87, and 1000 seeds weigh 40–45 g on average.

2.2 Preparation of Soil and Its Characterization

The soils used to establish the experiment were collected at an upper 0–30 cm depth from an agricultural field cultivated with grain crops. In order to characterize the soil for various chemical and physical characteristics, the soil was air dried, milled, and sieved through a 2-mm sieve. The soil specifications are as follows: silty textured soil, 38.5% sand; 42% silt; 19% clay; 0.04 ppm nitrogen; 255 ppm potassium; 27.30 ppm phosphorus; and 71.20 ppm silicon. After preparing the uniform soil, the pots were filled at a rate of 15 kg per pot. The pots were placed in the glass greenhouse, which had control conditions like 65–70% humidity, proper aeration at, was 20–25 °C for seed sowing of wheat, and photoperiods of 10–12 h.

2.3 Induction of Salt Stress and Silicon Dioxide Nanoparticles

In this experiment, NaCl salt was used for the salinity treatment. Also, the amount of salt required was calculated using the Salt Calc software. Then, salinity stress treatments were induced by irrigating plants with different concentrations (0, 35, 70, and 105 mM) of NaCl solution in two stages. Two stages were included: the 3–4 leaf stage and before the tillering stage. Also, SiO_2-NPs with different concentrations (0, 30, and 60 mg/L) were applied to the leaves in two stages of vegetative growth. Two stages were included the stem stage (54 days after planting) and before booting stage (70 days after planting).

2.4 Characteristics of Bio-fertilizers and Silicon Dioxide Nanoparticles

In this study, the fungus and bacteria used were *Glomus mosseae* (NCBI: 27,381), *Pseudomonas putida* strain 18,798 (NCBI: 1,295,133), and *Flavobacterium* (NCBI: 237), which were prepared by Zist Fanavar Turan corporation and isolated from the rhizospheres of wheat by the Research Institute of Soil and Water, Tehran, Iran, respectively. Colony-forming unit (CFU) was 10^8 live and active bacteria per gram. Seeds were covered with gum Arabic to act as an adhesive and then rolled into a bacterial mixture until uniformly coated. SiO_2-NPs were obtained from The American Corporation US Research Nanomaterial, which was prepared by Pishgaman Nanomaterials Company. The SiO_2-NPs characteristics were 98% purity and 20–30 nm particle size. The SiO_2-NPs powder was suspended in deionized water and sonicated using an ultra sonicator (100 watts) at 40 kHz for 30 min, causing a fairly homogeneous solution.

2.5 Physiological Traits of Wheat Leaf

2.5.1 Photosynthetic Pigments

Leaf chlorophyll and carotenoids content were assessed through Arnon (1949) method and through the absorption of the leaf extract at 470, 645, and 663 nm by spectrophotometer (Model UV-2100). In brief, 0.2 g of fresh leaves was crushed with liquid nitrogen, and 20 mL of 80% acetone was added. Then, the resulting mixture was centrifuged for 10 min at 4000 rpm at 4 °C, and the supernatant was used to assess chlorophyll content and carotenoids. Carotenoid and chlorophyll content (a, b, and total) were estimated using the following formulas:

$$Chla = [19.3(A663) - 0.86(A645)]V/(1000 \times W)$$

$$Chlb = [19.3(A645) - 3.6(A663)]V/(1000 \times W)$$

$$Total\ Chl = [20.2(A645) + 8.02(A663)]V/(1000 \times W)$$

$$Carotenoide = [1000(A470) - 1.8(Chla) - 85.02(Chlb)] + 1.198$$

where V and W are extracted sample volume and fresh weight, respectively.

2.5.2 Quantification of Anthocyanin

First, 0.1 g of fresh leaves sample was crushed with liquid nitrogen and 10 mL of acidified methanol with 1% HCl for 24 h at 4 °C with occasional shaking. Then, the resulting mixture was centrifuged for 10 min at 4000 rpm at 4 °C, and the supernatant was used to assess anthocyanin. Then, its absorbance was recorded at wavelength 550 nm using a spectrophotometer (Wagner 1979). Finally, the anthocyanin was estimated using the following formula:

$$A = \varepsilon bc$$

where ε is an extinction coefficient 33,000 cm/mol, A absorption, C is the concentration of the solution, and b is the cell's width (1 cm).

2.5.3 Chlorophyll Fluorescence Parameters

The photosynthetic efficiency of wheat leaf was determined by measuring the chlorophyll fluorescence (after 25 min dark adaptation) using a chlorophyll fluorometer, model Optic Science-OS-30 USA. To ensure the adaptation of the leaves to light, fluorescence values were measured between 9 and 11 a.m. on the first fully expanded leaf of each plant. The analyzed photosynthetic performance parameters mainly comprised of maximum fluorescence (F_m), minimum fluorescence (F_0), variable fluorescence (F_v), and also photosystem II potential quantum efficiency (F_v/F_m) were also estimated.

2.5.4 Chlorophyll Index and Leaf Area

Chlorophyll index (SPAD) was measured on the first fully expanded leaf of each plant using a chlorophyll meter (SPAD-502 model, Minolta Konica, Japan). The reading was taken in the middle third of the leaf blade of the first fully expanded leaf, which uses three light frequency ranges; thus, the optical measurement analyzes the absorption of light by the leaf by estimating the presence of chlorophyll. In addition, to measure plant leaf surface area, five plants were randomly sampled from replicates of each treatment to determine the leaf area index (LAI) of each plant using a leaf area meter.

2.5.5 Membrane Stability Index

The membrane stability index (MSI) was assessed through the Sofy et al. (2021)method. In brief, 10 fresh leaf sample disks were taken from young branches of identical size. The samples were washed in 25 cm^3 of deionized water and then moved to falcon tubes with deionized water. Samples were kept in falcon tubes for 24 h at 25˚ C, and their electrical conductivity was recorded using a conductivity meter (EC_1). This was followed by autoclaving each sample batch at 80 °C for 1 h, at which point each flask's final conductivity (EC_2) was recorded. Finally, the MSI was calculated as follows:

$$MSI = [1 - \left(\frac{EC1}{EC2}\right)] \times 100$$

where MSI is membrane stability index and EC_1 and EC_2 are electrical conductivity before and after heating.

2.6 Morphological Traits and Grain yield

To measure the grain yield, plant height, and spike length, at the end of the growth period, 10 plants of each pot randomly were harvested. Then, to measure dry weight, wheat plants were harvested and washed with tap water to remove soil particles from the plants' roots. The roots and shoots were separated and placed in a plant sample drying oven for 48 h at 75˚ C. Then the dry weight of the roots, were measured on an electrical weighing balance, and the data was recorded.

2.7 Chemical Analysis of Plants

After the end of the experiment and at harvest time, to measure Si, P, K^+, and Na^+ in leaves, the leaves were collected from the plants of each replication of each treatment and placed in drying oven for 48 h at 75˚ C and milled.

2.7.1 Determination of Silicon content

Aminomolybdate colorimetric method was used to measure the Si content of leaves. In such a way that the Si content in the leaf was measured by extracting the element according to the methodology described by Elliot and Snyder (1991), its absorbance was recorded at wavelength 650 nm using a spectrophotometer.

2.7.2 Determination of P, K^+, and Na^+ content

To assess P, K^+, and Na^+ concentrations of first leaf, 1 g milled plant leaf sample were heated in a furnace for 2 h at 550˚C. Then 5 ml of HCl was added to the sample and placed in a water bath for 10 min. Finally, phosphorus

absorbance was recorded at wavelength 660 nm using a spectrophotometer (Ahmadi et al. 2019). K^+ and Na^+ were measured with a flame photometry (model M410).

2.8 Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA). Mean comparison was performed using least significant difference (LSD) at the 0.05 and 0.01 probability level by SAS 9.4 software. The standard deviation of means ± was then calculated from the average of each treatment.

3 Results

3.1 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Photosystem Activities of Wheat Plants

Concerning photosynthetic pigments and photosystem activities data in Table 1 showed that the effects of silicon dioxide nanoparticles (SiO_2-NPs) and the inoculation of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) under salinity stress on chlorophyll a content (Chl a), chlorophyll b content (Chl b), total chlorophyll content (T Chl), carotenoid content, chlorophyll index (spad), and chlorophyll fluorescence parameters (F_0, F_m, F_v, and F_v/F_m) had a significant effect (Table 1).

3.1.1 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Photosynthetic Pigments

As can be seen in Fig. 1a–d, the photosynthetic pigments, such as chlorophyll a, b, total, and carotenoids contents, were negatively affected by salt stress. However, the application of the co-inoculation of AMF and PGPR and SiO_2-NPs under salinity stress showed a significant increase in the photosynthetic pigments in the leaves of wheat plants. In other words, co-inoculation of AMF and PGPR and the application of 60 mg/L SiO_2-NPs significantly improved the concentration of photosynthetic pigments under salt stress compared with the control (Fig. 1a–d). Indeed, plants treated with AMF/PGPR and SiO_2-NPs (60 mg/L) showed a significant increase in chlorophyll a, b, total, and carotenoid concentrations under 105 mM NaCl. Our results showed that the highest content of chlorophyll a, b, total chlorophyll, and carotenoids were 1.97, 0.821, 2.23, and 8.14 $mg.g^{-1}$ FW^{-1}, respectively, when 60 mg/L SiO_2-NPs and co-inoculation of AMF and PGPR were applied under non-saline conditions. Moreover, the lowest content of chlorophyll a, b, total, and carotenoids was 1, 0.456, 1.19, and 4.50 $mg.g^{1}$ FW^{-1}, respectively, under only 105 mM NaCl condition. Indeed, application of SiO_2-NPs at a concentration of 60 mg/L and co-inoculation of AMF and PGPR resulted in 96.8, 80, 87.3, and 80.8% increases in chlorophyll a, b, total, and carotenoid content, respectively, in leaves of wheat plants under non-saline stress conditions as compared to under 105 mM NaCl alone (Fig. 1a–d).

Table 1 Analysis of variance photosystem activities under salinity conditions, application of bio-fertilizers, and silicon dioxide nanoparticles

S.O.V	D.F	Mean squares								
		Chla[a]	Chlb[b]	T chl[c]	Carotenoid	F_m[d]	F_v[e]	F_v/F_m	F_0[f]	Spad
Replication	2	0.474	0.092	1.357	0.923	67,783.3	479,180.8	0.097	14,989.9	2747.7
Salinity (SA)	3	1.223**	0.055**	0.164*	7.495**	133,425.5**	20,411.8**	0.116**	16,047.7**	1304.1**
Bio-fertilizers (BF)	3	0.070 ns	0.060**	0.097 ns	0.386 ns	17,413.1**	15,897.1**	0.033**	713.52**	729.98**
Silicon dioxide (SiO_2-NPs)	2	0.280**	0.113**	0.031 ns	6.521**	134,563.1**	3574.1 ns	0.083**	82,157.11**	227.98**
SA × BF	9	0.223**	0.024**	0.315**	1.919 ns	14,377.6**	27,377.7**	0.039**	87.56 ns	28.32 ns
SA × SiO_2-NPs	6	0.194**	0.056**	0.412**	4.802**	30,226.1**	1246.9 ns	0.012**	405.23 ns	144.84**
BF × SiO_2-NPs	6	0.031 ns	0.011**	0.264**	0.833 ns	11,097.5*	19,994.6**	0.007 ns	221.65 ns	36.87 ns
SA × BF × SiO_2-NPs	18	0.094**	0.015**	0.142**	2.173**	14,505.5**	11,637.4**	0.010**	28.39 ns	19.81 ns
Error	94	0.031	0.003	0.061	1.053	4602.3	4177.6	0.0040	144.90	27.82
C.V (%)	-	13.77	9.21	15.36	17.35	10.33	8.86	9.05	5.65	10.27

ns, *, and ** indicating non-significant and significant at 5% and 1% levels of probability, respectively

[a]Chlorophyll a

[b]Chlorophyll b

[c]Total chlorophyll

[d]Maximum fluorescence

[e]Variable fluorescence

[f]Mminimum fluorescence

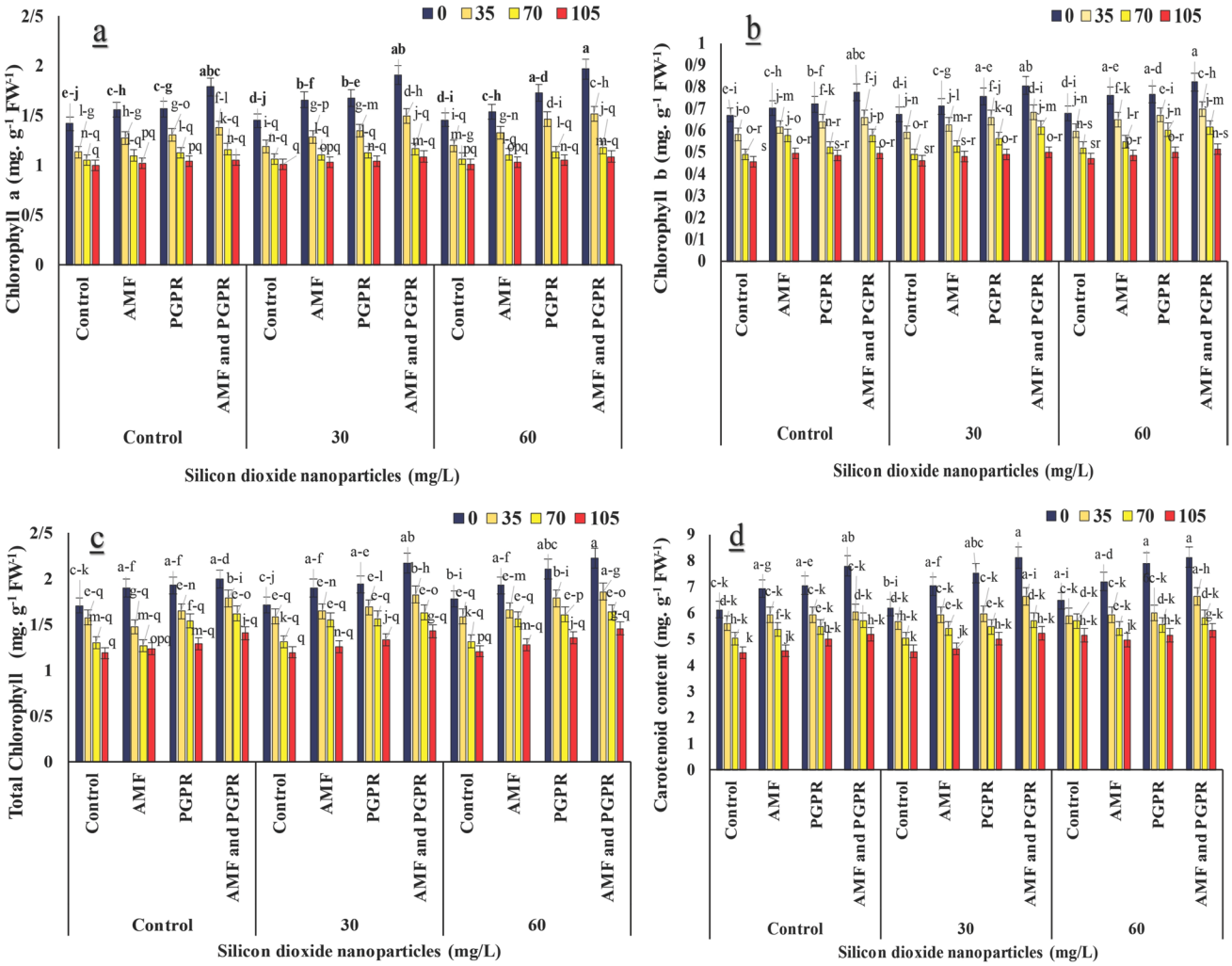

Fig. 1 The application of silicon dioxide nanoparticles as well as co-inoculation of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) on chlorophyll a (**a**), chlorophyll b (**b**), total chlorophyll (**c**), and carotenoid content (**d**) in the first expanded leaf of wheat growing under salinity stress

3.1.2 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Chlorophyll Fluorescence Parameters

In the present experiment, it was observed that chlorophyll fluorescence parameters were adversely affected under NaCl stress. However, seed bio-priming with AMF and PGPR and the application of SiO_2-NPs caused a significant increase in chlorophyll fluorescence parameters under NaCl stress (Fig. 2a–c). Our results showed that the highest F_m (937.2), F_v (703), and F_v/F_m (0.874) were obtained through the co-inoculation of AMF and PGPR and the application of 60 mg/L SiO_2-NPs under non-salinity conditions. Furthermore, the lowest F_m, F_v, and F_v/F_m were 531.1, 456, and 0.539, respectively, under 105 Mm NaCl stress and the absence of AMF/PGPR and SiO_2-NPs (Fig. 2a–c). In other words, our study showed that in plants under NaCl stress, the lowest chlorophyll fluorescence parameters were at the highest level of NaCl (105 mM), which recorded a decrease in F_m, F_v, and F_v/F_m of about 43.32%, 35.13%, and 38.32%, respectively, compared to co-inoculation of AMF and PGPR and 60 mg/L SiO_2-NPs (Fig. 2a–c). Also, under 70 and 35 Mm NaCl stress and the application of 60 mg/L SiO_2-NPs as well as co-inoculation of AMF/PGPR increased F_m (9.96%), F_v (7.16%), and F_v/F_m (18.36%) compared to non-application bio-fertilizers and SiO_2-NPs under 70 and 35 Mm NaCl stress (Fig. 2a–c) (Table 2).

Also, a significant difference was found for the individual effects of NaCl stress, the inoculation of AMF and PGPR, and the application of SiO_2-NPs treatments on minimum fluorescence (F_0), but there was no significant difference for any interaction effects on minimum fluorescence (Table 1). In fact, the highest minimum fluorescence (233.96±43.25) was observed when plants were treated with 105 mM NaCl as compared to controls, as the salt treatment

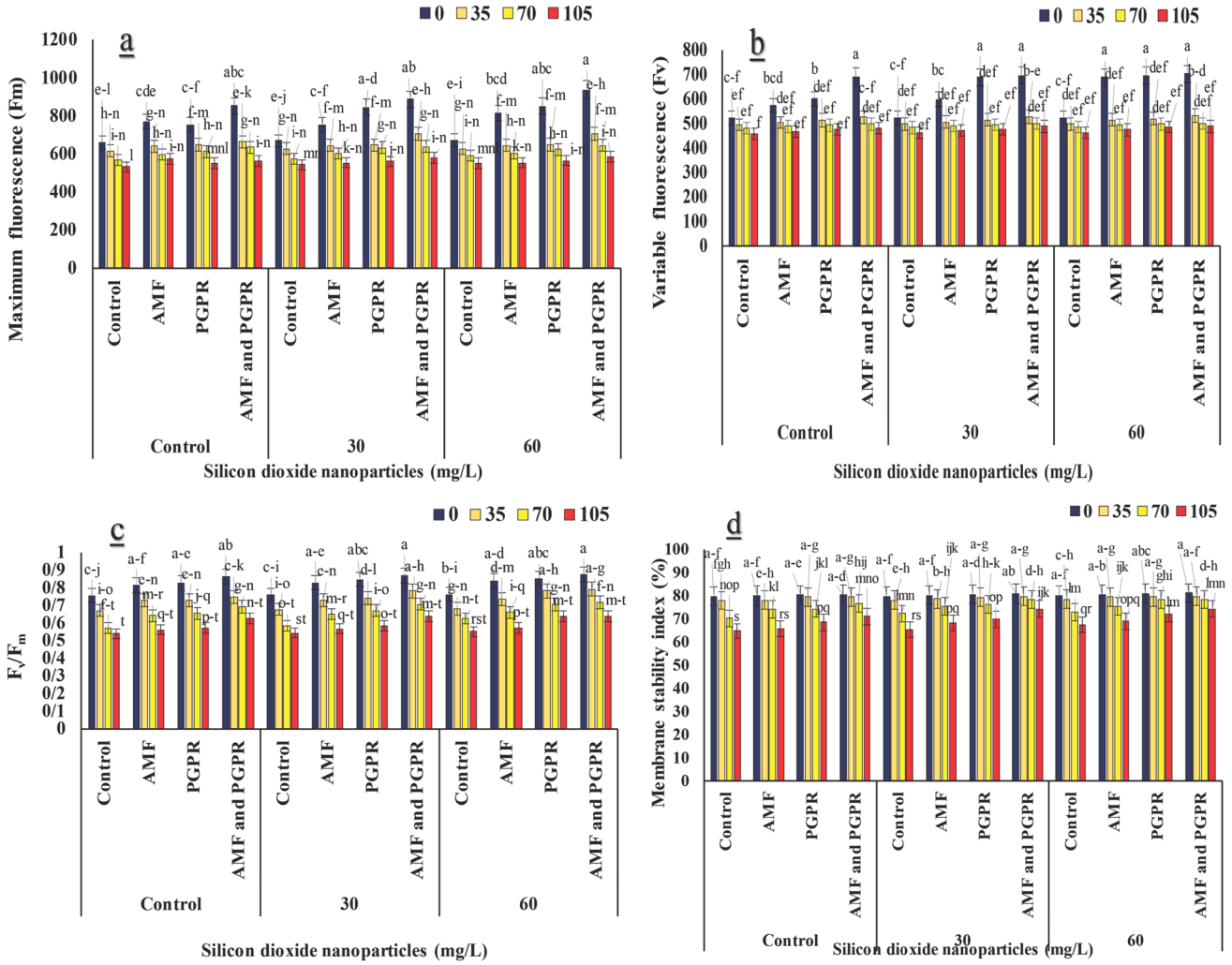

Fig. 2 The application of silicon dioxide nanoparticles as well as co-inoculation of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) on maximum fluorescence (**a**), variable fluorescence (**b**), photosystem II potential quantum efficiency (**c**), and membrane stability index (**d**) in the first expanded leaf of wheat growing under salinity stress

increased minimum fluorescence by 24.42% (Table 3). Furthermore, the comparison of the averages showed that the inoculation of AMF and PGPR led to an improvement in minimum fluorescence. The lowest F_0 (208.08 ± 38.82) was obtained when plants were inoculated with AMF and PGPR, which showed no significant difference from the inoculation of bacteria. Also, the comparison of the averages showed that the lowest $F_0$was obtained in 60 mg/L of SiO_2-NPs (172.95 ± 23.17), which showed a significant difference from the concentration of 30 mg/L (Table 3).

3.1.3 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Chlorophyll Index

The present study showed that all NaCl concentrations caused negative effects on plant chlorophyll index as compared with the control treatment. In the present study, it was shown that all NaCl concentrations (35, 70, and 105 mM) caused negative effects on plant chlorophyll index as compared with control treatment. The highest chlorophyll index was obtained in the absence of salinity stress, which increased by about 25.15% comparing with the 105 mM NaCl conditions (Table 3). On the other hand, application of almost all bio-fertilizers had a significant increase in chlorophyll index, especially by using co-inoculation of AMF and PGPR, which raised it to 56.21 ± 11.08, which boosted the leaves chlorophyll index to about 23.81% as compared to the control (Table 3). Also, the application of 60 mg/L SiO_2-NPs increased the content of chlorophyll index to 53.28 ± 12.07 as compared with the significant reduction in untreated plants (49 ± 8.79) (Table 3).

Table 2 Analysis of variance in some physiological and morphological traits, and grain yield of wheat plants growing under salinity conditions, treated with bio-fertilizers and silicon dioxide nanoparticles

S.O.V	D.F	Mean squares						
		Membrane stability index	Lead area index	Anthocyanin	Grain yield	Root dry weight	Plant height	Spike length
Replication	2	3.30	1.27	0.00041	5.27	17.02	7273.64	130.98
Salinity (SA)	3	938.32**	3.10**	0.00017**	4.48**	114.21**	1692.31**	3.47**
Bio-fertilizers (BF)	3	5.73*	2.12**	0.00018**	0.68**	30.77*	460.78**	4.79**
Silicon dioxide (SiO_2-NPs)	2	0.127^{ns}	1.77**	0.000012*	0.27**	125.65**	183.46**	0.384**
SA × BF	9	4.129*	0.095^{ns}	0.000005^{ns}	0.08^{ns}	13.50^{ns}	33.27**	0.952**
SA × SiO_2-NPs	6	0.748^{ns}	0.186^{ns}	0.000002^{ns}	0.06^{ns}	23.00*	18.14**	0.446**
BF × SiO_2-NPs	6	106.21**	0.072^{ns}	0.000001^{ns}	0.05^{ns}	23.65*	441.83**	1.72**
SA × BF × SiO_2-NPs	18	7.40**	0.092^{ns}	0.000002^{ns}	0.02^{ns}	14.90^{ns}	41.43**	0.596**
Error	94	2.15	0.146	0.000003	0.091	10.27	4.48**	0.080
C.V (%)	-	1.93	14.97	10.11	17.33	16.13	3.48	3.53

ns, *, and ** indicating non-significant and significant at 5% and 1% levels of probability, respectively

Table 3 Comparison of means of some physiological, yield and root dry weight traits of wheat plants growing under salinity conditions, application of bio-fertilizers and silicon dioxide nanoparticles

Treatments		Spad	Minimum fluorescence	Leaf area index	Anthocyanin (μmol.g^{-1} FW)	Grain yield (g)	Root dry weight (g)
Salt stress (mM)	0	57.76 ± 8.96^{a}	188.03 ± 33.09^{d}	3.11 ± 0.5^{a}	0.0159 ± 0.0027^{c}	2.20 ± 0.63^{a}	21.77 ± 4.23^{a}
	35	55.03 ± 10.79^{b}	203.27 ± 36.68^{c}	2.94 ± 0.40^{a}	0.0172 ± 0.0028^{b}	1.82 ± 0.32^{b}	20.63 ± 2.77^{ab}
	70	43.39 ± 9.68^{c}	226.41 ± 40.06^{b}	2.63 ± 0.33^{b}	0.0175 ± 0.0029^{b}	1.55 ± 0.27^{c}	19.40 ± 4.15^{b}
	105	45.15 ± 8.07^{c}	233.96 ± 43.25^{a}	2.46 ± 0.55^{b}	0.021 ± 0.0037^{a}	1.39 ± 0.31^{d}	17.61 ± 3.82^{c}
	Control	45.40 ± 9.31^{c}	217.18 ± 45.06^{a}	2.49 ± 0.54^{c}	0.0172 ± 0.0034^{b}	1.58 ± 0.37^{c}	18.67 ± 3.61^{b}
Bio-fertilizers	Fungi	51.07 ± 9.46^{b}	216.17 ± 45.34^{a}	2.70 ± 0.46^{b}	0.0178 ± 0.0034^{b}	1.70 ± 0.40^{bc}	21.13 ± 4.18^{a}
	Bacteria	52.65 ± 10.28^{b}	210.23 ± 43.18^{b}	2.87 ± 0.43^{ab}	0.0177 ± 0.0031^{b}	1.76 ± 0.49^{b}	20.89 ± 3.11^{ab}
	Fungi × Bacteria	56.21 ± 11.08^{a}	208.08 ± 38.82^{b}	3.06 ± 0.57^{a}	0.0189 ± 0.0041^{a}	1.91 ± 0.67^{a}	19.71 ± 4.94^{ab}
SiO_2-NPs (mg/L)	0	49.00 ± 8.79^{b}	255.56 ± 30.90^{c}	2.58 ± 0.50^{b}	0.0175 ± 0.0033^{b}	1.67 ± 0.39^{b}	18.15 ± 2.75^{c}
	35	51.72 ± 10.74^{a}	210.23 ± 24.76^{b}	2.97 ± 0.46^{a}	0.0178 ± 0.0035^{ab}	1.73 ± 0.57^{ab}	20.04 ± 4.58^{b}
	60	53.28 ± 12.07^{a}	172.95 ± 23.17^{a}	2.80 ± 0.59^{a}	0.0184 ± 0.0039^{a}	1.82 ± .0.54^{a}	21.37 ± 4.02^{a}

Means followed by similar letter (s) in each column are not significantly different by LSD test at 5% probability level

3.2 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Physiological and Morphological Traits and Grain Yield of Wheat Plants

Membrane stability index (MSI), leaf area index (LAI), and anthocyanin in wheat leaves, grain yield, and root dry weight parameters were selected to evaluate the effect of inoculation of AMF and PGPR as well as the application of SiO_2-NPs under salinity stress. The data shows that treatments had a significant effect on all of the parameters (Table 2).

3.2.1 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Membrane Stability Index

The results showed that the MSI of wheat leaves decreased significantly under NaCl stress. But these values increased after the application of SiO_2-NPs and co-inoculation of AMF and PGPR. So that, the absence of NaCl stress and

the application of 60 mg/L SiO_2-NPs as well as co-inoculation of AMF/PGPR increased 25.29% MSI compared to non-application bio-fertilizers and SiO_2-NPs under 105 Mm NaCl stress (Fig. 2d). As shown in Fig. 2d, the highest MSI content was 81.14%, which was obtained by applying 60 mg/L SiO_2-NPs as well as co-inoculating AMF and PGPR under non-saline conditions. Moreover, the lowest MSI percentage was 64.76% under only 105 mM NaCl stress conditions. In fact, the non-application of SiO_2-NPs as well as co-inoculation of AMF and PGPR resulted in a decrease of 12.91% under 105 mM NaCl stress compared with NaCl 105 mM stress and the application of 60 mg/L SiO_2-NPs as well as co-inoculation of AMF and PGPR (Fig. 2d).

3.2.2 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Leaf Area Index

As shown in Table 3, the application of SiO_2-NPs and inoculation with AMF or PGPR, or both, has a significant increase on the LAI of wheat leaf. Also, NaCl stress significantly decreased the LAI in wheat leaf. In fact, all NaCl concentrations caused negative effects on plant LAI as compared with the control treatment. Our study showed that in the absence of salinity, LAI increased by 26.42% as compared to NaCl 105 Mm. Also, the comparison of the averages showed that the highest LAI was obtained in 30 mg/L of SiO_2-NPs (2.97 ± 0.46), which showed no significant difference from the concentration of 60 mg/L. Indeed, non-application of SiO_2-NPs caused decrease 13.13% in LAI as compared to 30 mg/L SiO_2-NPs treatment (Table 3). On the other hand, our results showed that co-inoculation of AMF and PGPR caused an increase of 29.3% in LAI as compared to no-inoculation treatment (Table 3).

3.2.3 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Anthocyanin

Anthocyanin content was determined in wheat leaf. The results showed that the effects of NaCl stress led to an increase in the anthocyanin contents. In this study, it was shown that all NaCl concentrations (35, 70, and 105 mM) caused an increase in anthocyanin as compared with control treatment. The lowest anthocyanin (0.0159 ± 0.0027 µmol.g^{-1} FW) was obtained in the absence of salinity stress, which increased by about 24.28% comparing with the 105 mM NaCl treatment (Table 3). On the other hand, application of both bio-fertilizers had a significant increase in anthocyanin, especially by using co-inoculation of AMF and PGPR, which raised it to 0.0189 ± 0.0041 µmol.g^{-1} FW, which boosted the leaves anthocyanin content to about 9.88% compared to the control (Table 3). Also, the application of 60 mg/L Si-NPs increased the content of anthocyanin to 0.0184 ± 0.0039 µmol.g^{-1} FW which mean a significant reduction as compared with untreated plants. Using a 60 mg/L SiO_2-NPs concentration had no significant difference from a 30 mg/L SiO_2-NPs concentration (Table 3).

3.2.4 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Grain Yield and Root Dry Weight

The results of the present study have shown that salinity stress significantly decreased the wheat seed yield comparing with the control plants. Our results showed that all NaCl concentrations (35, 70, and 105 mM) caused a considerable reduction in grain yield as compared to the control treatment. In other words, under the low NaCl concentration (35 mM), the decrease in grain yield was 17.27%, while it was 29.54% and 36.81% under the high NaCl concentrations (70 and 105 mM), respectively, comparing with the control treatment. Nevertheless, the application of SiO_2-NPs and co-inoculation of AMF and PGPR individually or in combination led to a significant increase in the grain yield in wheat plants under saline stress in comparison with untreated salinized plants. Indeed, among the treatments, the best treatment was the 60 mg/L SiO_2-NPs and co-inoculation of AMF as well as PGPR, which resulted in an increase in grain yield by 8.98% and 20.88%, respectively, comparing with the control treatment (Table 3).

Also, the results showed that root dry weight decreased with increasing salinity level. As, the lowest root dry weight was obtained in the 105 mMNaCl stress treatment (17.61 ± 3.82 g) which decreased by about 19.1% compared with the absence of NaCl stress (Table 3). On the other hand, plants treated with the application of AMF, PGPR, its combination as well as SiO_2-NPs had higher root dry weight. In other words, the comparison of the averages showed that the highest root dry weight was obtained in the AMF (21.13 ± 4.18 g), which had no significant difference among PGPR its combination. On the other hand, the application of 60 mg/L SiO_2-NPs increased the root dry weight to 21.37 ± 4.02 g which increased by about 17.74% comparing with untreated plants (Table 3).

3.2.5 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Plant Height and Spike Length

The results showed that the plant height decreased significantly under NaCl stress. But these values increased after the application of SiO_2-NPs and the co-inoculation of AMF and PGPR. So that, the absence of NaCl stress and the application of 60 mg/L SiO_2-NPs, as well as the co-inoculation of AMF and PGPR, increased plant height by 68.9%

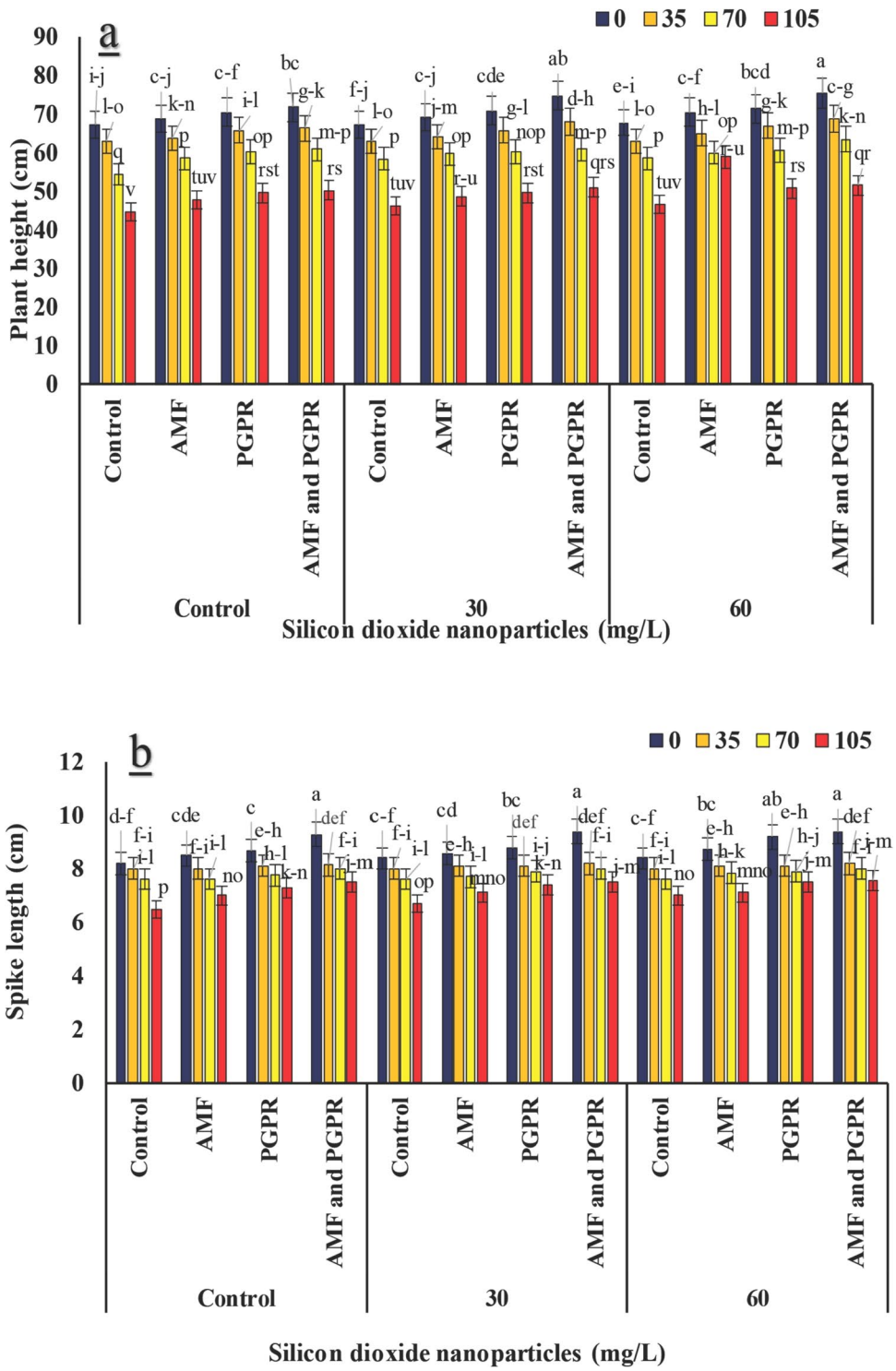

Fig. 3 The application of silicon dioxide nanoparticles as well as co-inoculation of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) on plant height (**a**) and spike length (**b**) under salinity stress

compared to the absence of bio-fertilizers and SiO_2-NPs under 105 Mm NaCl stress (Fig. 3a). Also, under 105 mM NaCl stress and the application of 60 mg/L SiO_2-NPs as well as co-inoculation of AMF and PGPR, plant height increased by 13.37% compared with NaCl 105 mM stress and the non-application of SiO_2-NPs as well as co-inoculation of AMF and PGPR (Fig. 3a). Indeed, as shown in Fig. 3a, the highest plant height was 75.5 cm, which was obtained by applying 60 mg/L SiO_2-NPs as well as co-inoculating AMF and PGPR under non-saline conditions. Moreover, the lowest plant height was 44.7 cm under only 105 mM NaCl stress conditions. (Fig. 3a).

Also, the results showed that spike length decreased with increasing salinity level. The comparison of means

showed that under salinity stress conditions, the application of SiO_2-NPs as well as co-inoculating AMF and PGPR resulted in a significant increase in spike length compared with the no application them. As shown in Fig. 3b, the lowest spike length was at the highest level of salinity (105 mM) and the no application of SiO_2-NPs as well as no co-inoculating AMF and PGPR. In other words, gradual increases in salinity and the use co-inoculating AMF and PGPR as well as 60 mg/L SiO_2-NPs improve spike length in wheat plant, the most significant of which were salinity 105 mM and the combined use of AMF/PGPR, as well as 60 mg/L SiO_2-NPs, which recorded an increase in spike length of about 13.9%, compared to control plants (Fig. 3b). In other words, the absence of NaCl stress and the application of 60 mg/L SiO_2-NPs as well as co-inoculation of AMF/PGPR increased 44.61% spike length compared to the no application of bio-fertilizers and SiO_2-NPs under 105 Mm NaCl stress (Fig. 3b).

3.3 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Uptake of Nutrient Elements of Wheat Plants

As shown in Table 4, the application of bio-fertilizers and SiO_2-NPs, as well as NaCl stress conditions treatments, had a significant effect on phosphorus (P), sodium (Na^+), potassium (K^+), and silicon (Si) concentrations in wheat leaves.

3.3.1 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Uptake of Phosphorus

In this study, the results showed that phosphorus content decreased with increasing NaCl levels (Table 4). As shown in Table 5, the lowest phosphorus uptake was 22.97 ± 4.63 mg.g^{-1} DW at 105 mM NaCl stress and the highest phosphorus uptake was 25.37 ± 6.01 mg.g^{-1} DW in the absence of NaCl

Table 4 Analysis of variance uptake of nutrient elements in leaves of wheat under salinity conditions, application of bio-fertilizers and silicon dioxide nanoparticles

S.O.V	D.F	Mean squares			
		Phosphorus (P)	Sodium (Na^+)	Potassium (K^+)	Silicon (Si)
Replication	2	71.53	931.65	611.56	2973.55
Salinity (SA)	3	37.10*	1664.44**	47.86**	1278.43**
Bio-fertilizers (BF)	3	158.78**	36.06**	8.29**	2668.22**
Silicon dioxide (SiO_2-NPs)	2	626.28**	32.53*	12.84**	209.59**
SA × BF	9	9.22^{ns}	8.25^{ns}	3.46^{ns}	15.35**
SA × SiO_2-NPs	6	20.64^{ns}	28.51**	0.268^{ns}	1.23^{ns}
BF × SiO_2-NPs	6	4.34^{ns}	50.85**	9.90**	38.44**
SA × BF × SiO_2-NPs	18	7.98^{ns}	2.14^{ns}	1.02^{ns}	0.211^{ns}
Error	94	12.00	9.20	2.30	3.58
C.V (%)	-	14.43	12.06	7.09	4.81

ns, *, and ** indicating non-significant and significant at 5% and 1% levels of probability, respectively

Table 5 Comparison of means uptake of nutrient elements in leaves of wheat under salinity conditions, application of bio-fertilizers and silicon dioxide nanoparticles

Treatments	(mg.g^{-1} DW)	P content	Na^+ content	K^+ content	Si content
Salt stress (mM	0	25.37 ± 6.01^{a}	17.28 ± 3.56^{d}	22.59 ± 3.23^{a}	33.74 ± 8.82^{d}
	35	24.06 ± 4.26^{ab}	22.97 ± 5.30^{c}	22.27 ± 3.04^{ab}	35.14 ± 9.19^{c}
	70	23.60 ± 4.54^{b}	26.82 ± 5.36^{b}	21.57 ± 4.05^{b}	42.17 ± 11.02^{b}
	105	22.97 ± 4.63^{b}	33.47 ± 5.48^{a}	20.00 ± 3.02^{c}	46.38 ± 12.13^{a}
Bio-fertilizers	Control	21.18 ± 4.55^{c}	26.34 ± 7.82^{a}	21.20 ± 3.17^{b}	31.69 ± 6.77^{d}
	Fungi	23.83 ± 4.33^{b}	24.32 ± 7.49^{b}	21.22 ± 3.47^{b}	34.67 ± 7.44^{c}
	Bacteria	24.87 ± 4.34^{ab}	25.57 ± 7.70^{ab}	21.86 ± 3.55^{ab}	39.82 ± 9.08^{b}
	Fungi × Bacteria	26.11 ± 5.26^{a}	24.30 ± 7.90^{b}	22.16 ± 3.78^{a}	51.25 ± 11.32^{a}
SiO_2-NPs (mg/L)	0	21.39 ± 4.27^{b}	25.91 ± 7.54^{a}	21.06 ± 3.46^{b}	37.21 ± 10.14^{c}
	35	22.48 ± 3.10^{b}	25.23 ± 7.99^{ab}	21.67 ± 3.64^{ab}	39.48 ± 11.74^{b}
	60	28.12 ± 4.43^{a}	24.27 ± 7.62^{b}	22.09 ± 3.36^{a}	41.38 ± 12.35^{a}

Means followed by similar letter (s) in each column are not significantly different by LSD test at 5% probability level

stress, which was not significant from 35 mM NaCl stress. Nevertheless, inoculation with AMF and PGPR and the application of SiO_2-NPs significantly increased the uptake of phosphorus in leaves of wheat plants. As shown in the comparison of the averages, the highest phosphorus uptake was obtained in the co-inoculation of AMF and PGPR, which resulted in an increase of 23.27% as compared to the control treatment. Also, the application of 60 mg/L SiO_2-NPs lead to an increase in phosphorus content by 17.74% as compared to the control (Table 5).

3.3.2 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Uptake of Na^+ and K^+

Salinity stress significantly affects Na^+ and K^+ uptake (Table 4). The results of our data show that salinity stress leads to a 93.69% increase in Na^+ uptake and an 11.46% decrease in K^+ content in wheat leaves. In other words, the highest Na^+ content was 33.47 ± 5.48 mg.g^{-1} DW at the highest NaCl level (105 mM), resulting in decreased K^+ content (20.00 ± 3.02 mg.g^{-1} DW). Also, application of SiO_2-NPs and co-inoculation of AMF and PGPR resulted in significant reduction of Na^+ accumulation in wheat leaves. The application of 60 mg/L SiO_2-NPs as well as co-inoculation of AMF and PGPR resulted in 6.32% and 19.13% reduction in Na^+ accumulation in wheat leaves, respectively, compared with the control treatment (Table 5). Also, the highest K^+ accumulation, 22.16 ± 3.78 and 22.09 ± 3.36 mg.g^{-1} DW, was obtained with the co-inoculation of AMF and PGPR as well as the application of 60 mg/L SiO_2-NPs, respectively, as compared with the control treatment (Table 5).

3.3.3 Responses of Wheat Plants to Salinity Stress Under the Effects of SiO_2-NPs as well as Co-inoculation of AMF/PGPR on Uptake of Silicon

Our results showed that the application of silicon and bio-fertilizer treatments resulted in an increase in silicon uptake as compared to control treatments when plants were exposed to salt stress. In fact, silicon uptake increased by 4.14, 24.95, and 37.46% at NaCl concentrations of 35, 70, and 105 mM, respectively, as compared to the control treatments (Table 5). On the other hand, the highest silicon uptake was 51.25 ± 11.32 and 41.38 ± 12.35 mg.g^{-1} DW when 60 mg/L SiO_2-NPs and co-inoculation of AMF and PGPR were applied, which was significantly difference from other treatments (Table 5). Indeed, as shown in Table 4, the application of 60 mg/L SiO_2-NPs and co-inoculation of AMF and PGPR increased silicon uptake by 11.17% and 61.72%, respectively, as compared with control treatments.

4 Discussion

Recently, wheat has been exposed to numerous stresses in different agricultural systems. Among these environmental stresses, salinity stress is one of the most important in the world because it has negative effects on plant growth and economic yield. On the other hand, increased soil salinity in the plant medium leads to a reduction of plant growth attributes, physiological characteristics, photosynthetic activity, and elements uptake, which ultimately disturb the economic yield of plants. So, in this study, we endeavored to use bio-fertilizers and silicon dioxide nanoparticles to improve the condition of the plant under salinity stress conditions. In the present study, the results of Fig. 1a–d showed that leaf chlorophyll a, b, total, and carotenoid content decreased with increasing salinity stress. On the other hand, the results of Table 3 showed that all NaCl concentrations (35, 70, and 105 mM) caused negative effects on plant chlorophyll index as compared with control treatment. So, the chlorophyll index was reduced in response to salinity stress. Indeed, salinity leads to a lack of water in the plant, and this causes the stomata to close and the destruction of chlorophyll and chloroplasts, resulting in a decrease in photosynthesis. Therefore, it seems that the decrease in chlorophyll content in this study can probably be attributed to the decrease in chlorophyll synthesis and the increase in its decomposition. It has been reported that the main inhibitory effect of salinity on plant growth parameters and wheat yield can be due to specific ion toxicity, osmotic effect, and nutritional imbalance, which results in a reduction in photosynthetic proficiency and other physiological disorders (Adil et al. 2022). Also, researchers reported that increasing salinity levels decreased the chlorophyll and total carotenoid contents and the chlorophyll index in canola and Iranian licorice leaves (Iqbal et al. 2022; Mousavi et al. 2022). Also, this study showed that under salt stress, application of bio-fertilizers like mycorrhizal fungi and growth-promoting bacteria can increase chlorophyll and carotenoid content and also the chlorophyll index due to improved water access and selective uptake of mineral elements, especially nitrogen and phosphorus (Table 5), as well as increased activity of the nitrate-reducing enzyme; nitrate reductase. Also, the chlorophyll and carotenoid content and the chlorophyll index were significantly increased as the application of SiO_2-NPs increased (Fig. 1a–d). Previous research has shown that inoculation with AMF and PGPR increases chlorophyll content under NaCl stress conditions (Hashem et al. 2018). Also, the chlorophyll content increased significantly as the application of SiO_2-NPs increased. It seems that the increase in chlorophyll and carotenoid content in the application of SiO_2-NPs as well as inoculation of AMF and PGPR is due to the reduction of hydrogen peroxide production, which

increases the chlorophyll content, the activity of bio-phosphate carboxylase, and the photosynthesis of leaves (Khan et al. 2023; Toubali and Meddich 2023). On the other hand, silicon, when placed in the apoplasm of the outer walls of the epidermal cells, in addition to making the leaf stronger, causes the formation of uneven tissue on both leaf surfaces, which delays the death of the leaf but increases the chlorophyll content and decreases the transpiration through stomata (Somaddar et al. 2022). Researchers reported that chlorophyll and carotenoid content increased under salinity stress when plants are treated with biostimulants and nanoparticles. In fact, inoculation with AMF/PGPR and the application of silicon improve these compounds' content by stimulating their synthesis pathways (Loo et al. 2022; Ouhaddou et al. 2022). Also, it has been reported that the application of silicon nanoparticles leads to an increase in the chlorophyll content of wheat (Mushtaq et al. 2019).

Moreover, the current study clearly showed that chlorophyll fluorescence parameters (F_m, F_v, F_v/F_m, and F_0) were negatively affected at three NaCl concentrations during the growth period. The results shown could be attributed to the deleterious effects of salinity on cell cytoplasm, cytoplasmic dehydration, and chlorophyll degradation (Fig. 1a–d), as well as decreased cell turbidity and photosynthetic rate (Stefanov et al. 2022). In the case, application of SiO_2-NPs as well as co-inoculation of AMF and PGPR resulted in improvement of F_m, F_v, F_v/F_m, and F_0 (Fig. 2a–c and Table 3). The photosystem II is very sensitive to environmental stress, and salt stress leads to damage of this center. In other words, the amount of fluorescence indicates the state of the thylakoid membrane and the relative efficiency of electron transfer from photosystem II to photosystem I. So, it seems that one of the reasons for improving chlorophyll fluorescence parameters is the co-inoculation of AMF and PGPR and the foliar spraying of SiO_2-NPs. Indeed, the application of all SiO_2-NPs with inoculation of AMF, PGPR, or both under salinity stress conditions leads to increases in F_m, F_v, and F_v/F_m and, in contrast, reduces F_0 compared to control treatment. It has been reported that the application of AMF and PGPR individually and together can improve the chlorophyll fluorescence parameters of the host plant due to the high potential for adaptation to a diverse range of environmental stresses (Tirry et al. 2022). In this concern, bio-fertilizers improve plant nutritional status, eliminate pathogenic bacteria, dissolve insoluble phosphate, and produce plant growth regulators, all of which contribute to leaf chlorophyll fluorescence metrics (Begum et al. 2022). On the other hand, Avestan et al. (2021) reported that the chlorophyll fluorescence parameters of wheat leaves decreased significantly under NaCl stress; in contrast, the use of SiO_2-NPs reduced the negative impacts of salinity stress by improving cell wall thickness, providing a higher content of water, and leading to increased chlorophyll content and fluorescence (Avestan et al. 2021).

Plant adaptation mechanisms to salinity stress are intricate. Indeed, cell membranes are the first place where stress conditions cause cell damage to appear (Kafi et al. 2011). The results of our study revealed that the membrane stability index decreased with increasing NaCl concentration (Fig. 2d). NaCl stress has been shown to have an indirect effect on plant growth via the generation of ROS in plant cells. Furthermore, increased ROS lowered cell membrane stability by reacting with it, causing electrolyte leakage, and ultimately destroying the thylakoid membrane (Aazami et al. 2023). Indeed, researchers reported that one of the important parameters for defense against NaCl stress is the membrane stability index. Because the negative effects of salinity stress first appear in the cell membranes and lead to a reduction of the membrane stability index (Parihar et al. 2020). However, the application of 60 mg/L SiO_2-NPs and co-inoculation of AMF and PGPR under NaCl 105 mM stress resulted in an increase of 14.82% compared to the control treatment (Fig. 2d). It has been reported that the application of bio-fertilizers increases the uptake of nutrients, the development of the root system, and the improvement of the water condition of the plants, which stabilizes the cell membrane in the plant (Azarmi-Atajan & Sayyari-Zohan 2020). Also, Taha et al. (2021) reported that the application of silicon led to an increase in the membrane stability index under salinity stress conditions. Similar findings have been reported about the use of silicon to increase the membrane stability index under salinity stress conditions (Haghighi & Pessarakli 2013). On the other hand, our results showed that when plants are placed under salinity stress, there is an increase in the anthocyanin content of their leaves. Also, the application of 60 mg/L SiO_2-NPs and co-inoculation of AMF and PGPR resulted in an increase in anthocyanin content under salinity stress (Table 3). In other words, another beneficial effect of bio-fertilizers and SiO_2-NPs under NaCl stress is related to the synthesis of non-enzymatic antioxidants, including anthocyanin, by regulating the phenylpropanoid pathway (Tisarum et al. 2020; Zaimenko et al. 2018). The increase in anthocyanin content under environmental stress is another mechanism of plants against stress-induced damage, and the reason for this increase may be attributed to the photoprotective role of anthocyanin by removing ROS under oxidative stress (Sun et al. 2021). Similar results have been reported on the use of Si-NPs (Alsaeedi et al. 2019) and co-inoculation of AMF and PGPR (Yasmeen et al. 2019) to increase anthocyanin content under salt stress conditions, which are similar to the results of the current study.

Furthermore, the current study clearly revealed that the application of SiO_2-NPs and inoculation of AMF and PGPR individually or in combination led to a significant increase in

grain yield, root dry weight, and LAI in wheat plants under saline stress in comparison with untreated plants (Table 3). So increasing leaf area index leads to increase photosynthesis which could reflect on increasing grain yield. Previous research has shown that the inoculation with AMF and PGPR are obviously beneficial to grain yield under NaCl stress conditions (Sagar et al. 2021). According to reports, salt stress has negative impacts on plant germination, development, and reproduction, lowering crop yields (Nadeem et al. 2019). However, inoculation with AMF and PGPR led to an increase in grain yield, so it has been reported that seed inoculation with AMF and PGPR helped to tolerate salinity stress by improving ionic homeostasis, modulating carbohydrate metabolism, and improving growth and yield (Bharti et al. 2016). Similar findings have been reported about the use of Si-NPs to improve the growth and grain yield of wheat under salinity stress conditions, which are similar to the results of the current study (Mushtaq et al. 2019). It seems that part of the increase in yield under salinity stress conditions with the application of SiO_2-NPs and inoculation of AMF and PGPR individually or in combination can be caused by the increase in chlorophyll and carotenoid content (Fig. 1a–d), LAI, root dry weight, anthocyanin, MSI (Table 3), uptake of nutrient elements such as phosphorus, K^+, and Si (Table 5), and reduction in Na^+ (Table 5), causing better plant tolerance to salinity stress conditions. Also, the researchers' studies showed that the application of Si-NPs and inoculation of AMF and PGPR increased LAI (Siddiqui et al. 2020) and root dry weight (Kaloterakis et al. 2021) under salinity stress, which is consistent with the results of the present study. Also, our study showed that the plant height and spike length decreased significantly under NaCl stress. But these values increased after the application of SiO_2-NPs and the co-inoculation of AMF and PGPR. So that, the absence of NaCl stress and the application of 60 mg/L SiO_2-NPs, as well as the co-inoculation of AMF and PGPR, increased plant height and spike length by 68.9% and 44.61%, respectively, compared to the absence of bio-fertilizers and SiO_2-NPs under 105 Mm NaCl stress (Fig. 3a and b). Generally speaking, we can deduce that the observed decrease in plant height due to NaCl stress treatment could be due to the negative impact of this NaCl on the rate of photosynthesis, as well as a decrease in the level of carbohydrates and growth hormones, both of which can lead to growth inhibition. Indeed, salt stress prevents the plant from developing its full genetic potential by disrupting photosynthetic processes and reducing the supply of nutrients to the growing parts. On the other hand, the use of bio-fertilizers increases nutrient absorption by the plant, improves soil qualities such as organic matter content, and increases the available nitrogen content of the soil. This is because plant height is influenced by the number of nodes and the length of the internodes, which increase when water and the necessary nutrients are available. It has been reported that the application of Si-NPs (Badem & Söylemez 2022) and bio-fertilizers (Shultana et al. 2022) led to improved plant height under salinity stress (Ismail et al. 2022). On the other hand, salinity stress, by changing the final capacity of the spike, causes a significant decrease in the spike length, the number of grains in the spike, and finally the grain yield (Table 3). Also, the application of SiO_2-NPs as well as co-inoculation of AMF and PGPR causes a significant decrease in the spike length (Table 3). Mushtaq et al. (2019) reported that the application of Si-NPs under salinity stress conditions increases the spike length (Mushtaq et al. 2019). Also, it has been reported that the application of bio-fertilizers leads to an increase in spike length under salinity stress conditions (Akat 2020), which is consistent with the results of the present study.

On the other hand, salinity stress can negatively impact the availability of essential plant nutrients, resulting in disturbed concentrations of some mineral nutrients (P, N, K, and Zn). In this study, it was observed that salinity stress reduced the uptake and accumulation of phosphorus and K^+, as well as increasing the uptake of Na^+. However, the application of SiO_2-NPs and inoculation with AMF and PGPR were found to increase nutrients concentration, as indicated in Table 5. Researchers reported that salinity stress leads to imbalanced the nutrient uptake and increased the entrance of Na^+ and Cl^- in plant cells, which cause physiological disorders in plants (Tolay 2021). The results of our data show that salinity stress leads to a 93.69% increase in Na^+ uptake and 11.46% decrease in K^+ content in wheat leaves (Table 5). It has been reported that Na^+ opposes cation uptake and Cl- opposes anion uptake by root transporters (Calero Hurtado et al. 2020). According to reports, plant tissues accumulate high concentrations of Na^+ under NaCl stress, which disrupts the equilibrium of other elements like K^+ and results in physiological difficulties and ion imbalances (Ahmad et al. 2018). In this context, several studies were performed to improve plant nutrition under saline conditions, and it was shown, for instance, that the application of bio-fertilizers and silicon reinforces plant nutrient supply under saline conditions. Abdel-Haliem et al. (2017) findings showed that the application of silicon to rice plants result in decrease in Na^+ concentration and increase in K^+ concentration under salinity stress. They reported that this indicates the role of silicon in alleviating salt nutritional negatively affect (Abdel-Haliem et al. 2017). Also, the results of our data show that the application of co-inoculation of AMF and PGPR resulting in a decrease in Na^+ uptake and an increase in K^+ content in wheat leaves (Table 5). It has been reported that inoculation of the plant with PGPR and AMF can reduce passive flow (apoplasmic) of Na^+ and thus limit the Na^+ uptake by the roots, resulting in an increased K^+ uptake under salinity stress (Solórzano-Acosta et al. 2023). On the other hand, our results showed that the application of SiO_2-NPs and

bio-fertilizer treatments resulted in increased silicon and phosphorus uptake as compared to control when plants were exposed to salt stress (Table 5). Lack of phosphorus uptake in environmental stress conditions can lead to disturbances in the synthesis of phosphate sugars and nucleotides in the structure of RNA and DNA molecules. For this reason, phosphorus absorption decreases under salinity stress conditions, but the application of AMF and PGPR individually or in combination, as well as the use of SiO_2-NPs, increased phosphorus uptake under salinity stress conditions as compared to the control. In this context, researchers reported that salt-tolerant plant growth-promoting rhizobacteria and mycorrhizal fungi are an alternative bio-fertilizer that mitigates salt stress by producing diverse bioactive secondary metabolites and phytohormones, including gibberellins (Adhikari et al. 2020). Similar findings have been reported about the use of silicon nanoparticles to increase phosphorus uptake under salinity stress conditions, which are similar to the results of the current study (Agostinho et al. 2017). Also, Adhikari et al. (2020) findings showed that the application of silicon to soybean plants, as well as, the inoculation of the plant with bio-fertilizers Si and P contents in plant shoots were significantly elevated under salinity stress (Adhikari et al. 2020). It has been reported that under environmental stress conditions, the plant uptake more silicon to increase the concentration inside the cell (Zhu et al. 2019). Also, it has been reported that the application of silicon nanoparticles led to an increase in the silicon content of leaves under environmental stress conditions (Alsaeedi et al. 2019). According to reports, the use of silicon resulting in increased silicon uptake in sugarcane under salinity stress conditions (Ashraf et al. 2010), which are similar to the results of the current study.

5 Conclusions

In the current study, we were able to improve our understanding of the beneficial role of the application of plant growth-promoting rhizobacteria (PGPR) as well as arbuscular mycorrhizal fungi (AMF) individually or in combination and the use of silicon dioxide nanoparticles (SiO_2-NPs) in mitigating the adverse effects of salinity on wheat. Overall, the application of SiO_2-NPs and co-inoculation of AMF and PGPR provides numerous benefits to plants, especially in reducing salt stress by improving photosynthesis, chlorophyll and carotenoid content, plant height, spike length, and nutrient management. The results also showed that higher NaCl concentrations lead to disruption of the uptake of nutrients, including potassium (K^+), silicon (Si), and phosphorus (P), as well as increased sodium (Na^+) uptake in the wheat plant. However, co-inoculation of AMF and PGPR and application of 60 mg/L SiO_2-NPs played ameliorative roles by increasing photosynthesis activities, leaf area index, membrane stability index, plant height, spike length, and reduction of anthocyanin in wheat under salinity stress, which reduced oxidative stress in plants and increased grain yield. According to the findings of this study, co-inoculation of AMF and PGPR as well as the application of 60 mg/L SiO_2-NPs can increase wheat grain yield under salinity stress by improving the physiological characteristics and uptake of nutrients.

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Data Availability The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Consent for Publication All authors have expressed explicit consent to submit this manuscript for publication.

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