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RESEARCH

Reduction of Salinity Stress in Wheat through Seed Bio-Priming with Mycorrhiza and Growth-Promoting Bacteria and its Effect on Physiological Traits and Plant Antioxidant Activity with Silicon Nanoparticles Application

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Abstract
The yield of crops is threatened significantly by environmental stresses in various parts of the world. So, the non-essential element silicon and seed priming with mycorrhiza and growth-promoting bacteria are known to improve plant fitness by alleviating the effects of environmental stress, particularly in crops. However, the potential role of the integrated application and their possible role in the exceptional tolerance of this variety of wheat to salinity have not been investigated. This project was designed to assess the potential role of silicon nanoparticles (Si-NPs) and bio-fertilizers in increasing wheat's tolerance to salinity stress by assessing antioxidant activity. The experiment was carried out as a factorial based on a randomized complete block design in greenhouse conditions. The experimental factors consisted of different levels of salt (0, 35, 70, and 105 mM), Si-NPs (0, 30, and 60 mg/L), and bio-fertilizers (control, mycorrhizal fungi, *Flavobacterium*, and *Pseudomonas* strain 18,798 bacteria, application of both bacteria and fungi). The results showed that, under salinity stress, the selected bio-fertilizers and Si-NPs enhanced plant leaf water potential (94.4%), soluble protein (58.61%), soluble sugar (57.54%), catalase (79%), peroxidase (95.87%), and polyphenol oxidase (90.93%) parameters. Both single and dual bio-fertilizers and Si-NPs application improved physiological parameters by stomatal conductance, electrical conductivity, electrolytic leakage and proline. Also, the highest grain yield and lowest malondialdehyde, hydrogen peroxide content were obtained in the combined application of bio-fertilizers and Si-NPs application (60 mg/L) under non-salinity stress. Therefore, obtained results indicated that Si-NPs and bio-fertilizers can improve wheat grain yield under salinity stress conditions by improving the physiological and biochemical traits.

Keywords Nanotechnology · PGPR · Environmental stresses · Hydrogen peroxide · Cereal

1 Introduction

Wheat (*Triticum aestivum* L.) is an important food crop that produces over 20% of the calories, proteins, minerals, and B vitamins consumed worldwide [1]. Environmental factors like drought, salt, and severe temperatures, on the other hand, have an impact on wheat development and yield [2]. In fact, environmental stresses are considered the most important limiting factor for agricultural production in all parts of the world. Furthermore, soil salinity is the most common abiotic stress among the various environmental stresses, and it is a major problem for agriculture, negatively affecting crop growth, development, and productivity [3]. Salinity causes osmotic stress, ion toxicity, turgescence, metabolic imbalance, membrane disorganization, and the generation of reactive oxygen species (ROS), all of which affect the integrity of biological membranes, cause oxidative damage to cells and metabolic processes, and ultimately reduce crop yield [4]. In other words, soil salinity causes several physiochemical abnormalities in sensitive plants, resulting in growth arrest and eventual death. In reality, salt causes oxidative damage, and the generation of ROS leads to membrane breakdown, reduced antioxidant enzyme activity (SOD, POD, APX,

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and CAT), and a reduction in function [5, 6]. According to ALKahtani et al. (2021), in salty-stressed plants, oxidative stress indicators such as hydrogen peroxide (H_2O_2), superoxide ($O^{\cdot-}$), and lipid peroxidation (MDA) are significantly increased [7]. A high salt concentration has been reported to cause osmotic and ionic stresses that damage the photosynthetic apparatus and physiology, such as closing the stomata and reducing the level of leaf expansion [8]. Furthermore, salinity is commonly related to oxidative damage caused by the formation of ROS, such as $O^{\cdot-}$ and H_2O_2, which promotes MDA in a variety of plants under various stress factors [9].

Several techniques have been devised to reduce the damaging effects of high salinity on plant growth. In fact, providing the required circumstances and the requirements to utilize more soil microorganisms and bio-fertilizers is one of the techniques for optimal crop output and environmental health. Among them, plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) have been suggested as sustainable strategies to combat salinity stress [10]. Salinity is mitigated by bio-fertilizers (PGPR and AMF) through a variety of mechanisms, including increased photosynthetic efficiency and nutrient acquisition, maintenance of ionic homeostasis and osmotic equilibrium in plants, and enhancement of the antioxidant system to prevent damage from reactive oxygen species [11]. Researchers has reported that after inoculation with the PGPR, which can reduce ROS production and mitigate the negative effects of salt stress, proline content, MDA, and antioxidant enzyme activities were decreased [12]. Several studies have found that mycorrhizal fungi mitigate the negative effects of salinity through a variety of mechanisms, including increased photosynthetic efficiency and nutrient acquisition, maintenance of ionic homeostasis and osmotic equilibrium in plants, and enhancement of the antioxidant system to protect against damage from ROS [13]. Also, Babaei et al. (2017) suggested that bio-fertilizer inoculation enhanced proline, antioxidant enzymes, and increased wheat yield under stress conditions [14].

Nanoparticles (NPs) are microscopic particles with a diameter of fewer than 100 nm. NPs have unique physicochemical characteristics, such as a large surface area, high reactivity, tunable pore size, and particle form, that enable them to be used in a variety of applications [15]. Silicon (Si) is a significant element that makes up about 28% of the lithosphere and is thought to be an important part of the soil because it effectively neutralizes the harmful effects of various stresses on plants, such as salt and various other stresses [16]. Si is never found in its pure form and is always mixed with other elements to produce oxides, which are then absorbed by plants as uncharged silicic acid [17]. The benefits of Si in improving plant tolerance to biotic and abiotic stresses in a variety of crops, as well as their significance to agriculture, have been widely reported. In this way, Si nanoparticles were reported to reduce oxidative stress in leaves by decreasing hydrogen peroxide and malondialdehyde production while enhancing superoxide dismutase and peroxidase activity [2]. Furthermore, Si nanoparticles (Si-NPs) have been shown to improve plant yield and growth under salinity stress conditions [18]. The co-application of silicon nanoparticles and bio-fertilizer has been reported as an environmentally friendly approach for improving salinity stress tolerance in crop plants. In a way, bio-fertilizer and Si applications have recently been discovered to boost salinity stress resistance, making them practical and promising for improving plant growth and yield under salinity stress [19]. Furthermore, higher levels of defensive enzymes may be provided via PGPR and Si fertilization, which may result in faster plant development [20]. The researchers reported that the usage of bio-fertilizers and silicone in saline stress conditions increased plant performance. On the other hand, it has enhanced the plant's antioxidant enzymes. As a result, the plant has grown in response to the salinity stress [19–21]. Also, Researchers have reported that under salinity stress, application of Si and AMF improved the growth, yield, and physiological traits of baby corn [21]. On the other hand, previous research has shown that the application of bio-fertilizer and Si improved the physiological traits of alfalfa, and increased antioxidant enzymes under salinity stress. So, the plant has grown in response to the salinity stress [22]. Reported that PGPR activities are currently stated as being enhanced silicon nanoparticles [23]. Nevertheless, a detailed examination of the mutual interaction of the bio-fertilizers (PGPR and AMF) and nano-compounds is needed for their investigation of sustainable agriculture plans. In fact, the integrated application of silicon nanoparticles with PGPRs and AMF may provide better results than applying them as an individual [24]. Because of the importance of bio-fertilizers and foliar utility with silicon nanoparticles in reducing the effects of abiotic stresses, and due to a lack of research on the interaction of those factors, the impact of bio-fertilizers and silicon on physiological traits and plant antioxidant activity of wheat under salinity was investigated.

2 Materials and Methods

2.1 Greenhouse Experiment and Experimental Factors

The objective of this experiment was to determine the appropriate concentration of silicon nanoparticles and wheat seed bio-priming with mycorrhiza and growth-promoting bacteria and evaluate their effect on physiological traits and plant antioxidant activity under salinity stress conditions. It was carried out in 2021–2022 at the greenhouse of the Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Iran. This experiment was conducted in the greenhouse for about seven months. The experiment was carried out as a factorial based on a randomized complete block design with three replications in greenhouse conditions.

The experimental factors were included different salt concentrations (Control, 35, 75, and 105 mM), foliar application of silicon nanoparticles (Si-NPs) (control, 30, and 60 mg/L) and bio-fertilizer (control, mycorrhiza application, both application of *Flavobacterium* and *Pseudomonas* strain 18,798, both application of *Flavobacterium* and *Pseudomonas* strain 18,798 with mycorrhiza). Salt stress treatments were done with irrigation water in two stages (at the 3–4 leaf stage and before the tillering stage). In furthermore, Si-NPs was applied to the leaves in two stages of vegetative growth (stem stage and before the booting stage). The wheat cultivare of Mihan was used in this study that 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. The soil Specification are as follows: silty soil textured, 38.5% sand; 42% silt and 19% clay. The experiment was carried out in natural conditions (photoperiods of 10–12 h, temperatures of 20–25˚C, and humidity levels of 65–70%).

2.2 Specifications of Bio-Fertilizers and Silicon Nanoparticles

The fungus used was *Glomus mosseae* (NCBI: 27,381), which was prepared by Zist Fanavar Turan Corporation. Also, *Psedumonas Putida* strain 18,798 (NCBI: 1,295,133) and *Flovobacterium* (NCBI: 237) of were isolated from the rhizospheres of wheat by Research Institute of Soil and Water, Tehran, Iran. Which contained 10^8 live and active bacteria per gram. Seeds were covered with gum Arabic to act as an adhesive, then rolled into a bacterial mixture until uniformly coated. Si-NPs were obtained from The American Corporation US Research Nanomaterial, which was prepared by Pishgaman Nanomaterials Company. The Si-NPs characteristics were: 98% purity and 20—30 nm particle size. The Si-NPs were suspended in water after ultrasonic vibrations (100 watts) at 40 kHz for 30 min, yielding a largely homogenous solution. The flag leaves of plants were separated in the middle of the booting stage for the following measurements [25].

2.3 Biochemical and Physiological Traits of Wheat Leaf

2.3.1 Relative Water Content (RWC)

Fresh leaves of the plant were recorded after its fresh weight sampling sample. It was then placed in distilled water for 24 h. After 24 h, the swollen weight was measured. Finally, the dry weight of the samples was measured for 24 h after being placed in an oven at 60 °C. Finally, the RWC was calculated using the following formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \quad (1)$$

Where RWC, FW, DW, and TW are relative water content, fresh weight, dry weight, and turgid weight, respectively.

2.3.2 Electrolyte Leakage Assay (EL %), Electrical Conductivity (EC), and stomatal conductance (Gs)

In 25 cm^3 of deionized water, ten fresh wheat leaf discs (1 cm^2) were placed in bottles. Bottles were shaken for 20 h, electrical conductivity (EC_1) was measured, then flasks were heated for 1 h at 80˚C, and the samples were shaken for another 20 h at 21˚C, at which point each flask's final conductivity (EC_2) was calculated. Finally, the electrolyte leakage assay was calculated using the following formula:

$$EL = \frac{EC1}{EC2} \times 100 \quad (2)$$

In this formula, EL is electrolytic leakage. EC_1 and EC_2 are electrical conductivity before and after heating. Also, prometer device was used to measure stomatal conductance of flag leaves.

2.3.3 Antioxidant Enzymes Activity

Catalase (CAT), Peroxidase (POX) and polyphenol oxidase (PPO) activity were assessed through [26] method and by spectrophotometer which were described as OD µg Protein min^{-1}. In a way, to determine enzyme activity, 0.2 gr of fresh flag leaf tissue was crushed with liquid nitrogen and one milliliter of Tris–HCl (0.05 M, pH = 7.5) buffer was added. The resulting mixture was centrifuged for 20 min at 13,000 rpm at 4 °C, and the supernatant was used to assess enzyme activity.

2.3.4 Measurement of Proline Content

To measure the proline content (µg g Fw^{-1}) in leaves was measured by the method of Bates et al. [27]. First, 0.5 gr of fresh flag leaf tissue was crushed with liquid nitrogen and 10 ml of Sulfosalicylic acid 5% was added were mixed together in an ice container. Then, centrifuged for 30 min at 4 °C at 7500 rpm. Its absorbance was recorded at wavelength 520 nm using a spectrophotometer.

2.3.5 Total Soluble Protein and Sugars Content

The Bradford [28] method was used to determine the amount of soluble protein (mg g Fw^{-1}) in leaf tissue. The leaf samples (0.2 gr of fresh flag leaf tissue) were mixed with one ml of Tris–HCl (0.05 M, pH = 7.5) buffer and centrifuged for 20 min at 4° C at 11,500 rpm. The supernatant was used for the assay of soluble proteins. Soluble protein was measured at 595 nm by spectrophotometer.

Also, Total soluble sugars (mg/g Fw^{-1}) were assessed through [29] method and was measured at 490 nm by spectrophotometer. 0.2 gr of the leaf sample was ground with two ml of sodium phosphate buffer and centrifuged for 20 min at 4° C at 10,000 rpm.

2.3.6 Measurement of Malondialdehyde (MDA) and Hydrogen Peroxide (H_2O_2)

Lipid peroxidation (µmol g Fw^{-1}) was recorded in terms of MDA content based on the thiobarbituric acid (TBA) method [30] and by spectrophotometer with absorbance was recorded at wavelength 532 and 600 nm. Also, H_2O_2 in plant leaf was determined by using the method [31], and is expressed as µg/g Fw^{-1} measured by spectrophotometry at 390 nm. In addition, to measure the grain yield, at the end of the growth period, 10 plants of each pot randomly were harvested.

2.4 Statistical Analysis of the Data

The data obtained were subjected to one-way Analysis of Variance (ANOVA). Each treatment was conducted with three replicates and comparisons concerning treatment tools were made by recruiting the least significant difference (LSD) at the 0.05 and 0.01 probability level by SAS 9.4 software.

3 Results and Discussions

3.1 Impact of Bio-Fertilizers and Silicon Nanoparticles Application under Salt Stress on Wheat Biochemical Characteristics

Catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) as an antioxidant defense, peroxide hydrogen (H_2O_2), proline, and malondialdehyde (MDA) as oxidative damage, and soluble protein, soluble sugar and proline content as a biochemical change in wheat leaves were selected to evaluate the effects of silicon nanoparticles (Si-NPs) and bio-fertilizers (BF) under salinity stress. The data shows that treatment had a significant effect on all of the parameters (Table 1).

3.1.1 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Activity of Antioxidant Enzymes of Wheat

Antioxidant enzymes CAT, POX, and PPO activity as an indicator of stress was more evident in wheat plants subjected to salinity stress compared to controls, resulting in significant increases in antioxidant enzyme activities in salinity-stressed plants (Fig. 1, A to C). As shown in Fig. 1, the highest content of activity of antioxidant enzymes was at the highest level of salinity (105 mM) and with the application of the combined use of arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) and Si-NPs (60 mg/L). In other words, gradual increases in salinity and the use combined of AMF/PGPR and Si-NPs increase the activity of antioxidant enzymes, the most significant of which were salinity 105 mM and the combined use of AMF/PGPR, and 60 mg/L Si-NPs, which recorded an increase in CAT, POX, and PPO of about 79%, 95.87%, and 90.93%, respectively, compared to control plants (Fig. 1, A to C). Indeed, one of the reasons for the increase in antioxidant enzyme activity in salinity stress conditions could be the effective coexistence of the application of AMF/PGPR together or individually versus their non-application. Because of the enhanced nutrient absorption by the plant, the application of bio-fertilizers increases the activity of antioxidant enzymes. So, previous studies have reported that mycorrhiza and PGPR applications have the greatest effect on enzymatic antioxidant activities under

Table 1 Analysis of variance biochemical traits under salinity conditions, application of bio-fertilizers and silicon nanoparticles

S.O.V	D.F	Mean squares							
		CAT	POX	PPO	soluble sugar	H_2O_2	MDA	Soluble protein	Proline
Replication	2	1.84	16,231.35	1.62	6979.26	0.168	0.0194	846.82	44.12
Salinity (SA)	3	1566.62**	16,685.33**	6196.36**	1171.95**	0.018**	0.00194**	281.81**	46.80**
Bio-fertilizers (BF)	3	335.47**	621.45**	943.64**	888.02**	0.002**	0.00058**	188.63**	9.96**
Nano-Silicon(Si-NPs)	2	201.34**	242.04**	227.10**	136.98**	0.001^{ns}	0.00022**	31.70**	10.50**
SA* BF	9	32.76**	378.90**	131.59**	11.67**	0.019**	0.00010**	9.23**	0.699^{ns}
SA*Si-NPs	6	14.61**	95.74**	29.38**	1.95^{ns}	0.002*	0.000015^{ns}	2.49**	0.331^{ns}
BF* Si-NPs	6	12.28**	22.92^{ns}	32.56**	15.64**	0.010**	0.000064**	2.09**	0.850^{ns}
SA* BF* Si-NPs	18	8.28**	30.05*	17.89*	11.55**	0.004**	0.000074**	0.77*	0.355^{ns}
Error	94	4.152	16.18	9.92	1.94	0.0006	0.000016	0.453	0.573
C.V (%)	-	4.42	4.37	5.24	2.31	8.49	4.12	3.20	10.82

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

environmental stress, such as salinity stress [32]. Recently, researchers reported that mustard plants exposed to salt stress during early development showed increased activity of antioxidant enzymes according to increasing salinity doses, while inoculation with AMF and PGPR increased the total amount of antioxidant enzymes [33]. In fact, an increase in the activity of antioxidant enzymes such as catalase, peroxidase, and polyphenol oxidase with the application of bio-fertilizers can be considered a cellular defense mechanism against oxidative damage under salinity stress [24]. On the other hand, the application of 60 mg/L Si-NPs increased the activity of antioxidant enzymes under salt stress conditions, according to our findings. Some of the increase in antioxidant enzyme activity appears to be due to the use of Si-NPs, which protects the cell membrane from lipid peroxidation and allows the plant to tolerate stress conditions, thereby increasing the activity of antioxidant enzymes like catalase and peroxidase. It has been reported that the increase in CAT, POX and PPO content under salt stress may be a result of improved biological activity of plants by the addition of bio-fertilizer and silicon [21]. Similar findings have been reported about the use of silicon to increase plant antioxidant activity in salinity stress conditions, which are similar to the results of the current study [34].

3.1.2 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Soluble Sugar of Wheat

As can be seen in Fig. 1, D, the highest soluble sugar was 72.80 mg/g FW obtained the absence of BF and Si-NPs under salinity 105 mM stress conditions. Furthermore, the lowest amount of soluble sugar was 46.21 mg/g FW in the application of the combined use of AMF/PGPR and Si-NPs of 60 mg/L under non-salinity stress conditions (Fig. 1, D). In fact, applying Si-NPs at 60 mg/L and BF resulted in a 57.54% increase under salinity 105 mM stress conditions, compared to not applying Si-NPs and AMF/PGPR under non-salinity stress conditions (Fig. 1, D). This study showed that at each stage of salinity stress, the content of soluble sugar also increased with the increase in stress intensity. It seems that the increase in soluble sugars due to salinity stress is due to raising the osmotic pressure inside the plant, because in this way the plant can absorb nutrients and water from the soil. Also, the use of bio-fertilizers in conditions of environmental stress by increasing the absorption of nitrogen and potassium and increasing the efficiency of these two elements in the process of plant photosynthesis has played a significant role, which increases the solubility

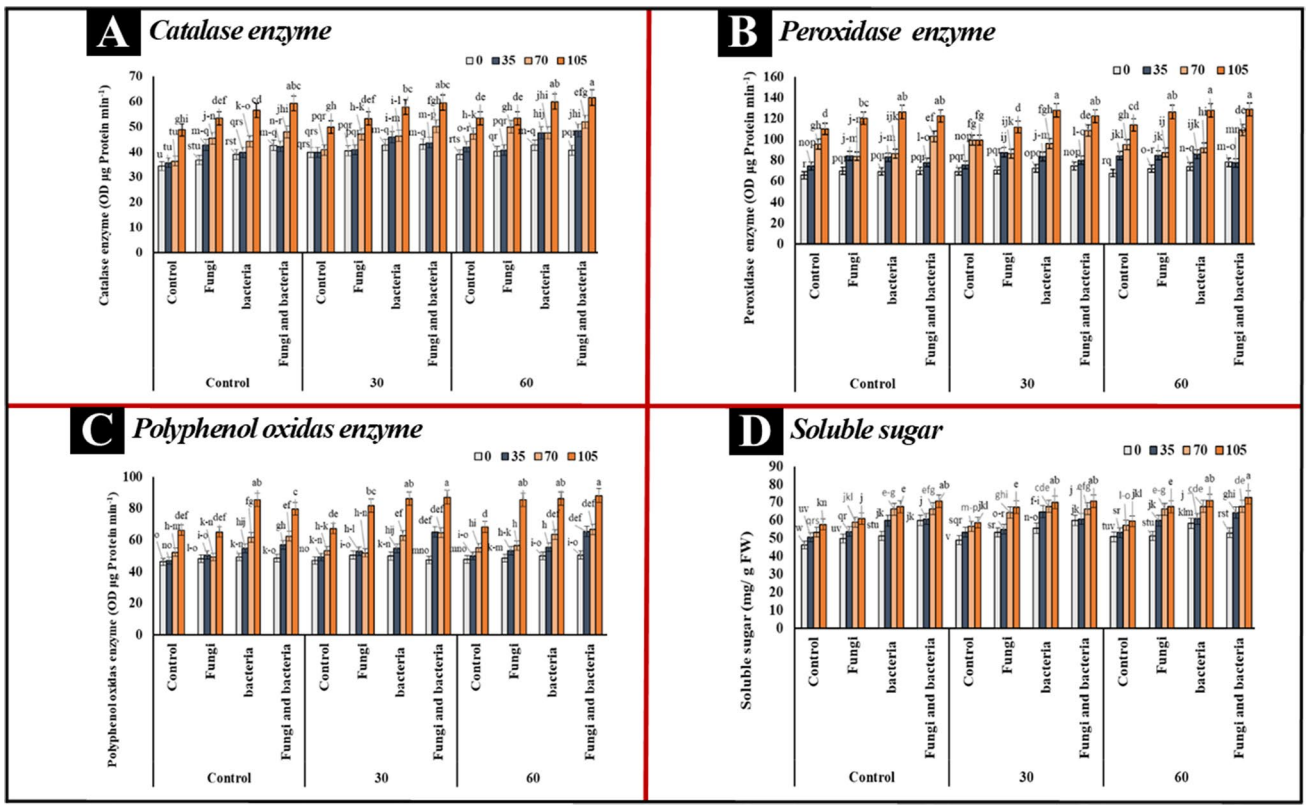

Fig. 1 Effect of bio-fertilizers and silicon nanoparticles on catalase (**A**), peroxidase (**B**), polyphenol oxidase (**C**), and soluble sugar (**D**) in wheat under salinity stress

of elements in the root environment and the absorption of nutrients. As a result, it increases the rate of photosynthesis and increases sugar production in the plant. On the other hand, by reducing oxidative stress, protecting chloroplasts and cell membranes, and protecting macromolecules such as proteins, silicon increases the sugars in the plant. Kerbab et al. (2021) reported that the application of bio-fertilizers under salinity stress conditions increases the content of soluble sugar. These researchers stated that an increase in the soluble sugar content following inoculation with bio-fertilizers significantly contributes to the growth of plants under salt stress by modulating defense strategies [12]. Also, it has been reported that the application of silicon nanoparticles in conditions of salinity stress also increases water absorption in the roots by accumulating soluble sugars and amino acids [35], which is consistent with the results of the present study.

3.1.3 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on H_2O_2 and MDA of Wheat

The comparison of means showed that under salinity stress conditions, application of Si-NPs and AMF/PGPR resulted in a significant reduction in H_2O_2 compared with the no application them. In fact, the maximum H_2O_2 was obtained at the highest level of salinity (105 Mm) and the absence of BF and Si-NPs, which caused a 78.63% increase in H_2O_2 compared to the use of AMF/PGPR and 60 mg/L of Si-NPs (Fig. 2, A). According to reports, the level of H_2O_2 increases during salinity stress, and antioxidant enzymes are the most important compounds in the deactivation of free radicals [32]. Also, the application of AMF/PGPR and Si-NPs causes a decrease in hydrogen peroxide by increasing the activity of some important enzymes of the oxidative defense system, such as catalase and peroxidase (Fig. 1, A and B) and decreasing the content of MDA (Fig. 2, B). In fact, Si-NPs and AMF/PGPR enhanced antioxidant machinery by acting as scavengers against H_2O_2 and O^-_2, ensuring ROS equilibrium at the cellular level, which promoted membrane stability and permeability [36]. The increase in H_2O_2 content under salt stress was also found in previous studies [37]. This increase depends on the severity of salt stress and the intensity of cell membrane damage. It was reported that the application of silicon nanoparticles and bio-fertilizers under salt stress conditions significantly reduced the H_2O_2 content. It seems that part of the decrease in H_2O_2 content in the case of application of silicon nanoparticles [38]and bio-fertilizers [39] is related to the increase in the activity of some important enzymes of the oxidative defense system, such as catalase (Fig. 1, A).

Also, our results showed that salinity stress significantly increased the MDA. In addition, it was found that application of Si-NPs and AMF/PGPR significantly reduction the MDA content in salinity stress conditions compared to no application them. Nonetheless, the results showed that applying Si-NPs (60 mg/L) and AMF/PGPR externally alleviated salt-induced oxidative injury by lowering MDA and H_2O_2 levels in wheat tissues. Thus, the highest MDA content was obtained under salinity stress conditions of 105 mM (0.1159 µmol/g FW) and without the use of bio-fertilizers and Si-NPs. Also, the lowest MDA content was obtained in the application of AMF/PGPR and Si-NPs, at 60 mg/L in non-salinity stress conditions (0.080 µmol/g FW). In fact, the increase in MDA suggests that under stress conditions, the membrane structure is damaged and its lipids are released, and these lipids are oxidised and MDA is formed due to the presence of ROS chemicals that increase under stress. It has been reported that the highest MDA content under salt stress is due to the large amount of hydrogen peroxide in plants exposed to salt stress [40], which may demonstrate the rate of lipid peroxidation of membranes. On the other hand, plants colonized with AMF/PGPR and Si-NPs have lower MDA content than control plants, suggesting that bio-fertilizer and silicon are involved in the metabolism of ROS. It seems that the application of Si-NPs and bio-fertilizers increases the activity of antioxidant enzymes (Fig. 1, A to C) and protects the cell membrane against lipid peroxidation, as well as increases compatible osmolytes such as proline (Fig. 2, B) and soluble sugars (Fig. 1, D), which leads to better plant tolerance to stress conditions and a decrease in MDA. In fact, oxidative damage (H_2O_2 and MDA content) induced by salinity stress was significantly reduced in inoculated with AMF and PGPR plants. It has been reported that the content of MDA increased under stress conditions, while plants inoculated with bio-fertilizers [41] and silicon [42] have lower MDA than control plants, implying that both bio-fertilizers and silicon are involved in the metabolism of ROS [43].

3.1.4 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Total Soluble Protein of Wheat

As can be seen in Fig. 2, C, the highest content of soluble protein was 30.09%, obtained from 60 mg/L of Si-NPs application and AMF/PGPR under non-salinity conditions. Furthermore, the lowest amount of soluble protein was 18.97% in non-applications of BF and Si-NPs under salinity 105 mM stress conditions (Fig. 2, C). In fact, applying Si-NPs at 60 mg/L and BF resulted in a 58.61% increase under non-salinity stress conditions, compared to not applying Si-NPs and BF under salinity 105 mM stress conditions (Fig. 2, C). Due to a reduction in protein synthesis and an increase in the activity of protein hydrolyzing enzymes, salinity typically results in a decrease in protein in stressed plants [44]. Also, under salt stress, use of the mycorrhizal fungi and

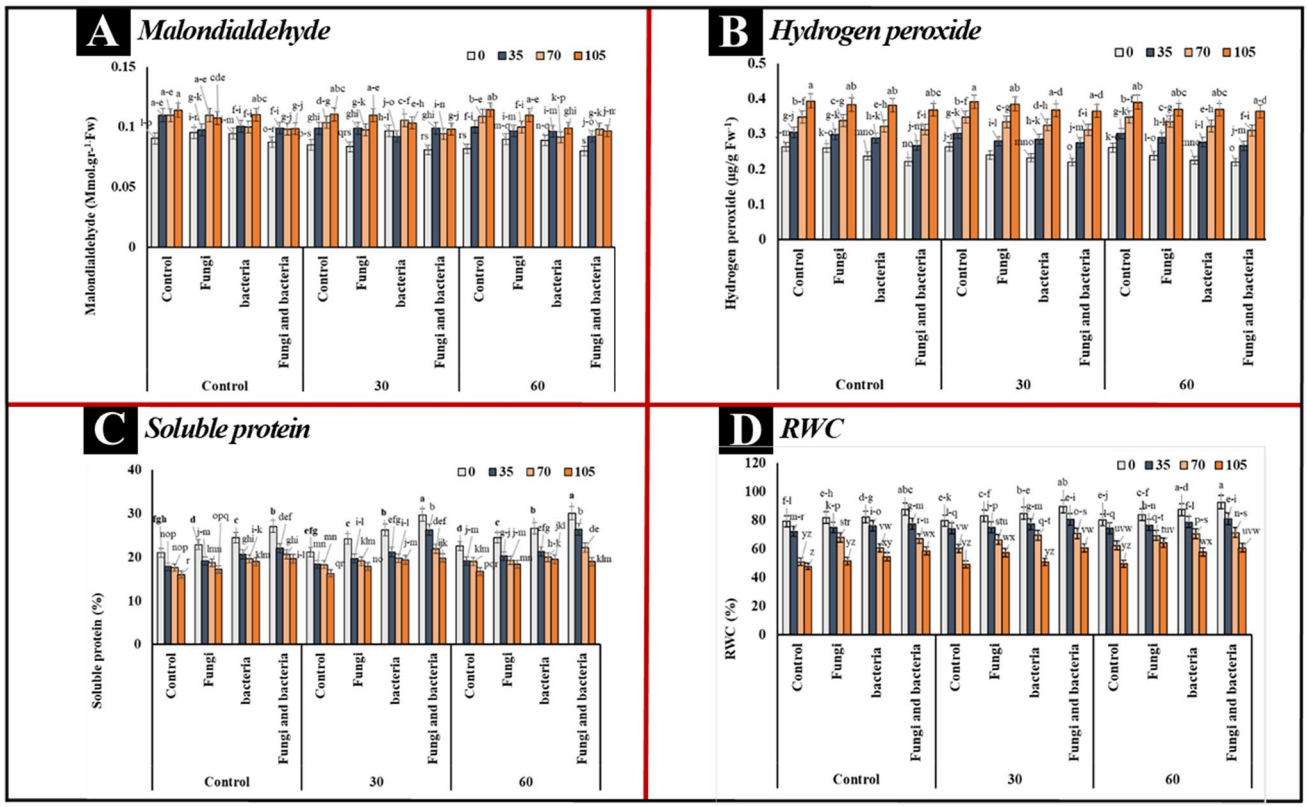

Fig. 2 Effect of bio-fertilizers and silicon nanoparticles on peroxide hydrogen (**A**), malondialdehyde (**B**), soluble protein (**C**), and relative water content (**D**) in wheat under salinity stress

growth-promoting bacteria can boost protein synthesis due to improved water access and selective absorption of mineral elements, especially nitrogen and phosphorus, as well as increased activity of the nitrate-reducing enzyme, nitrate reductase [45]. Previous research has shown that using mycorrhizal fungi and growth-promoting bacteria increases soluble protein under salinity stress conditions. Also, the amount of soluble proteins increased significantly as the use of silicon nanoparticles increased. It seems that the increase of soluble proteins in the application of silicon nanoparticles is caused by the synthesis of new proteins, the increase in the level of proteins related to stress tolerance such as proline, or the role of this element in dealing with oxidative stress [46]. The decrease in protein content under salinity stress appears to be caused by the reaction of protein with free radicals, which results in amino acid changes, a decrease in protein synthesis, an accumulation of free amino acids, including proline, and an increase in the activity of protein degrading enzymes. Furthermore, when 60 mg/L Si-NPs was used, the soluble proteins increased significantly. It seems that the increase of soluble proteins in the application of Si-NPs is due to the synthesis of new proteins, the increase in the level of proteins related to stress tolerance, such as proline, or the role of this nanoparticle in dealing with oxidative stress. Also, the effect of Si-NPs in preventing the structural and functional destruction of the cell membrane, increasing the stability of lipids in the cell membrane of crop plants exposed to salinity stress has also been reported by other researchers [47].

3.1.5 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Proline of Wheat

The comparison of the averages showed that the highest proline content was obtained in 60 mg/L of Si-NPs (7.41 µg/g leaf wet weight) and application of the combined use of AMF/PGPR (7.65 µg/g leaf wet weight) under 105 mM salinity stress conditions (8.06 µg/g leaf wet weight) (Table 3). In the present study, Under 105 mM salinity stress, the proline content increased by 45.75% when compared to non-salinity stress. Also, the application of 60 mg/L of Si-NPs and the combined effects of AMF and PGPR enhanced 14.17% and 25%, respectively, compared to non-application of these (Table 3). In actuality, the absence of AMF/PGPR and Si-NPs during salinity stress lowered proline content when compared to the use of 60 mg/L Si-NPs and bio-fertilizers during non-salinity stress circumstances. Increased proline concentration protects

Table 2 Analysis of variance physiological and yield traits under salinity conditions, application of bio-fertilizers and silicon nanoparticles

S.O.V	D.F	Mean squares				
		RWC	EC	EL	Gs	Grain yield
Replication	2	9390.42	14,613.78	648.70	1215.78	5.27
Salinity (SA)	3	2943.11**	28,706.08**	2634.14**	188.57**	4.48**
Bio-fertilizers (BF)	3	3814.87**	734.33**	430.17**	136.09*	0.68**
Nano-Silicon(Si-NPs)	2	417.02**	79.73*	167.77**	1346.04**	0.27**
SA* BF	9	80.43**	23.52**	63.95**	1.20^{ns}	0.08^{ns}
SA*Si-NPs	6	11.00^{ns}	8.88^{ns}	11.49*	3.27^{ns}	0.06^{ns}
BF* Si-NPs	6	46.25**	2.25^{ns}	4.51^{ns}	2.38^{ns}	0.05^{ns}
SA* BF* Si-NPs	18	12.00*	7.78^{ns}	1.60^{ns}	3.52^{ns}	0.02^{ns}
Error	94	6.35	22.27	4.92	45.86	0.091
C.V (%)	-	3.60	3.17	6.83	15.98	17.33

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

cell membranes, proteins, cytoplasmic enzymes, reactive oxygen species, and scavenges free radicals in many plants when cells are subjected to environmental stresses such as salinity stress. Plants, in reality, can tolerate stress by producing more proline, polyamine, and protein [14]. The enhanced accumulation of proline due to salinity stress has been reported by the researchers, who concluded that proline helps to stabilize membranes, sub-cellular structures, and cellular redox potential by destroying free radicals [48]. It has been reported that under salt stress, proline content increases and the application of silicon enhances these biochemical changes that occur under high stress conditions, up to a certain limit. Indeed, prolines act as osmolytes under salt stress conditions [49]. In addition, reported that salt-tolerant bio-fertilizers increase proline content and regulate phytohormone levels, nutrient uptake, redox potential, ion homeostasis, photosynthetic capacity, and expression of stress-sensitive genes that are positively correlated with salt tolerance [50]. Also, proline plays an effective role in preventing the destruction of enzymes, preventing the breakdown of macromolecules, and maintaining the strength of the cell wall during environmental stress; therefore, it seems that the application of Si-NPs and the combined of AMF/PGPR by increasing proline intensifies this effect and helps to increase the plant's tolerance against salinity stress [51].

3.2 Impact of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Wheat Physiological and Yield Traits

Concerning physiological and grain yield characteristics, Table 2 showed that the effects of silicon nanoparticles (Si-NPs) and bio-fertilizers (BF) under salinity stress on relative water content (RWC), electrolyte leakage (EL), electrical conductivity (EC), stomatal conductance (Gs), and grain yield had a significant effect (Table 2).

Table 3 Comparison of means of physiological and yield traits under salinity conditions, application of bio-fertilizers and silicon nanoparticles

Salinity levels (Mm)	Proline (µg/g FW)	EC (µsm^{-2})	EL (%)	Gs (mmol.m^{-2}.s)	Grain yield (gr)
Control	5.53^{c} ± 0.85	107.58^{d} ± 9.46	21.35^{d} ± 5.72	50.83^{a} ± 11.30	2.20^{a} ± 0.63
35	6.68^{b} ± 1.02	152.72^{c} ± 31.28	30.25^{c} ± 8.85	43.54^{b} ± 10.29	1.82^{b} ± 0.32
70	7.71^{a} ± 1.40	165.42^{b} ± 29.22	38.09^{d} ± 11.41	39.21^{c} ± 5.50	1.55^{c} ± 0.27
105	8.06^{a} ± 1.46	168.85^{a} ± 32.51	40.17^{a} ± 12.74	35.89^{d} ± 14.20	1.39^{d} ± 0.31
Bio-fertilizers					
Control	6.36^{c} ± 1.58	153.19^{a} ± 38.90	36.96^{a} ± 12.33	36.34^{d} ± 9.76	1.58^{c} ± 0.37
Fungi	7.01^{b} ± 1.59	150.69^{b} ± 34.50	32.71^{b} ± 13.12	41.18^{c} ± 8.17	1.70^{bc} ± 0.40
Bacteria	6.95^{b} ± 1.49	148.05^{c} ± 32.12	31.57^{c} ± 11.86	44.92^{b} ± 8.59	1.76^{b} ± 0.49
Fungi×Bacteria	7.65^{a} ± 1.39	142.64^{d} ± 34.28	28.62^{d} ± 8.93	47.02^{a} ± 10.78	1.91^{a} ± 0.67
Si-NPs (mg/L)					
Control	6.49^{c} ± 1.56	147.35^{b} ± 37.53	34.04^{a} ± 8.63	40.94^{b} ± 10.09	1.67^{b} ± 0.39
30	7.08^{b} ± 1.53	148.65^{ab} ± 30.06	32.95^{b} ± 9.21	42.63^{a} ± 8.87	1.73^{ab} ± 0.57
60	7.41^{a} ± 1.50	149.93^{a} ± 37.53	30.40^{c} ± 9.90	43.54^{a} ± 11.22	1.82^{a} ± 0.0.54

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

3.2.1 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on RWC of Wheat

In this study showed that all salinity concentrations caused negative effects on plant RWC content as compared with control treatment. Overall, plants treated with the combined application of AMF/PGPR and Si-NPs under non-stress and salt stress conditions had higher RWC values (Fig. 2, D). In saline plants, a substantial decrease in the RWC was detected. Nevertheless, with sole and combined treatments of Si-NPs and bio-fertilizers, a significant increase in the RWC was shown by plants. In other words, results showed that of the combined application of AMF/PGPR and 60 mg/L of Si-NPs under non stress, RWC contents increased by about 94.4% compared with the absence of AMF/PGPR and Si-NPs under 105 mM salinity stress (Fig. 2, D). The response of plants to osmotic stress starts to show up immediately after the salt concentration around the roots increases to a threshold level, this affects water uptake, which retards plant [52]. Singh et al. reported that the RWC of wheat leaves decreased significantly under salt stress, which this is attributed to the negative effect of salt stress on soil water uptake and reduced water availability, which affects the overall water status of the plant [53]. Although salt stress can lead to a significant decrease in the RWC of leaves, it seems that the application of Si-NPs increases the RWC by increasing the concentration of potassium and silicon ions inside the cell. Through deposition in the epidermis of the cells and also in the upper part of the cuticle of the leaf, silicon causes a decrease in the water exit from the leaves, and as a result, it leads to an increase in the RWC of the leaves and the tolerance of the plant to stress conditions [54]. It has been reported that the application of bio-fertilizers individually and together can improve the growth of the host plant due to the high potential for adaptation to a diverse range of stressed environments. In fact, the use of bio-fertilizers creates a more developed root system, which leads to an increase in the plant's ability to absorb water and an increase in the RWC [55, 56].

3.2.2 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Electrical Conductivity and Electrolyte Leakage of Wheat

This study showed that all salinity concentrations caused more effects on plant EC as compared with control treatment. Also, plants treated with the combined application of AMF/PGPR and Si-NPs had lower EC values. In other words, the comparison of the averages showed that the highest EC content was obtained in the absence of Si-NPs (147.35 μsm^{-2}) and non-BF (153.19 μsm^{-2}) under 105 mM salinity stress conditions (168.85 μsm^{-2}) (Table 3). It seems that the reason for the increase in EC under stress conditions can be caused by the production of ROS and the induction of oxidative stress. Because ROS lead to peroxidation of membrane lipids and changes in membrane permeability and damage to the cell, as a result of which the cell membrane is torn and causes an increase in ion leakage out of the cell. In this situation, electrical conductivity will increase due to cell membrane damage and the removal of cell electrolytes [14]. However, the use of bio-fertilizers increases the absorption of nutrients, the development of the root system and the improvement of the water status of plants (Fig. 2, D), which stabilizes the plant's cell membrane. It has been reported that under saline soils, there is a reduction in maize yield due to osmotic stress and ion toxicity caused by higher accumulation of Na + in the leaves, leading to severe wilting, and that the application of bio-fertilizers and silicon nanoparticles leads to an improvement in maize yield [57], which is consistent with the results of the present study.

Also, the results showed that EL% increased with increasing salinity level. As, the highest was obtained in the 105 mM salinity stress conditions which increased by about 88.1% compared with the absence of salinity stress (Table 3). On the other hand, plants treated with the combined application of AMF/PGPR and Si-NPs had lower EL% values. In other words, the comparison of the averages showed that the highest EL% was obtained in the absence of Si-NPs (34.04%) and non-biofertilizers (36.96%). It seems that salinity has caused an increase in electrical conductivity to the outside of the cell through damage to the cell membrane and destruction of its structure and the leakage of more intracellular substances. It has been reported that due to the overproduction of ROS in plant cells, membrane stability or integrity is disturbed, resulting in increased electrolyte leakage under stress conditions [53]. Also, researchers reported that electrolyte leakage increases under salinity stress conditions, while PGPR inoculation under salinity treatment remarkably reduced the electrolyte leakage assay in the leaves of pea seedlings [58]. Also in the current study showed that with the application of Si-NPs under salinity treatment remarkably reduced the electrolyte leakage. Some researchers have attributed the reason for this to the deposition of silicon in the cell membrane, its hardening and significant increase in its stability [59].

3.2.3 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Stomatal Conductance of Wheat

The comparison of the averages showed that the highest Gs content was obtained in 60 mg/L of Si-NPs (43.54 $mmol.m^{-2}.s$), which showed no significant difference from the concentration of 30 mg/L (Table 3). Also, in the present experiment the comparison of the averages showed that the highest Gs content was obtained in the combined application

of AMF/PGPR (47.02 mmol.m^{-2}.s) and non-salinity stress (50.83 mmol.m^{-2}.s) (Table 3). In other words, combined application of AMF/PGPR and Si-NPs caused increase 29.3% and 6.35% respectively, Gs compere to control treatment (Table 3). In fact, osmotic tolerance is one of the main salinity tolerance mechanisms in plants, and by the way stomatal conductance quickly decreased to preserve water. In this research, silicon nanoparticles treatmented samples and bio-fertilizers had more stomatal conductance than control. Researchers reported that silicon plays an effective role in maintaining water balance in plant tissues by increasing water uptake and increases stomatal conductance under stress conditions [60]. In addition, the use of bio-fertilizers that increase nutrient uptake such as phosphorus, leads to root expansion and improved access to water resources, a reduction in abscisic acid, and an increase in stomata conductance [50]. It has been reported that stomatal conductance reductions under salinity stress conditions, while the application of bio-fertilizers and silicon under salinity treatment remarkably increase the stomatal conductance in the leaves of plants [46, 61], which is consistent with the results of the present study.

3.2.4 The Effect of Bio-Fertilizer and Silicon Nanoparticles Application under Salt Stress on Grain Yield of Wheat

The results of the present study have shown that the salinity stress decreases the wheat grain yield. Our results revealed that all salinity levels (35 to 105 mM) caused a considerable reduction in the grain yield compared to the control treatment. In other words, obtained results in Table 3 revealed a statistically significant decrease in grain yield under salinity-stressed wheat (17.27%, 29.54%, and 36.81%) compared with the control treatment. Nevertheless, the application of bio-fertilizers and Si-NPs considerably augmented grain yield in wheat plants under saline stress in comparison with untreated plants. In fact, among the treatments, the combined application of AMF/PGPR and concentration of 60 mg/L of Si-NPs resulted in an increased grain yield of 20.88% and 8.98% respectively, compared with control treatment (Table 3). Researchers reported that under salt stress, the highest grain yield of rice was obtained by applying bio-fertilizer and silicon nanoparticles [24]. Also, It has been reported that the application of silicon nanoparticles, by increasing the accumulation of antioxidant enzymes and increasing the efficiency of photosynthetic devices and PGPR containing the enzyme ACC deaminase, improve the yield of wheat grains due to the reduction of ethylene level in the plant, growth and yield [62, 63]. It seems that part of the increase in yield in salinity stress conditions with the use of bio-fertilizers and Si-NPs can be caused by the increase in the activity of antioxidant enzymes (Figure 1, A to C), RWC (Figure 2, D), reduction in EC (Table 3), and MDA (Figure 2, B), which causes better plant tolerance to salinity stress conditions. The researchers' studies, revealed that, the treatment with Si-NPs [7] and bio-fertilizers [46] increased grain yield under salinity conditions, which is consistent with the results of the present study.

4 Conclusions

Plants try to maintain optimal conditions when they are stressed. As a result, many plant metabolites undergo quantitative and qualitative changes. What was observed in this project also confirms this fact. In the current study, we provided evidence of Si-NPs exogenous application and mycorrhiza fungi and growth-promoting bacteria seed treatment in the mitigation of salinity stress effects on wheat plants. The results showed that the bio-fertilizer inoculation and application of Si-NPs at 60 mg/L enhanced the biochemical and physiological mechanisms of wheat plants under salinity stress. The results also demonstrated that higher concentrations of salt cause osmotic disturbance in the wheat plant, and bio-fertilizer inoculation and application of Si-NPs played an ameliorating role by promoting osmolytes accumulation and the action of antioxidant enzymes under saline conditions, which reduced the oxidative stress in plants and enhanced the grain yield. According to the results of this study, application of bio-fertilizers and silicon nanoparticles at a concentration of 60 mg/L can improve wheat grain yield under salinity stress conditions by improving the physiological and biochemical traits.

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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

Competing interests The authors declare no competing interests.

Competing Interest The authors declare that they have no known competing financial. Interests or personal relationships that could have appeared to influence the work reported in this paper. Consent for Publication All authors have expressed explicit consent to submit this manuscript for publication.

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