



Effects of Nano Iron-Silicon Oxide on Yield and Some Biochemical and Physiological Characteristics of Triticale Under Salinity Stress

Fatemeh Aghaei¹ · Raouf Seyed Sharifi¹ · Salim Farzaneh¹

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Abstract

Salinity, as an important abiotic parameter, has a negative influence on crop productivity. The application of plant growth-promoting rhizobacteria and iron-silicon nanoparticles has been found to enhance plant growth and grain yield while also increasing its resistance to abiotic stresses. In this regard, a factorial experiment was carried out based on randomized complete block design with three repetitions under greenhouse conditions in 2021. Experimental factors included salinity in three levels (no salinity as control, salinity 35 and 70 mM) by NaCl, four plant growth-promoting rhizobacteria levels (no application as control, application of *Azospirillum*, *Pseudomonas*, both application of *Azospirillum* and *Pseudomonas*), and nanoparticles foliar application in four levels (foliar application with water as control, nano Fe, nano Si, foliar application of iron-silicon nanoparticles). The findings demonstrated that under salinity 70 mM, chlorophyll index (27.71%), quantum yield (23.8%), relative water content (43.69%) and grain yield (12.83%) increased in the dual application of plant growth-promoting rhizobacteria and nanoparticles foliar application compared to control level (no application of plant growth-promoting rhizobacteria and nanoparticles) in the same salinity level. However, under such conditions, electrolyte leakage, hydrogen peroxide and malondialdehyde content decreased by 38.93%, 35.34% and 35.13%, respectively, in comparison to the lack of plant growth-promoting rhizobacteria and nanoparticles applications under salinity 70 mM. Also, the usage of plant growth-promoting rhizobacteria and nanoparticles under 70 mM salinity increased the activity of catalase and peroxidase enzymes (42.1% and 73.14%, respectively), as well as proline and soluble sugar content (55.41% and 64.08%, respectively) in comparison to lack of plant growth-promoting rhizobacteria and nanoparticles applications under non-salinity conditions. According to the results of the current study, the application of plant growth-promoting rhizobacteria and nanoparticles could increase the grain yield of triticale under the highest salinity level due to improving physiological and biochemical traits.

Keywords Chlorophyll index · Electrolyte leakage · *Pseudomonas* · Peroxidase

1 Introduction

Triticale is a hybrid of rye and wheat that is harvested owing to a mixture of positive rye and wheat characteristics. Triticale can yield more grains than wheat because it is more tolerant of many soil types and environmental factors [1].

The most critical abiotic stress is salinity stress, which has an adverse influence agricultural productivity worldwide. Due to its detrimental effects on numerous physiological

and biochemical processes, this stress restricts the growth and development of plants [2, 3]. According to Hasanuzzaman et al. (2020), plants are affected by salinity through the osmotic and ionic effects, hormonal and nutritional imbalances, and the production of reactive oxygen species (ROS) [4]. Plant growth and productivity are reduced by salt stress because it interferes with physiological functions, particularly photosynthesis. The aggregation of intracellular sodium ions during salt stress alters the K:Na ratio, which may have an impact on photosynthesis's bio-energetic processes [5]. Further, plants aggregate certain suitable solutes, including sugars, proline (PRO), proteins, amino acids, inside vacuoles [6]. These solutes help sustain cellular homeostasis in plant cells by regulating osmotic pressure [7].

Developing methods and strategies to lessen the effect of salt stress on agricultural yield output is shown to be

✉ Fatemeh Aghaei
f.aghaei1994@gmail.com

¹ Department of Plant Production and Genetics, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran

essential in light of the world's expanding population. Many methods have been employed to help plants cope with the stress that comes from their soil's salinity. To mitigate the negative effects of salt stress, plant growth-promoting rhizobacteria (PGPR) and nanoparticles (NPs) containing iron (Fe) and silicon (Si) can be used. Given the rapid changes in the environment and the growing severity of the drought, NPs have emerged as a valuable tool for raising crop yields. Stress disrupts cellular membranes, the photosynthetic system, water and nutrient uptake, and antioxidant activities, all of which have a negative influence on plant development, physiological functions, and metabolic processes. Plant resistance to biotic and abiotic stressors is enhanced by NPs. Applying NPs helps plants grow more significantly under stress by preserving water interactions, protecting the membranes, and improving nutrient and water intake. When NPs are present, plants are more resilient to stress because they safeguard the photosynthetic system, improve photosynthetic efficiency, and promote the aggregation of hormones, phenolics, and osmolytes, as along with gene expression and antioxidant activities [8]. Plants can be shielded against oxidative damage by NPs via increasing antioxidant activities [9]. Moreover, NPs can lessen the effects of stress toxic by reducing malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) accumulation and keeping the effectiveness of the photosynthetic apparatus [9, 10].

By lowering the generation of ROS in wheat, barley, faba bean, strawberry, feverfew, mango, Mahaleb cherry, one of the micronutrients, Si, can assist plants in becoming more robust to a range of environmental challenges (e.g., salt) [11]. This element can strengthen antioxidant defense mechanisms against a range of environmental challenges, promote crop productivity, boost photosynthetic efficiency, and fix nitrogen in plants [12]. Si can also play a vital role in osmotic modifications through controlling the expression of genes linked to the biosynthesis of PRO. On different occasions, Si enhanced total antioxidant capacity and appeared PRO content in plants that were under salt stress conditions [13, 14]. In some reports, a noticeable Si-induced improvement in relative water content (RWC) and chlorophyll content and reduced electrolyte leakage (EL) and MDA has been found in stressed sugar beet plants [15].

As mentioned by Askary et al. (2017), Fe is considered an essential microelement that affects a wide range of biochemical and physiological procedures and is the fourth element in abundance with regard to its value; nonetheless, its quantity is inadequate or poor for plant needs [16]. Using NPs to treat Fe deficiency is an alternative technique because of the low mineral solubility, including Fe, and improves plant tolerance to various abiotic stressors [16]. Fe is necessary for healthy plant growth and development and is crucial for photosynthesis and enzyme activities. Moreover, it enhances the function of photosystems, RNA synthesis, DNA transcription,

and auxin activity [17]. Hasanuzzaman et al. (2017) found a decline in RWC in rapeseed in stress conditions; nonetheless, the administration of Fe NPs raised RWC, suggesting that by preserving osmolyte production, Fe NPs could enhance the water status of stressed plants [18]. Furthermore, it has been shown that PGPR inoculation enhances plant tolerance to abiotic stresses and promotes plant growth, development, and production [19]. By altering the structure and morphology of the root system, producing phytohormones, extracellular polysaccharides, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, volatile compounds, and osmolyte accumulation, the PGPR can increase a plant's ability to withstand stress. Additionally, they might stimulate the transcriptional control of stress response genes and strengthen the antioxidant defense system [20]. Through ion homeostasis, antioxidant synthesis, ACC deaminase, phytohormones, extracellular polymeric substances, volatile organic compounds, accumulation of osmolytes, activation of plant antioxidative enzymes, and improved nutrient uptake, PGPR can increase salt tolerance in plants [21]. Chlorophyll fluorescence indices were reported by Neshat et al. (2022), and RWC increased significantly in PGPR-inoculated treatments [22]. Their results showed that the inoculation with these bacteria led to an increase in antioxidant capacity, PRO, and antioxidant enzymes but a decrease in MDA and H_2O_2 . The impact of applying PGPR and NPs (Fe and Si) to triticale under salinity stress is not well understood. In order to lessen the negative impacts of salt stress, the current study evaluated the use of NPs and PGPR to enhance the biochemical and physiological traits as well as the activities of the antioxidant defense system in triticale plants. Furthermore, the current study sought to assess the possible advantages of using NPs to reduce the growth and productivity of triticale crops grown in salt stress (Fe, Si, and PGPR).

2 Materials and Methods

2.1 Greenhouse Experiment and Experimental Factors

In 2021, a study was conducted using a factorial-experimental, randomized complete block design with three repetitions at the Research Farm of Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili. One of the experimental settings was three salinity levels [no salinity as control (S_0), salinity 35 (S_1), 70 (S_2) mM]. The other factors were nanoparticles foliar application (NPs) at four levels (foliar application with water as control (N_0), nano Fe (N_1) ($1\text{ g}\cdot\text{L}^{-1}$), nano Si (N_2) ($50\text{ mg}\cdot\text{L}^{-1}$), and application of nano Fe-Si (N_3), and plant growth-promoting rhizobacteria application at four levels (no application as control (B_0), application of *Pseudomonas* (B_1), *Azospirillum* (B_2), and

the application of both *Azospirillum* and *Pseudomonas* (B_3). The greenhouse's maximum and lowest relative humidity levels were 60% and 67%, respectively, while its daytime temperature ranged from 20 to 30 °C to 18 to 21 °C. In this experiment, the triticale cultivar 'Sanabad' was employed. For this cultivar, 380 seeds.m⁻² is the ideal density. Thus, 50 seeds were planted in 41-cm-diameter pots filled with 16 kg of soil. After planting, the pots were immediately covered with water.

2.2 Specifications of PGPR and Nano Si-Fe O Particles

The Research Institute of Soil and Water in Tehran, Iran, identified *Pseudomonas* and *Azospirillum* from the rhizospheres of wheat. Gum Arabic was used as an adhesive to coat the seeds before they were rolled into the bacterial mixture for inoculation, until they were coated evenly. The microorganisms used as PGPR in this experiment had cell densities and strains of 1×10^8 colony-forming units. The utilized nano Si-Fe particles had a specific surface area of more than 30 m² and an average particle size of less than 30 nm.g⁻¹. The leftovers of US nanomaterial research were provided by Pishgaman Nanomaterials Company in Iran. For a better solution, deionized water was mixed with nano Si and nano Fe powder and placed on a shaker with ultrasonic equipment (100 W and 40 kHz). Two phases of period growth, BBCH 21 and 30, were used for the foliar application of nano Si and Fe.

2.3 Biochemical and Physiological Traits of Triticale Leaf

To assess certain biochemical features, the plants' flag leaves were separated at the mid-heading stage (BBCH 59). The samples were taken from a greenhouse onto an ice bath and wrapped in aluminum foil. Using techniques recommended by Sudhakar et al. (2001), the activity of antioxidant enzymes such as polyphenol oxidase (PPO), peroxidase (POD), and catalase (CAT) underwent measurement [23]. Further, flag leaf protein, soluble sugar content, and PRO content were determined by methods presented by Bradford (1976), Dubios et al. (1956), and Bates et al. (1973), respectively [24–26]. Furthermore, H₂O₂ content and MDA were estimated by the techniques of Alexieva et al. (2001) and Stewart and Beweley (1980), respectively [27, 28].

Likewise, at the stage of heading (BBCH 59), the plants' flag leaves were chosen for measuring chlorophyll index (SPAD), relative water content (RWC), electrical conductivity (EL), and quantum yield (Fv/Fm).

A portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan) was used to compute the chlorophyll index. With a high degree of precision, this device

can calculate the total chlorophyll levels in plant leaves [29]. Using a fluorometer (chlorophyll fluorometer; Optic Science-OS-30 U.S.A.), the quantum yield was determined on the flag leaves by the highest fool expanded leaf. RWC was computed based on formula (1) according to previous research [30]:

$$RWC (\%) = [(Fw - Dw) / (Tw - Dw)] \times 100 \quad (1)$$

where RWC, FW, DW, and TW represent relative water content, fresh weight, dry weight, and turgid weight, respectively.

In addition, the EL percentage (EL%) from the cell was estimated as follows:

$$El (\%) = (EC1 / EC2) \times 100 \quad (2)$$

Where EL, EC₁, and EC₂ represent electrolyte leakage, primary leakage from the cell, and secondary leakage, respectively.

Mean comparisons and analysis of variance (ANOVA) were performed to determine each plant's estimated grain output, six plants were randomly taken out of each pot.

2.4 Statistical Analysis of the Data

Using the SAS (version 9.1 computer software program). The least significant difference test was used at the 0.05 significance level to look at the interactions and main effects.

3 Results

3.1 Relative Water Content, Quantum Yield (Fv/Fm), Chlorophyll Content (SPAD), and Electrolyte Leakage

Concerning physiological features, the results revealed that the effects of Si and Fe NPs, Si and Fe, and PGPR (*Azospirillum* and *Pseudomonas*) under salinity stress on RWC, quantum yield (Fv/Fm), chlorophyll index (SPAD), and EL had a significant effect (Table 1).

3.1.1 Relative Water Content (RWC)

All salinity concentrations in the current investigation affected plant RWC negatively when compared to the control treatment. The application of salinity as S₂ decreased RWC by 18.69% compared to salinity as S₀. Under salt, the leaves' RWC sharply dropped (Table 2). On the other hand, under both salt-stress and non-stress circumstances, plants treated with a combination of NPs and PGPR showed greater RWC values (Table 2). There was a noticeable reduction in the RWC in saline plants. However, plants demonstrated a

Table 1 Analysis of variance related to physiological traits and activity of antioxidant enzymes under salinity conditions, as well as the application of PGPR and nano iron-silicon oxide

S. O.V	D.F	Mean squares							
		RWC	Fv/Fm	SPAD	EL	CAT	POX	PPO	
Replication	2	165.74**	0.0158**	342.66**	44.86**	1025.98**	3908.69**	291.44**	
Salinity (S)	2	861.57**	0.011**	153.19**	1443.67**	476.58**	4316.31**	866.77**	
Bio-fertilizers (B)	3	1249.94**	0.0205**	274.84**	597.72**	11.38 ^{ns}	307.06**	62.97 ^{ns}	
Foliar application of nanoparticles (N)	3	168.69**	0.0081**	72.87**	252.51**	16.07*	219.9**	13.37 ^{ns}	
S×B	6	28.5**	0.0055**	22.23**	11.98 ^{ns}	10.4 ^{ns}	250.94**	80.86**	
S×N	6	7.56 ^{ns}	0.0021 ^{ns}	21.97**	12.79 ^{ns}	34.94**	411.18**	73.53*	
B×N	9	23.79**	0.0058**	12.82**	8.61**	18.68**	174.18**	39.41 ^{ns}	
S×B×N	18	13.09*	0.0029**	12.57**	16.76**	15.4**	96**	28.55 ^{ns}	
Error	94	7.03	0.0011	3.46	6.23	6.19	34.33	29.31	
CV (%)	-	4.56	4.27	4.23	5.64	6.5	8.06	10.5	

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

S.O.V. Sources of variations; D.F Degrees of freedom; RWC Relative water content; Fv/Fm Ratio of variable to maximum fluorescence; SPAD chlorophyll index; EL Electrolyte leakage; CAT Catalase; POX Peroxidase; PPO Polyphenol oxidase; PGPR Plant growth-promoting rhizobacteria

notable increase in RWC when exposed to NPs and PGPR alone or in combination. Put otherwise, the findings indicated that when PGPR and NPs were applied together in a non-stressful situation, RWCs rose by roughly 52.6% as opposed to when they were not applied at all under salinity stress at 70 mM.

3.1.2 Quantum yield (Fv/Fm)

Salinity significantly decreased quantum yield in comparison with control plants. However, plants under both salt-stress and non-stress conditions treated with a combination of NPs and PGPR showed higher Fv/Fm values. The highest quantum yield (0.81) was obtained in salinity as S₀, the application of PGPR as B₃, and NPs as N₃, while the lowest values (0.63) were obtained in S₂B₀N₀ (Table 2). In other words, the results revealed that by the combined application of NPs and PGPR under non-stress conditions, Fv/Fm contents showed an increase of nearly 28.57% in comparison to the lack of NPs and PGPR under salinity stress conditions at 70 mM (Table 2).

3.1.3 Chlorophyll Index (SPAD)

Salinity stress significantly affected this parameter and decreased it. The lowest SPAD was observed at 70 mM salinity as S₂. Salinity as S₂ decreased the SPAD by 20.92% in comparison to no salinity (S₀) (Table 2). It seems that the low rates of the chlorophyll index at the highest salinity level can be attributed to the production of free radicals such as H₂O₂ and MDA (Table 5) and EL

(Table 2), which is in conformity with the results of Neshat et al. [22]. Salt stress plants when treated with PGPR and NPs as B₃N₃ demonstrated a considerable increase in the chlorophyll index compared to the salt-treated plants without PGPR and NPs as B₀N₀ (Table 2). More precisely, mean comparison revealed that the maximum chlorophyll index (51.1) was obtained in salinity as S₀, the application of PGPR as B₃, and NPs as N₃, while the lowest values (36.8) were obtained in S₂B₀N₀ (Table 2). An increase of about 38.85% was observed in the chlorophyll index at the lowest salinity level, the application of PGPR, and NPs as B₃N₃ in comparison with S₂B₀N₀ (Table 2).

3.1.4 Electrolyte Leakage (EL)

The findings demonstrated that as saline levels rose, EL% increased as well. When compared to the absence of salinity stress, the rate of leakage increased by approximately 18.82% in conditions of salinity 70 mM (Table 2). This outcome may be the consequence of salinity's detrimental effects on triticale plants, which also negatively impact membrane stability and selective permeability, leading to an increase in EL. Plants that received both PGPR and NPs at the same time naturally had lower EL% levels. To put it another way, the mean comparison represented that the application of non-PGPR as B₀ and non-NPs as N₀, together with salinity 70 mM as S₂, produced the highest EL% (55.49), while the lowest values (33.83) were obtained in S₀B₃N₃ (Table 2). There was an increase of about 64.02% in EL at the maximum salinity level, no application of PGPR, and NPs as B₀N₀ in comparison with S₀B₃N₃ (Table 2).

Table 2 Mean comparison of physiological traits and activity of antioxidant enzymes under salinity conditions, as well as the application of PGPR and nano iron-silicon oxide

Treatments	RWC (%)	Fv/Fm	SPAD	EL (%)	Catalase (OD µg Protein.min ⁻¹)	Peroxidase
S ₀ ×B ₀ ×N ₀	54.6 ^{m-r}	0.73 ^{b-i}	44.5 ^{g-m}	46.7 ^{h-m}	31.78 ^q	52.66 ^f
S ₀ ×B ₁ ×N ₀	59.1 ^{h-l}	0.73 ^{b-j}	42.9 ^{k-q}	39.8 ^{q-u}	34.57 ^{m-q}	54.83 ^{qr}
S ₀ ×B ₂ ×N ₀	61.2 ^{o-i}	0.71 ^{e-l}	40.9 ^{o-u}	44.43 ^{l-o}	33.48 ^{o-q}	55.61 ^{qr}
S ₀ ×B ₃ ×N ₀	68.7 ^{ab}	0.72 ^{c-k}	48.3 ^{a-f}	36.35 ^{t-x}	32.87 ^{pq}	57.76 ^{p-r}
S ₀ ×B ₀ ×N ₁	56.7 ^{l-o}	0.71 ^{d-l}	43.2 ^{j-o}	43.61 ^{m-q}	34.33 ^{m-q}	57.98 ^{p-r}
S ₀ ×B ₁ ×N ₁	63.3 ^{d-h}	0.77 ^{a-e}	47.3 ^{b-h}	40.21 ^{p-t}	35 ^{k-q}	66.33 ^{i-p}
S ₀ ×B ₂ ×N ₁	59.7 ^{g-k}	0.72 ^{c-l}	41 ^{o-u}	36.16 ^{u-x}	32.04 ^q	55.86 ^{qr}
S ₀ ×B ₃ ×N ₁	67.8 ^{a-c}	0.77 ^{a-e}	47.8 ^{b-f}	34.71 ^{wx}	33.7 ^{o-q}	63.11 ^{l-q}
S ₀ ×B ₀ ×N ₂	56.7 ^{k-o}	0.71 ^{d-l}	42.4 ^{m-r}	45.3 ⁱ⁻ⁿ	38.85 ^{e-k}	62.27 ^{m-q}
S ₀ ×B ₁ ×N ₂	63.9 ^{e-g}	0.74 ^{b-h}	41.7 ^{m-s}	35.49 ^{v-x}	41.04 ^{b-g}	78.36 ^{b-h}
S ₀ ×B ₂ ×N ₂	64.9 ^{b-f}	0.77 ^{a-d}	45.9 ^{e-k}	36.69 ^{l-x}	36.84 ^{h-p}	68.2 ^{i-o}
S ₀ ×B ₃ ×N ₂	68.8 ^{ab}	0.75 ^{a-f}	50.2 ^{ab}	33.86 ^x	33.77 ^{o-q}	56.62 ^{qr}
S ₀ ×B ₀ ×N ₃	55.6 ^{k-p}	0.74 ^{b-h}	45.7 ^{e-k}	40.93 ^{o-s}	39.64 ^{b-i}	76.84 ^{d-i}
S ₀ ×B ₁ ×N ₃	66.6 ^{a-d}	0.79 ^{ab}	48.7 ^{a-e}	35.41 ^{v-x}	33.29 ^{o-q}	60.01 ^{o-r}
S ₀ ×B ₂ ×N ₃	68 ^{a-c}	0.76 ^{a-e}	46.8 ^{d-i}	34.43 ^x	34.98 ^{k-q}	70.74 ^{h-m}
S ₀ ×B ₃ ×N ₃	70.2 ^a	0.81 ^a	51.1 ^a	33.83 ^x	37.08 ^{e-o}	73.31 ^{f-j}
S ₁ ×B ₀ ×N ₀	50.8 ^{r-l}	0.68 ^{h-m}	42.1 ^{m-r}	52.31 ^{a-c}	35.05 ^{k-q}	62.55 ^{l-q}
S ₁ ×B ₁ ×N ₀	51.7 ^{p-t}	0.69 ^{f-m}	41.2 ^{n-t}	49.6 ^{d-i}	40.46 ^{b-h}	87.37 ^{ab}
S ₁ ×B ₂ ×N ₀	49.8 ^{s-u}	0.72 ^{c-l}	45.7 ^{e-k}	48.18 ^{f-l}	34.79 ^{l-q}	63.29 ^{n-q}
S ₁ ×B ₃ ×N ₀	65.3 ^{b-e}	0.74 ^{b-h}	49.9 ^{a-c}	41.73 ^{n-r}	40.94 ^{b-g}	77.58 ^{e-i}
S ₁ ×B ₀ ×N ₁	49.9 ^{s-u}	0.72 ^{c-l}	43.3 ^{j-o}	50.78 ^{b-g}	41.24 ^{a-e}	85.55 ^{a-d}
S ₁ ×B ₁ ×N ₁	55.2 ^{l-q}	0.71 ^{e-l}	38.7 ^{s-v}	41.33 ^{n-s}	39.01 ^{d-k}	81.87 ^{a-f}
S ₁ ×B ₂ ×N ₁	53.6 ^{a-s}	0.7 ^{f-l}	39.7 ^{r-v}	48.78 ^{e-k}	38.61 ^{e-l}	70.71 ^{h-m}
S ₁ ×B ₃ ×N ₁	65.3 ^{b-f}	0.75 ^{a-g}	44.2 ⁱ⁻ⁿ	38.49 ^{r-w}	41.89 ^{a-e}	84.07 ^{a-d}
S ₁ ×B ₀ ×N ₂	49 ^{tu}	0.68 ^{h-m}	40 ^{q-u}	44.94 ^{k-o}	33.89 ^{o-q}	61.1 ^{n-r}
S ₁ ×B ₁ ×N ₂	56.1 ^{k-o}	0.77 ^{a-e}	46.8 ^{d-i}	43.79 ^{m-q}	40.05 ^{b-i}	85.15 ^{a-d}
S ₁ ×B ₂ ×N ₂	56 ^{no}	0.72 ^{c-l}	46.9 ^{d-i}	46.73 ^{g-m}	34.16 ^{n-q}	59.26 ^{o-r}
S ₁ ×B ₃ ×N ₂	61.2 ^{e-i}	0.75 ^{a-f}	48.6 ^{a-f}	37.31 ^{s-x}	38.33 ^{e-m}	73.38 ^{f-j}
S ₁ ×B ₀ ×N ₃	51.5 ^{p-t}	0.72 ^{c-l}	42.6 ^{l-r}	49.49 ^{d-i}	40.09 ^{b-i}	71.56 ^{g-m}
S ₁ ×B ₁ ×N ₃	61.03 ^{f-j}	0.73 ^{b-j}	40.1 ^{p-u}	43.86 ^{m-p}	36.3 ^{i-p}	69.6 ^{h-m}
S ₁ ×B ₂ ×N ₃	57.6 ⁱ⁻ⁿ	0.75 ^{a-h}	48 ^{b-f}	39.15 ^{r-v}	38.18 ^{f-n}	56.92 ^{p-r}
S ₁ ×B ₃ ×N ₃	67 ^{a-d}	0.77 ^{a-e}	49.1 ^{a-d}	36.47 ^{l-x}	35.3 ^{j-q}	72.78 ^{f-k}
S ₂ ×B ₀ ×N ₀	46 ^u	0.63 ^m	36.8 ^v	55.49 ^a	39.49 ^{b-i}	71.98 ^{g-l}
S ₂ ×B ₁ ×N ₀	48.7 ^{tu}	0.67 ^{i-m}	38.9 ^{s-v}	53.23 ^{a-d}	41.4 ^{a-f}	84.93 ^{a-d}
S ₂ ×B ₂ ×N ₀	50 ^{s-u}	0.66 ^{k-m}	38.8 ^{s-v}	53.4 ^{a-d}	39.8 ^{b-i}	83.62 ^{a-d}
S ₂ ×B ₃ ×N ₀	64.2 ^{c-f}	0.72 ^{c-l}	44.4 ^{h-m}	49.11 ^{e-j}	42.35 ^{a-e}	83.28 ^{a-e}
S ₂ ×B ₀ ×N ₁	49.2 ^{tu}	0.69 ^{g-m}	38.6 ^{t-v}	54.82 ^{ab}	43 ^{a-d}	83.27 ^{a-e}
S ₂ ×B ₁ ×N ₁	50.6 ^{r-t}	0.71 ^{e-l}	41.5 ^{m-t}	50.08 ^{d-i}	42.34 ^{a-e}	83.11 ^{a-e}
S ₂ ×B ₂ ×N ₁	58.2 ^{i-m}	0.66 ^{lm}	41.1 ^{o-t}	50.62 ^{c-h}	40.91 ^{b-g}	78.85 ^{b-h}
S ₂ ×B ₃ ×N ₁	61.7 ^{e-i}	0.73 ^{b-i}	45.6 ^{g-l}	45.3 ⁱ⁻ⁿ	40.2 ^{b-i}	80.64 ^{b-g}
S ₂ ×B ₀ ×N ₂	48.7 ^{tu}	0.67 ^{i-m}	38 ^{uv}	54.13 ^{a-c}	43.42 ^{ab}	84.18 ^{a-d}
S ₂ ×B ₁ ×N ₂	52.6 ^{o-t}	0.71 ^{d-l}	43.1 ^{k-p}	50.37 ^{c-i}	39.32 ^{c-j}	74.04 ^{e-j}
S ₂ ×B ₂ ×N ₂	49.5 ^{s-u}	0.73 ^{c-k}	40.7 ^{o-u}	53.33 ^{a-d}	43.1 ^{a-c}	83.81 ^{a-d}
S ₂ ×B ₃ ×N ₂	64.8 ^{b-f}	0.74 ^{b-h}	47.6 ^{b-f}	43.84 ^{m-q}	39.15 ^{c-j}	73.84 ^{e-j}
S ₂ ×B ₀ ×N ₃	51.1 ^{q-t}	0.68 ^{h-m}	39.9 ^{q-u}	51.45 ^{a-f}	40.71 ^{b-h}	84.9 ^{a-d}
S ₂ ×B ₁ ×N ₃	57.7 ⁱ⁻ⁿ	0.74 ^{b-h}	46.2 ^{d-j}	41.73 ^{n-r}	41.44 ^{a-f}	86.46 ^{a-c}
S ₂ ×B ₂ ×N ₃	59.8 ^{g-k}	0.76 ^{a-f}	43 ^{k-o}	46.35 ^{i-m}	42.12 ^{a-f}	85.83 ^{a-d}
S ₂ ×B ₃ ×N ₃	66.1 ^{a-d}	0.78 ^{a-c}	47 ^{c-h}	39.94 ^{p-u}	45.16 ^a	91.18 ^a
LSD	4.3	0.06	3.01	4.04	4.03	9.49

S₀, S₁, and S₂: indicate without salinity or control, 35 mM, and 70 mM salinity, respectively. B₀, B₁, B₂, and B₃ indicate no application of biofertilizers, application of *Pseudomonas*, *Azospirillum*, *Azospirillum* + *Pseudomonas*. N₀, N₁, N₂ and N₃ denote no foliar application, nano iron oxide foliar application, nano silicon, nano iron-silicon
Means with similar letters in each column are not significantly different based on the least significant difference test. RWC Relative water content; Fv/Fm Ratio of variable to maximum fluorescence; SPAD chlorophyll index; EL Electrolyte leakage; PGPR Plant growth-promoting rhizobacteria

3.2 Biochemical Characteristics of Triticale

In triticale leaves, the following biochemical alterations were identified: soluble sugar, soluble protein, and PRO content (as a biochemical alteration); PRO, peroxide hydrogen (H_2O_2), and MDA (as oxidative damage); and POX, CAT, and PPO (as an antioxidant defense); all of which were examined in relation to PGPR (*Azospirillum* and *Pseudomonas*) and nano Fe-Si oxide under salinity stress conditions. The statistics showed that every aspect was significantly impacted by the treatment.

3.2.1 Activity of Catalase (CAT), Polyphenol Oxidase (POX) and Peroxidase (PPO) Enzymes

ANOVA revealed a noteworthy interaction impact between “salinity \times PGPR \times NPs” on the antioxidant enzyme activity (CAT and POX) in triticale (Table 2). To protect against oxidative damage, plants have evolved robust antioxidant defense systems that comprise enzymes like POX, CAT, and PPO activities. First line of defense against reactive oxygen species (ROS) such as H_2O_2 is provided by these systems. Antioxidant enzymes PPO, POX, and CAT activity was more pronounced as a stress indicator in triticale plants subjected to salinity stress compared to controls. As a result, the salinity-stressed plants’ antioxidant enzyme activity increased significantly (Tables 2 and 3). Based on the findings (Tables 2 and 3), at the greatest salinity level (70 mM) and when PGPR and

NPs (Si and Fe) were applied together, antioxidant enzyme activity was at its peak. Stated differently, the two that showed the most increases in CAT and POX, with increases of around 42.1% and 73.14%, respectively, in comparison to control plants as $S_0B_0N_0$, were salinity 70 mM and the combined usage of B_3N_3 . These results imply that the use of PGPR and NPs like B_3N_3 and progressive increases in salinity may improve the activity of antioxidant enzymes (Table 2). In addition, ANOVA demonstrated a significant treatment combination among “salinity \times PGPR, salinity \times NPs” on PPO (Table 1). The average comparison represented that the highest level of PPO (56.37 and 56.27 OD $\mu\text{g protein}\cdot\text{min}^{-1}$, respectively) was obtained by the application of the combined use of PGPR \times salinity and NP \times salinity (Table 3).

3.2.2 Proline and Soluble Sugar Content

Salinity stress promotes the accumulation of compatible osmolytes (PRO and soluble sugars) (Table 4), which it is essential for cells to adjust to saline environments, so that, under salinity stress 70 mM as S_2 , PRO and soluble sugar content increased by 39.25% and 60.34%, respectively, when compared to non-salinity stress as S_0 (Table 5). Based on average comparisons, applying a combination of NPs (Si and Fe) and PGPR under salinity stress at 70 mM represented the highest PRO and soluble sugar content (8.75 and 130.38 $\mu\text{mol}\cdot\text{gFW}^{-1}$, Table 5). In fact, steady rises in salinity and the combined use of NPs and PGPR as B_3N_3 could increase soluble sugar content and PRO, the most important of which were the combined use of B_3N_3 and salinity 70 mM, indicating an increase in soluble sugar content and PRO of about 64.08% and 55.41%, respectively, in comparison to control plants as $S_0B_0N_0$ (Table 5).

3.2.3 Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA) Content

The H_2O_2 and MDA content noticeably increased in salinity conditions (Table 5). The enhanced level of H_2O_2 following severe salinity stress was accompanied by increased amounts of MDA (Table 5) and EL (Table 3), indicating severe oxidative stress in triticale under salinity stress. To support our finding, Ghorbanpour et al. [31] reported a significant accumulation of H_2O_2 and MDA in the leaves of barley (*Hordeum vulgare* L.) under salinity stress [30]. The mean comparison demonstrated that, under salt stress conditions, applying NPS (Si and Fe) and PGPR caused a noticeable decline in H_2O_2 and MDA in comparison to their lack of application. In reality, the highest levels of H_2O_2 and MDA were achieved at the greatest salinity level (70 mM and in the absence of PGPR and NPs as B_0N_0 , leading to a 63.5% and 57.05% increase in H_2O_2 and MDA, respectively, compared to the application of PGPR and NPs as B_3N_3 (Table 5).

Table 3 Mean comparison of PPO in salinity conditions, as well as the usage of PGPR and nano iron-silicon oxide

Polyphenol oxidase (OD $\mu\text{g Protein}\cdot\text{min}^{-1}$)				
Plant growth-promoting rhizobacteria				
Salinity	B_0	B_1	B_2	B_3
S_0	46.33 ^c	47.53 ^c	47.97 ^c	45.63 ^c
S_1	48.61 ^c	55.45 ^a	49.55 ^{bc}	56.37 ^a
S_2	56.33 ^a	54.92 ^a	53.4 ^{ab}	56.09 ^a
LSD	4.53			
Iron-silicon oxide				
Salinity	N_0	N_1	N_2	N_3
S_0	43.75 ^e	45.71 ^{de}	48.83 ^{cd}	49.16 ^{cd}
S_1	52.49 ^{a-c}	55.66 ^a	51.8 ^{a-c}	49.99 ^{b-d}
S_2	55.57 ^a	53.84 ^{ab}	55.1 ^a	56.27 ^a
LSD	4.64			

LSD Least significant difference; PGPR Plant growth-promoting rhizobacteria

S_0 , S_1 , and S_2 indicate without salinity or control, 35 mM, and 70 mM salinity, respectively. B_0 , B_1 , B_2 , and B_3 indicate no application of biofertilizers, application of *Pseudomonas*, *Azospirillum*, application of *Azospirillum* + *Pseudomonas*. N_0 , N_1 , N_2 and N_3 denote no foliar application, nano iron oxide foliar application, nano silicon, nano iron-silicon oxide

Table 4 Analysis of variance related to the biochemical characteristics of the grain yield in salinity conditions, as well as the usage of PGPR and nano iron-silicon oxide

S.O.V	D.F	Mean squares					
		Proline	Soluble sugar	MDA	H ₂ O ₂	Soluble protein	Grain yield
Replication	2	7.88**	3991.7**	0.0817**	0.0181**	7.82**	5.99**
Salinity (S)	2	23.13**	3493.26**	0.00627**	0.0426**	15.78**	1.04**
Bio-fertilizers (B)	3	3.64**	510.25**	0.00753**	0.0457**	23.83**	0.20**
Foliar application of nanoparticles (N)	3	0.887**	436.16**	0.00401**	0.0236**	10.63**	0.06**
S×B	6	0.477 ^{ns}	379.48**	0.000207 ^{ns}	0.0005 ^{ns}	0.334 ^{ns}	0.02**
S×N	6	0.328 ^{ns}	168.04*	0.000299*	0.0008 ^{ns}	0.889*	0.04**
B×N	9	1.32**	226.66**	0.000183 ^{ns}	0.0009*	1.209**	0.01*
S×B×N	18	1.8**	787.37**	0.000298**	0.00103**	0.338 ^{ns}	0.01**
Error	94	0.249	65.21	0.00014	0.0004	0.341	0.007
CV (%)	-	6.85	7.47	5.94	5.83	5.17	4.75

^{ns} * and ** represent non-significant and significant probability levels at 5% and 1%, respectively

S.O.V. Sources of variations; D.F: Degrees of freedom; MDA Malondialdehyde; H₂O₂ Hydrogen peroxide; PGPR Plant growth-promoting rhizobacteria

3.2.4 Protein Content

Salinity significantly decreased the protein content in comparison with control plants. However, plants that received both PGPR and NPs at the same time showed higher values for protein content. Based on the findings (Table 6), the highest content of protein (12.91% and 12.59%, respectively) was achieved under non-salinity and application of PGPR and PGPR × NPs, while the lowest values belonged to S₁N₀ and B₀N₀ (Table 6). In fact, NP application could increase 24.97% in non-salinity stress in comparison to their lack of application under salinity 35 mM stress conditions (Table 6). Additionally, applying NPs and PGPR resulted in a 29.79% increase compared to their lack of application (Table 6).

3.3 Grain Yield

ANOVA results indicated significant interaction effects among “salinity × PGPR × NPs (Si and Fe)” on triticale grain yield (Table 4). The results of the present study demonstrated that salt stress may reduce the yield of triticale grains. According to our findings, grain yield was significantly lower at a salt level of 70 mM than it was in the control treatment. To put it another way, the application of salinity 70 mM as S₂ decreased the grain yield by 26.35% in comparison to non-salinity as S₀ (Table 5). However, when compared to untreated plants, the administration of PGPR and NPs could considerably increase the grain production in triticale plants under saline stress. As a matter of fact, when PGPR and NPs were applied together as B₃N₃, the grain yield rose by 44.59% compared to S₂B₀N₀, the control treatment (Table 5).

4 Discussion

The decrease RWC in leaves can be attributed to the negative impact of salinity stress on soil water absorption and reduced water availability, which in turn affects the plant's overall water status [2, 32]. Neshat et al. (2022) reported that RWC represented a noticeable increase in PGPR-inoculated treatments. A plant's ability to absorb water increases and its root system gets more established when PGPR is used, which raises the RWC [22]. Moreover, it seems that PGPR can inhibit the synthesis of ethylene and promote root development by producing auxin. Additionally, bacteria secrete extracellular polymeric compounds known as exopolysaccharides, which provide the right soil texture for water absorption. Previous studies indicated that when PGPR and nano Si were applied together, RWC increased the most because it was more effective than when applied separately on untreated plants [33]. Applying Si can help triticale leaf tissue that has been stressed by salt and lessen its succulency, which will increase the RWC. These findings may be the consequence of Si's integrative function in regulating water status in salinity-stressed triticale plants. Si can also lessen water loss, protect leaves from transpiration, and thicken leaves [34]. Hasanuzzaman et al. (2017) found that when rapeseed was stressed, RWC demonstrated a decrease. Nevertheless, when nano Fe were applied, RWC increased, suggesting that nano Fe might maintain osmolyte production and thereby improve the water status of stressed plants [18].

The Fv/Fm value is an important indicator of environmental changes such as salinity. Fv/Fm may decrease as a result of D1 protein degradation, light harvesting center damage, and suppression of osmotically driven water uptake in salt stress. In salinity stress, ion imbalance and low

Table 5 Mean comparison of biochemical characteristics in salinity conditions, as well as the usage of PGPR and nano iron-silicon oxide

Treatments	Soluble sugar mg.g FW ⁻¹	Proline ($\mu\text{mol.gFW}^{-1}$)	MDA ($\mu\text{mol.gFW}^{-1}$)	H ₂ O ₂	Grain yield (g per plant)
S ₀ ×B ₀ ×N ₀	79.46 ^u	5.63 ^w	0.207 ^{c-h}	0.386 ^{c-i}	1.87 ^{lm}
S ₀ ×B ₁ ×N ₀	113.08 ^{e-l}	6.76 ^{m-u}	0.194 ^{f-o}	0.361 ^{g-m}	1.91 ^{i-l}
S ₀ ×B ₂ ×N ₀	85.08 ^w	6.7 ^{n-v}	0.195 ^{f-n}	0.354 ^{i-o}	1.88 ^{k-m}
S ₀ ×B ₃ ×N ₀	112.87 ^{d-l}	7.03 ^{k-q}	0.177 ^{n-q}	0.296 ^{s-v}	1.97 ^{d-h}
S ₀ ×B ₀ ×N ₁	103.65 ^{j-p}	6.07 ^{t-w}	0.205 ^{d-i}	0.368 ^{f-l}	1.91 ^{i-l}
S ₀ ×B ₁ ×N ₁	87.87 ^{r-u}	6.2 ^{r-w}	0.188 ^{i-p}	0.335 ^{l-r}	2 ^{b-f}
S ₀ ×B ₂ ×N ₁	109.78 ^{f-m}	7.8 ^{e-k}	0.185 ^{i-p}	0.34 ^{k-p}	1.96 ^{e-i}
S ₀ ×B ₃ ×N ₁	90.18 ^{q-u}	6.27 ^{q-w}	0.176 ^{n-q}	0.292 ^{s-v}	2.03 ^{bc}
S ₀ ×B ₀ ×N ₂	114.95 ^{c-k}	6.96 ^{l-r}	0.208	0.386 ^{c-i}	1.87 ^{k-m}
S ₀ ×B ₁ ×N ₂	87.64 ^{s-u}	6.18 ^{r-w}	0.218 ^{b-d}	0.364 ^{g-m}	2.01 ^{b-e}
S ₀ ×B ₂ ×N ₂	101.92 ^{k-q}	5.99 ^{u-w}	0.176 ^{n-q}	0.289 ^{l-v}	1.95 ^{f-j}
S ₀ ×B ₃ ×N ₂	94.46 ^{o-t}	7.19 ^{j-p}	0.172 ^{pq}	0.281 ^{uv}	1.93 ^{g-j}
S ₀ ×B ₀ ×N ₃	101.44 ^{l-q}	6.52 ^{o-v}	0.182 ^{k-q}	0.304 ^{r-v}	2.05 ^b
S ₀ ×B ₁ ×N ₃	82.73 ^{tu}	6.15 ^{s-w}	0.175 ^{o-q}	0.29 ^v	2.11 ^a
S ₀ ×B ₂ ×N ₃	118.33 ^{a-h}	7.33 ^{h-n}	0.175 ^{o-q}	0.28 ^{uv}	2.02 ^{b-d}
S ₀ ×B ₃ ×N ₃	91.45 ^{o-u}	5.95 ^{vw}	0.163 ^q	0.274 ^v	2.14 ^a
S ₁ ×B ₀ ×N ₀	124.36 ^{a-d}	8.03 ^{b-i}	0.235 ^b	0.44 ^a	1.6 ^{r-u}
S ₁ ×B ₁ ×N ₀	91.34 ^{p-u}	6.46 ^{p-v}	0.226 ^{bc}	0.416 ^{a-d}	1.76 ^{op}
S ₁ ×B ₂ ×N ₀	125.33 ^{a-d}	8.18 ^{a-g}	0.217 ^{b-d}	0.401 ^{b-f}	1.78 ^{op}
S ₁ ×B ₃ ×N ₀	120.89 ^{a-g}	7.48 ^{g-n}	0.193 ^{f-o}	0.346 ^{j-o}	1.87 ^{n-p}
S ₁ ×B ₀ ×N ₁	108.57 ^{g-m}	6.52 ^{o-v}	0.232 ^b	0.434 ^{ab}	1.78 ^{op}
S ₁ ×B ₁ ×N ₁	104.53 ^{i-o}	6.85 ^{l-t}	0.2 ^{d-l}	0.401 ^{b-f}	1.74 ^p
S ₁ ×B ₂ ×N ₁	94.89 ^{n-t}	6.31 ^{q-w}	0.201 ^{d-j}	0.356 ^{h-n}	1.79 ^{n-p}
S ₁ ×B ₃ ×N ₁	110.56 ^{e-l}	8.46 ^{a-e}	0.185 ^{i-p}	0.321 ^{o-t}	1.91 ^{i-l}
S ₁ ×B ₀ ×N ₂	100.86 ^{l-r}	6.76 ^{m-v}	0.216 ^{b-e}	0.389 ^{c-h}	1.65 ^{qr}
S ₁ ×B ₁ ×N ₂	126.9 ^{a-c}	7.26 ^{i-p}	0.189 ^{h-p}	0.325 ^{n-r}	1.84 ^{mn}
S ₁ ×B ₂ ×N ₂	117.21 ^{b-i}	7.97 ^{c-j}	0.217 ^{b-d}	0.382 ^{e-i}	1.87 ^{lm}
S ₁ ×B ₃ ×N ₂	128.98 ^{ab}	7.89 ^{d-j}	0.181 ^{l-q}	0.305 ^{q-v}	1.92 ^{h-k}
S ₁ ×B ₀ ×N ₃	103.17 ^{j-q}	7.48 ^{g-n}	0.201 ^{d-i}	0.376 ^{e-j}	1.68 ^{op}
S ₁ ×B ₁ ×N ₃	123.17 ^{a-e}	6.75 ^{m-v}	0.189 ^p	0.337 ^{k-r}	1.81 ^{no}
S ₁ ×B ₂ ×N ₃	86.33 ^{s-u}	8.14 ^{a-h}	0.182 ^{l-q}	0.31 ^{p-u}	1.91 ^{i-l}
S ₁ ×B ₃ ×N ₃	107.55 ^{h-n}	7.25 ^{j-p}	0.178 ^{m-q}	0.307 ^{p-v}	1.99 ^{c-g}
S ₂ ×B ₀ ×N ₀	127.41 ^{a-c}	7.84 ^{e-k}	0.256 ^a	0.448 ^a	1.48 ^w
S ₂ ×B ₁ ×N ₀	126.22 ^{a-c}	7.55 ^{f-m}	0.230 ^b	0.429 ^{ab}	1.55 ^{uv}
S ₂ ×B ₂ ×N ₀	95.03 ^{n-t}	8.53 ^{a-e}	0.205 ^{d-i}	0.384 ^{d-i}	1.6 ^{r-u}
S ₂ ×B ₃ ×N ₀	126.37 ^{a-c}	7.91 ^{d-j}	0.197 ^{e-m}	0.356 ^{h-n}	1.63 ^{q-s}
S ₂ ×B ₀ ×N ₁	121.11 ^{a-g}	6.26 ^{q-w}	0.235 ^b	0.442 ^a	1.54 ^v
S ₂ ×B ₁ ×N ₁	92.87 ^{o-t}	8.8 ^{ab}	0.226 ^{bc}	0.419 ^{a-c}	1.64 ^{q-s}
S ₂ ×B ₂ ×N ₁	102.3 ^{j-q}	7.21 ^{j-p}	0.220 ^{b-d}	0.392 ^{c-g}	1.53 ^{vw}
S ₂ ×B ₃ ×N ₁	124.36 ^{a-d}	7.95 ^{c-j}	0.187 ^{i-p}	0.338 ^{k-q}	1.64 ^{q-s}
S ₂ ×B ₀ ×N ₂	122.01 ^{a-f}	8.67 ^{a-d}	0.216 ^{b-d}	0.417 ^{a-d}	1.57 ^{t-v}
S ₂ ×B ₁ ×N ₂	103.16 ^{j-q}	6.96 ^{l-s}	0.205 ^{d-i}	0.387 ^{c-i}	1.62 ^{q-t}
S ₂ ×B ₂ ×N ₂	119.74 ^{a-h}	8.35 ^{a-f}	0.204 ^{d-j}	0.362 ^{g-m}	1.64 ^{qr}
S ₂ ×B ₃ ×N ₂	118.02 ^{a-h}	9.8 ^a	0.189 ^{g-p}	0.331 ^{m-r}	1.67 ^q
S ₂ ×B ₀ ×N ₃	98.6 ^{m-s}	8.25 ^{a-g}	0.209 ^{c-f}	0.402 ^{b-e}	1.58 ^{s-v}
S ₂ ×B ₁ ×N ₃	115.19 ^{c-j}	7.3 ^{i-o}	0.202 ^{d-j}	0.37 ^{e-k}	1.64 ^{q-s}
S ₂ ×B ₂ ×N ₃	112.87 ^{e-k}	7.62 ^{f-l}	0.189 ^{h-p}	0.323 ^{n-t}	1.66 ^q
S ₂ ×B ₃ ×N ₃	130.38 ^a	8.75 ^{a-c}	0.185 ^{i-p}	0.331 ^{m-r}	1.67 ^q
LSD	13.09	0.8	0.019	0.033	5.05

S₀, S₁, and S₂ indicate without salinity or control, 35 mM, and 70 mM salinity, respectively. B₀, B₁, B₂, and B₃ indicate no application of biofertilizers, application of *Pseudomonas*, *Azospirillum*, *Azospirillum* + *Pseudomonas*. N₀, N₁, N₂ and N₃ denote no foliar application, nano iron oxide foliar application, nano silicon, nano iron-silicon oxide
 MDA Malondialdehyde; H₂O₂ Hydrogen peroxide; PGPR Plant growth-promoting rhizobacteria; LSD Least significant difference

Means with similar letters in each column are not significantly different based on the least significant difference test

Table 6 Mean comparison of protein in salinity conditions, as well as the usage of PGPR and nano iron-silicon oxide

Salinity	Protein (%)			
	iron-silicon oxide nanoparticles			
	N ₀	N ₁	N ₂	N ₃
S ₀	11.3 ^{bc}	11.73 ^b	11.74 ^b	12.91 ^a
S ₁	10.33 ^d	10.79 ^{cd}	11.31 ^{bc}	11.89 ^b
S ₂	10.69 ^{cd}	10.58 ^{cd}	10.73 ^{cd}	11.3 ^{bc}
LSD	0.784			
PGPR	N ₀	N ₁	N ₂	N ₃
B ₀	9.7 ⁱ	9.87 ⁱ	10.24 ^{hi}	11.18 ^{d-f}
B ₁	10.42 ^{g-i}	10.81 ^{f-h}	11.6 ^{c-e}	11.85 ^{b-d}
B ₂	11.11 ^{e-g}	11.48 ^{c-f}	10.75 ^{f-h}	12.51 ^{ab}
B ₃	11.85 ^{b-d}	11.97 ^{a-c}	12.45 ^{ab}	12.59 ^a
LSD	0.729			

LSD Least significant difference; PGPR Plant growth-promoting rhizobacteria

S₀, S₁, and S₂: indicate without salinity or control, 35 mM, and 70 mM salinity, respectively. B₀, B₁, B₂, and B₃ indicate no application of biofertilizers, application of *Pseudomonas*, *Azospirillum*, *Azospirillum*+*Pseudomonas*. N₀, N₁, N₂ and N₃ denote no foliar application, nano iron oxide foliar application, nano silicon, nano iron-silicon oxide

Means with similar letters in each column are not significantly different based on the least significant difference test

mineral nutrition induce non-photochemical quenching [35]. Salt stress plants, when treated with PGPR as B₃, indicated a noticeable increase in quantum yield in comparison to the salt-treated plants without PGPR. The current investigation confirmed a beneficial overall effect on the host plants' Fv/Fm levels under salinity conditions, representing that PGPR application increased photosynthetic efficiency [22] due to decreased EL (Table 2) and improve leaf SPAD and RWC (Table 2) under stress conditions.

When applied singly or in combination, NPs (Si and Fe) reduced the impact of salt stress and regulated photosynthetic processes by decreasing MDA and H₂O₂ production (Table 5), which inhibits pigments involved in photosynthetic processes in part. In addition, NP application could enhance antioxidant enzymes' activities (Table 2), leaf SPAD (Table 2), and RWC (Table 2). Additionally, it can stop chlorophyll degradation in plants that have been grown in stress conditions [34, 36].

Neshat et al. (2022) found that PGPR ameliorated the chlorophyll index in canola (*Brassica napus* L.) under salinity stress conditions [22]. They also reported that in the PGPR-inoculated plants, higher contents of SPAD and RWC contributed to greater photosynthetic activity and maintained growth. Part of the enhancement in SPAD can be attributed to the effect of PGPR on increasing the Fv/Fm (Table 2) and RWC (Table 2). Similar results were obtained

in the application of nano Si and nano Fe on the chlorophyll content. Furthermore, the application of Si could increase the chlorophyll content of triticale due to an improvement in RWC (Table 2) while decreasing EL (Table 2) and MDA (Table 5) under salinity stress. Evidence suggests that the presence of Si can increase the chlorophyll content due to improving leaf RWC and decreasing EL [15]. The positive impacts of nano Fe on the chlorophyll content under stress are in line with previous research [37].

EL is calculated to estimate how salinity stress affects membrane permeability [38]. It seems that the main reason for the low EL due to the application of PGPR is decreasing the damage due to salinity stress on the cytoplasmic membrane in several ways, including improving the Fv/Fm and increasing the RWC (Table 2), which lessens the influence caused by salinity stress and consequently decreases EL (Table 2). Pritesh et al. (2020) found that when rice is subjected to salt stress, PGPR with the capacity to solubilize potassium and phosphorus can reduce EL [39]. Moreover, Hafez et al. (2021) concluded that EL decreased with the soil application of PGPR + Si [33]. Adrees et al. (2020) have observed similar findings with wheat, indicating that EL from leaf membranes in plants under stress was reduced by applying nano Fe [10]. This could be clarified by the reality that NPs can maintain cells through reduced lipid peroxidation (Table 5) and improve compatible osmolytes (Table 5), increasing access to the cell by antioxidative enzymes (Table 2) [15]. Additionally, Si has a protective function against stress; it aids in raising calcium concentrations, which are crucial for enhancing membrane integrity and activating certain enzymes that reduce ROS buildup and enhance the electron transport chain [40].

Antioxidant enzymes efficiently scavenge ROS, which are elevated in salinity-exposed cells. According to several studies, antioxidant enzyme activity increased with increases in salinity [41]. In fact, one reason for the rise in antioxidant enzyme activity under salinity stress may be the efficient coexistence of the application of *Pseudomonas* and *Azospirillum*, either separately or together, against their nonapplication. According to earlier research, plants exposed to salinity stress in their early development showed increased activity of antioxidant enzymes in response to increasing salinity doses, whereas plants inoculated with PGPR showed a rise in antioxidant enzymes such as POX, CAT, and PPO [41]. Additionally, PGPR improved the canola and sweet corn antioxidant systems against salinity stress conditions, respectively [42, 43]. Moreover, NP treatment improved antioxidant enzymatic activity such CAT, POX, and PPO, increasing cell membrane stability and integrity while reducing oxidative damage. Therefore, in line with Hafez et al. (2021), it appears that Si can enhance RWC and osmotic regulation (Table 2) under salinity stress [33]. In other words, the application of Si not only maintains RWC

shields the plant by maintaining the macromolecules' shape and function [2]. Further, Alexandre et al (2017) confirmed the impact of nano Fe oxide treatment on wheat plants' antioxidant enzyme activity during stress [44]. Antioxidant enzymes can shield a plant from the harmful effects of salt stress.

Plants often use an increase in organic osmolytes as a defense against damage to cellular organelles caused by stress. By preserving cell redox potential, lowering ROS such as the H_2O_2 level, and lowering lipid peroxidation, PRO preserves membrane integrity (Table 5). Furthermore, this amino acid is thought to be essential for the upkeep of RWC, the protection of cellular membrane structures, and the operation of ROS-scavenging enzymes [45]. Soluble sugar contents are the other influential osmolytes that aggregate under salinity stress conditions. To maintain osmoregulation, soluble carbohydrates are essential. These sugars have an indirect influence on plant growth and development in addition to the osmotic impact by controlling the metabolism of carbohydrates in the presence of salt stress [19]. According to previous studies, PRO effectively contributes to stopping enzyme destruction, preventing macromolecule breakdown, and keeping cell wall strength during environmental stress; hence, PGPR application intensifies this effect by increasing PRO and helps enhance the plant's resistance to salt stress [22]. Moreover, numerous studies documented the aggregation of soluble sugars in plants subjected to salt stress; among them, some highlighted the beneficial role of PGPR in the accumulation of soluble sugars [46], which conforms to the findings of the present study.

Additionally, through the biosynthetic regulation of osmolytes and some plant hormones, introducing NPs (Fe and Si) to triticale plants under salt conditions enhanced the accumulation of these osmolytes (PRO and soluble sugar levels), hence improving triticale's tolerance to salinity stress. The protective impact of osmolytes on triticale plants under stress may be ascribed to their function in maintaining membrane stability and averting plant cell physiological drought [38]. Based on the results of previous data, Si may have contributed to the rise in soluble sugar concentration by enhancing photosynthesis, which in turn encourages the synthesis of soluble sugar [47]. In addition, the positive effects of nano Fe oxide on PRO and soluble sugar contents under salinity stress conform to previous reports [48]. Therefore, it appears that applying NPs and PGPR strengthens the plant's resistance to salt stress by raising the levels of PRO and soluble sugar.

The application of PGPR and NPs decreased the EL (Table 2), which would have further led to a decrease in the MDA concentration. Additionally, the combo therapy enhanced and increased antioxidant enzymatic activity (Table 2), and thus could decrease MDA contents (Table 5). Similar results have been found by Hafez et al. [33]. In addition, it is evident that a reduction in the H_2O_2 level leads to

a decrease in membrane damage, leading to a decline in the MDA content and EL (Table 2). The same pattern was demonstrated in the canola (*Brassica napus* L.) by Nashat et al. (2022) and in the rice (*Oryza sativa* L.) plant by Pritesh et al. (2020) with PGPR application [22, 39].

The H_2O_2 and MDA content significantly decreased when applying NP (Si and Fe). In this context, previous studies revealed that when subjected to salt stress, Si can lessen oxidative damage by controlling the functions of the antioxidant system [34]. Additionally, application of Si decreased MDA during salinity stress; this may have been because Si is involved in controlling osmolytes and preserving membrane integrity (PRO and soluble sugar contents) and H_2O_2 in the plants [2]. In addition, nano Fe application could significantly reduce H_2O_2 and MDA contents in triticale plants. Researchers reported that micronutrients can lessen the environmental stressors' impressions, including salinity stress [16]. It appears that applying PGPR and NPs stimulates antioxidant enzyme activity and shields the cell membrane from lipid peroxidation. Additionally, it raises suitable osmolytes, including soluble sugars and PRO (Table 5), improving plant resistance to stress and lowers H_2O_2 and MDA (Table 5).

Salinity usually causes a decrease in protein in stressed plants because it inhibits protein synthesis and increases the activity of enzymes that hydrolyze proteins [49]. Furthermore, it might have been brought on by an increase in oxidative stress brought on by an excess of ROS, such as H_2O_2 (Table 5), which can affect plants by causing protein breakdown and DNA damage [50]. The use of PGPR encourages the build-up of proteins that are directly responsible for transferring salt tolerance and can increase protein synthesis by selectively absorbing mineral elements and improving water access [46]. Moreover, adding NP (Fe and Si) to triticale plants under salinity conditions increased in the protein content. Si application improves protein by reducing oxidative damage brought on by elevated ROS, such as H_2O_2 (Table), during salinity stress [51]. Singh et al. (2022) reported similar findings in wheat [2].

Previous research revealed that the application of Si (NPs) enhanced the aggregation of antioxidant enzymes and the efficiency of photosynthetic devices and PGPR containing the enzyme ACC deaminase, resulting in an improvement in maize grain production because it decreased the level of ethylene in the plant and enhanced growth and yield [33, 52]. By increasing SPAD (Table 2), decreasing oxidative damage, controlling compatible osmolytes and phytohormones, improving RWC (Table 2), boosting antioxidant enzyme activities, and lowering oxidative stress, the application of Si to salinity-stressed triticale plants mitigates the negative effects of salinity [38, 53]. According to certain research, nano FeO effectively reduces the negative effects of salt on wheat, increasing grain production [10]. Therefore, when applying PGPR and NPs under salt stress conditions,

the enhanced activity of antioxidant enzymes, SPAD, and RWC may contribute to a portion of the yield gain. However, it causes a decrease in EI (Table 2) and MDA (Table 5), which results in improved plant tolerance to circumstances of salinity stress.

5 Conclusion

Due to the induction of osmotic stress, ionic toxicity, and excessive generation of toxic ROS that adversely influence cell functional integrity and cause the oxidation of cell molecules such as proteins, lipids, and chlorophyll, salty stress can have a negative impact on the growth and yield of triticale plants. Our research showed that ionic disruption causes physiological and biochemical alterations in triticale plants under salt stress. According to our findings, applying PGPR (*Azospirillum* and *Pseudomonas*) and nano Fe-SiO (NPs) under salinity stress often reduced these damage by strengthening the defensive mechanisms, particularly antioxidant enzymes and the build-up of soluble sugars and PRO. In summary, our results indicated that NP and PGPR applications upgrade plant physiology and trigger the cellular defense of triticale plants against high salt stress (70 mM). Based on the findings of this study, applying NPs and PGPR could enhance physiological and biochemical characteristics, hence increasing triticale grain yields under salinity stress conditions.

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Data Availability Data is provided within the manuscript or supplementary information files.

Declarations

Ethics Approval Not applicable.

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Competing Interests The authors declare no competing interests.

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