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Biogenic Titanium Dioxide Nanoparticles Ameliorate the Effect of Salinity Stress in Wheat Crop

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Abstract: Crop productivity worldwide is being hampered by salt stress. Nanotechnology and its applications in agriculture are noteworthy and fruitful. The current work investigates the potential significance of TiO_2 NPs in alleviating salt stress in wheat cultivars. TiO_2 NPs were prepared by green synthesis; their characterizations were carried out by UV–visible spectroscopy, SEM, and EDX. In the greenhouse control condition, different concentrations of salt (NaCl) with and without TiO_2 NPs were administered to wheat crops. Plants treated with TiO_2 NPs showed an enhanced germination rate, morphological, and metabolic profiling with and without salt stress. Different concentrations of TiO_2 NPs (25, 50, 75, and 100 µg/mL) and salt solutions (NaCl 50, 100, and 150 mM) were used. TiO_2 NP concentrations of 25 µg/mL and 50 µg/mL improved the germination attributes, physio-morphic parameters: plant length, the fresh and dry weight of plants, the number of leaves, the leaf area, the RWC, the MSI, and different chlorophyll contents at all saline conditions. These two concentrations also enhanced the biochemical attributes: free amino acids, soluble sugar content, proline content, SOD, and POD, of wheat varieties at all salinity levels. Wheat subjected to salt stress responded best to the application of TiO_2 NPs at a concentration of 50 µg/mL. However, the highest concentrations (75 and 100 µg/mL) of TiO_2 NPs showed detrimental effects on germination, agronomic, physiological, and biochemical attributes, and caused stress in both wheat varieties (Pirsabak-05 and NARC-09) under control and saline conditions. The outcomes of the current research work are notable, and NPs with such capabilities may be a useful supply of fertilizer in the agricultural industry.

Keywords: Nano-titanium; green synthesis; abiotic stress; wheat; physiological attributes

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1. Introduction

Wheat is considered to be a rich source of carbohydrates. It is categorized as the third leading crop, after corn and rice. Wheat is a universal staple food crop and its derivatives are used in different parts of the world, including South Asian countries such as Pakistan, Bangladesh, Sri Lanka, India, and Nepal. Wheat has a high potential to resolve food security problems in underdeveloped countries globally. It is considered to be the most extremely researched crop [1]. Various abiotic environmental factors such as salt stress, temperature frequency, drought stress, and flooding often affect the productivity of crops worldwide. Among all these abiotic stresses, salt stress has the most disastrous effects on wheat crop plants, resulting in low yields [2].

Salinity has detrimental effects on climatic change. Saline irrigated water and soil both reduce productivity and reduce crop efficiency. Probably soils of arid and semi-arid regions are saline and have more impact on agriculture production [3]. According to the CSSRI (Central Soil Salinity Research Institute) report of 2014, about 33% percent of irrigated farming lands and 20% of cultivated lands worldwide suffer from the problem of salinity stress, and this is predicted to increase at an alarming rate up to the year of 2050 [4]. Soil salinity has become a great issue nowadays for declining crop quality and yields [2]. Salt stress causes the obstruction of various developmental processes, such as stomatal processing, shoot biomaterial, root length, the photosynthetic mechanism rate, and the leaf water potential.

A high accumulation of salt in different parts of the plant causes a reduction of the relative water content and a decrease of the crop production rate, the number of grains on the spike, and a decline in the plant size [5]. A high storage of salt particles in plants leads to the production of ROS (reactive oxygen species) in large amounts, that is why the production of proline and water-soluble carbohydrates is enhanced in plants [6]. Different mechanistic processes in plants, e.g., photosynthesis, respiration, and photorespiration result in the formation of a reduced form of oxygen (ROS), which causes rupturing of cell membranes and destruction of different functional biomolecules, like proteins, DNA, lipids, and photosynthetic pigments [7].

For better production of wheat crops in saline regions, various technologies have been used, but due to the high cost of those technologies now simple, eco-friendly, and economically convenient technologies have been established [8]. Currently, an engineered nanoparticle has been industrialized worldwide. So, metallic nanoparticles can significantly affect the developmental processes and growth of various crops detrimental to food production [9]. In addition, nanotechnologists have used bio-synthesized nanoparticles because of their cheap accessibility, eco-friendly nature, high biocompatibility rate, high diffusion in plant cells, small particle size, and surface nature, that leads to their characteristic physiochemical properties [10].

Compared with other nanoparticles, TiO_2 NPs have much significance as they improve plant growth and crop yield. They are also used in the cosmetics industry. TiO_2 NPs are described as being more beneficial for the physiological, morphological, and biochemical parameters of various crop plants [11]. TiO_2 NPs have also been identified as accelerating the photosynthetic rate, chlorophyll activation, the accumulation of antioxidant enzymes, and rubisco activity. The productive influence of TiO_2 NPs on broad bean plants in response to saline conditions has also been reported [12]. They were shown to help in promoting plant growth, increase proline contents, provide strong antioxidant defense mechanism, and cause reductions in H_2O_2 and MDA contents in broad bean plants. Besides this, another study reported that the exogenous application of TiO_2 NPs on maize crops caused a decrease in salt stress, enhancement in chlorophyll contents, phenolic production, agronomical parameters, antioxidant formation, and crop production [13].

Following this review of the literature, it is seen that TiO_2 NPs have manifested both useful and harmful impacts on crop plants. High doses of TiO_2 nanoparticles are harmful to plants due to the accumulation of ROS molecules and their causing a reduction in chlorophyll contents [11], while low doses enhance the growth attributes and crop productivity [14]. The current study was designed to explore the role of TiO_2 NPs on wheat under salt stress, because fewer data are available on the beneficial and toxicological influence of bio-synthesized TiO_2 NPs on wheat crops in saline conditions. This study shows that biogenic TiO_2 NPs play a vital role in plant tolerance under different stress conditions. Limited information is available on the molecular mechanisms of these NPs. Further studies are needed to explore the molecular mechanisms of these NPs in plants.

2. Materials and Methods

2.1. Preparation of Leaf Extract

Fresh and healthy plant leaves of *Moringa oleifera* were taken and rinsed with tap water, then washed by distilled water to remove all the remains of unwanted particles such as dust. The leaves were dried at 25 °C and cut into pieces. About 10–20 g of leaves were weighed and transferred to beakers containing 100 mL distilled water, and were boiled for 10–20 min in an oven. To get a clear solution, free of particulates, the extract was filtered three times through Whatman no. 21 filter paper before being stored at 4 °C in a 250 mL measuring flask. To ensure accurate results, the experiment was conducted under sterile conditions at all times [15].

2.2. Green Synthesis of Titanium Dioxide Nanoparticles

TiO_2 NPs were synthesized by mixing plant extracts with salt solution. A solution of titanium dioxide (3.5 mM) was prepared by dissolving Titanium dioxide salt in distilled water to reach a concentration of 0.27 g/L. The solution was stirred for 4 h at 50 °C using a magnetic stirrer, and the solution was reduced by adding plant extract drop wise. When the color of the solution had changed to a milky white, the solution was then poured into an Eppendorf tube and centrifuged at 14,000 rpm for 10 min. The supernatant was discarded and TiO_2 NPs pellets were suspended in deionized water followed by centrifuging at 14,000 rpm for 10 min. This process was repeated three times for the removal of non-reacted salt and plant extracts. The resulting TiO_2 NPs were suspended in deionized water and subjected to characterization [16].

2.3. Characterization of Nanoparticles

Characterization of the bio-synthesized TiO_2 NPs (nanoparticles) was carried out using different characterization techniques, i.e., using the UV–visible spectrophotometer (Halo DB-20 Spectrophotometer) having the wavelength range of 200–600 nm. Scanning electron microscopy (Sigma model) was used for morphological analysis of TiO_2 NPs, and the elemental analysis was carried out by energy-dispersive X-ray (EDX) [17].

2.4. Plant Materials and Growth Conditions

Seeds of two varieties of *Triticum aestivum* L. (Pirsabak-05 and NARC-09) were purchased from NARC (National Agriculture Research Center Islamabad, Pakistan; an institution that provides seeds to farmers and scientists with prior permission to use them for research or food reasons). The seeds were surface sterilized by using 1% Sodium Hypochlorite solution, and then washed several times with distilled water. Experimentation was done in the glass house of the Department of Botany PMAS Arid Agriculture University Rawalpindi Pakistan. Sowing was done in November. The wheat seeds were sown in a particular pot with a 24 cm width and 22 cm length, filled with soil (4.5 kg sandy loam) containing sand 42.8%, silt 5.7%, and clay 51.5%; 8–10 seeds were sown in each pot. The soil was analyzed for measuring EC, pH, organic matter, available phosphorous, potassium, and saturation. Fertilizers were not used throughout the experiment.

2.5. Experimental Design and Application of Treatments

Both wheat varieties were subjected to four different concentrations of TiO_2 NPs (25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL) and three different concentrations of salt solutions (50 mM, 100 mM, and 150 mM). Details of treatments have been represented in Table 1. The experimental design was completely randomized design (CRD) and each treatment was carried out for three different biological replicates. After three weeks of germination, three different concentrations of NaCl salt (50 mM 100 mM, 150 mM) were applied up to the end of each experiment. After every third day salt stress was applied, and on the third time of salt treatment water was applied. The exogenous application of different concentrations of TiO_2 NPs was performed after 10-15 days of salt stress application.

Table 1. Different treatments of TiO_2 NPs and NaCl on two wheat varieties (Pirsabak-05 and NARC-09) under salinity stress.

Treatments	Description	
TiO_2 NPs treatment	**T0**	0 µg/mL of TiO_2 NPs
	T1	25 µg/mL of TiO_2 NPs
	T2	50 µg/mL of TiO_2 NPs
	T3	75 µg/mL of TiO_2 NPs
	T4	100 µg/mL of TiO_2 NPs
Salt Stress (NaCl)	**T5**	Salt stress (NaCl) 50 mM
	T6	Salt stress (NaCl) 100 mM
	T7	Salt stress (NaCl) 150 mM
TiO_2 NPs + 50 mM Salt Stress	**T8**	Salt stress (NaCl) 50 mM + 25 µg/mL of TiO_2 NPs
	T9	Salt stress (NaCl) 50 mM + 50 µg/mL of TiO_2 NPs
	T10	Salt stress (NaCl) 50 mM + 75 µg/mL of TiO_2 NPs
	T11	Salt stress (NaCl) 50 mM + 100 µg/mL of TiO_2 NPs
TiO_2 NPs + 100 mM Salt Stress	**T12**	Salt stress (NaCl) 100 mM + 25 µg/mL of TiO_2 NPs
	T13	Salt stress (NaCl) 100 mM + 50 µg/mL of TiO_2 NPs
	T14	Salt stress (NaCl) 100 mM + 75 µg/mL of TiO_2 NPs
	T15	Salt stress (NaCl) 100 mM + 100 µg/mL of TiO_2 NPs
TiO_2 NPs + 150 mM Salt Stress	**T16**	Salt stress (NaCl) 150 mM + 25 µg/mL of TiO_2 NPs
	T17	Salt stress (NaCl) 150 mM + 50 µg/mL of TiO_2 NPs
	T18	Salt stress (NaCl) 150 mM + 75 µg/mL of TiO_2 NPs
	T19	Salt stress (NaCl) 150 mM + 100 µg/mL of TiO_2 NPs

2.6. Analysis of Germination Parameters

The germination experiment was established in the Plant Biotechnology Lab in the Department of Botany, PMAS-Arid Agriculture University Rawalpindi. In Petri dishes, ten healthy, uniform seeds were sown. The seeds were subjected to different concentrations of TiO_2 NPs and were irrigated with distilled water daily. The Petri dishes without salt stress and nanoparticles treatment served as a control.

2.6.1. Germination Percentage (Germination %)

The germination percentage of the wheat seedlings was recorded after 7 days by using the method of [18].

$$\text{Germination \%} = \frac{\text{Total seeds germinated}}{\text{Total no. of seeds planted}} \times 100$$

2.6.2. Germination Index (G.I.)

The germination index was determined by using the protocol of Abdul-Baki [18]. Germination Index = n/d (Where, *n* = numbers of seedlings emerged on a given day, and d = days after planting).

2.6.3. Seedling Vigor Index (S.V.I.)

The vigor index of the seedlings was calculated by using the following equation, as designed by [18].

$$\text{S.V.I.} = \text{seedling length (mm)} \times \text{germination \%}$$

2.7. Determination of Morphological Parameters

The pH and EC of the soil were checked properly throughout the experiments. After the completion of three months, different morphological and physiological attributes were analyzed using a random sampling method. Uprooted plants were washed with distilled water. The root length and shoot length of the plants were measured by using a scale. Three plants were taken from each wheat variety and thoroughly washed with tap water, then by distilled water, to count the number of leaves per plant. The fresh weight of plants was determined by using an electric analytical weight balance. The samples were placed in oven at 70 °C for 7 days to obtain a constant dry weight [19].

2.8. Determination of Physiological Parameters

2.8.1. Leaf Relative Water Content

To determine the relative water content in a leaf disc of the wheat varieties, approximately 0.2 g of a leaf was cut into a disk from each treatment. First of all, the fresh weight of the leaf was measured, followed by incubation for 24 h in a Petri dish filled with water. After measurement of the turgid weight of the leaves, they were then placed in an oven for one week at 70 °C, and then the dry weight was measured after one week [20]. The relative water content was determined by the formula given below.

$$\text{RWC} = \frac{(\text{Fresh weight} - \text{Dry weight}) \times 100}{(\text{Saturated weight} - \text{Dry weight})}$$

2.8.2. Membrane Stability Index

From each sample, a leaf was taken. The leaves were cut into small pieces (100 mg) and washed carefully twice using distilled water. The small discs were added to test tube and then placed in a water bath at 40 °C for 30 min. The electrical conductivity (C_1) was measured by an EC meter after 30 min. The test tube was kept again at 100 °C for 10 min in the water bath. After 10 min, the electric conductivity (C_2) was recorded by using the formula for the membrane stability index calculation protocol as provided by [21].

$$\text{Membrane stability index} = [1 - C_1/C_2] \times 100$$

2.8.3. Leaf Chlorophyll Content

The leaf chlorophyll content was analyzed by using a UV-visible spectrophotometer following the procedure of [22]. Leaves taken from each sample of 0.2 g were ground in 10 mL of acetone. In another set of test tubes filtrate was obtained. The absorbance was observed at wavelengths of 645, 652 and 663 nm, and the chlorophyll content was calculated with the help of the following equations.

$$\text{Chlorophyll a content} = 12.7(A_{663}) - 2.7(A_{645})$$

$$\text{Chlorophyll b content} = 22.9(A_{645}) - 4.7(A_{663})$$

$$\text{Total chlorophyll content} = (A_{652} \times 1000/34.5)$$

2.9. Determination of Biochemical Parameters

2.9.1. Proline Content

For the determination of the proline content, the methodology of [23] was used. About 0.2 g of fresh leaf samples was ground in 4 mL of 3.0% sulfosalicylic acid. Filtrate of 2 mL, taken in separate test tubes, was mixed with 2 mL of ninhydrin reagent, and allowed to react with 2 mL of glacial acetic acid. The test tubes were then placed in a 65 °C water bath to boil until a color appeared. To halt the reaction, these test tubes were placed on ice, followed by the addition of 4 mL of toluene. The mixture was properly shaken until the appearance of an upper colored layer. The supernatant was transferred to a new reaction

tube, and the absorbance was recorded at 520 nm using a spectrophotometer. The following formula was used to calculate the proline content.

$$\text{Total Proline} = \frac{(\text{Sample absorbance} \times \text{Dilution. factor} \times \text{K value})}{\text{Fresh weight of plant tissue}}$$

2.9.2. Free Amino Acids

The ninhydrin method was used for estimation of the quantity of free amino acids [24]. Fresh leaf material of 0.2 g was ground in a sodium phosphate buffer for the preparation of the leaf extract. The leaf extract was filtered to obtain 1 mL of filtrate in separate test tubes. A solution of 1 mL of 10% pyridine and 1 mL of 2% ninhydrin solution was added to them. The test tubes were then placed for 30 min in a water bath. Afterward, the mixture was diluted to the requisite concentration and was observed at 570 nm of absorbance. For the calculation of free amino acids (mg/g fresh weight), the following formula was used.

$$\text{Total free amino acids} = \frac{(\text{Reading of Sam.} \times \text{Sam. Vol.} \times \text{Dil. factor})}{\text{Weight of fresh plant tissue} \times 1000}$$

2.9.3. Soluble Sugar Content

The phenol sulphuric acid method was used for the determination of the soluble sugar content [25]. From each sample, a fresh leaf weighing 0.5 g was taken and ground in 10 mL of ethanol (80%). For 1 hr test tubes were heated in a water bath at 80 °C. A sample extract of 0.5 mL was taken in another set of test tubes, and 1 mL of 18% phenol was added. The mixture was allowed to incubate at room temperature. After incubation, 2.5 mL of sulphuric acid was added. After proper shaking of the mixture, the absorbance was observed at 490 nm wavelengthm using a spectrophotometer, using the following equation.

$$\text{Soluble sugar} = \frac{(\text{Sample absorbance} \times \text{Dilution factor} \times \text{K value})}{\text{Weight of fresh plant tissue}}$$

2.9.4. Superoxide Dismutase (SOD) Activity

The SOD activity was measured by adding 0.6 mL of plant extract to 0.2 mL of 130 mM methionine, 0.2 mL of 1 mM EDTA, 0.78 mL of 50 mM phosphate buffer (pH 7), 0.2 mL of 0.75 mM NBT, and 0.02 mL of 0.02 mM riboflavin in test tubes. The reaction mixture was kept under fluorescent light for 7 min and the absorbance was recorded at 560 nm using a spectrophotometer. The SOD activity was calculated using the Beer–Lambert law [26].

$$A = \varepsilon LC$$

2.9.5. Peroxidase Dismutase (POD) Activity

The POD activity was observed by adding 0.2 mL of plant leaf extract to 200 µL of 100 mM guaiacol, 200 µL of 27.5 mM hydrogen peroxide, 400 µL of phosphate buffer, and 1000 µL of distilled water, for the formation of the solution. Another solution was prepared in the same way, but instead of leaf extract, phosphate buffer was added. The reaction mixture and blank solution were placed under florescent light for 7 min and the absorbance was noted at 470 nm using a spectrophotometer [27].

2.10. Research Involving Plants

The plants use in the study were cultivated and provided by the National Agriculture Research Center Islamabad Pakistan with prior permission for research purposes.

2.11. Statistical Analysis

All the experiments were performed in triplicate, using three different biological replicates. The data were analyzed statistically using SPSS version 20 software for the

analysis of variance (ANOVA) and the mean significant differences were separated using the least significance difference test (LSD).

3. Results

3.1. Biosynthesis and Characterization of TiO_2 NPs

Plant based TiO_2 NPs were synthesized by using plant leaf extract of *Moringa oliefera* L., which has been proved experimentally to be an effective extract for the reduction of TiO_2 salt to nanoparticles. The plant leaf extract was added to a salt solution of titanium dioxide, and stirred until the color changed from off white to a pinkish brown color, indicating the synthesis of TiO_2 NPs. In the present research, the characterization of the TiO_2 NPs was performed using the UV-visible spectrum. The results showed that characterization peaks for biosynthesized TiO_2 NPs appeared in the wavelength range 360–450 nm (Figure 1a). The structural analysis of the biologically synthesized TiO_2 NPs was performed through scanning electron microscopy (SEM). The recorded images of SEM showed a spherical shape of the TiO_2 NPs. Some TiO_2 NPs were fused, and formed aggregates of tiny structures. Most of the nano-forms ranged from 25 to 110 nm in size. The dense synthesis of TiO_2 NPs obtained through the leaf extract, showed the titanium nanostructures' development (Figure 1b). The quantitative and qualitative elemental analysis of TiO_2 NPs was determined through EDX spectroscopy and confirmed the existence and purity of TiO_2 NPs (Figure 1c).

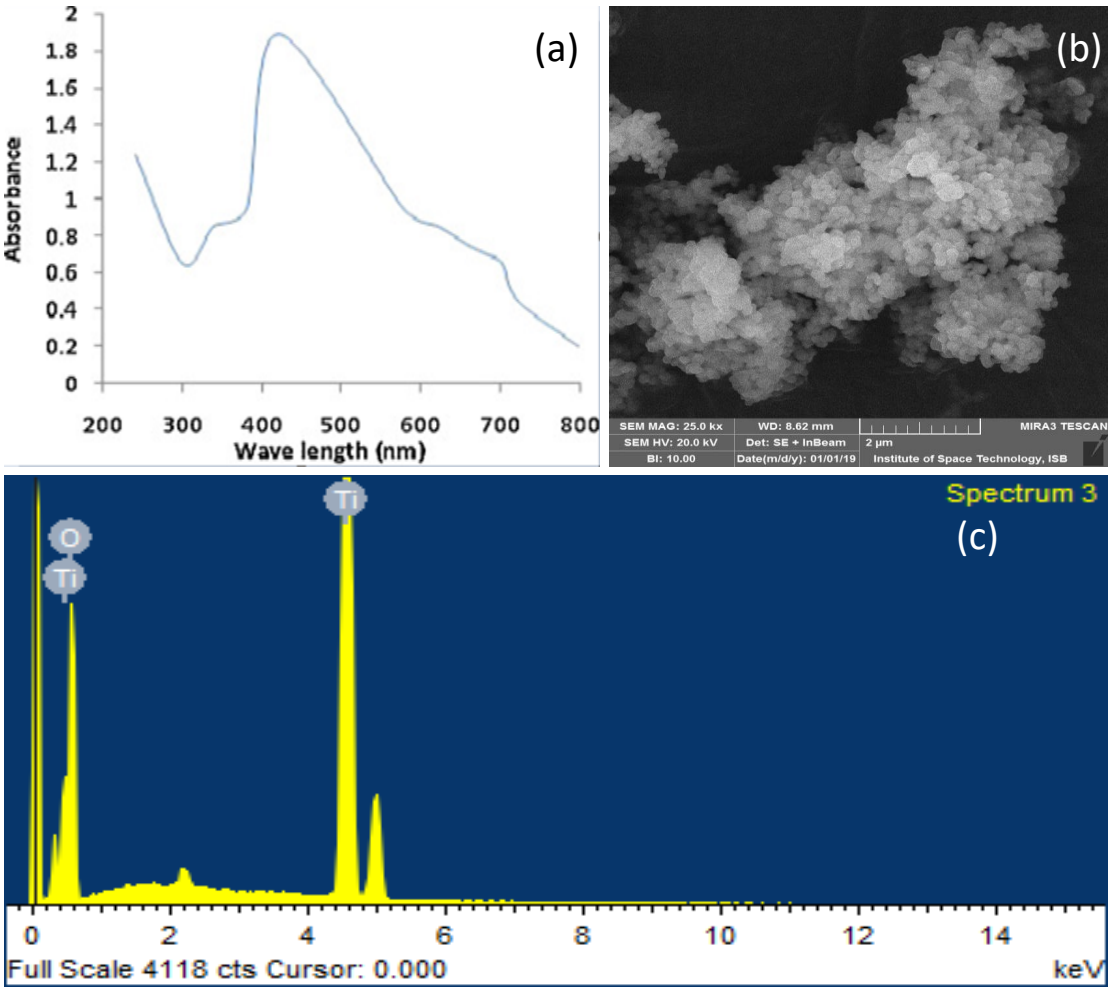


Figure 1. Green synthesized TiO_2 NPs were characterized using (**a**) UV-visible spectrum (**b**) scanning electron microscope (SEM) (**c**) energy dispersive x-ray (EDX) spectroscopy.

3.2. Germination Parameters

Data showing the effect of TiO_2 NPs on the germination attributes of two different wheat varieties, under saline and control conditions, was collected and analyzed (Table 2). It was observed that salinity stress negatively affects the germination parameters (germination percentage, germination index, seedling vigor index, seedling length, and fresh weight) of both wheat varieties, while plant-based TiO_2 NPs were found to improve the germination attributes under both conditions (control and saline). Application of a 50 mM salt solution caused a reduction in the germination percentage (77.23% and 79.54%), germination index (70.76% and 74.31%), seedling vigor index (41.43% and 43.21%), seedling length (34.12% and 38.08%), and seedling fresh weight (52.32% and 59.41%) in both wheat varieties, respectively. Application of a 100 mM NaCl solution caused a pronounced reduction in the germination percentage (87.01%, 89.33%), germination index (81.14%, 85.24%), seedling vigor index (46.44%, 48.19%), seedling length (41.35% and 45.25%), and seedling fresh weight (65.22%, 72.15%) of both wheat varieties, respectively. The application of a salt solution at a concentration of 150 mM revealed no germination in both wheat varieties. It was observed that germination parameters of both wheat varieties decreased under salt stress.

Treatment with plant based TiO_2 NPs enhanced the germination attributes of wheat plants under salinity stress. The application of 50 µg/mL TiO_2 NPs at 50 mM NaCl solution showed increases of 72.26% and 68.11% in the germination percentage, 67.28% and 61.35% in the germination index, 58.12% and 51.18% in the seedling vigor index, 34.32% and 27.21% in the seedling length, and 30.24% and 25.42% in the fresh weight, for both wheat varieties, respectively. Similarly, treatment with 50 µg/mL TiO_2 NPs at 100 mM salt solution increased the germination percentage by 65.04% and 59.21%, the germination index by 61.14% and 49.33%, the seedling vigor index by 45.04% and 39.10%, the seedling length by 28.42% and 22.24%, and the fresh weight by 26.16% and 19.26%, for both wheat varieties, respectively. The application of 50 µg/mL TiO_2 NPs at 150 mM NaCl solution, showed a 45.13% and 41.27% increase in the germination percentage, a 35.76% and 27.92% increase in the germination index, a 30% and 27% increase in the seedling vigor index, a 25.63% and 21.29% increase in the seedling length, and a 17.72% and 12.27% increase in the fresh weight, of both wheat varieties, respectively. In addition, application of 50 µg/mL TiO_2 NPs alone, showed a remarkable increase in the germination percentage (15.82% and 10.16%), the germination index (10.25% and 7.31%), the seedling vigor index (12.15% and 9.55%), the seedling length (15.36% and 11.59%), and the seedling fresh weight (8.35% and 6.78%), for both wheat varieties, respectively, compared to their respective controls.

3.3. Morphological Parameters

The data showing the effect of TiO_2 NPs on the growth of both wheat varieties in well-watered and saline conditions were collected and analyzed (Table 3). It was observed that the application of salt solutions caused a significant reduction in morphological attributes of both wheat varieties. Application of the 50 mM salt solution caused a reduction in the plant length (22.34% and 28.67%), plant fresh weight (33.56% and 37.67%), plant dry weight (17.24% and 22.63%), and the number of leaves and leaf area (21.25%, 28.67%, 38.14%, and 42.42%) of both wheat varieties, respectively, compared to the respective controls. Similarly, application of the 100 mM salt solution caused a significant reduction in the plant length (30.21% and 37.66%), plant fresh weight (48.45% and 57.52%), plant dry weight (26.16% and 34.03%), and the number of leaves and leaf area (30.98%, 41.54%, 52.92%, and 58.81%) of both wheat varieties, respectively, in comparison with the respective controls. A higher concentration of salt solution (150 mM) caused an inhibitory effect on the morphological growth of both wheat varieties, and reduced the plant length (44.23% and 49.56%), fresh weight plant (60.02% and 66.53%), plant dry weight (30.12% and 40.45%), and the number of leaves and area of the leaf (55.67%, 67.34%, 63.12%, and 70.56%) of both wheat varieties, respectively, compared to the respective controls.

Table 2. Effects of foliar application of TiO_2 NPs (25, 50, 75, and 100 µg/mL) on germination parameters of wheat varieties (Pirsabak-05 and NARC-09) under different salt stresses (50, 100, and 150 mM NaCl) treatments.

Treatments	Germination % Age		Germination Index		Seedling Vigor Index		Seedling Length (cm)		Fresh Weight (g)	
	Pirsabak-05	NARC-09	Pirsabak-05	NARC-09	NARC-09	Pirsabak-05	NARC-09	Pirsabak-05	NARC-09	Pirsabak-05
T0	82.00 ± 5.88 ^{b}	80.00± 5.75 ^{b}	2.21 ± 0.065 ^{a}	2.11 ± 0.08 ^{a}	154.62 ± 2.23 ^{a}	152.43 ± 2.01 ^{a}	14.20 ± 0.34 ^{a}	14.16 ± 0.44 ^{a}	0.59 ± 0.028 ^{a}	0.57 ± 0.018 ^{a}
T1	73.00 ± 5.01 ^{c}	70.00 ± 4.92 ^{c}	1.47 ± 0.055 ^{b}	1.34 ± 0.03 ^{b}	144.25 ± 2.31 ^{a}	142.21 ± 2.11 ^{a}	13.43 ± 0.28 ^{a}	12.92 ± 0.22 ^{a}	0.51 ± 0.022 ^{a}	0.49 ± 0.016 ^{ab}
T2	91.00 ± 3.82 ^{a}	90.00 ± 3.17 ^{a}	2.41 ± 0.088 ^{a}	2.35 ± 0.05 ^{a}	169.51 ± 2.01 ^{a}	167.52 ± 2.45 ^{a}	15.91 ± 0.22 ^{a}	15.71 ± 0.18 ^{a}	0.64 ± 0.032 ^{a}	0.62 ± 0.023 ^{a}
T3	51.00 ± 3.25 ^{d}	50.00 ± 2.88 ^{d}	1.27 ± 0.095 ^{b}	1.21 ± 0.05 ^{b}	142.46 ± 2.88 ^{a}	140.34 ± 2.18 ^{a}	12.88 ± 0.31 ^{a}	12.64 ± 0.41 ^{a}	0.41 ± 0.035 ^{ab}	0.39 ± 0.038 ^{ab}
T4	22.00 ± 2.88 ^{fg}	20.00 ± 2.22 ^{g}	0.96 ± 0.044 ^{c}	0.92 ± 0.07 ^{c}	103.48 ± 3.09 ^{a}	101.46 ± 2.81 ^{a}	8.89 ± 0.37 ^{b}	8.55 ± 0.45 ^{b}	0.31 ± 0.029 ^{ab}	0.29 ± 0.032 ^{b}
T5	18.00 ± 2.21 ^{gh}	17.00 ± 2.25 ^{gh}	0.63 ± 0.045 ^{cd}	0.57 ± 0.05 ^{cd}	89.33 ± 3.36 ^{b}	87.32 ± 3.02 ^{b}	9.31 ± 0.35 ^{b}	8.61 ± 0.25 ^{b}	0.28 ± 0.023 ^{b}	0.37 ± 0.019 ^{ab}
T6	10.00 ± 2.76 ^{i}	9.00 ± 2.18 ^{i}	0.38 ± 0.032 ^{d}	0.32 ± 0.04	81.31 ± 3.77 ^{b}	79.47 ± 3.9 ^{c}	8.35 ± 0.43 ^{b}	8.11 ± 0.37 ^{b}	0.21 ± 0.015 ^{b}	0.19 ± 0.14 ^{c}
T7	0.00 ± 0.00 ^{j}	0.00 ± 0.00 ^{j}	0.00 ± 0.00 ^{e}	0.00 ± 0.0 ^{e}	0.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{f}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}
T8	30.00 ± 3.11 ^{e}	27.00 ± 2.89 ^{ef}	1.11 ± 0.081 ^{b}	1.04 ± 0.07 ^{b}	75.85 ± 3.62 ^{c}	71.76 ± 3.42 ^{c}	10.47 ± 0.44 ^{b}	10.26 ± 0.34 ^{b}	0.34 ± 0.018 ^{ab}	0.32 ± 0.022 ^{ab}
T9	50.00 ± 3.64 ^{d}	45.00 ± 3.14 ^{d}	1.53 ± 0.07 ^{b}	1.34 ± 0.05 ^{b}	89.87 ± 3.98 ^{b}	87.86 ± 3.88 ^{b}	13.64 ± 0.49 ^{a}	13.11 ± 0.39 ^{a}	0.42 ± 0.029 ^{ab}	0.37 ± 0.035 ^{ab}
T10	20.00 ± 2.01 ^{g}	17.00 ± 1.88 ^{gh}	1.02 ± 0.05 ^{b}	0.97 ± 0.05 ^{c}	75.23 ± 2.88 ^{c}	73.47 ± 2.99 ^{c}	9.91 ± 0.29 ^{b}	9.32 ± 0.35 ^{b}	0.29 ± 0.035 ^{b}	0.21 ± 0.018 ^{b}
T11	10.00 ± 2.66 ^{i}	8.00 ± 2.02 ^{i}	0.85 ± 0.03 ^{c}	0.82 ± 0.03 ^{c}	71.26 ± 2.28 ^{c}	69.26 ± 2.42 ^{d}	8.87 ± 0.18 ^{b}	8.23 ± 0.22 ^{b}	0.17 ± 0.039 ^{cd}	0.13 ± 0.041 ^{c}
T12	20.00 ± 2.95 ^{g}	18.00 ± 2.31 ^{gh}	1.04 ± 0.06 ^{b}	0.98 ± 0.05 ^{c}	68.36 ± 3.01 ^{d}	66.48 ± 2.36 ^{d}	9.83 ± 0.17 ^{b}	9.14 ± 0.11 ^{b}	0.25 ± 0.024 ^{b}	0.18 ± 0.02 ^{c}
T13	30.00 ± 1.95 ^{e}	25.00 ± 1.44 ^{ef}	1.37 ± 0.03 ^{b}	1.36 ± 0.03 ^{b}	77.16 ± 2.95 ^{c}	73.35 ± 2.86 ^{c}	10.20 ± 0.11 ^{b}	9.81 ± 0.15 ^{b}	0.31 ± 0.018 ^{ab}	0.24 ± 0.012 ^{b}
T14	15.00 ± 1.77 ^{h}	13.00 ± 1.99 ^{hi}	0.96 ± 0.04 ^{c}	0.82 ± 0.05 ^{c}	66.41 ± 4.22 ^{d}	64.43 ± 4.15 ^{d}	9.25 ± 0.23 ^{b}	9.03 ± 0.19 ^{b}	0.14 ± 0.02 ^{c}	0.11 ± 0.026 ^{c}
T15	10.00 ± 2.12 ^{i}	9.30 ± 2.44 ^{i}	0.72 ± 0.07 ^{cd}	0.65 ± 0.07 ^{cd}	64.13 ± 4.01 ^{d}	62.13 ± 3.72 ^{d}	8.32 ± 0.23 ^{b}	8.28 ± 0.26 ^{b}	0.11 ± 0.022 ^{c}	0.09 ± 0.031 ^{cd}
T16	10.00 ± 2.65	8.70 ± 2.17 ^{i}	0.49 ± 0.04 ^{d}	0.41 ± 0.04 ^{d}	62.28 ± 3.99 ^{d}	60.13 ± 4.07 ^{d}	8.34 ± 0.24 ^{b}	8.24 ± 0.29 ^{b}	0.19 ± 0.013 ^{c}	0.15 ± 0.016 ^{c}
T17	25.00 ± 2.11 ^{ef}	22.30 ± 2.01 ^{fg}	0.62 ± 0.031 ^{cd}	0.54 ± 0.02 ^{cd}	66.89 ± 3.11 ^{d}	63.46 ± 3.02 ^{d}	9.56 ± 0.32 ^{b}	9.46 ± 0.22 ^{b}	0.24 ± 0.009 ^{b}	0.17 ± 0.006 ^{c}
T18	10.00 ± 3.17 ^{i}	9.50 ± 2.77 ^{i}	0.38 ± 0.024 ^{d}	0.27 ± 0.02 ^{d}	52.13 ± 2.88 ^{e}	50.16 ± 2.82 ^{e}	5.83 ± 0.39 ^{c}	5.56 ± 0.31 ^{c}	0.08 ± 0.019 ^{cd}	0.05 ± 0.022 ^{cd}
T19	0.00 ± 0.00 ^{j}	0.00 ± 0.00 ^{j}	0.00 ± 0.00 ^{e}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{f}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}

The data represented in the columns in the form of means having similar letters are identical, and dissimilar letters are significantly different, where p was kept $p \leq 0.05$.

Table 3. Effects of foliar application of TiO_2 NPs (25, 50, 75, and 100 µg/mL) on morphological parameters of wheat varieties (Pirsabak-05 and NARC-09) under different salt stresses (50, 100, and 150 mM NaCl) treatments.

	Plant Length (cm)		Plant Fresh Weight (g)		Plant Dry Weight (g)		Number of Leaves		Leaf Area (cm^2)	
Treatments	**Pirsabak-05**	**NARC-09**	**Pirsabak-05**	**NARC-09**	**Pirsabak-05**	**NARC-09**	**Pirsabak-05**	**NARC-09**	**Pirsabak-05**	**NARC-09**
To	76.34 ± 2.21 ^{a}	75.54 ± 1.84 ^{a}	6.29 ± 0.08 ^{a}	6.23 ± 0.07 ^{a}	1.51 ± 0.06 ^{ab}	1.49 ± 0.07 ^{b}	7.91 ± 0.28 ^{a}	7.71 ± 0.32 ^{a}	125.16 ± 1.83 ^{a}	120.57 ± 0.98 ^{a}
T1	74.01 ± 2.93 ^{ab}	73.01 ± 2.73 ^{ab}	5.21 ± 0.06 ^{a}	5.15 ± 0.05 ^{a}	1.53. ± 0.04 ^{ab}	1.48 ± 0.05 ^{b}	7.53 ± 0.33 ^{a}	6.54 ± 0.41 ^{b}	114.52 ± 1.65 ^{b}	110.47 ± 0.91 ^{b}
T2	80.23 ± 1.88 ^{a}	78.23 ± 1.98 ^{a}	6.91 ± 0.09 ^{a}	6.81 ± 0.06 ^{a}	2.17 ± 0.18 ^{a}	2.02 ± 0.46 ^{a}	8.96 ± 0.39 ^{a}	8.61 ± 0.17 ^{a}	139.34 ± 1.98 ^{a}	133.63 ± 1.13 ^{a}
T3	68.57 ± 1.75 ^{b}	67.32 ± 1.65 ^{b}	4.38 ± 0.11 ^{b}	4.31 ± 0.08 ^{b}	2.06 ± 0.32 ^{a}	1.98 ± 0.59 ^{ab}	7.51 ± 0.19 ^{a}	6.54 ± 0.14 ^{b}	109.13 ± 2.11 ^{b}	108.36 ± 2.38 ^{b}
T4	58.25 ± 1.66 ^{c}	57.17 ± 1.52 ^{c}	3.55 ± 0.07 ^{b}	3.51 ± 0.09 ^{b}	1.16 ± 0.30 ^{b}	1.14 ± 0.63 ^{b}	6.59 ± 0.15 ^{b}	5.53 ± 0.11 ^{b}	95.23 ± 1.98 ^{c}	92.21 ± 2.52 ^{c}
T5	59.23 ± 1.71 ^{c}	58.29 ± 1.63 ^{c}	4.21 ± 0.08 ^{b}	4.16 ± 0.34 ^{b}	1.44 ± 0.38 ^{b}	1.15 ± 0.34 ^{b}	4.53 ± 0.26 ^{c}	3.59 ± 0.22 ^{c}	75.65 ± 2.44 ^{d}	70.37 ± 1.59 ^{d}
T6	52.14 ± 2.11 ^{cd}	51.01 ± 2.02 ^{cd}	3.16 ± 0.15 ^{b}	3.31 ± 0.11 ^{b}	1.17 ± 0.75 ^{b}	1.08 ± 0.67 ^{b}	5.54 ± 0.22 ^{b}	4.55 ± 0.29 ^{c}	60.22 ± 2.88 ^{e}	55.17± 3.24 ^{e}
T7	41.43 ± 2.35 ^{e}	40.52 ± 2.46 ^{e}	2.46 ± 0.21 ^{c}	2.4 ± 0.18 ^{c}	1.11 ± 0.06 ^{b}	0.97 ± 0.07 ^{c}	3.51 ± 0.37 ^{c}	2.57 ± 0.45 ^{d}	45.56 ± 1.98 ^{f}	40.32 ± 1.22 ^{fg}
T8	66.32 ± 2.98 ^{b}	65.24 ± 3.11 ^{b}	5.06 ± 0.09 ^{a}	5.02 ± 0.12 ^{a}	1.43 ± 0.19 ^{b}	1.31 ± 0.48 ^{b}	5.56 ± 0.41 ^{b}	4.51 ± 0.37 ^{c}	80.12 ± 1.75 ^{d}	74.54 ± 2.62 ^{d}
T9	69.85 ± 2.63 ^{b}	68.67 ± 2.73 ^{b}	5.77 ± 0.08 ^{a}	5.64 ± 0.09 ^{a}	1.88 ± 0.47 ^{ab}	1.75 ± 0.66 ^{ab}	6.52± 0.32 ^{b}	5.56 ± 0.31 ^{b}	89.42 ± 2.22 ^{c}	85.37 ± 3.15 ^{c}
T10	56.26 ± 2.11 ^{c}	55.23 ± 2.01 ^{c}	3.04 ± 0.04 ^{b}	3.02 ± 0.46 ^{b}	1.52 ± 0.24 ^{ab}	1.46 ± 0.51 ^{b}	4.57 ± 0.27 ^{c}	3.51 ± 0.21 ^{c}	84.29 ± 2.65 ^{c}	82.59 ± 1.88 ^{c}
T11	52.14 ± 1.55 ^{cd}	51.12 ± 1.65 ^{cd}	3.02 ± 0.58 ^{b}	2.92 ± 0.55 ^{c}	1.12 ± 0.06 ^{b}	1.01 ± 0.07 ^{bc}	4.21 ± 0.44 ^{c}	3.22 ± 0.39 ^{c}	80.17 ± 3.19 ^{d}	78.13 ± 2.11 ^{d}
T12	48.33 ± 1.33 ^{d}	47.39 ± 1.43 ^{d}	4.22 ± 0.16 ^{b}	4.19 ± 0.12 ^{b}	1.23 ± 0.22 ^{b}	1.21 ± 0.43 ^{b}	5.58 ± 0.29 ^{b}	4.52 ± 0.22 ^{c}	72.25 ± 3.024 ^{d}	70.47 ± 2.34 ^{d}
T13	44.67 ± 1.11 ^{e}	43.67 ± 1.28 ^{e}	5.01 ± 0.16 ^{a}	4.34 ± 0.19 ^{b}	1.32 ± 0.29 ^{b}	1.27 ± 0.23 ^{b}	6.11 ± 0.20 ^{b}	5.12 ± 0.16 ^{b}	75.31 ± 2.18 ^{d}	73.23 ± 1.66 ^{d}
T14	49.41 ± 1.2 ^{d}	48.48 ± 1.11 ^{d}	3.02 ± 0.21 ^{b}	2.99 ± 0.12 ^{c}	1.11 ± 0.18 ^{b}	1.09 ± 0.31 ^{b}	5.54 ± 0.19 ^{b}	4.57 ± 0.26 ^{c}	61.14 ± 1.22 ^{de}	62.7 ± 2.16 ^{de}
T15	41.22 ± 1.56 ^{e}	40.20 ± 1.41 ^{e}	2.98 ± 0.38 ^{c}	2.77 ± 0.26 ^{c}	1.02 ± 0.07 ^{bc}	0.63 ± 0.17 ^{cd}	3.55 ± 0.16 ^{c}	2.62 ± 0.28 ^{d}	57.22 ± 1.32 ^{e}	55.09 ± 1.21 ^{e}
T16	43.35 ± 1.43 ^{e}	42.35 ± 1.53 ^{e}	3.67 ± 0.44 ^{b}	3.24 ± 0.34 ^{b}	1.09 ± 0.04 ^{bc}	0.86 ± 0.08 ^{c}	2.58 ± 0.23 ^{d}	2.51 ± 0.31 ^{d}	52.19 ± 1.53 ^{e}	49.4 ± 2.36 ^{f}
T17	44.67 ± 1.13 ^{e}	43.93 ± 1.25 ^{e}	5.11 ± 0.57 ^{a}	4.21 ± 0.18 ^{b}	1.31 ± 0.15 ^{b}	0.94 ± 0.10 ^{c}	5.51 ± 0.34 ^{b}	4.36 ± 0.39 ^{c}	65.43 ± 1.89 ^{de}	61.17 ± 2.11 ^{de}
T18	39.23 ± 1.28 ^{ef}	38.22 ± 1.44 ^{ef}	2.77 ± 0.66 ^{c}	2.57 ± 0.11 ^{c}	0.86 ± 0.08 ^{c}	0.73 ± 0.05 ^{cd}	2.54 ± 0.36 ^{d}	2.23 ± 0.29 ^{d}	48.08 ± 2.015 ^{f}	46.05 ± 1.24 ^{f}
T19	34.01 ± 1.09 ^{f}	33.01 ± 1.26 ^{f}	2.72 ± 0.81 ^{c}	2.25 ± 0.09 ^{c}	0.69 ± 0.05 ^{cd}	0.58 ± 0.03 ^{cd}	2.21 ± 0.26 ^{d}	1.82 ± 0.19 ^{de}	44.13 ± 1.33 ^{f}	42.26 ± 2.24 ^{f}

The data represented in the columns in the form of means having similar letters are identical, and dissimilar letters are significantly different, where p was kept $p \leq 0.05$.

However, the application of biogenic TiO_2 NPs under salinity stress conditions increased the agronomic attributes of both wheat varieties. The application of plant-based TiO_2 NPs at 50 µg/mL led to a remarkable increase in the plant length (33.21% and 18.56%), fresh weight of the plant (43.23% and 35.76%), plant dry weight (36.14% and 23.54%), number of leaves and leaf area (40.13%, 30.34%, 48.56%, and 40.23%) under 50 mM salt solution in both wheat varieties, respectively. The application of 50 µg/mL of TiO_2 NPs under 100 mM NaCl solution caused an increase in the plant length (24.34% and 17.62%), plant fresh weight (33.44% and 29.20%), dry weight of plant (28.11% and 21.19%), number of leaves and leaf area (33.21%, 22.45%, 37.56%, and 29.92%), for both wheat varieties, respectively, compared to their respective controls. Similarly, biogenic titanium dioxide nanoparticles at 50 µg/mL enhanced the plant length (18.17% and 14.26%), fresh weight of the plant (27.18% and 22.42%), dry weight of the plant (24.26% and 18.18%), number of leaves and the area of leaf (27.53%, 17.83%, 29.17%, and 21.76%) at 150 mM of NaCl solution in both wheat varieties, respectively, compare to the non-stressed plants. In addition, treatment of the plants with TiO_2 NPs alone, at 50 µg/mL, increased the plant length (8% and 5%), plant fresh weight (26.23% and 19.47%), dry weight of plant (17.14% and 15.83%), number of leaves and leaf area (12.63%, 11.32%, 15.56%, and 10.31%) in both wheat varieties, respectively, compared to their respective controls. In the present research, biogenic TiO_2 NPs improved agronomic parameters of both wheat varieties under salt stress, and there is little work done on the application of titanium dioxide nanoparticles on different crops under stress conditions. However, the positive effect of 50 µg/mL TiO_2 NPs was observed on the morphological parameters of the plants in the current study, as represented in Table 3.

3.4. Physiological Parameters

3.4.1. Membrane Stability Index

The results showed that the application of TiO_2 NPs caused a significant increase ($p < 0.05$) in physiological parameters, while without the addition of NPs saline conditions negatively affected the physiology of both wheat varieties are mentioned in Figure 2. Application of TiO_2 NPs at 50 µg/mL with no additional salt stress, considerably increased the MSI (24.13% and 20.53%) of both wheat varieties, respectively, compared to the control (Figure 2a). Application of a 50 mM salt solution caused a reduction in the MSI (42.12% and 49.24%), while a salt solution of 100 mM also decreased the MSI (49.22% and 55.64%), and a 150 mM salt solution led to reductions of (61.28% and 64.46%) in both wheat varieties, respectively, compared to the control (Figure 2b). Biogenic TiO_2 NPs mitigate the effect of salt stress and cause a significant improvement in physiological parameters. Application of 50 µg/mL TiO_2 NPs caused an increase in the MSI of 64.62% and 61.29% under a 50 mM salt solution, this increased to 52.10% and 47.12% at 100 mM salt stress, and 16.13% and 13.29% at 150 mM saline condition, for both wheat varieties, respectively, compared to their respective controls (Figure 2c–e).

3.4.2. Relative Water Content

The relative water content was significantly increased by the use of TiO_2 NPs in both wheat varieties. Application of TiO_2 NPs alone, at 50 µg/mL, caused increased relative water contents (7% and 5%) for both wheat varieties, respectively, compared to the control (Figure 3a). Salinity significantly decreased the relative water content (Figure 3b). At 50 mM NaCl solution, the relative water contents decreased by 23.33% and 27.93%, by 40.32% and 46.18% at 100 mM salt solution, and by 52.17% and 57.90% under 150 mM salt solution, for both wheat varieties, respectively. Moreover, TiO_2 NPs increased the relative water content in plants under salinity stress. Our results showed that the treatments with the TiO_2 NPs at 50 µg/mL, in combination with 50 mM salt stress, significantly increased RWC (55.36% and 53.71%), while under 100 mM saline solution, RWC increased by 45.12% and 30.35%, and under application of 150 mM saline solution, RWC increased by 34.47% and 29.28%, in both wheat varieties, respectively, as compared with the non-treated plants (Figure 3c–e).

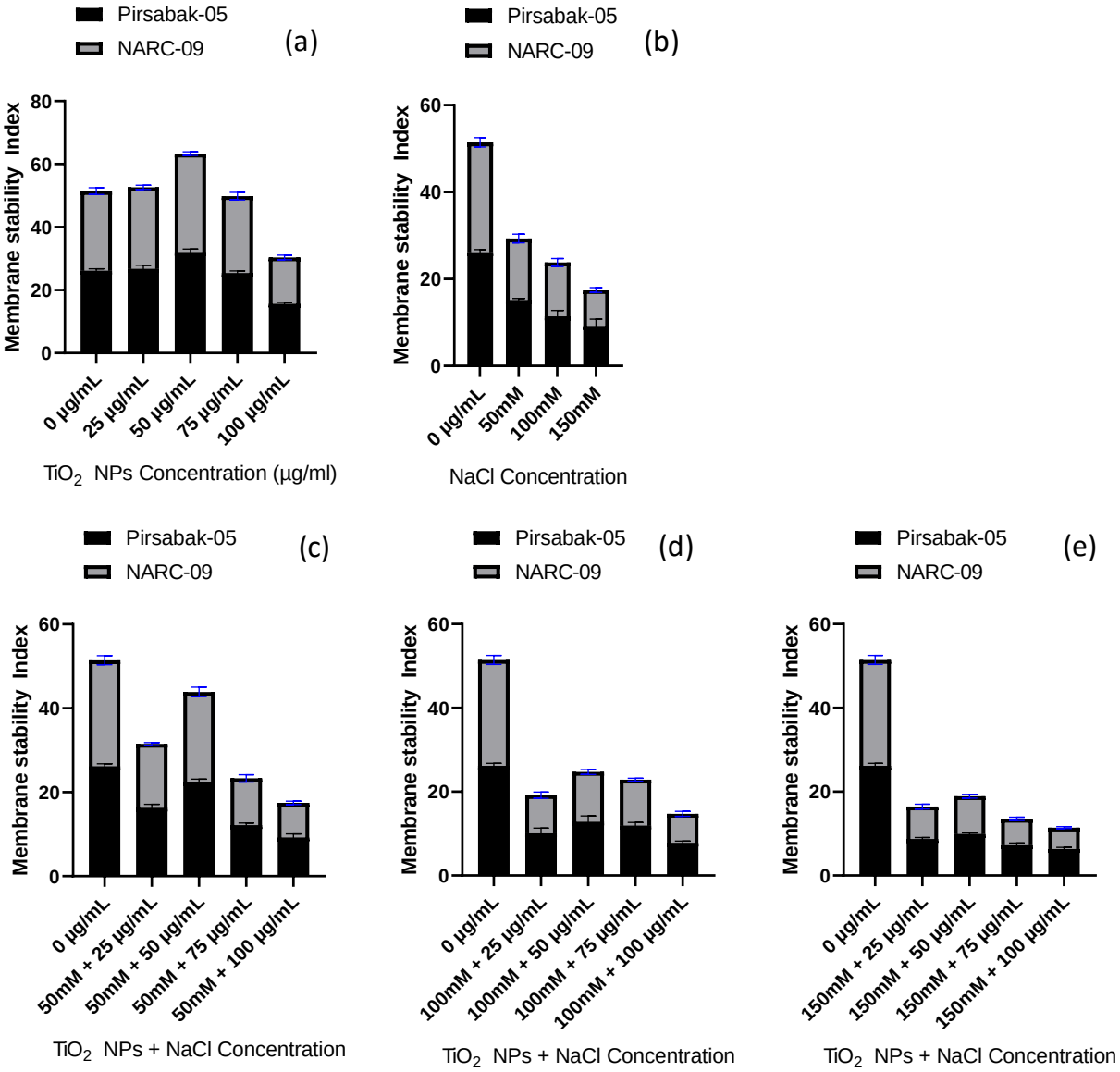


Figure 2. Effects of biogenic TiO_2 NPs on the membrane stability index of selected wheat varieties under salinity stress conditions; (**a**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties, (**b**) Effect of different concentrations of NaCl on wheat varieties, (**c**–**e**) Effect of different concentrations of TiO_2 NPs under varied concentrations (i.e., 50 mM, 100 mM, and 150 mM of NaCl). The data are represented in the form of means, while p was kept at $p \leq 0.05$.

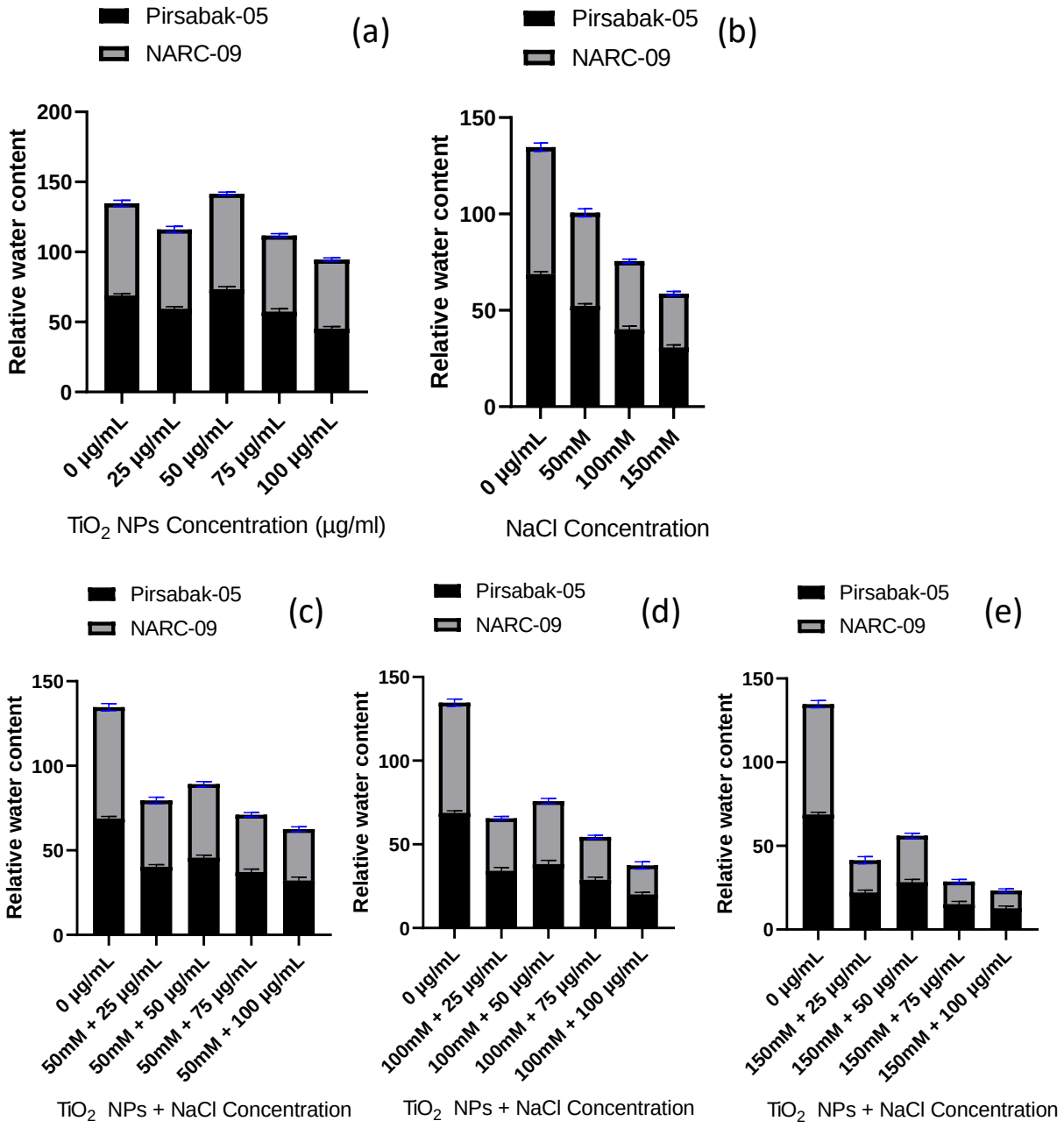


Figure 3. Effects of biogenic TiO_2 NPs on the relative water content of selected wheat varieties under salinity stress conditions; (**a**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties, (**b**) Effect of different concentrations of NaCl on wheat varieties, (**c**–**e**) Effect of different concentrations of TiO_2 NPs under varied concentrations (i.e., 50 mM, 100 mM, and 150 mM of NaCl). The data are represented in the form of means, while p was kept at $p \leq 0.05$.

3.4.3. Chlorophyll a. Content

The results showed that the application of TiO_2 NPs caused a significant increase ($p < 0.05$) in the chlorophyll content, while saline conditions alone negatively affected the photosynthetic pigments in the leaves of both wheat varieties mentioned in Figure 4. Treatment with TiO_2 NPs at 50 µg/mL, with no additional salt stress, caused an enhancement in the chlorophyll a. contents (13.22% and 9.27%) in both wheat varieties, respectively, compared to the controls (Figure 4a). Treatment with the salt solution of 50 mM caused a reduction in the chlorophyll a. contents of 38.11% and 45.82%, with the 100 mM salt solution this was 49.35% and 56.45%, and with the 150 mM salt solution, 61.48% and 67.47%, for both wheat varieties, respectively, compared to the controls (Figure 4b). Biogenic TiO_2 NPs

mitigated the effect of salt stress, and caused a significant improvement in the physiological parameters (Figure 4c–e). Application of 50 µg/mL TiO_2 NPs caused an increase in Chl. a contents of 44.45% and 35.58% under the 50 mM salt solution, 42.53% and 31.23% under the 100 mM salt solution, and 14.23% and 10.12% under the 150 mM salt solution, in both wheat varieties, respectively, compared with the non-treated plants.

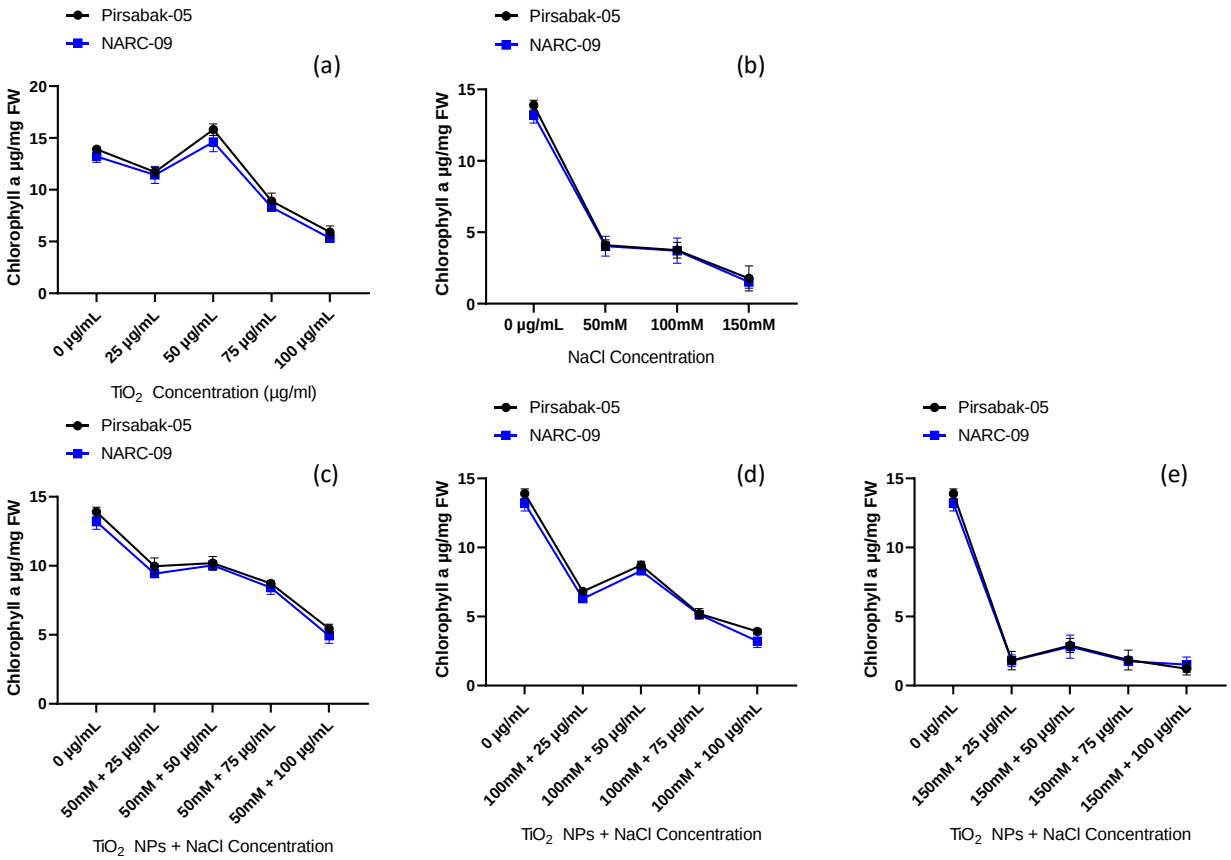


Figure 4. Effects of biogenic TiO_2 NPs on the chlorophyll a. content of selected wheat varieties under salinity stress conditions; (**a**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties, (**b**) Effect of different concentrations of NaCl on wheat varieties, (**c**–**e**) Effect of different concentrations of TiO_2 NPs under varied concentrations (i.e., 50 mM, 100 mM, and 150 mM of NaCl). The data are represented in the form of means, while p was kept at $p \leq 0.05$.

3.4.4. Chlorophyll b. Content

Application of plant-based TiO_2 NPs to the plants, at 50 µg/mL, with no salt solution, increased the chl. b level by 10.46% and 7.84% for both wheat varieties, respectively, compared to the controls (Figure 5a). For plants grown in saline soil, the Chl. b content decreased by 20.12% and 23.34% with the 50 mM salt solution, 31.45% and 33.34% with the 100 mM salt solution, and 36.34% and 46.83% with the 150 mM salt solution for both wheat varieties, respectively, compared with their respective controls (Figure 5b). At optimum concentrations, plant-mediated TiO_2 NPs, combined with salt stress, had a positive effect on chlorophyll content and increased the Chl. b. content. Exposure to 50 g/mL TiO_2 NPs under 50 mM salt solution increased the Chl. b. contents by 25.38% and 21.12%, at 100 mM salt solution the Chl. b. contents increased by 33.29% and 25.12%, while treating with 150 mM salt solution increased the level of Chl. b. contents by 10% and 7%, for the varieties Pirsabak-05 and NARC-09, respectively (Figure 5c–e).

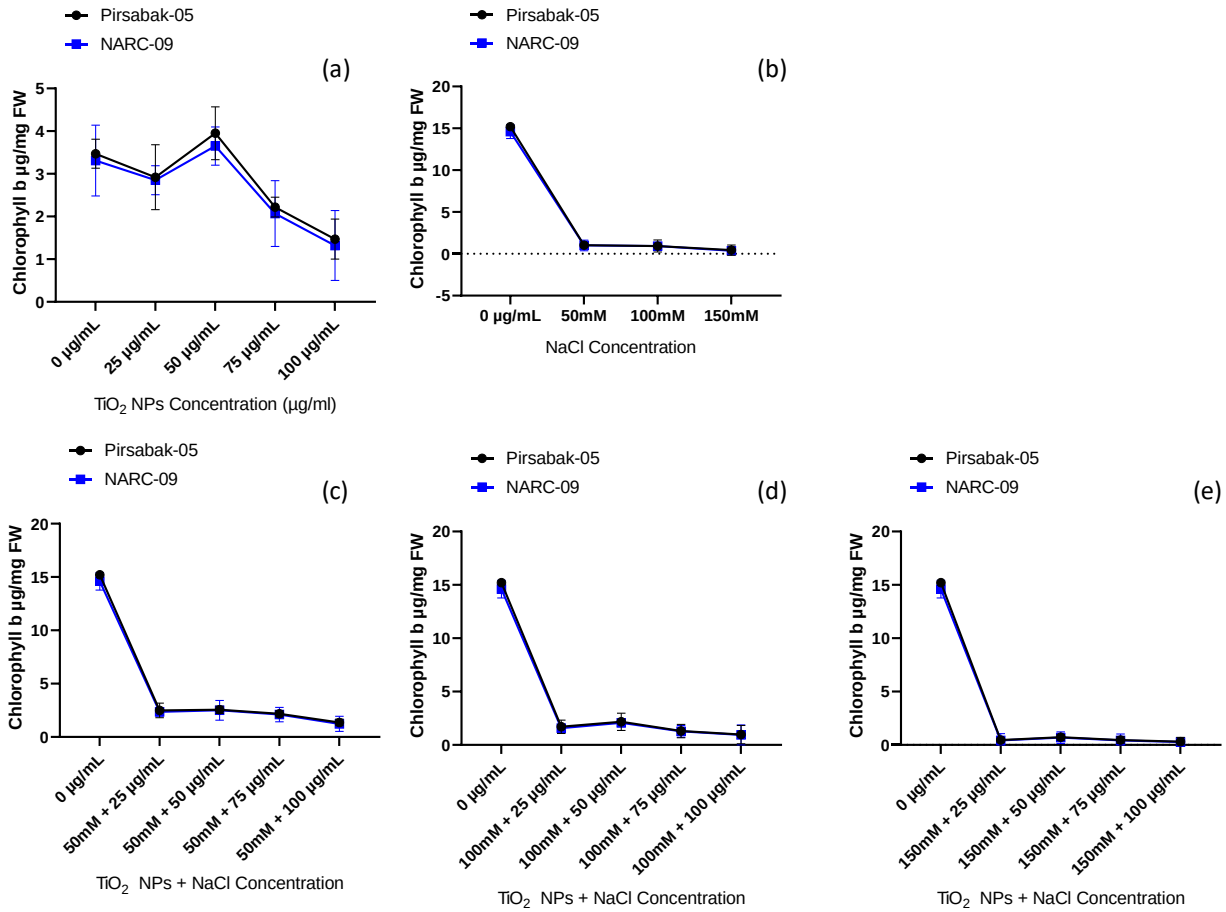


Figure 5. Effects of biogenic TiO_2 NPs on the chlorophyll b. content of selected wheat varieties under salinity stress conditions; (**a**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties, (**b**) Effect of different concentrations of NaCl on wheat varieties, (**c**–**e**) Effect of different concentrations of TiO_2 NPs under varied concentrations (i.e., 50 mM, 100 mM, and 150 mM of NaCl). The data are represented in the form of means, while p was kept at $p \leq 0.05$.

3.4.5. Total Chlorophyll Content

The results showed that the application of plant based TiO_2 NPs to the wheat plants caused a significant increase ($p < 0.05$) in the total chlorophyll content, while saline conditions alone negatively affected the photosynthetic pigments in the leaves of both of the wheat varieties mentioned in Figure 6. Application of the plant-based 50 µg/mL TiO_2 NPs, with no salt solution, considerably increased the total Chl. contents (15.42% and 12.56%, respectively) for both wheat varieties as compared to the controls (Figure 6a). Application of the 50 mM salt solution caused a reduction in the total Chl. contents (35.23% and 41.12%, respectively) for both wheat varieties. A salt solution of 100 mM decreased the total Chl. contents by 45.55% and 52.76%, respectively, and application of the 150 mM salt solution reduced the total Chl. contents by 55.28% and 60.11%, respectively, for both wheat varieties (Pirsabak-05 and NARC-09) compared to the non-treated plants (Figure 6b). Biogenic TiO_2 NPs alleviated the effect of salinity and caused an increase in the total Chl. content. Application of 50 µg/mLTiO_2 NPs increased the total Chl. contents (42.45% and 37.34%) under 50 mM salt solution for both wheat varieties, respectively, compared to their respective controls. Application of 50 µg/mL of TiO_2 NPs under 100 mM salt solution promoted the growth of both wheat varieties and caused enhancements in the total Chl. contents of 40.76% and 36.42%, respectively, and under the 150 mM salt solution, the total Chl. contents increased by 14.65% and 12.32%, respectively, for both wheat varieties compared to their respective controls (Figure 6c–e).

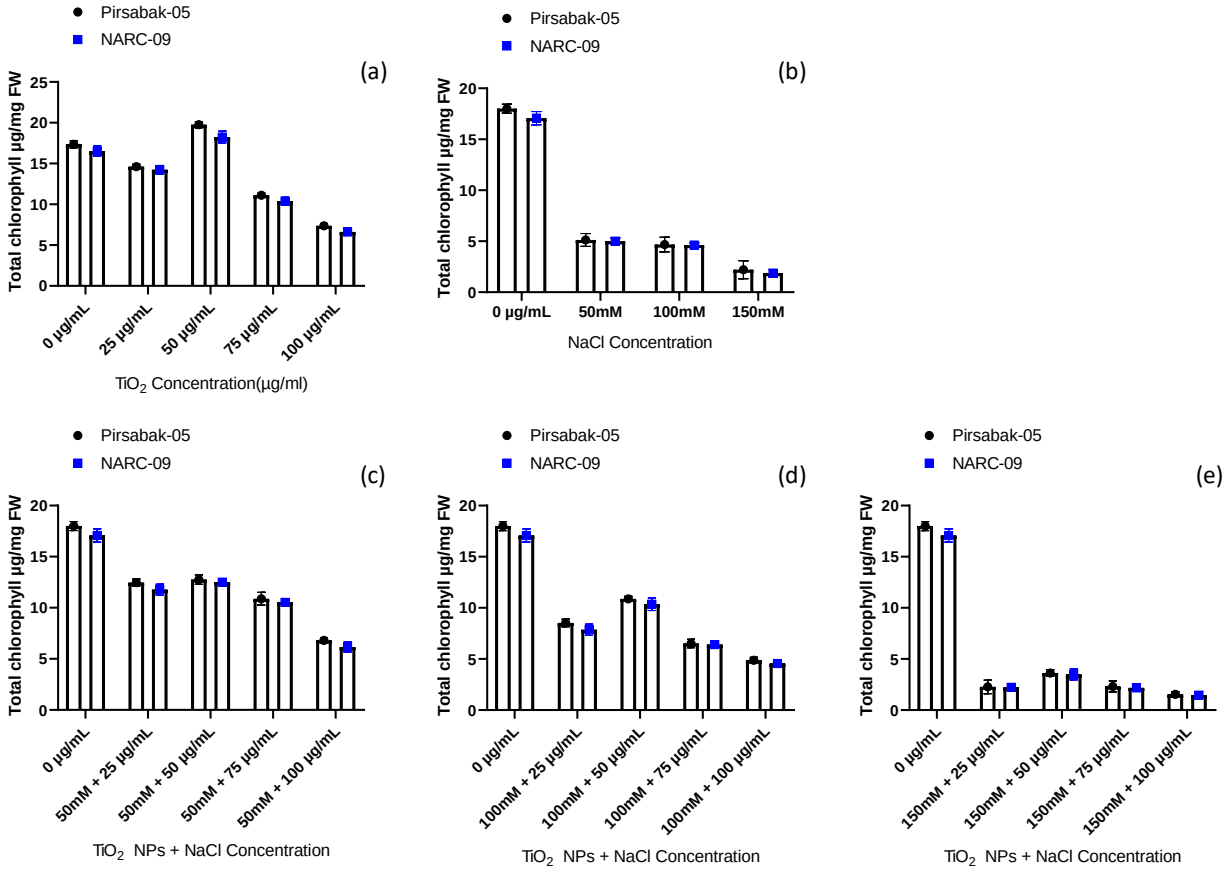


Figure 6. Effects of biogenic TiO_2 NPs on the total chlorophyll content of wheat varieties under salinity stress conditions; (**a**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties, (**b**) Effect of different concentrations of NaCl on wheat varieties, (**c**–**e**) Effect of different concentrations of TiO_2 NPs under varied concentrations (i.e., 50 mM, 100 mM, and 150 mM of NaCl). Details of treatments have been shown in Table 1. The data are represented in the form of means, while p was kept at $p \leq 0.05$.

3.5. Biochemical Parameters

3.5.1. Proline Content

Proline production occurs under stress conditions and plays an important role in protecting proteins from denaturation, thereby stabilizing normal cellular metabolism [28]. Both wheat varieties showed significantly higher proline contents when treated with 50 g/mL of TiO_2 NPs (Figure 7a,b). The proline contents were higher at T2 (50 µg/mL) of TiO_2 NPs caused a remarkable increase of (29.35% and 19.23%) for (Pisabak-05 and NARC-09), respectively, compared to the controls. Salinity significantly increased the proline content in both wheat varieties (Figure 7c,d). Application of the 50 mM salt solution caused an increase in the proline contents of 40.13% and 34.10%, the 100 mM salt solution caused an increase of 49.03% and 43.38%, and the 150 mM salt solution caused an increase of 66.34% and 59.12%, for both wheat varieties, respectively, compared with the non-stressed plants. Treatment of the plants with TiO_2 NPs resulted in a higher level of proline relative to treatment with the 50, 100, and 150 mM NaCl solutions alone. Moreover, TiO_2 NPs increased the proline content even under salinity stress. Our results showed that the treatment of the plants with 50 µg/mL of TiO_2 NPs and the 50 mM salt solution increased the proline contents by 44% and 41%, with the 100 mM salt solution this was 56.22% and 51.95%, and with the 150 mM salt solution, 77.34% and 71.84%, in both wheat varieties (Pirsabak-05 and NARC-09), respectively, compared with the non-treated plants (Figure 7e–j).

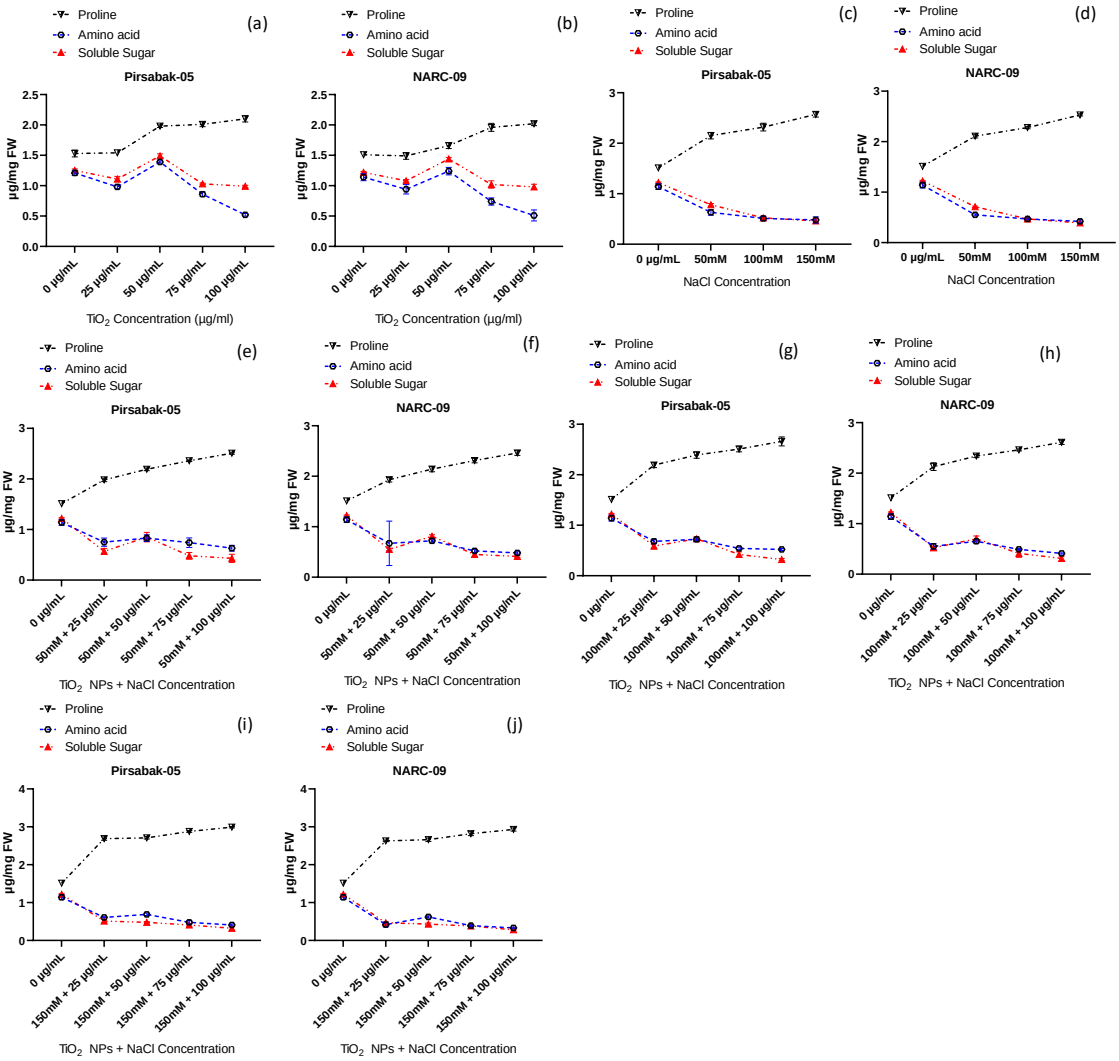


Figure 7. Effects of biogenic TiO_2 NPs on different biochemical traits (proline content, free amino acid content and soluble sugar content) of selected wheat varieties under salinity stress conditions; (**a**,**b**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties Pirsabak-05 and NARC-09, (**c**,**d**) Effect of different concentrations of NaCl on wheat varieties Pirsabak-05 and NARC-09, (**e**,**f**) Effect of different concentrations of TiO_2 NPs under 50 mM of NaCl on wheat varieties Pirsabak-05 and NARC-09, (**g**,**h**) Effect of different concentrations of TiO_2 NPs under 100 mM of NaCl on wheat varieties Pirsabak-05 and NARC-09, (**i**,**j**) Effect of different concentrations of TiO_2 NPs under 150 mM of NaCl on wheat varieties Pirsabak-05 and NARC-09. The data are represented in the form of means, while p was kept at $p \leq 0.05$.

3.5.2. Free Amino Acid Contents

Free amino acids play a key role in protein synthesis, and L amino acid contributes to the production of plant metabolic proteins. Treatment of plants with 50 µg/mL TiO_2 NPs increased the free amino acid contents of both wheat varieties (14.45% for Pirsabak-05 and 11.23% for NARC-09) (Figure 7a,b). Salinity caused a decrease in the free amino acid content in both wheat varieties, shown in (Figure 7c,d). Application of the 50 mM salt solution alone decreased the free amino acid contents by 37.45% and 41.24%, the 100 mM salt solution decreased the free amino acid contents by 44.24% and 48.65%, and the exposure of the plant to the 150 mM saline solution caused a pronounced decrease of 55.18% and 57.23% in both varieties (Pirsabak-05 and NARC-09), respectively, relative to the non-treated plants. Treating plants with the biogenic TiO_2 NPs mitigated the effect of salt stress, and increased the free amino acid content for the synthesis of proteins. The free amino acid contents

were higher at 50 µg/mL of TiO_2 NPs concentration under different salt concentrations. Application of 50 g/mL TiO_2 NPs to plants, in combination with the 50 mM salt solution, increased the production of free amino acids by 36.53% and 31.87%, for the 100 mM salt solution this was 45.43% and 42.16%, and for the 150 mM salt solution the free amino acid contents of both wheat varieties were higher by 52.27% and 49.34%, respectively, as compared to the controls (Figure 7e–j).

3.5.3. Soluble Sugar Contents

The application of 50 g/mL TiO_2 NPs to the plants increased the biochemical contents, resulting in the increased accumulation of soluble sugar contents by 21.23% and 17.46% in both wheat varieties (Pirsabak-05 and NARC-09), respectively (Figure 7a,b). Treatment of the plants with just the 50 mM salt solution decreased the soluble sugars contents by 40.45% and 43.23%, the 100 mM salt solution decreased the the soluble sugars contents by 55.57% and 58.23%, and the 150 mM salt solution decreased the soluble sugars contents by 63.27% and 67.62%, in the two wheat varieties, respectively, as compared with the non-stressed controls (Figure 7c, d). The application of plant-based TiO_2 NPs, with the addition of salt solution showed a significant effect on the biochemical parameters. Soluble sugar contents were increased at optimum concentrations. By application of 50 µg/mL of TiO_2 NPs and the 50 mM salt solution, promoted biochemical contents and increased the accumulation of the soluble sugar contents by 33.27% and 29.18%, the 100 mM salt increased the soluble sugar contents by 41.12% and 39.22%, and the 150 mM salt solution increased the soluble sugar contents by 64.78% and 61.42%, for both wheat varieties, respectively, compared to the controls (Figure 7e–j).

3.5.4. Superoxide Dismutase (SOD) Activity

The application of biogenic TiO_2 NPs increases the activities of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase dismutase (POD) significantly (Figures 8 and 9) in salt stress-facing plants. Superoxide dismutase is the primary defense mechanism against oxidative stress and causes catalysis of H_2O_2 into water and molecular oxygen species by changing the amount of hydrogen peroxide and oxygen [29]. The current findings showed that application of TiO_2 NPs alone, at 50 µg/mL, significantly increased the SOD activity (50% and 47%) in comparison with the non-treated plants (Figure 8a). The application of just the NaCl solution of 50 mM caused an increase in SOD activity of 30.24% and 26.16%, the 100 mM NaCl solution increased SOD activity by 45.55% and 41.86%, and the 150 mM NaCl solution increased the SOD activity by 75% and 70%, for both wheat varieties, respectively, compared with non-stressed plants (Figure 8b). In addition, TiO_2 NPs 50 µg/mL, in combination with the 50 mM NaCl solution significantly increased the SOD activity (by 39.82% and 31.48%), the 100 mM NaCl solution by 58.47% and 54.15%, and the 150 mM NaCl solution by 69.58% and 62.28%, for both varieties (Pirsabak-05 and NARC-09), respectively, as compared with their respective controls (Figure 8c–e).

3.5.5. Peroxidase Dismutase (POD) Activity

Titanium dioxide nanoparticles have been shown to boost antioxidant activity. The highest values of peroxidase dismutase (POD) activity (38.87% and 33.33%) were obtained by applying TiO_2 NPs at a concentration of 50 µg/mL (Figure 9a) in the absence of saline solution. While, application of NaCl solution alone, at 50 mM, caused an increase in POD activity of 13% and 9%, 100 mM NaCl solution increased POD activity by 34.28% and 26.35%, and 150 mM NaCl solution caused an increase in POD activity of 51.04% and 48.18%, respectively, as compared to the non-stressed controls (Figure 9b). Treatment of the plants with 50 µg/mL TiO_2 NPs, in addition to the 50 mM NaCl solution, increased POD activity by 33.27% and 24.23%, with the 100 mM NaCl solution POD activity increased by 49.24% and 42.46%, and with the 150 mM NaCl solution, POD activity increased by 60.29% and 54.68%, respectively, for both wheat varieties compared to the controls (Figure 9c–e). Our current study also demonstrated that the addition of 50 µg/mL TiO_2 NPs was able to

significantly increase the biochemical content and plant growth under saline soil conditions. This is the first report that indicates the concentration-dependent effects of TiO_2 NPs application in the promotion of two wheat varieties grown under both normal and saline soil conditions. With 50 µg/mL TiO_2 NPs application, along with salt stress, antioxidant activities were remarkably increased in the treated plants as compared with the plants treated with the saline solution alone.

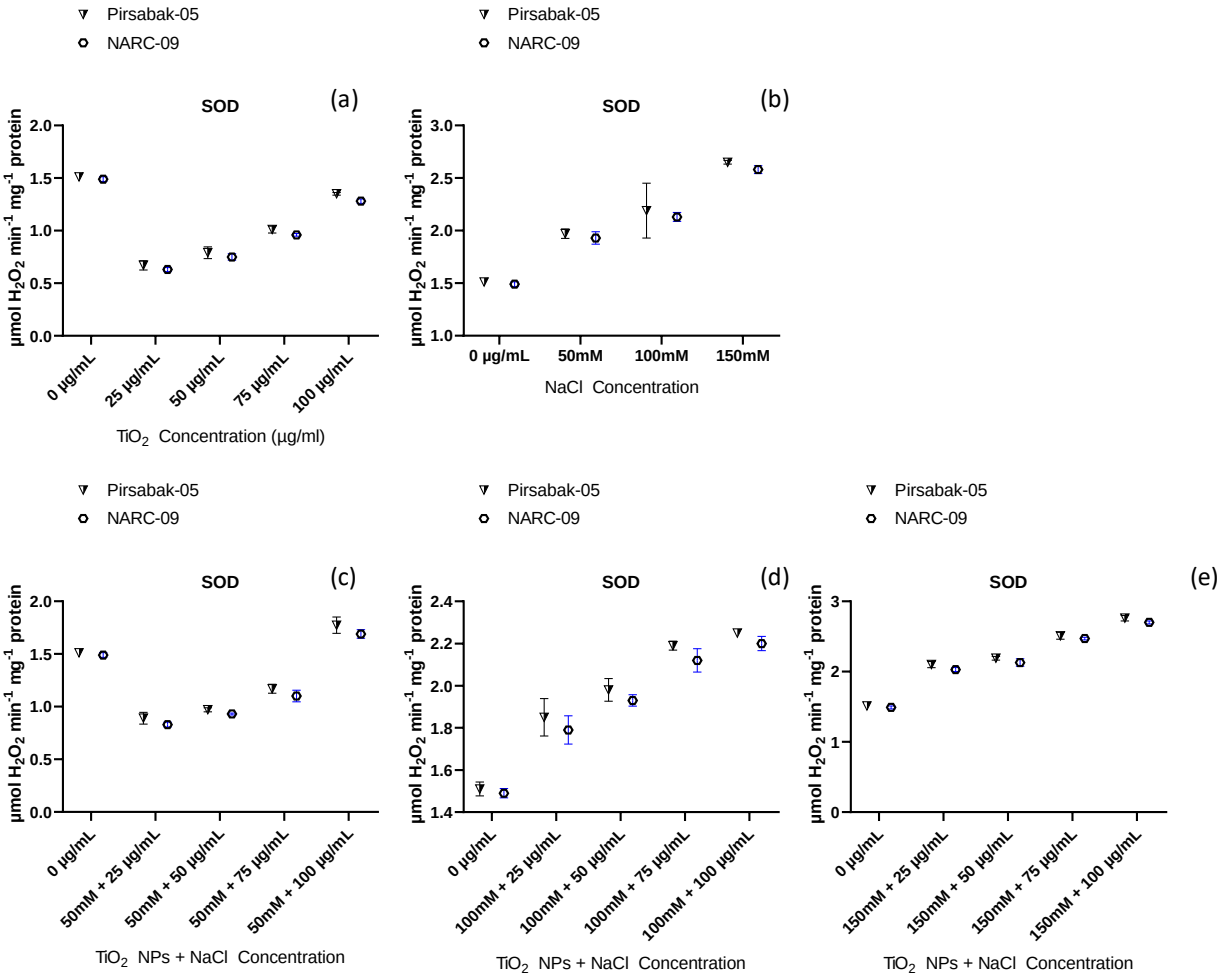


Figure 8. Effects of biogenic TiO_2 NPs on superoxide dismutase (SOD) activity on wheat varieties under salinity stress conditions; (**a**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties, (**b**) Effect of different concentrations of NaCl on wheat varieties, (**c**–**e**) Effect of different concentrations of TiO_2 NPs under varied concentrations (i.e., 50 mM, 100 mM, and 150 mM of NaCl). The data are represented in the form of means, while p was kept at $p \leq 0.05$.

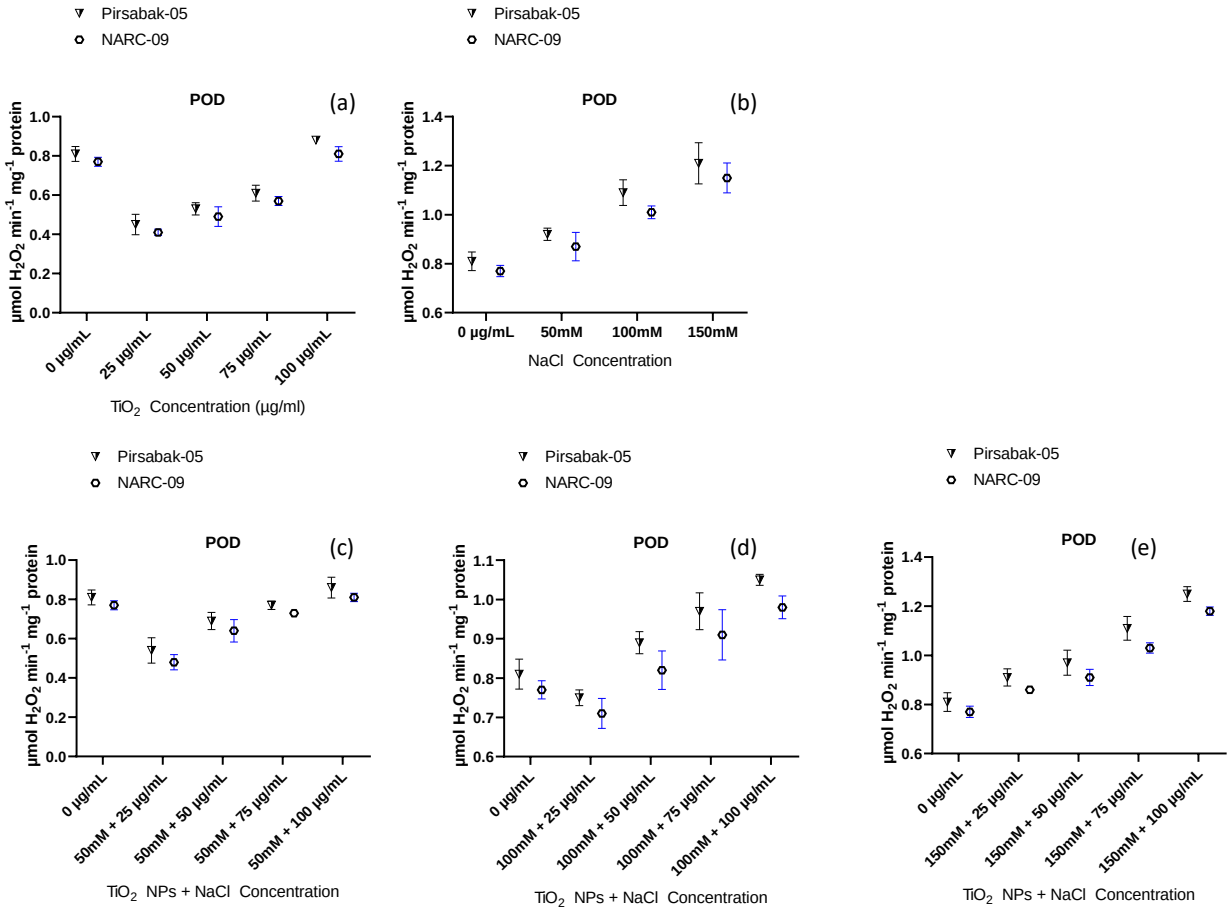


Figure 9. Effects of biogenic TiO_2 NPs on peroxidase dismutase (POD) activity for wheat varieties under salinity stress conditions; (**a**) Effect of different concentrations of biogenic TiO_2 NPs on wheat varieties, (**b**) Effect of different concentrations of NaCl on wheat varieties, (**c**–**e**) Effect of different concentrations of TiO_2 NPs under varied concentrations (i.e., 50 mM, 100 mM, and 150 mM of NaCl). The data are represented in the form of means, while p was kept at $p \leq 0.05$.

4. Discussion

Among all abiotic stresses, salinity stress is the most threatening stress which has a drastic effect on plant growth and development, limiting plant performance and production. Salinity stress affects a plant's growth and its physiochemical properties as well [30]. Nanoparticles help the plant to tolerate salt stress. In the current research, the application of TiO_2 NPs enhanced the growth of plants, and it may be linked to antioxidant enzyme production and osmotic adjustments in plants. Nanotechnology plays a significant role in agriculture and helps in crop management. Plant based extracts are very economical and simple to use to synthesize nanoparticles, containing naturally diverse phytochemicals that can be used as reducing and stabilizing agents for the synthesis of nanoparticles. Titanium dioxide nanoparticle biosynthesis is the more acceptable method, as it acts as a growth promoter and shows a remarkable effect on plant growth under salinity stress. Different concentrations of TiO_2 NPs were used, to check their responses on different plants under unfavorable conditions [11,31].

Salinity stress severely affects healthy plant growth and germination [32]. This study, showed that germination parameters were critically affected by salinity stress. All salt concentrations harmed plant germination of both wheat varieties, but 150 mM salt solution showed a severe effect on all germination phases. Our results agree with the findings of [33], who stated that seed germination and seedling growth of pakchoi were reduced under salt stress due to inhibition of water absorption by seeds. In addition to this, [34] identified that the decrease in germination is due to a reduction of H_20 in an emerging embryo and a delay

in the breakdown of biomolecules stored in endosperm. Our results are consistent with the findings of [35], who stated that the application of an appropriate concentration of TiO_2 NPs increased seedling growth of wheat, while a higher concentration of TiO_2 NPs caused a reduction in seed germination parameters of both wheat varieties. From the results of this research, it can be concluded that a higher concentration of salt stress caused a pronounced reduction in seedling growth of both wheat varieties, which was supported by [36] shown in Table 2.

Salt stress caused a remarkable reduction in wheat growth and biomass due to the non-availability of nutrients or high translocation of Na from the roots toward the shoots [2]. In the present work, a decrease in plant length was observed under salt stress, relative to the control, in both wheat varieties. Our result is similar to the findings of [30], which stated that salinity stress harms shoot length and root length at higher concentrations. High salt stress caused decreases in the fresh weight and dry weight of maize plants. Similarly, salinity stress caused a decrease in the shoot and root fresh weight of wheat plants [2]. Application of TiO_2 NPs increased macro- and micro-nutrient absorption, improved plant growth characteristics (e.g., root and shoot length, root and shoot fresh weight, and leaf numbers), and reduced the negative effects of salinity, such as disturbing photosynthesis and the absorption of essential elements [37].

The current research concluded that salt stress causes a decline in the MSI in both wheat varieties. This is due to osmotic reduction and the production of ABA hormones causing the closing of stomata, reducing the uptake of water through roots, and resulting in a low availability of water in the cell [38]. TiO_2 NPs increased the MSI and played a significant role in maize-water relations under salt stress, and stabilized the plants' water retention capacity, aiding against the effects of salinity [34]. Treatment with TiO_2 NPs has also been shown to increase the RWC in *stevia*, in addition to the maintained water status of the cells, over-accumulation of proline might provide several benefits concerned with scavenging ROS, preventing a sustained oxidative burst and stabilizing membranes to prevent electrolyte leakage [39].

Under salt stress, leaf chlorophyll contents are significantly reduced, this is due to the inhibition of chlorophyll biosynthesis [40]. Mediated nanoparticles cause an improvement in plant growth parameters such as chlorophyll a and b, and total chlorophyll contents under salinity conditions, as deduced from the present findings. Regarding TiO_2 NPs, the affirmative action of the relevant NPs was also noted for Moldavian balm and broad bean, which were correlated with the contribution to the chlorophyll development and Rubisco activities [11]. Our results, coinciding with the report of [41], stated that salinity reduces photosynthetic pigments due to an excessive accumulation of sodium ions, which causes severe changes in their function and structure.

Plant-mediated TiO_2 NPs in combination with salt stress showed a positive effect on the chlorophyll content and increased the Chl. b. content at optimum concentrations. The current research concluded that TiO_2 NPs increased photosynthetic pigments under saline conditions in wheat varieties, which is in line with the studies of [12], stating that TiO_2 NPs increased the chlorophyll b. content and carotenoid content in broad bean plants facing salinity conditions, and alleviated the harsh effect of salinity on plants. Salinity induces ROS production, which causes oxidative damage to photosynthetic pigments. TiO_2 NPs can cause a reduction in ROS production, which enhances photosynthetic pigments and prevents photosynthetic pigment destruction [42]. Under salt stress, leaf chlorophyll contents are significantly reduced, this is due to the inhibition of chlorophyll biosynthesis [40]. The current research evaluated that the optimum concentration of TiO_2 NPs, 50 μg/mL, in response to salinity stress, causes a significant increase in leaf chlorophyll contents for both wheat varieties. Our findings are supported by [12], which stated that Zn nanoparticles promoted photosynthetic pigments in *Lupine stermis* plants in response to salinity stress; similar results were also identified on *Osimum basillicum* by the application of silica nanoparticles under saline conditions.

Saline soil increased the accumulation of proline [12], this is due to a decrease in water uptake and an increased osmolarity of soil solution [43]. An increase in the proline content under salinity provides multiple benefits to plants, including maintaining the turgidity of the cell, and stabilizing the cell membrane to prevent scavenging ROS and leakage of different electrolytes [5]. The current findings are also consistent with the results indicating that the application of TiO_2 NPs increased the proline content in bitter melon and strawberry under salinity stress conditions. However, this caused an elevation in the proline content in plants by increasing the activity of nitrate reductase [12].

Mesoporous silica nanoparticles increased the free amino acid contents in *Vigna radiate* [44]. Our results agree with the findings of [45], which stated that TiO_2 NPs increased the free amino acid content of plants under salinity stress to improve the plants' nitrogen status. In the present study, TiO_2 NPs improved the carotenoid content in wheat varieties under salinity stress, which is in line with [41], who described the analogous findings in marigolds under salt stress conditions. This high production of soluble sugar contents is an important organic solute that may also help in cell homeostasis [46]. In the current study, all concentrations of saline solution caused the production of proline and carotenoids in both wheat plants. So our findings agree with [19], who reported similar results by applying TiO_2 nanoparticles on *Phaseolus vulgaris* L. (Fabaceae) and *Dracocephalum moldavica* L. (Lamiaceae) crops affected by salinity stress. The increase in the concentration of proline and carotenoids is due to TiO_2 NPs, which act as osmoprotectants in the salinity-exposed plants, by protecting enzymes from denaturation and membrane stabilization.

The application of TiO_2 NPs increased the SOD activity in the Moldavian balm plant under saline conditions [11]. This finding was supported by the fact that many abiotic stress-related genes were up-regulated by TiO_2 NPs treatments [47]. This is the first report that indicates the concentration-dependent effect of TiO_2 NPs application in the promotion of two wheat varieties' growth under both normal and saline soil conditions. At 50 µg/mL TiO_2 NPs application, in addition to salt stress, antioxidant activities in treated plants increased remarkably as compared to plants treated with salt alone. This amelioration of salt stress is not only limited to TiO_2 NPs, but CeO_2NPs have also been shown to enhance salt tolerance in *Brassica napus*, as reported by [48]. The increase of TiO_2 NPs concentration causes an increase in the anti-oxidant enzymes SOD and POD, which help to control the level of ROS production and keep the plant from damage [49]. The plants also retain a well-defined detoxification system by different enzymatic and non-enzymatic antioxidants to maintain ROS levels. These antioxidants are SOD, catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). SOD works as the first line of defense in the case of oxidative damage. It converts SO radicals to H_2O_2 according to [50], although all antioxidant enzymes increased significantly under salinity conditions. In this study, salinity stress increased the SOD and POD content in both wheat varieties, whereas TiO_2 NPs reduced SOD and POD content production under salinity stress, indicating that TiO_2 NPs are somehow helping the plant to overcome salinity stress in such conditions [30].

5. Conclusions

Plant-based TiO_2 NPs improved the physio-morphological and biochemical properties of wheat germination when the crop was subjected to salt stress. We reported using *Moringa oliefera* plant extracts as a reducing agent during the synthesis of TiO_2 NPs, which is an eco-friendly and more efficient and cost-effective process for obtaining phytogenic TiO_2 NPs. The effects of synthesized nanoparticles were investigated using two locally available wheat varieties, i.e., Pirsabak-05 and NARC-09. The exogenous application of biogenic TiO_2 NPs boosted salt tolerance in wheat plants. Green synthesized TiO_2 NPs at 50 g/mL improved wheat physio-morphic and biochemical features, whereas salt stress alone, and higher concentrations of TiO_2 NPs (75 and 100 g/mL), harmed wheat crops. It was concluded that TiO_2 NPs have the potential to reduce the negative impacts of salinity by improving all aspects of wheat in salty environments. Furthermore, when subjected to salt stress, wheat types Pirsabak-05 and NARC-09 differed in their sensitivity to TiO_2

NPs. There is a compelling need to use nanotechnology to uncover a unique molecular mechanism of TiO_2 NPs in plants subjected to salinity stress.

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