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Can Different Salt Formulations Revert the Depressing Effect of Salinity on Maize by Modulating Plant Biochemical Attributes and Activating Stress Regulators through Improved N Supply?

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Abstract: Salinity is a major constraint in improving agricultural productivity due to its adverse impact on various physiological and biochemical attributes of plants, and its effect on reducing nitrogen (N) use efficiency due to ion toxicity. To understand the relationship between sodium chloride (NaCl) and increased N application rates, a pot study was performed in which the ammonical (NH_4^+) form of N was applied as urea to maize crops at different rates (control, 160, 186, 240, 267, 293, and 320 kg N ha^{-1}) using two salinity levels (control and 10 dS m^{-1} NaCl). The results indicate that all biochemical and physiological attributes of the maize plant improved with increased concentration of N up to 293 kg ha^{-1}, compared to those in the control treatment. Similarly, the optimal N concentration regulated the activities of antioxidant enzymes, i.e., catalase activity (CAT), peroxidase activity (POD), and superoxide dismutases (SOD), and also increased the N use efficiencies of the maize crop up to 293 kg N ha^{-1}. Overall, our results show that the optimum level of N (293 kg ha^{-1}) improved the salinity tolerance in the maize plant by activating stress coping physiological and biochemical mechanisms. This may have been due to the major role of N in the metabolic activity of plants and N assimilation enzymes activity such as nitrate reductase (NR) and nitrite reductase (NiR).

Keywords: sodium chloride; nitrogen levels; biochemical attributes; antioxidant enzymes; nitrogen use efficiencies; maize

1. Introduction

Salinity is a major environmental limitation, causing extensive crop losses worldwide [1]. The increase in salinity has reduced the average yield of field crops by more than 50%, and these losses are of great concern, especially for agricultural countries [2]. Among abiotic stresses, salinity has an eminent position in reducing yield and quality by showing its damaging impact on global sustainable agriculture [3,4]. Salinization is increasing daily due to climate change and the poor quality of irrigation water [2,3]. Under the root zone of plants, a harmful condition is created by the high concentration of soluble salts such as sodium chloride (NaCl), which ultimately contribute to the inhibition of biochemical and physiological processes in plants, reducing their growth, quality, and productivity [5]. Based on their ability to survive under a saline environment, two types

of plants are distinguished, glycophytes and halophytes. Halophytes (e.g., smooth cordgrass and pickleweed) can tolerate high salinity due to compartmentalization, osmolyte production, and compatible solutes, while, glycophytes (e.g., rice, sunflower, sugar beets, and maize) cannot tolerate high salinity at both the cellular and plant level [6]. Most of the terrestrial plant population are glycophytes including field crops [3,7]. High salinity causes many physical and metabolic alterations such as osmotic stress, specific ion toxicity and ion imbalance, and production of reactive oxygen species (ROS), and these changes lead to decreased plant growth and productivity [3,8]. The osmotic effect is also known as the rapid effect because the accumulation of soluble salts under the root zone, reduces the ability of plants to absorb water, disturbing the stability index of the membranes and causing plant death. Similarly, ion toxicity and nutrient imbalance are secondary or later effects because of the accumulation and distribution of soluble salts within plant bodies in high concentrations [9]. A close relationship has been observed between soluble salts and essential plant nutrients, for example, sodium (Na^+) interacts with calcium (Ca^{2+}), potassium (K^+), magnesium (Mg^{2+}), and ammonium (NH_4^+), while, chloride (Cl^-) interacts with sulfate (SO_4^{2-}) and nitrate (NO_3^-) ions [10]. These interactions under high salinity disrupt ion uptake, causing plants to become nutrient-deficient, and eventually lead to poor plant yield and quality. Salt accumulation also destabilizes the stomatal opening of plant leaves, disrupting the influx and efflux ratios of carbon dioxide (CO_2) and oxygen (O_2) and restricting the fixation of CO_2 in plants [11]. The exchange of gases within plants inversely relates to the concentration of Na^+ and Cl^- ions and other plant attributes such as transpiration, the conductance of stomata, and photosynthetic processes, adversely affected by a saline environment [12,13]. In the same way as under saline conditions, plants produce compatible solutes such as sugar alcohols and proline content in order to scavenge ROS. These compatible solutes are hydrophilic in nature [11].

Among plant essential nutrients, N is considered to be one of the dominant nutrient abundantly absorbed by plants because it is a major component of numerous compounds synthesized within the plant body, such as proteins, amino acids, polyamides, and quaternary ammonium compounds involved in protection of plants under stress conditions; however, high salinity causes a reduction in N uptake and deregulates all functions mediated by N uptake [14]. For instance, N uptake under salinity is reduced due to the antagonistic effect of N forms (i.e., NO_3^- and NH_4^+) with salt ions. In Pakistani soils, Na^+ and Cl^- ions are more dominant, and soluble ions are due to alkaline calcareous soils. Sodium ions compete with NH_4^+, and Cl^- ions compete with the NO_3^- form of N, which causes a reduction in N uptake [15]. Therefore, the optimal supply of N under a saline environment could be a significant strategy to combat the negative effects of salinity and obtain optimal productivity from saline soils. Various studies have evaluated the relationship between N nutrition and plant growth responses in salt-affected soils, with increased salinization, shortage of good-quality irrigation water, and overpopulation are imposing major threat to food security [16–18]. Adequate N application under salt-affected soils can minimize the deleterious effects of salinity to plant growth and development by regulating osmotic stress [19], improving antioxidant activities [20], increasing stomatal conductance [21], mitigating ion toxicity [22], and improving photosynthetic processes [23], and by triggering the activities of N assimilation enzymes such as nitrate reductase (NR) and nitrite reductase (NiR) [24], and improving N use efficiency [25].

Similarly, an adequate supply of N under a saline environment also improves the plant biomass and root system. Due to the proliferation of plant roots, nutrient and water uptake increases, which subsequently improves the ability of plants to survive under stress conditions [26]. An adequate level of N also adjusts the selectivity of plant roots for nutrient uptake and increases the exclusion ability of salt ions through leaves to improve the tolerance capacity of plants under salt stress [27,28]. All these improvements in plant growth and development through higher N application under salt-affected soils improve the tolerance ability of plants, leading to a sustainable yield of field crops.

By keeping in view all these improvements of N nutrition in plants under salinity stress, we conducted an experiment using maize as a test crop because maize is the third staple crop after wheat and rice in Pakistan, and it is moderately sensitive to salinity. Specifically, we evaluated the interactive effect of salinity and different N application rates to determine the best N level for maize crops under salt stress.

2. Materials and Methods

2.1. Experimental Site

A pot study was conducted to evaluate the relation between different nitrogen rates and salinity stress on physiological and biochemical attributes of the maize plant in wire house conditions at the research area, College of Agriculture, University of Sargodha, Sargodha (latitude 32.082° N, longitude 72,669° E, and altitude 190 m) Punjab, Pakistan, during 2016.

2.2. Pot Experiment

A pot study was performed for optimization of N application rates at two different salinity levels. The study comprised a (7 × 2) factorial completely randomized design (CRD), with four repeats per treatment. The experiment had seven different N levels (control, 160, 186, 240, 267, 293, and 320 kg ha^{-1}) and two different salinity levels (control, 10 dS m^{-1}).

Sandy clay loam soil was used in this study, which belongs to the Lyallpur soil series (Aridisols fine silty, hyperthermic haplic vermosols according to FAO Soil Classification System), and it was collected from the fields of the College of Agriculture, University of Sargodha, Sargodha. Ten different soil samples from a depth of 0–15 cm were collected and hand sorted to remove any visible stones in the field and complete/intact dead and/or live vegetation before they were thoroughly mixed to develop a composite soil sample. The composite soil sample was air dried, then sieved through a 2 mm mesh screen, and analyzed for physicochemical and biochemical properties (Table 1).

Table 1. Physicochemical properties of soil to be used for experiments of this study.

Soil Properties	Value	References
Physical properties		
Sand (g kg^{-1})	581.0	Bouyoucos [29]
Silt (g kg^{-1})	293.0	
Clay (g kg^{-1})	126.0	
Textural class	Sandy clay loam	
Saturation percentage	34	
Chemical properties		
pH	7.85 ± 0.06	Jackson [30]
Electrical conductivity (µS cm^{-1})	1114 ± 39.23	
Organic matter (g kg^{-1})	9.97 ± 1.14	Walkley and Black [31]
Total organic C (g kg^{-1})	5.87 ± 0.31	
Dissolved organic C (mg kg^{-1})	45.90 ± 4.65	
Total N (mg kg^{-1})	264.04 ± 9.40	Bremner and Tabatabai [32]
Available P (mg kg^{-1})	8.94 ± 0.67	Olsen et al. [33]
Available K (mg kg^{-1})	189.4 ± 9.42	Hanway and Heidel [34]

After this, pots were filled with 15 kg soil that had dimensions of 36 × 54 cm, and salinity levels were developed at the time of soil filling. The recommended dose of chemical (PK) fertilizers at 80:60 kg ha^{-1} was applied as diammonium phosphate (DAP) and sulphate of potash (SOP) in all treatments at the time of filling and mixing of soil in pots, while different levels of N (control, 160, 186, 240, 267, 293, and 320 kg ha^{-1}) as urea were applied to select the most effective level of N at salinity of EC 10 dS m^{-1} using salt (NaCl) under pot conditions. However, a full dose of P and K fertilizers was applied as a basal dose at the time of soil filling, one third (1/3rd) of the recommended dose of N was applied at the

time sowing, and the remaining quantity of N was top dressed into three equal splits at different growth stages of maize plants.

Then, each pot was irrigated with water to develop optimal moisture conditions (70% WHC (water holding capacity)) for sowing. Four seeds of hybrid maize (cv. FH-1046, Pioneer Pakistan Seed Ltd.) per pot were sown, and only one plant was maintained in each pot for further development. After 15 days of germination, the uprooted plants were incorporated in the soil of the same pot. Good-quality irrigation water was applied throughout the research that had EC 0.075 dS m^{-1}, SAR 0.34 (mmol L^{-1})$^{1/2}$, and zero RSC. In addition, all agronomic practices were used to overcome crop diseases and weeds during the study.

All the biochemical parameters including chlorophyll contents (a, b, and total), leaf area, relative water content, membrane stability index, electrolyte leakage, photosynthetic N use efficiency, proline content, carotenoid content, malondialdehyde content, catalase activity, peroxidase activity, superoxide dismutase activity, N harvest index, N use efficiency, N yield efficiency, and physiological N efficiency were determined after the harvest of crops using standard procedures. After harvesting, the physicochemical and biochemical analyses of soils were performed in the laboratory.

2.3. Soil Analysis

Soil samples were collected at the depth of 0–30 cm from the research area in order to measure the physicochemical properties of the soil. For the determination of soil texture, the hydrometer method was used [29]. Finally, a textural triangle was used to find the textural class of the soil [35]. Soil pH was determined by using a pH meter (JENWAY-3510). For this, a suspension ratio of soil and water (1:5) was prepared to analyze the pH of the soil. Electrical conductivity (EC) of soil was measured using the conductivity bridge method explained by Jackson [30], while the soil saturation percentage was determined by using a prescribed formula. A fraction of soil saturated paste was oven dried at 105 °C to a constant weight. Then, the saturation percentage was determined by using the following formula.

$$\text{Water content } (\%) = \frac{\text{Loss in weight on drying}}{\text{Oven dry weight of soil}} \times 100 \quad (1)$$

For the determination of N from the soil, two steps are involved (digestion and distillation) in the estimation of total N from the soil using Kjeldhal's distillation apparatus [32]. Total organic C content in the soil was determined by using dichromate oxidation followed by titration with acidified ferrous ammonium sulphate ($FeH_8N_2O_8S_2$) using a modified Walkley and Black method [31], while dissolved organic C (DOC) of the sampled soil was extracted with distilled water (1:5 *w/v* ratio), and the amount of the C concentration was determined as described by Walkley and Black [31].

Soil available phosphorus was estimated by the sodium bicarbonate ($NaHCO_3$) extraction method [33]. For this, 3.0 g air-dried soil was properly mixed with 0.5 M $NaHCO_3$ (60 mL) into a 150 mL Erlenmeyer flask followed by shaking at 150 rpm for 30 min. The amount of available P in the filtrate was determined spectrophotometrically (λ = 880 nm) using ascorbate–molybdate reagent [33]. Soil available K was measured using the ammonium acetate (CH_3COO–NH_4) extraction method [34]. For this, 5.0 g air-dried soil in 50 mL of 1 M CH_3COO–NH_4 solution (pH 7) was shaken for 30 min at 275 rpm. The soil solution suspension was allowed to settle, and the extract was filtered through Whatman No. 2. The available K concentration in the soil extract was determined by flame spectrometry (Flame photometer PFP7).

2.4. Plant Analysis

Different physiological parameters (i.e., relative water content, membrane stability index, electrolyte leakage, chlorophyll contents (a, b, and total), carotenoid content, nitrogen use efficiency (NUE), nitrogen yield efficiency (NYE), physiological nitrogen efficiency (PNE), photosynthetic N use efficiency (PNUE), and N harvest index (NHI)) were taken

after 45 days of treatments application. In the case of biochemical attributes, proline content, and antioxidant enzymes were determined after 60 days of germination.

The percentage of relative water content (RWC) was determined according to the technique of Turner [36] using following formula.

$$\text{RWC } (\%) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100 \quad (2)$$

The electrolyte leakage (EL) percentage was found according to the procedure described by Lutts et al. [37] and estimated according to the following equation.

$$\text{EL } (\%) = \frac{\text{EC1}}{\text{EC2}} \times 100 \quad (3)$$

The membrane stability index (MSI) percentage was determined according to the equation given by Singh et al. [38].

$$\text{MSI } (\%) = \frac{1 - \text{EC1}}{\text{EC2}} \times 100 \quad (4)$$

Chlorophyll and carotenoid contents were analyzed according to the methods explained by Shabala et al. [39] and Lichtenthaler [40], respectively. The leaf area of plants was calculated using a leaf area meter (CI-202). Proline content was determined using the method described by Bates et al. [41] with a spectrophotometer at a 528 nm wavelength. Malondialdehyde (MDA) concentration was spectrophotometrically determined from leaf samples at a wavelength of 532 nm [42].

2.5. Determination of Antioxidant Enzymatic Activities

For this, a leaf sample was arranged according to method elucidated by Mukherjee and Choudhuri [43]. Catalase activity (CAT) was examined via a reaction solution of 50 mM phosphate buffer, H_2O_2 (30%), and 0.5 mL solution of enzymatic extract [44]. For the determination of peroxidase (POD) activity, the method of Maehly and Chance [45] was used. For this, guaiacol was oxidized in the presence of hydrogen peroxide (H_2O_2), while the activity of superoxide dismutase (SOD) was estimated by the technique illustrated by Dhindsa et al. [46].

2.6. Nitrogen Harvest Index

The ratio of nitrogen uptake by seeds to total plant biomass is known as the nitrogen harvest index [47]. It is determined by the following equation.

$$\text{NHI} = \frac{\text{NY}}{\text{NYt}} \quad (5)$$

where NY = nitrogen uptake by seeds, and NYt = nitrogen taken up by total plant biomass.

2.7. Nitrogen Use Efficiency

Nitrogen use efficiency (NUE) was predicted by applying the following equation [48].

$$\text{NUE} = \frac{\text{N}_{\text{ui}} - \text{N}_{\text{uc}}}{\text{N}_{\text{fi}} - \text{N}_{\text{fc}}} \quad (6)$$

where N_{ui} and N_{uc} = nitrogen uptake by seeds and straw in various treatments and control, and N_{fi} and N_{fc} = nitrogen in the form of fertilizers in various treatments and control.

2.8. Nitrogen Yield Efficiency

Nitrogen yield efficiency (NYE) was estimated to find the inputs of nitrogen utilization in response to nitrogen application [49]. It can be calculated using the formula

$$NYE = \frac{Y_i - Y_c}{N_{fi} - N_{fc}} \quad (7)$$

where Y_i = nitrogen in seed yield in various treatments, and Y_c = nitrogen in control treatment. For the calculation of NYE, the amount of nitrogen applied and the yield of seeds should be determined.

2.9. Physiological Nitrogen Efficiency

Physiological nitrogen efficiency (PNE) was also calculated, by using the following equation [50].

$$PNE = \frac{Y_i - Y_c}{N_{ui} - N_{uc}} \quad (8)$$

where N_{ui} and N_{uc} = nitrogen uptake by seeds and straw in various treatments and control, respectively.

2.10. Photosynthetic Nitrogen Use Efficiency

Photosynthetic nitrogen use efficiency can be determined from the readings of the photosynthetic rate and leaves' N content using the formula described by Poorter and Evans [51].

$$PNUE = \frac{RPL}{SNL} \quad (9)$$

where RPL = rate of photosynthesis in leaf (mmol m^{-2} s^{-1}), and SNL = specific N content in leaf (g m^{-2}). Overall, the PNUE is described in the unit of μmol $g^{-1}s^{-1}$.

2.11. Statistical Analysis

The data were analyzed using the Statistix 10 statistical package [52], and to compare the difference among the treatment means, we used the least significant difference (LSD) test at 0.05 P [53].

3. Results

In this study, seven different N levels (control, 160, 186, 240, 267, 293, and 320 kg N ha^{-1}) were tested to evaluate the optimum N application rate for the physiological and biochemical attributes of maize, as well as different N use efficiencies under saline conditions.

3.1. Physiological Stress Indicators of Plants

The results of the relative water content (RWC) show that the RWC in the plants was significantly affected by all salt levels (SL), nitrogen levels (NL), and their interaction in the two-way ANOVA test (Table 2). In the control treatment, an increase in the RWC was observed with an increase in the N concentration. The highest RWC appeared at the N5 level, which was 70.57%, and a low RWC (59.68%) was observed in N0 (control) at a salinity level of 10 dS m^{-1}, while N1, N2, and N3 showed nonsignificant results across the control pots.

Table 2. Effect of different N application rates on physiological stress indicators of maize plants under salt stress in pot conditions.

Nitrogen Levels	Relative Water Content (%)		Membrane Stability Index (%)		Electrolyte Leakage (%)		Proline Content (μmol g^{-1} DW)		Malondialdehyde Content (mg g^{-1})	
	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}
N0: control (untreated)	74.41 c	59.68 f	33.37 d	17.65 h	66.63 d	82.35 a	4.25 d	12.11 a	19.11 e	37.49 a
N1: 160 kg ha^{-1}	75.53 bc	62.55 ef	33.51 cd	19.41 fg	66.49 d	80.59 ab	4.02 d	11.39 a	17.73 ef	35.81 abc
N2: 186 kg ha^{-1}	77.01 abc	62.63 ef	34.28 bcd	20.27 f	65.72 d	79.73 ab	4.08 d	11.72 a	17.64 ef	35.15 bc
N3: 220 kg ha^{-1}	77.39 abc	63.61 e	34.35 abc	20.29 f	65.65 d	79.71 ab	3.97 d	11.65 a	17.40 efg	35.71 abc
N4: 267 kg ha^{-1}	78.07 ab	68.66 d	35.55 ab	23.44 e	64.45 d	76.56 bc	3.92 d	10.27 b	16.05 fg	34.07 cd
N5: 293 kg ha^{-1}	79.15 a	70.57 d	36.81 a	26.04 e	63.19 d	73.96 c	3.86 d	8.90 c	15.44 g	32.38 d
N6: 320 kg ha^{-1}	74.47 c	61.14 ef	33.18 cd	16.86 gh	66.82 d	83.14 a	4.17 d	11.83 a	18.09 ef	36.53 ab
Interactions between salinity levels and nitrogen levels										
SL	342.77 ***		908.86 ***		219.37 ***		1779.17 ***		1923.38 ***	
NL	10.24 ***		16.34 ***		3.20 *		8.05 ***		7.18 ***	
SL × NL	1.94 *		2.38 *		0.73 ns		5.37 ***		0.22 ns	
CV	3.58		8.33		4.83		8.29		5.82	

Values are means of four replicates. For each parameter, under each column, values sharing different letters differ significantly from each other at $p < 0.05$. NS: nonsignificant, *: $p < 0.05$, ***: $p < 0.001$, SL: salt level, NL: nitrogen levels. Degrees of freedom (df) for SL: 1, NL: 6, and SL × NL: 6.

The data regarding the membrane stability index (MSI) demonstrate that SL, NL, and their interaction affected the MSI markedly (Table 2). All treatments showed a significant difference among each other over the untreated control (N0). In control pots, and at 10 dS m^{-1} salinity, all treatments had an increased MSI with an increase in the N level, except for the N6 level (320 kg N ha^{-1}). The highest MSI at 10 dS m^{-1} was recorded as 26.01% as a result of the N5 level (293 kg N ha^{-1}), while the lowest MSI (up to 17.65%) was obtained in the untreated control (N0).

In the case of electrolyte leakage (EL), both SL and NL depicted a significant effect on the EL content; however, their interaction (SL × NL) effect was found to be nonsignificant (Table 2). Across the control pots, no considerable differences in EL were recorded between the N0 and N6 treatments. In the case of the 10 dS m^{-1} level of salinity, the N5 treatment was able to induce a significant reduction in EL of 73.96%, as compared to the untreated control (N0) treatment.

The data regarding the proline content reveal that both SL and NL depicted a significant effect on the proline content, while their interaction was also found to be significant (Table 2). In the case of the control, no considerable differences in the proline content were recorded between the N0 and N6 treatments. Meanwhile, in the case of 10 dS m^{-1} salinity, the N5 (293 kg N ha^{-1}) treatment was able to induce a significant decrease in the proline content of up to 8.90 µmol g^{-1} DW, as compared to the untreated control treatment (N0).

Both SL and NL showed a significant effect on the malondialdehyde (MDA) content; however, their interaction effect was found to be nonsignificant in this experiment (Table 2). Across the control pots, a considerable difference in the MDA content was recorded between the N0 and N6 treatments. In the case of the control and 10 dS m^{-1} salinity, the N5 treatment was able to induce a significant reduction in the MDA content (32.38 mg g^{-1}), as compared to the N0 treatment, whereas a high value of the MDA content was recorded up to 19.11 mg g^{-1} as a result of the N0 (control) treatment.

3.2. Leaf Area and Physiological Attributes of Maize Plants

The leaf area of maize plants indicated that SL, NL, and the interaction of both SL × NL affected the leaf area significantly (Table 3). All N levels increased the leaf area of maize plants markedly in normal and salinity-formulated soils at 10 dS m^{-1}. The highest leaf areas of 436.42 and 374.86 cm^2 of maize plants were recorded due to application of the N5 treatment in normal and salinity-formulated soils at 10 dS m^{-1}, respectively.

The results regarding the chlorophyll a content show that SL and NL affected the chlorophyll a content in maize plants significantly, but their interaction exhibited a nonsignificant impact on the chlorophyll *a* content (Table 3). Across the control and 10 dS m^{-1} soil pots, a significant increase in the chlorophyll a content was recorded with an increase in N levels up to N5. Similarly, the chlorophyll b content in plant leaves revealed that SL and NL affected the chlorophyll b content in maize plants significantly, but a nonsignificant effect on the chlorophyll b content was found by their interaction (Table 3). Across the control and 10 dS m^{-1} soil pots, a significant increase in the chlorophyll b content was recorded due to an increase in N levels up to N5 (293 kg N ha^{-1}), while the lowest value was observed at the N0 level of N. However, the results regarding the total chlorophyll content show that SL and NL affected the total chlorophyll content in maize plants significantly, but a nonsignificant effect was observed by their interaction (Table 3). In all control and 10 dS m^{-1} soil pots, a significant increase in the total chlorophyll content was recorded with an increase in N levels up to N5 (293 kg N ha^{-1}), while the lowest chlorophyll content was observed in the untreated control (N0) treatment.

The salinity level (SL) and nitrogen level (NL) affected the carotenoid content significantly according to the ANOVA test of this parameter (Table 3). The interaction of both SL × NL showed a nonsignificant impact on the carotenoid content. A significant increase in the carotenoid content was observed in all treatments over the control (N0). The highest content of carotenoids was observed at the N5 (293 kg N ha^{-1}) treatment in normal control pots and in pots at a salinity level of 10 dS m^{-1}.

Table 3. Effect of different N application rates on leaf area and physiological attributes of maize plants under salt stress in pot conditions.

Nitrogen Levels	Leaf Area (cm^2)		Chlorophyll a Content ($mg\ g^{-1}$)		Chlorophyll b Content ($mg\ g^{-1}$)		Total Chlorophyll Content ($mg\ g^{-1}$)		Carotenoid Content ($mg\ g^{-1}$)	
	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}
N0: control (untreated)	361.11 f	286.10 i	1.95 c	1.23 g	1.15 d	0.73 g	3.10 c	1.96 f	0.605 c	0.442 e
N1: 160 kg ha^{-1}	375.91 de	303.91 h	2.06 bc	1.34 fg	1.20 cd	0.77 fg	3.26 bc	2.11 ef	0.612 bc	0.448 de
N2: 186 kg ha^{-1}	384.75 cd	313.75 g	2.15 ab	1.35 fg	1.25 bc	0.78 fg	3.40 ab	2.13 e	0.615 bc	0.455 de
N3: 220 kg ha^{-1}	389.75 c	315.75 g	2.17 ab	1.38 ef	1.26 abc	0.80 f	3.43 a	2.18 e	0.619 abc	0.459 de
N4: 267 kg ha^{-1}	423.69 b	366.96 ef	2.21 a	1.51 de	1.29 ab	0.88 e	3.50 a	2.39 d	0.636 ab	0.470 d
N5: 293 kg ha^{-1}	436.42 a	374.86 e	2.24 a	1.59 d	1.33 a	0.92 e	3.56 a	2.51 d	0.646 a	0.474 d
N6: 320 kg ha^{-1}	359.75 f	289.84 i	2.00 c	1.31 fg	1.16 d	0.76 fg	3.15 c	2.07 ef	0.601 c	0.443 e
Interactions between salinity levels and nitrogen levels										
SL	1547.38 ***		827.81 ***		1192.67 ***		1267.08 ***		1026.30 ***	
NL	198.80 ***		11.31 ***		16.70 ***		17.43 ***		4.63 **	
SL × NL	2.19 *		0.82 ns		0.83 ns		1.05 ns		0.11 ns	
CV	1.83		5.42		4.53		4.38		3.55	

Values are means of four replicates. For each parameter, under each column, values sharing different letters differ significantly from each other at $p < 0.05$. NS: nonsignificant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, SL: salt level, NL: nitrogen levels. Degrees of freedom (df) for SL: 1, NL: 6, and SL × NL: 6

3.3. Antioxidant Enzyme Activities

In this study, catalase activity (CAT) in maize plants was significantly affected by both SL and NL, while their interaction, SL × NL, showed a nonsignificant effect (Table 4). Across the control pots, and at 10 dS m^{-1} salinity, CAT activity in maize was decreased significantly with increased levels of N. The lowest CAT activity of the respective antioxidant enzymes in maize was recorded at the N5 level of N (23.36 and 40.50 U mg^{-1} protein), whereas the highest CAT activity (30.11 and 48.45 U mg^{-1} protein) was found in the control (N0) treatment. The same behavior was observed in the peroxidase (POD) activity in maize plants, which was significantly affected by SL and NL, but their interaction, SL × NL, showed a nonsignificant impact on the POD activity (Table 4). Across the control pots, and at 10 dS m^{-1} salinity, the POD activity in maize plants was decreased significantly with an increase in the N level, except for the N6 (320 kg N ha^{-1}) treatment. The lowest activity of POD in maize plants was recorded at the N5 level of N (293 kg N ha^{-1}), which was 42.49 U mg^{-1} protein, whereas the highest activity was found in the control (N0) treatment (51.74 U mg^{-1} protein) at a salinity level of 10 dS m^{-1}.

Table 4. Effect of different of N application rates on antioxidant enzymes in maize plants under salt stress in pot conditions.

Nitrogen Levels	Catalase Activity (U mg^{-1} Protein)		Peroxidase Activity (U mg^{-1} Protein)		Superoxide Dismutase activity (U mg^{-1} Protein)	
	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}
N0: control (untreated)	30.11 e	48.45 a	32.91 e	51.74 a	35.93 d	54.76 a
N1: 160 kg ha^{-1}	28.65 efg	47.09 ab	30.50 efg	48.94 ab	33.03 ef	52.24 ab
N2: 186 kg ha^{-1}	28.03 efg	45.39 abc	29.67 fg	47.83 bc	32.88 ef	51.91 b
N3: 220 kg ha^{-1}	26.43 fgh	44.84 bc	28.32 fgh	47.23 bc	31.18 fg	50.08 b
N4: 267 kg ha^{-1}	25.35 gh	42.76 cd	27.63 gh	44.78 cd	29.53 gh	46.61 c
N5: 293 kg ha^{-1}	23.36 h	40.50 d	25.46 h	42.49 d	27.22 h	43.90 c
N6: 320 kg ha^{-1}	28.86 ef	46.04 abc	31.28 ef	49.44 ab	33.98 de	52.70 ab
Interactions between salinity levels and nitrogen levels						
SL	768.89 ***		939.66 ***		1281.69 ***	
NL	8.65 ***		12.55 ***		24.33 ***	
SL × NL	0.13 ns		0.23 ns		0.58 ns	
CV	6.63		5.75		4.66	

Values are means of four replicates. For each parameter, under each column, values sharing different letters differ significantly from each other at $p < 0.05$. NS: nonsignificant, ***: $p < 0.001$, SL: salt level, NL: nitrogen level. Degrees of freedom (df) for SL: 1, NL: 6, and SL × NL: 6.

The results regarding the superoxide dismutase (SOD) activity depict that both SL and NL had a significant effect, while their interaction showed a nonsignificant effect on the SOD activity (Table 4). In both types of control and 10 dS m^{-1} saline pots, a significant reduction was recorded in the SOD activity at the N5 (293 kg N ha^{-1}) treatment, and the highest activity was observed in the control (N0) treatment.

3.4. Nitrogen Use Efficiencies of Maize Plants

The results regarding the N harvest index (NHI) reveal that the NHI was markedly affected by both SL and NL, but their interaction (SL × NL) showed nonsignificant results (Table 5). In all control pots, an increase in the NHI was observed up to the N5 (293 kg N ha^{-1}) level, and in the case of pots of 10 dS m^{-1} salinity, the same N level (N5) was found, but a nonsignificant increase in the NHI was observed at N levels of N1, N2, N3, and N6, whereas the lowest NHI (10.03%) was recorded in the control (N0) treatment.

Table 5. Effect of different N application rates on N harvest index and various N efficiencies of maize plants under salt stress in pot conditions.

Nitrogen Levels	N Harvest Index (%)		NUE (g DM g^{-1} N)		NYE (g DM g^{-1} N)		PNE (g DM g^{-1} N)		PNUE (g DM g^{-1} N)	
	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}	Control	10 dS m^{-1}
N0: control (untreated)	13.92 e	10.03 f	-	-	-	-	-	-	6.10 ef	4.49 g
N1: 160 kg ha^{-1}	27.49 c	22.51 d	1.71 e	1.37 f	8.78 d	5.20 g	9.11 cd	5.61 e	8.35 cd	5.60 f
N2: 186 kg ha^{-1}	29.28 bc	22.53 d	1.85 cd	1.44 f	9.49 cd	5.71 fg	9.50 c	5.80 e	8.98 bc	6.56 e
N3: 220 kg ha^{-1}	31.39 b	24.21 d	1.91 bc	1.48 f	10.34 b	6.01 f	9.61 bc	5.90 e	9.49 b	6.78 e
N4: 267 kg ha^{-1}	36.02 a	29.63 bc	2.03 ab	1.74 de	11.79 a	7.36 e	10.21 b	6.07 e	11.73 a	8.06 d
N5: 293 kg ha^{-1}	37.55 a	31.28 b	2.10 a	1.81 cde	12.16 a	7.74 e	11.83 a	8.66 d	11.96 a	8.52 cd
N6: 320 kg ha^{-1}	29.78 bc	22.57 d	1.85 cd	1.46 f	9.63 bc	5.79 bc	9.23 cd	5.66 e	8.40 cd	6.58 e
Interactions between salinity levels and nitrogen levels										
SL	168.83 ***		183.54 ***		771.59 ***		661.64 ***		396.58 ***	
NL	137.41 ***		24.07 ***		43.08 ***		39.58 ***		92.34 ***	
SL × NL	0.99 ns		0.84 ns		1.10 ns		0.85 ns		6.43 ***	
CV	6.68		5.29		6.08		6.03		6.22	

Values are means of four replicates. For each parameter, under each column, values sharing different letters differ significantly from each other at $p < 0.05$. NS: nonsignificant, ***: $p < 0.001$, SL: salt level, NL: nitrogen level. Degrees of freedom (df) for SL: 1, NL: 6, and SL × NL: 6. NUE: nitrogen use efficiency; NYE: nitrogen yield efficiency; PNE: physiological N efficiency; PNUE: photosynthetic N use efficiency.

The data regarding the nitrogen use efficiency (NUE) reveal that the NUE was significantly affected by SL and NL; however, their interaction effect was found to be nonsignificant for the NUE (Table 5). The highest NUE across control pots was recorded in the N5 (293 kg N ha^{-1}) treatment, while N1 depicted the lowest NUE (1.71 g DM g^{-1} N) among all N treatments. However, at a salinity level of 10 dS m^{-1}, a nonsignificant increase in the NUE was observed with N fertilization, except for the N5 and N4 treatments that showed a significantly higher NUE (1.81 g DM g^{-1} N) in maize plants.

The results of the nitrogen yield efficiency (NYE) show that the effect of both SL and NL was significant, while the effect of their interaction was observed to be nonsignificant for the NYE (Table 5). The highest NYE was recorded in N5 (293 kg N ha^{-1}) followed by the N4 and N3 treatments, while N1 returned the lowest NYE (8.78 g DM g^{-1} N) among all N treatments. Similarly, at 10 dS m^{-1} salinity, a significant increase in the NYE was also found with N fertilization at the N5 (7.74 g DM g^{-1} N) and N4 (7.36 g DM g^{-1} N) treatments, resulting in a significantly higher NYE in maize plants.

The results of the physiological N efficiency (PNE) depict that both SL and NL had a significant effect on the PNE in maize plants, while the interaction of both salt and N levels did not reveal any significant effect on the PNE of the plants (Table 5). Both saline (10 dS m^{-1}) and control soil pots elucidated that highest PNE (8.66 and 11.83 g DM g^{-1}, respectively), which was observed at the N5 (293 kg N ha^{-1}) level of N, whereas N1 showed the lowest value of the PNE in maize plants.

The results of the photosynthetic N use efficiency (PNUE) show that SL, NL, and their interaction (SL × NL) had a significant effect on the PNUE in maize plants (Table 5). In the control and 10 dS m^{-1} pots, the highest value of the PNUE was recorded up to 8.52 g DM g^{-1} due to application of N5 and N4, respectively, with respect to the control (N0) treatment, where no N was applied.

4. Discussion

Among the biotic stresses, salinity always exerts a significant effect on the growth and yield of crop plants by inducing changes in metabolic activities, such as a specific ion effect, decreased nutrient intake, reduced water potential and chlorophyll contents, and increased antioxidant activities [54–56]. Nitrogen can enhance plants adaptation to tolerate salinity stress, since N is a key growth essential nutrient due to its significant contribution to the synthesis of proteins, polyamines, amino acids, and amides [9,57,58].

In this study, the rate of N fertilization was an important determinant of plant physiological and biochemical responses under salinity. Importantly, an improvement in plant physiological and biochemical attributes was very evident when plants were fed with nitrogen level N5 (293 kg N ha^{-1}); however, N6 (320 kg N ha^{-1}) led to a decrease in the plant physiological and biochemical parameters because of the nitrogen toxicity in the plants, as the most stable reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) generation can contribute to oxidative stress by disrupting the membrane permeability and increasing lipid peroxidation [59,60].

Contrariwise, electrolyte leakage was significantly reduced in stressed plants following N fertilization, and these valuable effects are attributed to the positive role of the adequate N supply on membrane stability that, in turn, aided Na exclusion and, consequently, improved the water supply across different plant compartments. Additionally, the presence of sufficient N improved the plant metabolic functions that are best reflected by the increase in CO_2 assimilation during photosynthesis, and the improvement in the chlorophyll and carotenoid contents [61,62].

Plants express both enzymatic and nonenzymatic antioxidant activities to thwart stress-induced oxidative damage [63]. In this study, the N5 treatment prompted a decrease in the antioxidant enzymes' activity (CAT, POD, and SOD), with concomitant low proline and malondialdehyde (MDA) contents, which might be associated with the level of salinity exposure. With optimum nitrogen nutrition, the adverse effects of salinity in maize plants were relegated, as evident by the decrease in the MDA concentration. In addition, the

proline contents and antioxidant enzyme activities were regulated in order to limit the production of ROS, eventually invoking stronger N-mediated plant defense against salinity stress [64,65].

Similarly, Nadian et al. [16] also noted that urea application at three different N rates alleviated the salinity-related negative effects on sugarcane. Although the minimization of salinity-related detrimental effects was associated with the overapplication of N fertilizers, it positively affected the leaf proline content and upregulated K metabolism as a marker of stress adaptation.

The extensive use of N fertilizers and the decline in N utilization efficiencies have caused widespread concern across agroecosystems [66,67]. Our current work proved that maize plants with an optimal N supply (N5) could improve various nitrogen use efficiency attributes, including NUE, NYE, PNE, PNUE, and NHI. These results also illustrate the higher antagonistic interaction of N with Na and Cl that might have improved the assimilatory function of the nitrogenous biochemical machinery, leading to an increase in the nitrogen yield efficiency, physiological nitrogen efficiency, photosynthetic nitrogen use efficiency, and nitrogen harvest index. This indicates that the increased concentration of N balanced the photosynthetic system under salinity stress through assimilation of carbon dioxide to increase stomatal conductivity in the C plant skeleton [25,68,69]. Similar results were also reported by Song et al. [70], attributing improved NUE to optimal N supply in oat plant under salinity stress.

5. Conclusions

The results of this study show that the interaction between salinity and N application improved all biochemical and physiological characteristics of maize plants at 10 dS m^{-1} salt stress. Higher N content (293 kg N ha^{-1}) in the form of urea was found to be effective in reducing the deleterious effects of salt (NaCl) on biochemical and physiological properties as well as antioxidant enzyme activity and N use efficiency of maize plants. In the present study, it is evident that increased N concentration up to 293 kg N ha^{-1} under salt stress can increase crop yield by enhancing tolerance mechanisms and regulating plant metabolism under salt stress. Although the main limitation of this study is that it was conducted in pots under greenhouse conditions and these treatments were not tested under field conditions, this study still provides baseline data which could be useful to conduct this study under field conditions with the same treatments in different ecological zones of Pakistan to establish the real authenticity of the results.

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