agronomy

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Enhancement of Morphological and Physiological Performance of *Zea mays* L. under Saline Stress Using ZnO Nanoparticles and 24-Epibrassinolide Seed Priming

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Abstract: Salinity is one of the most devastating environmental factors limiting crop productivity worldwide. Therefore, our study investigates the effect of seed priming with zinc oxide nanoparticles (ZnO NPs: 0, 50, and 100 mg L^{-1}), 24-epibrassinolide (EBL: 0.0, 0.2, and 0.4 µM), and their combined treatments on maize (*Zea mays* L.) grown with different levels of saline stress (i.e., control, 5, 10 dS m^{-1}) under semi-controlled conditions. Higher saline stress (10 dS m^{-1}) negatively influenced the growth traits, physiological attributes, and elemental (i.e., Zn and K) uptake for both roots and shoots of maize, whereas it increased Na^+ accumulation and Na^+/K^+ ratio in comparison to other treatments. However, seed priming with ZnO NPs and EBL as well as their combinations showed amelioration of the detrimental effects of saline stress on the growth and physiological and biochemical performance of maize. In general, seed priming with combined treatments of ZnO NPs and EBL were significantly more effective than either ZnO NPs or EBL as individual treatments. A combination of 100 mg L^{-1} ZnO NPS + 0.2 µM EBL resulted in the highest values of root length, root surface area, stem diameter, relative leaf water contents, total chlorophyll, net rate of photosynthesis, zinc accumulation, and K^+ uptake, while it resulted in the lowest Na^+ and Na^+/K^+ ratio, especially under the highest saline-stress treatment. Thus, we concluded that seed priming with combined ZnO NPs and EBL can effectively mitigate the saline-stress-mediated decline in the morphological, physiological, and biochemical traits of maize.

Keywords: saline stress; maize; seed priming; nanoparticles; EBL

Citation: Ahmad, A.; Tola, E.; Alshahrani, T.S.; Seleiman, M.F. Enhancement of Morphological and Physiological Performance of *Zea mays* L. under Saline Stress Using ZnO Nanoparticles and 24-Epibrassinolide Seed Priming. *Agronomy* **2023**, *13*, 771. https://doi.org/10.3390/agronomy13030771

Received: 17 February 2023
Revised: 4 March 2023
Accepted: 6 March 2023
Published: 7 March 2023

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1. Introduction

Saline stress is one of the major abiotic stresses which can limit the agricultural productivity of agronomic crops [1]. More than 6% of global land is already salt-affected, and this percentage is expected to rise in the coming decades. This occupies about 20% of the total cultivated land, out of which approximately 50% is irrigated [2–4]. Salinity stress can significantly affect the performances of agricultural plants; however, the mechanism of its action could be divided into two major processes. Firstly, osmotic stress, in which it can limit water absorption by creating a negative water potential in the rhizosphere and, hence, can inhibit plant growth. Secondly, ionic toxicity; once it enters the transpiration stream, the excessive Na^+ and Cl^- damage the plant cellular structures and alter the molecular composition, resulting in the impairment of homeostasis, physiological processes, cell division, growth, and production of primary and secondary metabolites [5,6]. Saline stress can disturb the photosynthetic process at different levels, such as (a) at the molecular level by decreasing the production of photosynthetic pigments, dysfunction of proteins involved, and denaturation of enzymes and structural molecules, (b) at the stomatal level by reducing the update of K^+, an essential ion to regulate the stomatal opening and closing by altering the osmotic pressure of guard cells, and (c) at the transpiration level by creating negative water potential and deficit supply of nutrients [2,4,5,7,8]. Several strategies have been developed to minimize salinity in soil and its negative effects, as well as Na^+ absorption by plants, and to aid plants to mitigate the higher concentration of Na^+ in cells. Agronomically, assisting plants in stress-coping mechanisms is a potential key to improving plant health, growth, and yield, for which multidimensional approaches are being explored; seed priming with nanoparticles (NPs) and/or phytohormones is one of those.

Zinc (Zn) is a well-recognized essential micronutrient for plants. It serves as a cofactor of several regulatory enzymes and thus significantly contributes to a number of physiological processes such as photosynthesis, biosynthesis of various metabolites, and homeostatic response [9]. Synthetic zinc fertilizers are considered a less efficient source of Zn when applied in the agricultural system [10]. Therefore, NPs can be effective tools to mitigate the critical problems of salinity in agriculture [11]. For instance, the application of zinc oxide nanoparticles (ZnO NPs) is considered one of the most promising approaches that can be used to mitigate abiotic stresses [12,13]. In this respect, Srivastav et al. [14] investigated a wide range of ZnO NPs (0–200 mg L^{-1}) on wheat and maize for growth and biochemical responses. They reported that the application of 100 mg ZnO NPs L^{-1} significantly improved growth and antioxidant enzymes, but caused a decline variably at higher levels (i.e., 150–200 mg L^{-1}) in plants.

On the other hand, attention has been paid to auxins, gibberellins, cytokinin, abscisic acid, ethylene, and recently on brassinosteroids (BRs) as plant hormones that can be used to mitigate abiotic stresses. BRs can have a significant role in photosynthesis, transpiration, and ion uptake, and they can induce specific changes in leaf anatomy and chloroplast structure [15]. Moreover, they can control other plant hormones by using biochemical crosstalk. 24-Epibrassinolide (EBR) is one among those BRs that can be isolated from different plant species. EBR can be chemically synthesized to be used for mitigating environmental stress [12,16]. BRs are nontoxic, ecofriendly, and naturally existing steroidal regulators and hence can be used in agriculture to improve crop yield and its quality by enhancing plant growth against biotic and abiotic stresses [12,17–19]. In this concern, Azhar et al. [20] investigated the effects of different concentrations (i.e., 0, 0.1, 0.25 and 0.5 mg L^{-1} EBL) on the seed germination of barley stressed with 0, 150, and 300 mM NaCl. They reported that the application of 0.25 mg L^{-1} EBL significantly enhanced root, shoot, and relative water content of seedlings stressed with 150 mM NaCl. Moreover, EBL application significantly reduced the content of Na^+ in shoots by approximately 50% compared to stressed seedlings.

Maize is the third most important cereal that occupied 205 M ha of cultivated agricultural land in 2020 [21]. An annual average of 1.2 billion tons of maize grain was recorded in 2021, which was significantly higher than in the past few years [22]. Its multipurpose nature, genetic diversity, and high adaptability to different ecological environments made its cultivation possible in more than 115 countries and over 170 climatic regions worldwide [23]. In addition to its agronomic importance and vibrant contribution in the food chain, maize has been used as a model plant for genetic and physiological studies for nearly a century [24]. However, maize is still generally categorized as “salt moderately sensitive”, and can hence severely be affected by saline stress [21]. Thus, addressing the mitigation strategies of saline stress in maize is a global issue to ensure a sustainable food supply for the next generations.

Therefore, the aim of the current study was to investigate the effects of zinc oxide nanoparticles (ZnO NPs: 0, 50, and 100 mg L^{-1}), 24-epibrassinolide (EBL: 0.0, 0.2, and 0.4 μM), and their combined treatments as seed priming on maize (*Zea mays* L.) grown under different levels of saline stress (i.e., control, 5, 10 dS m^{-1}).

2. Materials and Methods

2.1. Plant Materials, Treatments, and Experimental Design

Stock solutions of ZnO NPs at 100 mg L^{-1} and EBL at 0.4 µM were prepared and stored in a refrigerator at +4 °C until further use. Nine seed-priming solutions were prepared from stock solutions as individual and combined treatments of ZnO NPs and EBL, as shown below (Table 1). Seeds of maize (TWC 310) were placed in labeled conical flasks, and 250 mL of each priming solution was added to respective conical flasks. The flasks were covered with tape and incubated at 28 ± 1 °C in the dark on a reciprocal shaker (Phoenix, RS-OS20, UK) at 60 rpm. After 24 h of shaking, seeds were filtered through 2 mm steel mesh.

Table 1. Saline-stress and seed-priming treatments' detail of individual and combined applications of ZnO NPs and EBL.

(A) Saline Stress (Main Factor)	
Control	Control, normal soil
Moderate	5 dS m^{-1}
High	10 dS m^{-1}
(B) Seed-Priming Treatments (Sub-Factor)	
Treatment	Treatment Details: Concentrations and Combinations of ZnO NPs and EBL
T1	Control (zero EBL and zero ZnO NPs)
T2	EBL (0.2 µM)
T3	EBL (0.4 µM)
T4	ZnO NPs (50.0 mg L^{-1})
T5	ZnO NPs (100.0 mg L^{-1})
T6	ZnO NPs (50.0 mg L^{-1}) + EBL (0.2 µM)
T7	ZnO NPs (100.0 mg L^{-1}) + EBL (0.2 µM)
T8	ZnO NPs (50.0 mg L^{-1}) + EBL (0.4 µM)
T9	ZnO NPs (100.0 mg L^{-1}) + EBL (0.4 µM)

The soil was collected from the experimental farm of King Saud University; then, the soil was air-dried and sieved through a 2 mm steel mesh. The soil was divided into three equal portions and each one of those was treated with an additional amount of sodium chloride (NaCl). Three groups of soil were treated with 0 dS m^{-1}, 5 dS m^{-1}, and 10 dS m^{-1} for control and medium and high salinity, respectively (Table 1).

Saline soil was filled in conical cylinders (length 21.5 cm, upper diameter 4 cm, lower diameter 1.7 cm). The triangular drainage holes at the bottom of the cylinders were covered with cotton plugs before filling the soil. The treatment units with three replications consisted of 81 cylinders (9 treatments of ZnO NPs, EBL, and their combinations × 3 salt-stress treatments × 3 replications). The experiment was laid out as a completely randomized complete-block design (RCBD) with a factorial arrangement where saline-stress treatments were used as the main factor while seed-priming treatments were randomized within each block as a subfactor. Three seeds were sown in each treatment unit at a depth of 1 cm, which were thinned to one seedling per cylinder after full germination. The experiment was conducted in a semi-controlled glasshouse with 14 h of light and 10 h of darkness. The temperature was maintained with an artificial cooling system at 28 ± 1 °C during the day and 22 ± 1 °C during the night. The relative humidity ranged between 52 and 58%. All treatments were irrigated with 25 mL of water every 5 days for the first three weeks, and then every 3 days until the end of the experiment (45 DAS).

2.2. Measurements

2.2.1. Soil Analysis

Soil samples were collected for physiochemical analysis (i.e., soil texture, soil pH, electric conductivity (EC), and cation exchange capacity (CEC)). Soil texture was measured using Bouyoucos's method [25]. Soil samples were analyzed according to Richard's protocol [26], and pH meter (WTW-pH523, G417DL, Germany), EC meter (EC-YSI, Model 35, Columbus, OH, USA), and Flame photometer (Corning 400, Sherwood Scientific Ltd., Cambridge, UK) were used to measure pH, EC, and CEC, respectively. The soil analysis was EC 1.51 dS m^{-1}, CEC 6.55 cmol kg^{-1}, Na 431.11 mg kg^{-1}, K 209.31 mg kg^{-1}, Zn 15.92 mg kg^{-1}.

2.2.2. Root Traits

At 45 DAS, roots were separated from the whole plants, and the soil was removed from the roots using a soft brush, followed by washing using distilled water. The roots were then placed on towel paper to remove extra water from the surface and weighed for fresh root weight using an electric balance, and root length was measured using a wooden meter rod. They were then stained with food color for 24 h in sealed plastic bags. Roots were then dried with towel papers and scanned using a root scanner. The photographs were then analyzed using WinRHIZO software (v5.0, Regent Instruments, Quebec City, QC, Canada) to measure the total number of root tips, root volume, total surface area, average diameter, and total root length. To record root dry weight, stained roots were then placed in paper bags and heated at +65 °C in an electric oven until the constant weight was achieved.

2.2.3. Physiological Traits of Maize Seedlings

The physiological traits of maize seedlings grown in saline soils were recorded at 21, 28, 35, and 42 DAS between 10 and 12 am. Photosynthesis (Pn), transpiration rate (Trmmol), and intercellular CO_2 concentration (Ci) were measured using the portable LI-6400XT (LI-COR, Li-COR, Lincoln, NE, USA). Leaf green index (SPAD values) was recorded for plants in each pot using SPAD 502 Plus (Spectrum Technologies, Bridgend, United Kingdom). Chlorophyll fluorescence was measured as maximal photochemical efficiency of PSII (Fv/Fm) using a portable non-modulated fluorimeter Plant Efficiency Analyser (Handy PEA; Hansatech Instruments Ltd. Norfolk, UK) with a 30 min dark adaptation using light-withholding clips and the peak at a wavelength of 650 nm.

2.2.4. Growth Analysis

(i) *Stem diameter and shoot length:*

Stem diameter and shoot length of plants were recorded at 42 DAS. Vernier calipers were used to measure stem diameter at 5 cm above the soil surface.

(ii) *Leaf area and relative leaf water contents (RLWC):*

Plants were collected at 45 DAS, and green leaves area was measured using LI-3000C leaf area meter (LI-COR, Lincoln, NE, USA). In order to measure RLWC, a disc diameter of 1.5 cm was taken from each leaf, and fresh weight was measured using an electric balance. The leaf disc was then placed in distilled water (5 mm) in a Petri dish until it reached a constant weight and was fully turgid [27] (about 12 h). The discs were then removed and cleaned with paper towel to remove surface water, and turgor weight was recorded. To obtain a constant dry weight, leaf discs were placed in an electric oven at +65 °C for 72 h. The RWC were calculated using the following equation.

$$RLWC = \frac{Turgor\ weight - Fresh\ weight}{Turgor\ weight - Dry\ weight} \times 100 \quad (1)$$

(iii) *Whole plant dry weight:*

At 45 DAS, the whole plant was oven-dried, and dry weight was recorded, including stem, root, and leaves dry weight.

2.2.5. Mineral Profiling of Leaves and Roots

Dry samples from both roots and leaves as triplicates of all maize-seed-priming treatments grown under salt-stress treatments were grounded manually. A constant weight (i.e., 200 mg) of plant samples was digested following Wolf's method [28]. Sample extracts were used for analyzing Na and K contents using a Flame photometer (Corning 400, Sherwood Scientific Ltd., Cambridge, UK). ICP-OES (PerkinElmer Optima 4300 DV ICP-OES, Cambridge, MA, USA) was used for the analysis of trace elements (i.e., Zn).

2.3. Statistical Analysis

The raw data obtained from the different measurements as a result of seed-priming treatments with ZnO NPs, EBL, and their combination on maize grown under different salt-stress treatments, were subjected to an analysis of variance (ANOVA) using PASW statistics 21.0 (IBM Inc., Chicago, IL, USA). Different means of different treatments for each parameter were compared by a Duncan multiple-range test to show the significant differences at $p \leq 0.05$.

3. Results

3.1. Root Morphological Performance

Root morphological markers are of prime importance in saline-stress evaluation, as roots are the only plant structures that are in direct contact with salinity in soil. The highly significant deleterious effect of saline stress was observed in this study for all studied root parameters except root average diameter (Table 2). High salinity (10 dS m^{-1}) reduced root length, total root length, root total surface area, root total volume, and number of root tips per plant by −32.50%, −73.72%, −50.52%, −58.36%, and −44.09%, respectively, compared to control. Seed-priming treatments with various concentrations of ZnO NPs and EBL, solo as well as combined, showed significant improvement in aforesaid root parameters by mitigating the deleterious effects of saline stress. Maize root length was increased by +44.48% and +36.33% compared to control (T1) in T9 and T7 seed-priming treatments. Similarly, T7 and T6 improved the total root length by +97.24% and +68.27%, respectively. For root total surface area, T5 to T8 were statistically the same but showed significant improvement compared to the control (133.12 cm^2). Seed-priming treatments from T5 to T9 depicted no significant differences among themselves in the number of root tips per plant; however, they resulted in a significant increase compared to the control, with a maximum of +186.15% by T8. Moreover, T8 and T5 improved the root total volume by three times compared to the control. A similar trend was also seen in root average diameter, where again, T8 and T5 outperformed all other seed-priming treatments by increasing the root average diameter by +65.11% and +43.40%, respectively (Table 2).

Table 2. Effect of saline stress and seed priming with ZnO NPs and EBL on root length, total length of roots, root average diameter, root total surface area, root volume, and number of root tips of maize plants gown under semi-controlled greenhouse conditions.

Treatment	Root Length (cm)	Total Root Length (cm)	Root Total Surface Area (cm^2)	Root Average Diameter (mm)	Root Total Volume (cm^3)	Number of Root Tips
			Saline Stress			
Control	36.55 A	414.32 A	319.48 A	2.67	24.76 A	2487.94 A
5 dS m^{-1}	27.46 B	286.95 B	198.68 B	2.65	14.21 B	1590.06 B
10 dS m^{-1}	24.67 C	238.32 C	158.08 C	2.35	10.31 B	1391.11 B
SEM$_{0.05}$	0.438	15.624	13.512	0.203	2.083	114.162
Significance	***	***	***	NS	***	***

Table 2. *Cont.*

Treatment	Root Length (cm)	Total Root Length (cm)	Root Total Surface Area (cm^2)	Root Average Diameter (mm)	Root Total Volume (cm^3)	Number of Root Tips
Seed-Priming Treatments						
T1	24.28 e	234.84 d	133.12 d	2.35 bc	7.37 c	850.17 d
T2	26.41 de	301.86 cd	182.08 cd	2.44 bc	13.49 c	1506.00 c
T3	27.13 cd	263.95 d	172.18 cd	2.24 c	10.64 c	1686.67 bc
T4	29.27 c	306.85 cd	181.92 cd	2.01 c	9.78 c	1450.17 c
T5	31.81 b	243.01 d	259.91 ab	3.37 ab	27.71 ab	2031.67 abc
T6	26.54 d	395.16 ab	266.52 ab	2.31 bc	16.89 c	2229.17 ab
T7	33.10 ab	463.19 a	326.06 a	2.27 bc	18.88 bc	2303.17 ab
T8	32.44 b	251.54 d	274.96 ab	3.88 a	30.02 a	2432.83 a
T9	35.08 a	358.38 bc	231.96 bc	2.14 c	13.06 c	1917.50 abc
SEM$_{0.05}$	0.758	27.061	23.403	0.352	3.608	197.735
Significance	***	***	***	**	***	***

For abbreviations, see Table 1; SEM= standard error of means; *** = $p \leq 0.001$; ** = $p \leq 0.01$; NS = non-significant; Different letters for saline or seed-priming treatments are significantly differed at $p \leq 0.05$.

The interactive effect of saline stress and seed priming with ZnO NPs and EBL was also computed in this study, and the results showed remarkable significance in all studied root parameters (Table 3). Root length ranged between 17.97 cm and 42.33 cm in 27 combinations of interactions, with a maximum root length (42.33 cm) in saline control × T8 and minimum in 10 dS m^{-1} saline stress × T3. Total root length was the lowest (157.39 cm) with high salinity (10 dS m^{-1}) in the TI seed-priming combination, which was −51.77% lower than the double control (no salinity × T1). The saline control × T7 combination resulted in the maximum total root length (729.16 cm), +123.45% higher than the double-control combination treatment. Even though both the saline-stress and seed-priming treatments showed notable variations in root surface area, saline stress significantly lowered it regardless of the seed-priming treatment. The maximal increase in root surface area was observed in T7 (+195.55%) and T5 (+195.18%), compared to double control, whereas high saline stress in combination with T5 resulted in a −38.14% reduction. The interactive effect of saline stress and seed priming was significant for root average diameter. Interestingly, a three-fold increase in root average diameter was observed in high saline stress × T8 with respect to the control (1.61 mm).

Among all the studied root morphological characteristics, root total volume depicted the widest range, with a lowest value of 4.96 cm^3 and a highest of 58.07 cm^3. The seed-priming treatment T5 exhibited nine-fold higher root total volume in control salinity compared to T1. Contrarily, no significant reduction in root total volume was noted. Likewise, root volume and number of root tips per plant also portrayed a wide range, where a +302.93% increase compared to double control resulted from the T6 seed-priming treatment with no saline stress. The lowest number of root tips (−22.80%) was noted in T1 in combination with high saline stress (Table 3). In brief, the data revealed deleterious effects of saline stress on root morphological parameters, whereas the seed-priming treatments with ZnO NPs and EBL alleviated the extent of saline stress.

3.2. Growth Performance

Saline stress significantly inhibited plant growth performance in maize plants for all studied parameters (Table 4). Exposure to high salinity (10 dS m^{-1}) reduced stem diameter, shoot length, whole plant dry weight, average leaf area, and RLWC by −37.40 mm,

−48.10 cm, −64.26 g, −63.51 cm^2, and −20.36 %, respectively, compared to control. However, seed priming with ZnO NPs and EBL ameliorated the saline-stress-mediated decline in aforesaid growth parameters significantly. Seed priming with T4 to T7 improved stem diameter up to +45.42% compared to control (T1). The maximal shoot length was observed in T3, which stood at +41.88% higher than the control. Whole plant dry weight and average leaf area were elevated by +129.11% and +60.88%, respectively, by T6. A relatively less pronounced but significant increase of up to +7.11% in RLWC was recorded in T5, T6, and T7 seed-priming treatments (Table 4).

Table 3. Interactive effect of saline stress and seed priming with ZnO NPs and EBL on root length, total length of roots, root average diameter, root total surface area, root volume, and number of root tips of maize plants gown under semi-controlled greenhouse conditions.

Salinity	Treatment	Root Length (cm)	Total Root Length (cm)	Root Surface Area (cm^2)	Root Average Diameter (mm)	Root Total Volume (cm^3)	Number of Root Tips (Number)
Control	T1	32.53	326.32	164.99	1.61	6.64	870.50
	T2	32.97	472.80	234.18	1.67	11.57	2085.50
	T3	39.00	372.64	198.90	1.89	11.32	2250.50
	T4	36.13	359.27	236.76	2.36	14.84	2764.00
	T5	40.30	353.60	487.02	4.36	58.07	2871.00
	T6	37.17	463.63	384.86	3.01	29.17	3507.50
	T7	34.50	729.16	487.62	2.07	26.45	2687.00
	T8	42.33	306.21	412.96	4.37	46.32	3044.50
	T9	34.03	345.28	268.04	2.67	18.45	2311.00
5 dS m^{-1}	T1	22.13	220.81	130.24	3.24	9.62	1008.00
	T2	27.47	204.11	192.61	3.97	23.73	1485.00
	T3	24.43	252.04	200.91	2.52	12.75	1450.50
	T4	29.10	351.23	186.39	1.63	8.02	851.50
	T5	31.37	169.04	190.64	3.86	20.09	2197.00
	T6	23.40	487.13	250.12	1.57	11.28	2237.00
	T7	28.57	293.97	271.86	2.86	20.13	2216.50
	T8	28.80	297.52	197.74	2.54	14.66	1324.50
	T9	31.90	306.71	167.65	1.67	7.62	1540.50
10 dS m^{-1}	T1	18.17	157.39	104.14	2.21	5.83	672.00
	T2	18.80	228.67	119.46	1.67	5.17	947.50
	T3	17.97	167.17	116.73	2.31	7.86	1359.00
	T4	22.57	210.06	122.62	2.04	6.48	735.00
	T5	23.77	206.40	102.06	1.89	4.96	1027.00
	T6	19.07	234.71	164.59	2.36	10.23	943.00
	T7	36.23	366.46	218.72	1.88	10.07	2006.00
	T8	26.20	150.88	214.19	4.73	29.07	2929.50
	T9	39.30	423.16	260.20	2.09	13.11	1901.00
	SEM$_{0.05}$	1.313	46.871	40.536	0.609	6.249	342.486
	Significance	***	***	**	*	**	**

For abbreviations, see Table 1. SEM= standard error of means; *** = $p \leq 0.001$; ** = $p \leq 0.01$; * = $p \leq 0.05$.

Table 4. Effect of saline stress and seed priming with ZnO NPs and EBL on stem diameter, shoot length, whole plant dry weight, average leaf area per plant, and RLWC of maize plants grown under semi-controlled greenhouse conditions.

Treatment	Stem Diameter (mm)	Shoot Length (cm)	Whole Plant Dry Weight (g)	Average Leaf Area (cm^2)	RLWC (%)
Saline Stress					
Control	7.62 A	92.02 A	2.35 A	117.29 A	81.63 A
5 dS m^{-1}	5.38 B	65.59 B	1.02 B	53.19 B	73.53 B
10 dS m^{-1}	4.77 C	47.76 C	0.84 C	42.80 C	65.01 C
SEM$_{0.05}$	0.048	0.625	0.009	0.654	0.188
Significance	***	***	***	***	***
Seed-Priming Treatments					
T1	4.69 e	54.96 f	0.79 g	53.01 f	70.32 d
T2	4.68 e	60.02 e	1.20 e	63.11 e	72.58 bc
T3	5.59 c	77.98 a	1.19 e	61.09 e	72.46 bc
T4	6.64 a	67.98 cd	1.54 d	82.32 ab	74.66 a
T5	6.62 a	73.97 b	1.65 c	73.07 d	75.11 a
T6	6.82 a	72.68 b	1.81 a	85.28 a	75.32 a
T7	6.82 a	68.87 c	1.72 b	81.47 b	75.26 a
T8	6.19 b	74.60 b	1.68 bc	78.25 c	73.19 b
T9	5.27 d	65.07 d	1.01 f	62.24 e	71.63 c
SEM$_{0.05}$	0.083	1.086	0.015	1.133	0.325
Significance	***	***	***	***	***

For abbreviations, see Table 1. SEM = standard error of means; *** = $p \leq 0.001$; NS = non-significant; Different letters for saline or seed-priming treatments are significantly differed at $p \leq 0.05$.

The resulting data revealed that the interaction between saline stress and seed priming with ZnO NPs and EBL is highly significant for growth parameters such as stem diameter, shoot length, whole plant dry weight, and RLWC. When compared with double control (no saline stress and T1), T8 in non-stressed soil resulted in a +48.69% increase in stem diameter, whereas a −49.67% reduction was noted in high salinity in combination with T1. Seed-priming treatments in combination with no saline stress (control) showed notable enhancement in shoot length, where T3 yielded +49.55% improvement. Oppositely, a maximal depression in stem height (−52.23%) was observed in 10 dS m^{-1} saline stress in T1 seed priming.

The maximal increase (+185.09%) in whole plant weight compared to double control was found in maize seeds primed with T8 when sown under no saline stress, whereas the greatest reduction (−53.51%) was noted in the combined effect of T1 and high saline stress. Seed-priming treatments with combined ZnO NPs and EBL effectively increased the average leaf area in maize regardless of the existence of saline stress. Therefore, T6 and T4 without saline stress (control) resulted in a +65.18% and +50.72% upsurge in average leaf area, respectively. Despite the evidence that seed priming ameliorated the deleterious effects of saline stress, the lowest (−70.67%) average leaf area was recorded in the combined treatment of T3 and high saline stress. Saline stress continued its legacy of hampering plant growth and lowered the RLWC to a significant extent with a maximum depress of −21.42% in high saline stress in combination with T1 as a seed-priming treatment. On the other hand, seed-priming treatment T5 in non-stress soil (control) only managed to improve RLWC by +6.46% compared to double control (Table 5).

Table 5. Interactive effect of saline-stress and seed-priming treatments on stem diameter, shoot length, whole plant dry weight, average leaf area per plant, and RLWC of maize plants gown under semi-controlled greenhouse conditions.

Salinity	Treatment	Stem Diameter (mm)	Shoot Length (cm)	Whole Plant Dry Weight (g)	Average Leaf Area (cm^2)	RLWC (%)
Control	T1	6.10	74.47	1.14	88.21	79.30
	T2	5.97	85.07	2.34	107.55	80.67
	T3	7.63	111.37	2.08	116.69	78.58
	T4	8.13	92.50	2.47	132.95	82.36
	T5	7.83	104.03	2.82	115.34	84.42
	T6	8.87	94.80	2.92	145.71	81.12
	T7	8.03	89.47	2.65	114.96	84.24
	T8	9.07	97.17	3.25	130.04	83.76
	T9	6.97	79.30	1.48	104.18	80.21
5 dS m^{-1}	T1	4.90	54.83	0.69	42.90	69.33
	T2	4.90	54.43	0.73	50.81	72.82
	T3	4.77	70.70	0.92	40.95	74.41
	T4	5.97	68.87	1.23	72.99	75.52
	T5	7.00	72.83	1.35	60.76	76.89
	T6	5.67	70.53	1.42	53.46	78.17
	T7	6.03	62.57	1.29	74.73	73.70
	T8	4.97	70.87	0.90	42.13	70.65
	T9	4.20	64.70	0.64	39.95	70.29
10 dS m^{-1}	T1	3.07	35.57	0.55	27.93	62.31
	T2	3.17	40.57	0.53	30.97	64.25
	T3	4.37	51.87	0.60	25.61	64.40
	T4	5.83	42.57	0.93	41.02	66.11
	T5	5.03	45.03	0.79	43.13	64.01
	T6	5.93	52.70	1.08	56.69	66.65
	T7	6.40	54.57	1.21	54.71	67.83
	T8	4.53	55.77	0.91	62.59	65.15
	T9	4.63	51.20	0.91	42.58	64.41
	$SEM_{0.05}$	0.144	1.881	0.026	1.963	0.564
	Significance	***	***	***	***	***

For abbreviations, see Table 1. SEM= standard error of means; *** = $p \leq 0.001$.

3.3. Physiological Performance

The photosynthetic rate of maize was measured once the seedlings were fully established (21 DAS) and were tracked weekly until the end of the experiment (42 DAS). The results obtained showed a significant but consistent decline in rate of photosynthesis throughout the evaluation period; −20.11%, −16.09%, −19.22%, and −19.26% after 21, 28, 35, and 42 DAS, respectively, compared to unstressed soil (control). Contrarily, seed-priming treatments both as individual ZnO NPs and EBL and their combinations in various concentrations significantly alleviated the saline-stress-mediated decline in the rate of photosynthesis throughout the study period. Most of the seed-priming treatments showed a consistent increase in rate of photosynthesis in maize between 21 and 35 DAS, except T1 and T2. However, this ascending trend was only maintained by T5, T7, and T9, even under moderate and high saline stress (Figure 1). A combined treatment of ZnO NPs at 100 mg L^{-1} and EBL at 0.4 µM (T9) resulted in the highest improvement in rate of photosynthesis, as a +120.47%, +110.28%, +125.46%, and +178.72% increase was noted after 21, 28, 35, and 42 DAS, respectively, compared to T1 (control). At the end of the experiment, 42 days after sowing, T9 showed a remarkable increase compared to its control (T1); +130.31%, +189.08%, and +247.42% under no saline stress, moderate salinity, and high salinity, respectively.

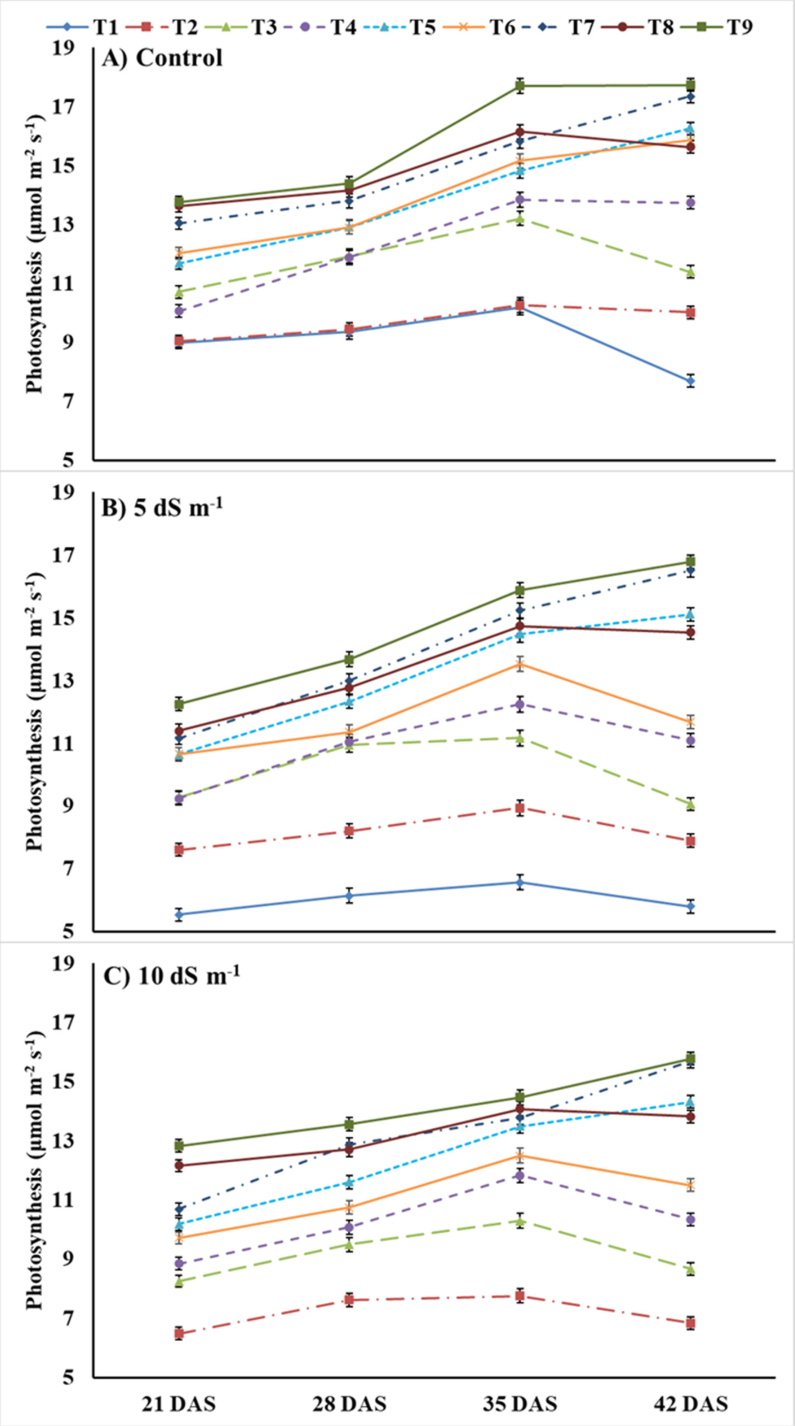

Figure 1. Effect of saline stress and seed priming with ZnO NPs and EBL on photosynthetic rate of maize plants grown under semi-controlled greenhouse conditions.

Exposure to saline stress negatively but significantly affected the transpiration rate in maize seedlings throughout the experiment. High saline stress (10 dS m^{-1}) decreased the transpiration rate by −17.71%, −18.07%, −24.50%, and −27.81% after 21, 28, 35, and 42 DAS, respectively, when compared with control. On the other hand, seed-priming treatments with both ZnO NPs and EBL, solo as well as combined, maintained significantly higher transpiration rates compared to plants from untreated seeds (T1). Remarkably, among all seed-priming treatments, T3 (EBL at 0.4 µM) compared to control (T1) repetitively maintained the highest transpiration rate, with an increase of +44.77%, +50.41%, +45.08%, and +67.39% after 21, 28, 35, and 42 DAS, respectively (Figure 2). In general, transpiration rate was decreased at 35 DAS in the control (0 dS m^{-1}) as well as moderate stress (5 dS m^{-1}), while under high saline stress (10 dS m^{-1}), the transpiration rate was reduced a little earlier (i.e., 28 DAS). However, seed-priming treatments with EBL either T2 and/or T3 resulted in a higher transpiration rate compared to all other seed-priming treatments (Figure 2), regardless of saline stress. Figure 2 showed a comprehensive comparison between individual factors and their interactions, which clearly portrayed a significantly higher performance of T3 under all saline-stress conditions, including control.

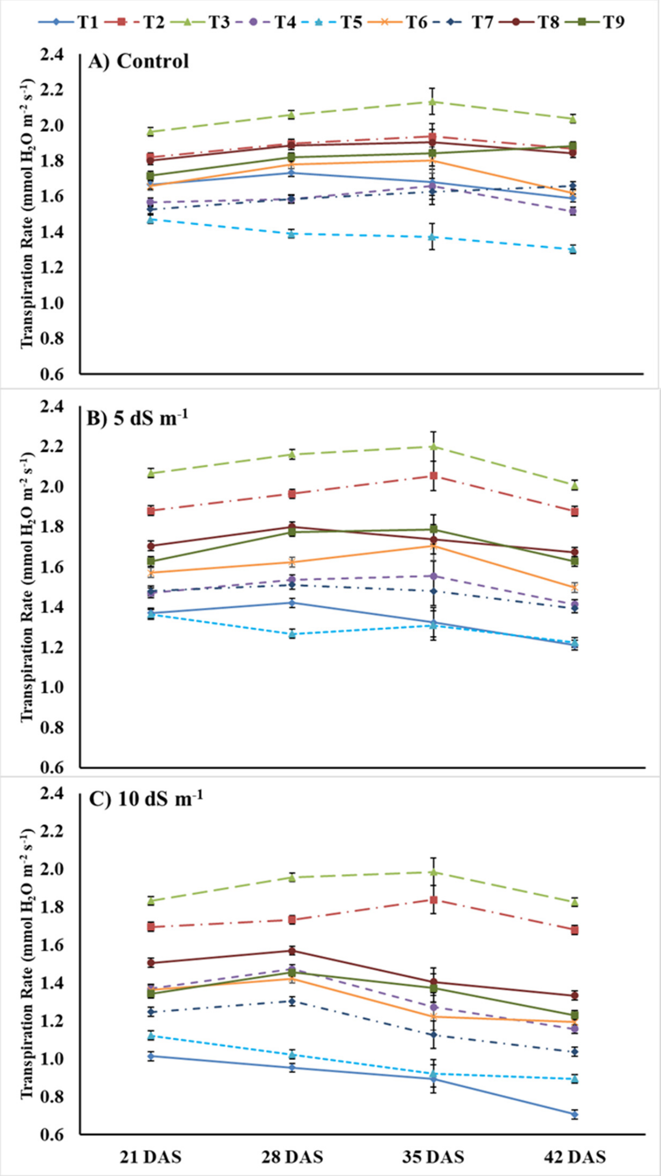

Figure 2. Effect of saline stress and seed priming with ZnO NPs and EBL on transpiration rate of maize plants grown under semi-controlled greenhouse conditions.

Near the end of experiment (42 DAS), the mean differences in seed-priming treatments showed that T3 resulted in +28.92%, +65.84%, and +158.49% higher transpiration rate compared to control (T1) under saline control and moderate and high salinity, respectively.

Intercellular CO_2 concentration (Ci) was significantly but gradually lowered with increasing saline stress. Results showed that saline stress continuously kept the Ci lower than the control, and high saline stress showed a −21.11%, −16.87%, −20.36%, and −20.63% decline compared to control when measured at 21, 28, 35, and 42 DAS. Figure 3 demonstrates a general descending trend for all treatments as the experiment proceeded; however, seed-priming treatments maintained significantly higher Ci compared to control in all saline-stress levels. Seed priming with T3 (EBL at 0.4 μM) surpassed all other treatments and resulted in a minimum saline-stress-mediated decline in Ci. When measured weekly at 21, 28, 35, and 42 days after sowing, the maximum Ci in T3 unswervingly stood higher compared to control; +35.39%, +40.59%, 42.62%, and +55.96%, respectively. The detailed comparison of treatments given in Figure 3 shows that, in general, the treatments with higher EBL concentrations were relatively more effective in managing the higher Ci under both moderate and high saline stress. At the end of experiment (42 DAS), T3 resulted in a maximum Ci +27.48% higher than the control (T1) for maize plants under no saline stress (control). However, interestingly, in moderate salinity, T8 overstepped the T3 and caused a +48.56% higher Ci compared to the control at 42 DAS. Once again, T3 ranked most effective under high saline stress (10 dS m^{-1}), with a remarkable Ci +131.03% higher than the control 42 days after sowing.

The leaf green index measured as SPAD reading showed a significant but negative effect of saline stress. Results depicted that high salinity (10 dS m^{-1}) decreased the leaf green index by −33.45% compared to control (no saline stress). However, seed priming with various individuals as well as combined treatments of ZnO NPs and EBL significantly minimized the saline-stress-mediated decline in SPAD reading in maize seedlings (Figure 4). In general, seed-priming treatments with ZnO NPs at 100 mg L^{-1} (T5, T7, and T9) showed relatively more pronounced results compared to all other treatments. The maximum SPAD reading produced by T9 was +34.88% higher than the control (T1), whereas the lowest was produced by the control itself. The interaction between saline stress and seed priming was also found highly significant in leaf green index. Compared to the control (T1, no saline stress), the maximum improvement in SPAD reading (+9.14%) was recorded in T5 under non-stressed saline treatment. Seed priming with T1 was the lowest in green leaf index under high saline stress (10 dS m^{-1}), with a maximal depression of −55.02% compared to the control (Figure 4).

Chlorophyll fluorescence was measured as maximal photochemical efficiency of PSII (Fv/Fm) in dark-adapted leaves. The results obtained showed a significant decline in Fv/Fm in response to saline stress with a maximum decrease of −10.16% under 10 dS m^{-1} compared to non-stressed plants. Seed priming with ZnO NPs and EBL as well as combinations of their various concentrations were found highly significant in mitigating the adverse effects of saline stress and showed significant improvement in Fv/Fm (Figure 5). The highest increase in Fv/Fm (+16.20%) compared to control (T1) was produced by T9 seed priming. In general, seed-priming treatments with high EBL concentrations (0.4 μM) comparatively performed more effectively in mitigating the saline-stress-mediated decrease in Fv/Fm (Figure 5). Furthermore, the interaction between saline stress and seed-priming treatments with ZnO NPs and EBL was highly significant for the maximal photochemical efficiency of PSII. The maximum improvement of +8.23% in Fv/Fm compared to double control (T1, no saline stress) was noted for T9 in saline control, whereas the maximum decrease of −26.64% in Fv/Fm resulted from the interaction of T1 and 10 dS m^{-1} saline stress.

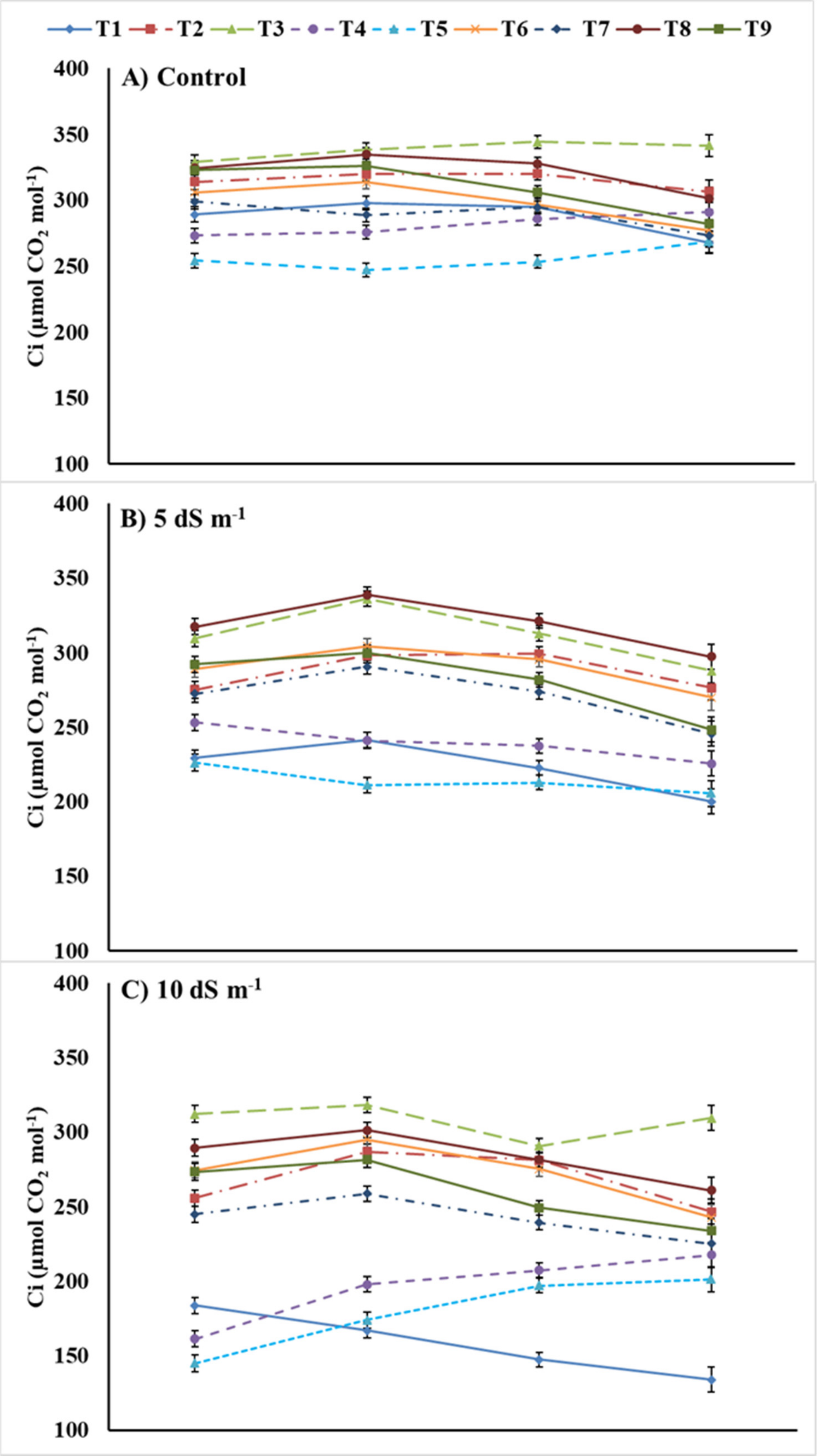

Figure 3. Effect of saline stress and seed priming with ZnO NPs and EBL on intercellular CO_2 concentration of maize plants grown under semi-controlled greenhouse conditions.

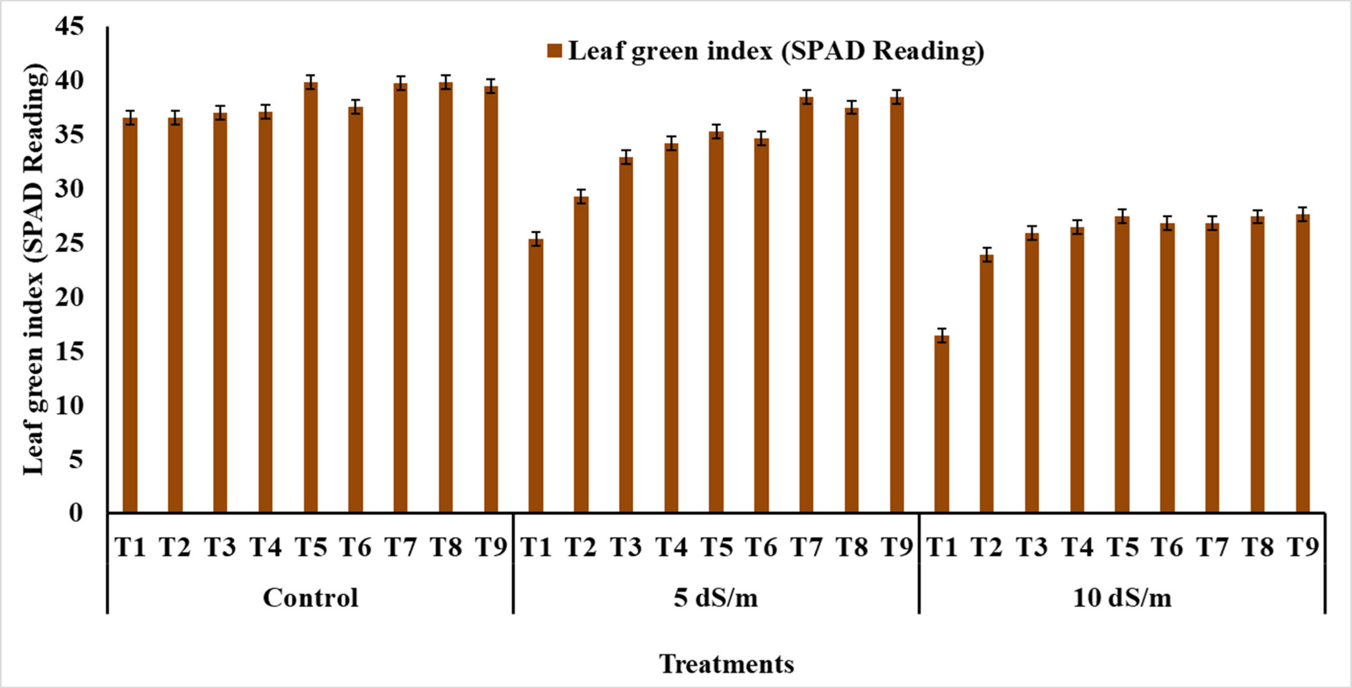

Figure 4. Effect of saline stress and seed priming with ZnO NPs and EBL on leaf green index (SPAD reading) of maize plants grown under semi-controlled greenhouse conditions.

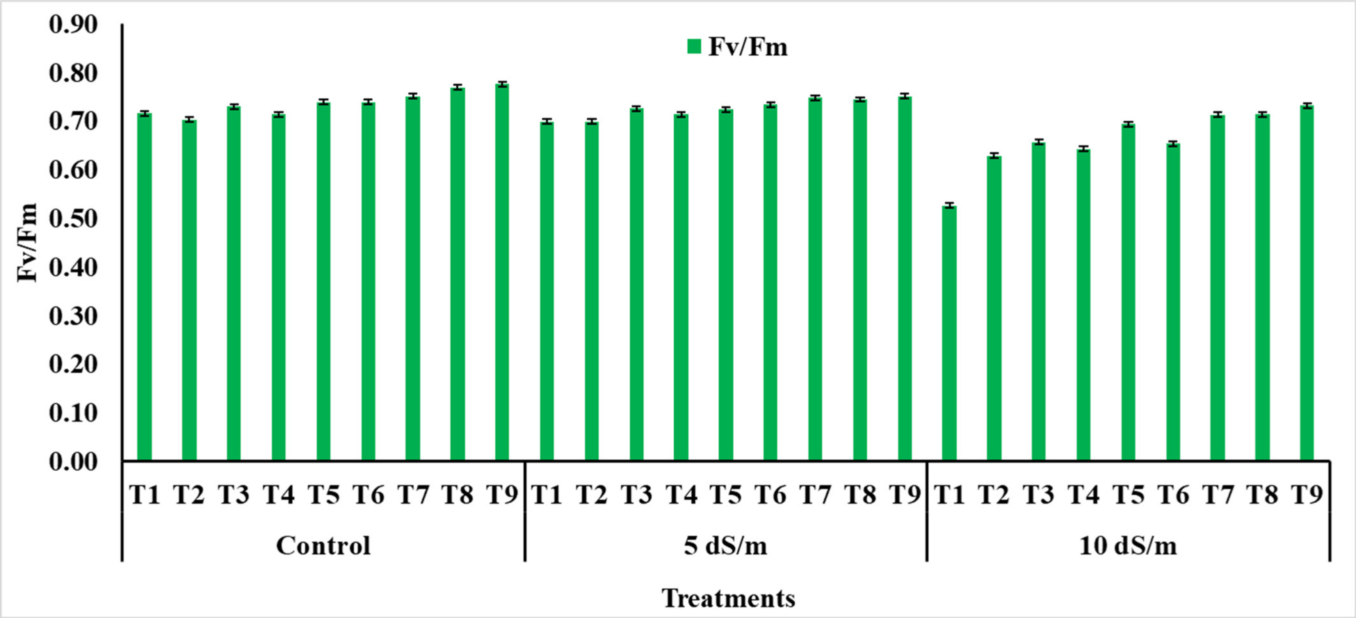

Figure 5. Effect of saline stress and seed priming with ZnO NPs and EBL on Fv/Fm of maize plants grown under semi-controlled greenhouse conditions.

The gradual intensification of saline stress lowered the Zn contents both in roots and leaves significantly. The high saline stress (10 dS m^{-1}) decreased the Zn in roots by −28.57% and in leaves by −42.11% compared to control. Seed-priming treatments played a high significant role in alleviating the saline stress; however, some treatments showed consistent dominance over others. Seed-priming treatments with ZnO NPs at 100 mg L^{-1} both individually (T5) and/or in combination with EBL (T7 and T9) showed notably higher Zn contents in leaves, with a maximum increase of +125.14% by T7 and +93.89% in leaves by T9. The results depicted that the interaction between seed priming and saline stress for Zn concentration in roots was significant, while in leaves it was non-significant. Even though data presented in Figure 6 show variations where ZnO-NPs-containing seed-priming treatments outperformed all other individual and combined treatments within each saline stress, the overall interaction stood non-significant for Zn contents in leaves. The interaction of saline stress and seed priming for Zn content in roots showed significant results; a maximum of +84.91% by T9 in saline control (no salinity), +131.63% by T7 in moderate saline stress, and +209.93% by T9 in high saline stress were observed (Figure 6).

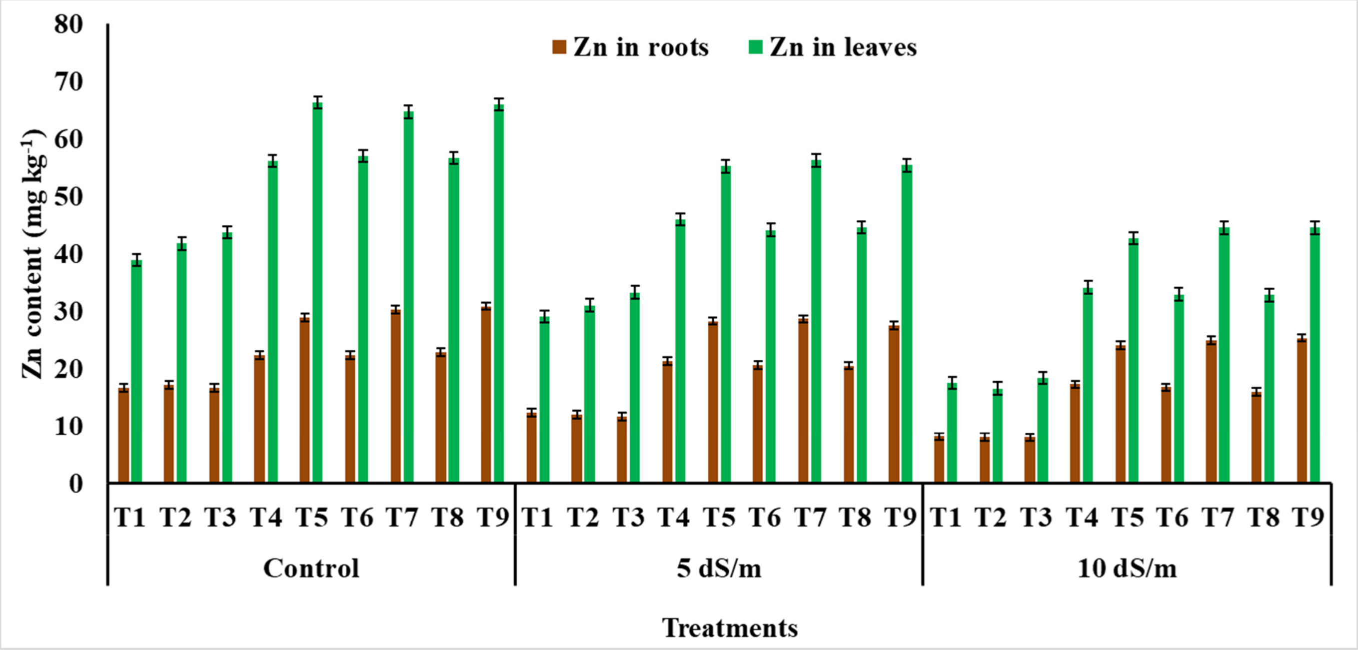

Figure 6. Effect of saline stress and seed priming with ZnO NPs and EBL on Zn contents in roots and leaves of maize plants grown under semi-controlled greenhouse conditions.

Root and leaf Na contents were increased gradually with an increase in intensity of saline stress from 0 to 10 dS m^{-1}. High salinity alleviated Na accumulation by +260.28% and +330.92% in leaves and roots, respectively. However, the seed-priming treatments with ZnO NPs and EBL in various individual and combined solutions significantly lowered the saline-stress-mediated Na accumulation in both roots and leaves. Seed treatment T7 lowered the Na$^+$ concentration in roots and leaves by −38.75% and −35.55%, respectively, compared to the control. Likewise, the individual effects of saline-stress and seed-priming treatments and their interaction were also highly significant. Data depicted that seed priming with ZnO NPs and EBL notably mitigated the saline-stress-mediated Na$^+$ ion accumulation in roots and leaves. Interestingly, Na contents in roots were not affected by seed-priming treatments under no saline stress (control), whereas in leaves, T7 sufficiently decreased the Na$^+$ concentration by −22.97% compared to its control (T1). Speed priming with T7 outperformed all other treatments by managing a lower Na content under both moderate and high saline stress. T7 resulted in a significant decline in Na content in roots by −38.83% in moderate and by −46.30% in high saline stress compared to control (T1). A similar trend was also noted in leaf Na contents; once again, treatment T7 lowered in by −19.21% and –45.51% in moderate and high saline stress, respectively (Figure 7).

As the closest chemical relatives, K and Na have a competitive analogy; the K$^+$ uptake in maize was significantly disrupted by high Na$^+$ concentration in the root zone due to saline stress. The K contents of roots were lowered by −11.94% in moderate salinity and by −26.83% in high saline stress. A similar trend in leaf K content was also noted, with a decline of −17.00% in moderate and −24.97% in high salinity compared to control (Figure 8). On the other hand, seed priming with ZnO NPs and EBL as individual and combined treatments significantly alleviated the saline-stress-mediated decrease in K content in both roots and leaves. T7 resulted in a highly significant increase of +34.01% in root and +35.31% in leaf K content compared to control (T1). Furthermore, the interaction treatments of saline stress and seed priming were also highly significant for K accumulations both in roots and leaves. The maxima of +15.07% and +30.11% increases in K content were recorded for T7 in roots and leaves, respectively, compared to double control (T1 in no saline stress), whereas the lowest K content in roots (−38.65%) resulted from the combination of T and high saline stress. Similarly, the minimal (−35.63%) K content in leaves compared to double control was again recorded for T1 under high saline stress (Figure 8). Briefly, where saline stress hampered the K absorption by maize plants, T7 seed priming significantly maintained a higher K accumulation regardless of the intensification of saline stress.

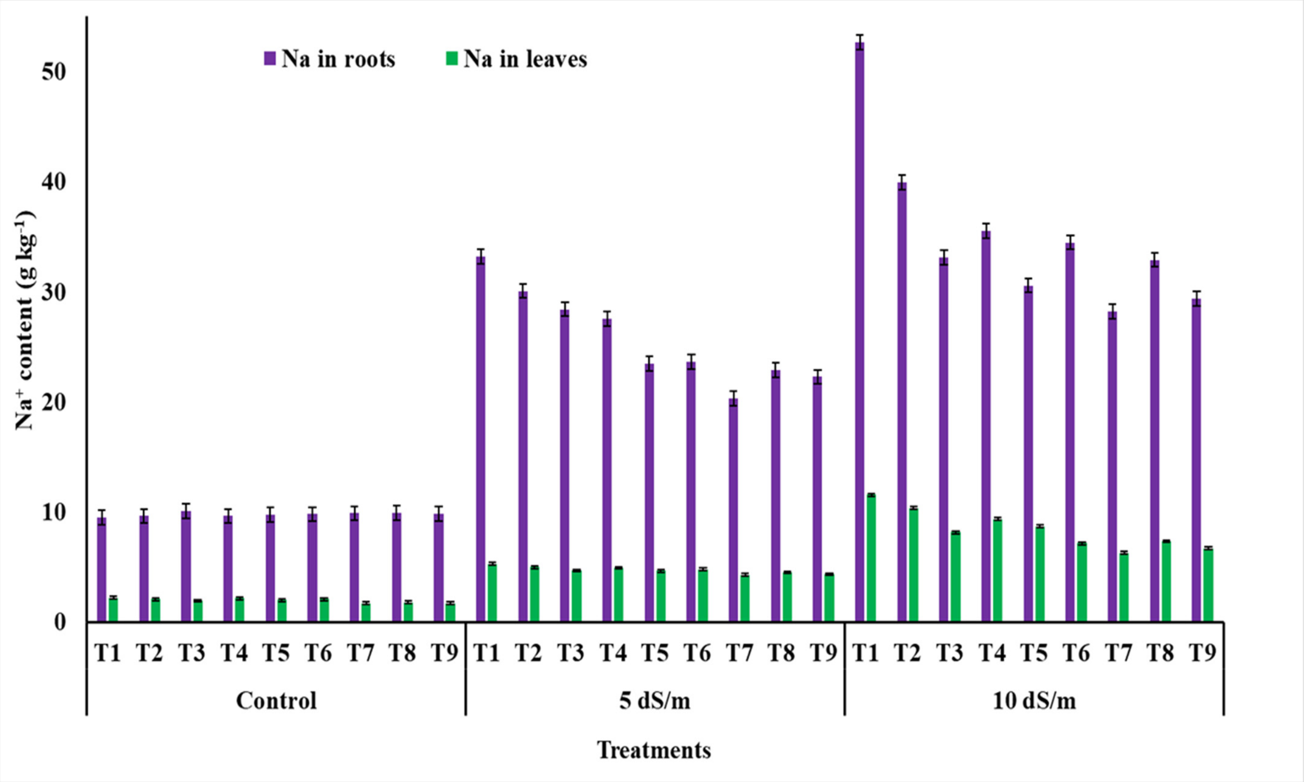

Figure 7. Effect of saline stress and seed priming with ZnO NPs and EBL on Na contents in roots and leaves of maize plants grown under semi-controlled greenhouse conditions.

The sodium to potassium ratio (Na^+/K^+) reflects the relative change in both ions as a stress indicator. Our results showed that the increase in saline stress intensity widened the Na^+/K^+ bond in roots and shoots. High saline stress elevated Na^+/K^+ by up to four-fold and five-fold in roots and shoots, respectively (Figure 9). However, seed-priming treatments significantly interrupted the saline-stress mechanism and lowered the Na^+/K^+. Maize seed priming with a combined treatment of ZnO NPs and EBL, as T7 showed, decreased Na^+/K^+ by −56.97% and −52.66% in roots and shoots, respectively, compared to control (T1). Moreover, the interaction between seed-priming treatments and saline stress was also highly significant for Na^+/K^+ in both roots and shoots. Even though the exposure to saline stress widens the Na^+/K^+ ratio, the application of ZnO NPs and EBL as priming significantly intermittently dominated the role of saline stress and maintained a lower Na^+/K^+, even under high saline stress (Figure 9). Seed priming with T7 declined the Na^+/K^+ in roots by −56.14% in moderate saline stress compared to T1 and by −65.96% in high saline stress compared to T1. Following the similar trend in leaves, T7 resulted in a −43.91% and −67.74% decline in Na^+/K^+ in moderate and high saline stress, respectively, compared to the control (T1) in both saline levels independently.

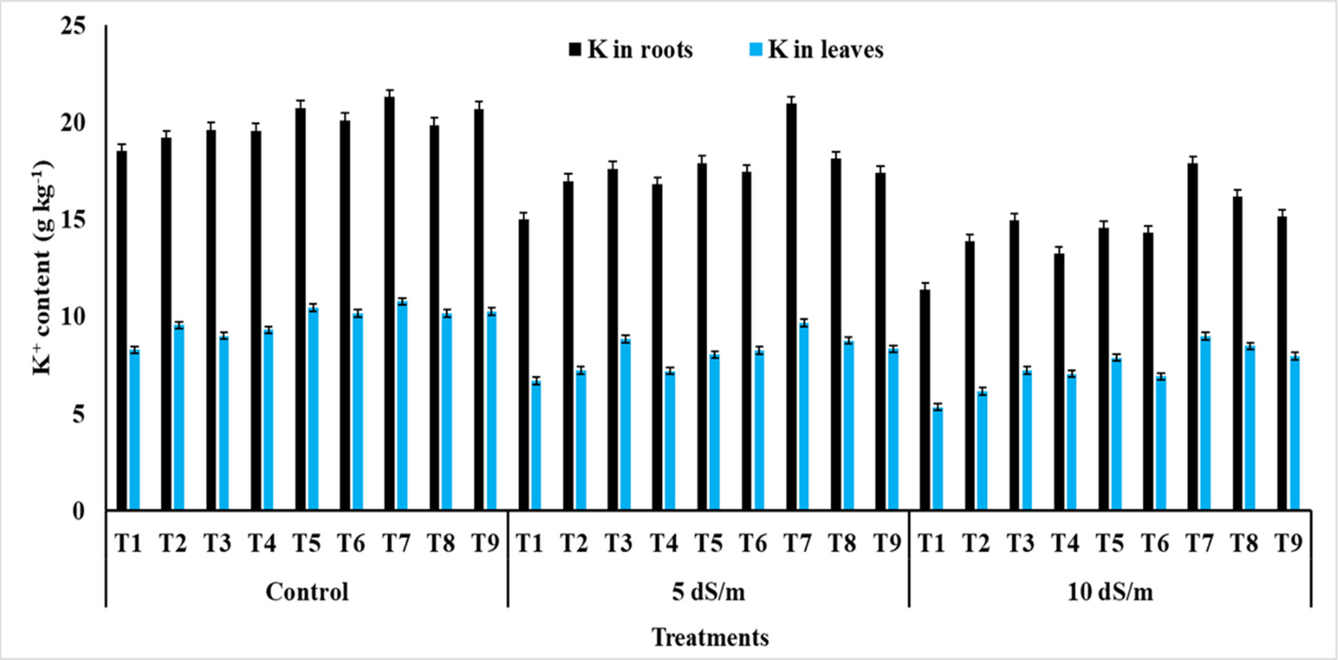

Figure 8. Effect of saline stress and seed priming with ZnO NPs and EBL on K contents in roots and leaves of maize plants grown under semi-controlled greenhouse conditions.

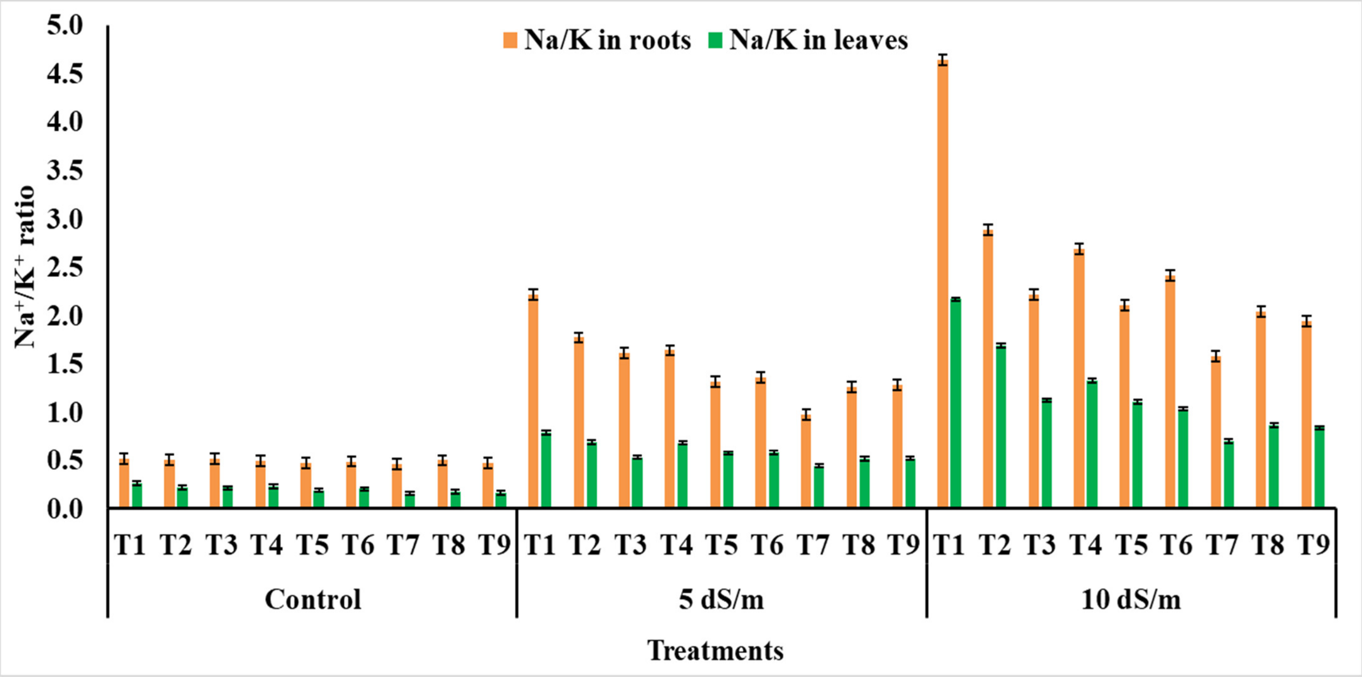

Figure 9. Effect of saline stress and seed priming with ZnO NPs and EBL on Na/K ratio content in roots and leaves of maize plants grown under semi-controlled greenhouse conditions.

4. Discussion

Along with the natural phenomena of salinization, climate change, and most importantly anthropogenic activities are making salinity a compelling environmental issue worldwide, posing serious threats to sustainable food production [29]. Taking into account the prime importance of maize in future food security and the continually expanding saline stress [30], the present study aimed to explore in depth the aid of ZnO NPs and EBL as a seed-priming technique to mitigate the deleterious effects of saline stress on maize morphology, growth, and physiology.

4.1. Root Characteristics

Our results showed that saline stress, particularly 10 dS m^{-1}, negatively affected root morphological traits such as root length, number of root tips, surface area, and root volume, which consequently resulted in lower root growth of maize in comparison to those obtained from the control treatment/unstressed plants (Tables 2 and 3). However, the application of ZnO NPs, EBL, and/or their combinations as seed priming, particularly T6–T9, mitigated the negative effects of salt stress on root morphological and growth traits (Tables 2 and 3).

Higher concentrations of salt in the root zone disrupt the osmotic balance between cell cytoplasm and soil solution, resulting in physiological drought and, hence, plants cannot take their desired amount of water. Furthermore, Na^+ in a root cell cannot be easily sequestrated into the vacuoles, which consequently produces ionic toxicity [31]. Salinity-induced ionic toxicity, due to Na^+ and Cl^- accumulation in the cell, induces oxidative stress, accelerates lipid peroxidation, and promotes the overproduction of reactive oxygen species (ROS) as a negative feedback mechanism [32]. The elevated lipoxygenase activity in saline-stressed plants results in the decomposition of fatty acids of phospholipids of the cell membrane, which results in the structural disability and functional instability of cellular membranes [33]. West et al. [34] reported a significant reduction in CYCB1;2 promoters and cyclin-dependent kinase; both of these are involved in regulating the cycle and growth of smaller meristems. Thus, Na^+ denatures cellular membranes, interfering with energy metabolism and various synthesis-associated processes, which ultimately affects cell division, cell elongation, biomass accumulation, and, hence, root morphological and growth characteristics [29,35].

On the other hand, the enhanced root traits in the current study as a result of seed priming with ZnO NPs, EBL, and their combinations can be attributed to their role as saline-stress mitigators. Zinc is an essential micronutrient which plays an important role in plant development. Recent studies have reported that Zn plays a supportive role in the biosynthesis of auxin [36]. The optimum production of auxin accelerates cell expansion and cell division; both of these processes enhance plant growth and biomass production. Furthermore, Zn binds with sulfhydryl groups and phospholipids and thus regulates cell membrane structure and its stability, especially under abiotic stresses [37,38]. The microprobe X-ray fluorescence of *Phaseolus vulgaris* seeds primed with ZnO NPs showed that most Zn was trapped in the seed coat, while a little amount was reported in cotyledon. Further analysis proved a slow release of Zn^{2+} ions in germinating seeds compared to $ZnSO_4$, and, hence, lower toxicity and higher root and seedling growth [39]. Seed priming with a 0.3% solution of Zn nanoparticles showed significant improvement in root length and root dry weight in spinach under saline-stress conditions [40]. The enhancement of root parameters in response to seed priming with EBR can be explained by its role in the cell cycle. Saline stress impaired root growth by creating osmotic stress as well as ionic toxicity, which ultimately decreased cell elongation, cell division, and osmotic balance. EBL is involved in the proliferation and elongation of meristematic tissues in roots and plays a crucial role in the optimum control of the cell cycle and propagation [41].

4.2. Growth Characteristics

Maize seedling growth and morphological characteristics such as stem diameter, shoot length, whole plant dry weight, average leaf area per plant, and RLWC were significantly hampered when exposed to saline stress, especially 10 dS m^{-1} in this study. However, seed priming with various combinations of ZnO NPs and EBL, particularly T6–T9, significantly improved the aforesaid growth parameters by ameliorating the saline-stress-mediated decline (Tables 4 and 5).

Under higher salinity, the maintenance of apoplastic and symplastic pathways to control entry of Na^+ and Cl^- in the transpiration stream plays a key role in minimizing ionic toxicity in aerial parts of the plant [29]. This mass flow of saline water through transpiration exaggerates Na^+ and Cl^- accumulation in different cell compartments in leaves up to noxious levels [42]. The existence of such ions beyond a certain limit interferes with protein synthesis, limits metabolic activity, damages cellular components, and even causes cell death [35]. Furthermore, it induces reactive oxygen species (ROS) production, which upon accumulation leads to the oxidative damage of nucleic acid, the denaturation of the cellular membrane, and finally, electrolyte leakage, which in turn affects osmotic pressure, cellular elongation, and cell division [29,43]. Indirectly, saline stress inhibits K^+ uptake and the Na^+/K^+ ratio in the intercellular spaces and cytoplasm, which disturb the stomatal regulations and eventually impair photosynthesis, progressively affecting plant growth attributes [44]. Most plants are capable of achieving desired osmotic adjustments by synthesizing compatible organic electrolytes, but it costs them 10-fold higher energy compared to the uptake of inorganic electrolytes [45]. Relying on K^+ for osmoregulation, and efficient sequestration of Na^+ in the vacuole, exerts extra energy pressure on cellular metabolism, which consequently results in lower plant growth [46].

The application of ZnO NPs enhances plant growth by providing resistance against abiotic stress [47]. ZnO NP application in plants substantially contributes to the cellular production of plant hormones such as IAA and GA3, thus promoting plant growth by enhancing cell division, cell elongation, membrane satiability, and activation of enzymes. It provides plants with a tolerance mechanism against abiotic stresses such as salinity [48]. Zn nanoparticles alleviated saline stress by regulating the biochemical activities in stressed plants and promoted the optimum biosynthesis of photosynthetic pigments, which in return accelerated plant growth such as shoot weight, shoot length, and total dry weight in spinach [40]. El-Badri et al. [49] reported a significant increase in shoot length under 150 mM NaCl stress caused by ZnO NP seed priming as compared to control in *Brassica napus* L.

Our results showed significant improvement in plant-growth-related parameters by seed priming with EBL alone (T3; for shoot length), as well as in combination with ZnO NPs (T5, T6, and T7; for RLWC), even under high saline stress (10 dS m^{-1}). This outcome can be explained by the role of EBL in regulating cell elongation, turgor pressure, and cell division [50]. Furthermore, EBL regulates cell differentiation and controls xylem generation and photomorphogenesis, which improves vegetative growth and results in a higher dry mass under stress conditions [51]. Plant osmotic balance and adjustments play an important role in plant growth; however, the accumulation of Na^+ and Cl^- under saline stress disrupts this balance and decreases the RLWC. The exogenous application of EBL replenishes RLWC by efficient osmotic adjustments at the cellular level [52]. Shahzad et al. [25] reported that the foliar application of EBL improved plant growth and biomass production in rice under 200 mM NaCl saline stress compared to the control. EBL application in wheat showed higher biomass production, plant growth, and grain yield by elevating the salt tolerance under saline-stress conditions [53]. A nearly similar finding was also reported for increased growth rate and shoot and root lengths by EBL application under ionic stress, such as Pb and Cd [38].

4.3. Physiological Performance

The maize seedlings' physiological performance was measured in this study using photosynthetic rate, transpiration rate, intercellular CO_2 concentration (Ci), leaf green index, and photochemical efficiency of PSII (Fv/Fm) as indicators. Our results depicted a significant decline in all studied physiological parameters in response to saline stress, particularly high salinity (10 dS m^{-1}). However, seed priming with ZnO NPs, EBL, and their combinations in various concentrations showed significant improvement in physiological activities by mitigating the saline-stress-mediated decline (Figures 1–5).

Yue et al. [54] reported that Na^+ accumulation within chloroplast resulted in irregular and looser thylakoid and swollen chloroplast in *R. pseudoacacia* L. when exposed to higher saline stress for 15 days. In addition to the reduction/denaturation of photosynthetic pigments, high salinity impairs photosynthetic efficiency by lowering the electron transport rate (ETR) and quantum yield of photosystem II [35]. Furthermore, reduced non-photochemical quenching (NPQ) in response to higher Na^+ accumulation induces photo-inhibition in plants by the overexcitation of energy [55]. Osmotic stress, higher ion accumulation in the photosynthesis apparatus, causes physiological drought at the cellular level, resulting in an indirect reduction in the photosynthetic rate [56]. Furthermore, under saline stress due to a lack of fully functional osmoregulation and lower K^+ uptake, the incapability of guard cells leads to stomatal closure, which directly reduces the photosynthetic activity [46].

The enhanced physiological performance of maize seedlings in response to ZnO NP application can be attributed to its role in chlorophyll production by ameliorating the negative effects of abiotic stresses [57]. Furthermore, the stabilization of photosynthetic pigments in response to the exogenous application of ZnO NPs improved the net photosynthetic rate by minimizing the oxidation damage imposed by abiotic stress, such as salinity or heavy metal ion accumulation in wheat [58]. Faizan et al. [38] reported substantial improvement in fluorescence, chloroplast structures, and photosynthesis-related parameters in response to ZnO NPs in rice. The ZnO NPs could improve the rate of photosynthesis under saline stress in multiple ways, such as minimizing the physiochemical damage of photosynthetic machinery, enhancing the biosynthesis of photosynthetic pigments, and/or neutralizing the ionic toxicity. Photosynthesis-associated ZnO NP seed priming in *Triticum aestivum* L. alleviated the saline-stress-mediated damage of leaf ultrastructures by reducing Na^+-induced changes in the electrophoretic profile of shoot proteins, which ultimately improved chlorophyll synthesis and photosynthetic efficiency [59].

Seed priming with EBL in our study showed significant improvement in the physiological attributes of maize seedlings, both as solo treatments and in combination with ZnO NPs, which further amplified the effect of ZnO NPs' positive outcome. The substantially higher physiological performance in response to EBL could be attributed to its role as "master hormone", as it manipulates the expression, biosynthesis, and receptor response of other hormones via biochemical crosstalk [51]. Moreover, the exogenous application of EBL maintained a higher photosynthesis rate by mediating the saline-stress-induced damage/changes in chloroplast structures, thylakoid membrane, and photochemical efficiency of PSII by lowering Na^+ toxicity [25]. Improved photosynthetic efficiency is linked to maximum Fv/Fm, NPQ, and efficient electron transport chains in response to EBL [54]. Shahzad et al. [25] reported significant improvement in photosynthesis-associated parameters in rice in response to EBL under saline stress, where the rate of photosynthesis, stomatal conductance, transpiration rate, Fv/Fm, and NPQ showed remarkable enhancement compared to stress plants.

4.4. Metal Ions and Ionic Toxicity

In addition to osmotic stress, salinity affects plant growth and physiological performance by imposing ionic stress by altering the ionic homeostasis. Hence, in this study, particular attention was devoted to ionic uptake and accumulation both in the leaves and roots of the maize seedlings. Results showed a significant effect of saline stress, especially 10 dS m^{-1}, on Na^+, K^+, Na^+/K^+, and Zn^{2+} (Figures 6–9). However, highly significant results were observed for Na^+, K^+, Na^+/K^+, and Zn^{2+} in response to seed priming with ZnO NPs and EBL, as well as their combined treatments, compared to control. Having a similar hydrated radius, Na^+ competes with K^+ at transport sites for entry into the symplast, resulting in a lower K^+ concentration under saline stress and a wider Na^+/K^+ ratio [60]. Under saline stress, salt is absorbed by the roots along with water, and transported to aerial parts via a transpiration stream, which ultimately accumulates Na^+ in leaves in most plants [61]. Abdelaziz et al. [62] reported the downregulation of genes related to K^+ affinity and inward-rectification channels in saline-stressed Arabidopsis roots compared to non-stress plants.

The seed priming with ZnO NPs significantly recovered mineral ions' uptake and accumulation both in roots and shoots by mediating the saline-stress-induced regulations.

At the cellular level, mineral ions, especially K^+, play an important role in maintaining the cell turgidity and are essentially required to activate enzymes associated with various metabolic activities. Saline stress creates an ionic imbalance, K^+ deficiency, and a high Na^+/K^+ ratio, which limits plant growth both at a cellular and organismic level [63]. However, ZnO NP application promotes tolerance against saline stress in plants by lowering the Na^+ uptake, facilitating K^+ accumulation, and balancing a lowing Na^+/K^+ ratio, which improves plant growth even under saline-stress conditions [64,65]. El-Badri et al. [49] reported that seed priming *Brassica napus* L. with ZnO nanoparticles reduced Na^+ toxicity in the cell cytosol by substituting Na^+ with K^+, Ca^{2+}, Zn^{2+}, and other micronutrients. Moreover, EBL application to *Robinia pseudoacacia* L. showed a significant decline in Na^+ contents and improved K^+ and Ca^{2+} in response to 200 mM NaCl saline stress. Seed priming with EBL showed a significant improvement in plant health under various ionic stresses such as Hg and Cd by regulating the uptake of mineral ions [38,66].

5. Conclusions

Saline stress, particularly 10 dS m^{-1}, enhanced Na^+ and restricted K^+ uptake by maize plants, which caused ionic imbalance, cellular damage, and metabolic impairment. Stress mediated lower chlorophyll contents, photochemical efficiency of PSII, and rate of photosynthesis, consequently resulting in a significant reduction in physiological and growth attributes of both roots and shoots compared to control. However, seed priming with various concentrations of ZnO NPs, EBL, and their combinations resulted in the significant amelioration of the saline-stress-mediated decline in physiological and growth attributes of maize. Moreover, seed priming with combined treatments of ZnO NPs and EBL outperformed the individual application of either ZnO NPs or EBL. The beneficial effect of seed priming was more pronounced at 100 mg L^{-1} ZnO NPs and 0.2 µM EBL combined treatment compared to all other seed-priming treatments, as well as control. It upsurged K^+ and discouraged Na^+ accumulation, which ultimately minimized ionic toxicity, regulated osmotic balance, and hence improved metabolic activity and growth. It also showed relatively higher chlorophyll contents (SPAD), rate of photosynthesis, rate of transpiration, photochemical activity of PSII (Fv/Fm), and lower intercellular CO_2, which indicates a higher physiological performance of maize plants compared to control, even under 10 dS m^{-1} saline stress. This study demonstrated significant improvement in ionic regulation, osmotic balance, metabolic activities, physiological performance, and growth in maize plants grown under saline stress.

Author Contributions: Conceptualization, M.F.S., E.T. and A.A.; methodology, M.F.S. and A.A.; validation, M.F.S.; formal analysis, M.F.S. and A.A.; investigation, M.F.S., E.T. and A.A.; data curation, M.F.S. and T.S.A.; writing—original draft preparation, A.A. and M.F.S.; writing—review and editing M.F.S., E.T., T.S.A. and A.A. All authors have read and agreed to the published version of the manuscript.

Funding: The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project no. (IFKSURG-2-591).

Data Availability Statement: All data are presented within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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