



# Article Zinc Seed Priming Alleviates Salinity Stress and Enhances Sorghum Growth by Regulating Antioxidant Activities, Nutrient Homeostasis, and Osmolyte Synthesis

Muhammad Umair Hassan<sup>1</sup>, Muhammad Umer Chattha<sup>2</sup>, Imran Khan<sup>2</sup>, Tahir Abbas Khan<sup>1</sup>, Mohsin Nawaz<sup>3</sup>, Haiying Tang<sup>4</sup>, Mehmood Ali Noor<sup>1</sup>, Tahani A. Y. Asseri<sup>5</sup>, Mohamed Hashem<sup>6</sup> and Huang Guoqin<sup>1,\*</sup>

- Research Center on Ecological Sciences, Jiangxi Agricultural University, Nanchang 330045, China; muhassanuaf@gmail.com (M.U.H.); tahirsargani@gmail.com (T.A.K.); mehmood2017@gmail.com (M.A.N.)
- <sup>2</sup> Department of Agronomy, University of Agriculture, Faisalabad 38000, Pakistan; drumer@uaf.edu.pk (M.U.C.); drimran@uaf.edu.pk (I.K.)
- <sup>3</sup> Institute of Environment and Ecology, School of Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013, China; mohsinnawaz2676@gmail.com
- <sup>4</sup> School of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology, Loudi 417000, China; thy39661026@sina.com
- <sup>5</sup> Department of Biology, College of Science, King Khalid University, Abha 61413, Saudi Arabia; tasseri@kku.edu.sa
- <sup>6</sup> Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71516, Egypt; mhashem@aun.edu.eg
- Correspondence: hgqmail441@sohu.com

Abstract: Salinity is a serious abiotic stress that limits crop production and food security. Micronutrient application has shown promising results in mitigating the toxic impacts of salinity. This study assessed the impacts of zinc seed priming (ZSP) on the germination, growth, physiological and biochemical functioning of sorghum cultivars. The study comprised sorghum cultivars (JS-2002 and JS-263), salinity stress (control (0 mM) and 120 mM)), and control and ZSP (4 mM). Salinity stress reduced germination and seedling growth by increasing electrolyte leakage (EL: 60.65%), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>: 109.50%), malondialdehyde (MDA; 115.30%), sodium (Na), and chloride (Cl) accumulation and decreasing chlorophyll synthesis, relative water contents (RWC), total soluble proteins (TSPs), and potassium (K) uptake and accumulation. Nonetheless, ZSP mitigated the deleterious impacts of salinity and led to faster germination and better seedling growth. Zinc seed priming improved the chlorophyll synthesis, leaf water contents, antioxidant activities (ascorbate peroxide: APX, catalase: CAT, peroxidase: POD, superoxide dismutase: SOD), TSPs, proline, K uptake and accumulation, and reduced EL, MDA, and H<sub>2</sub>O<sub>2</sub> production, as well as the accumulation of toxic ions (Na and Cl), thereby promoting better germination and growth. Thus, these findings suggested that ZSP can mitigate the toxicity of salinity by favoring nutrient homeostasis, antioxidant activities, chlorophyll synthesis, osmolyte accumulation, and maintaining leaf water status.

Keywords: antioxidants; germination; oxidative stress; salinity; zinc

# 1. Introduction

Agricultural productivity is affected by various abiotic stresses, including salinity, heat, drought, and heavy metals [1]. Soil salinity is a serious concern and a major abiotic stress that causes huge yield losses [2]. Its intensity is increasing over time due to intensive agricultural practices and climate change [3,4]. Globally, more than 1125 million hectares are affected by salinity, and this extent is increasing at a rate of 1.5 million hectares per year, highlighting the attention needed to tackle this serious problem [5]. Salinity stress hampers plant growth and development and accounts for a 50–60% yield reduction in various crops [6–8]. Salinity stress causes osmotic stress by reducing water availability, thus



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**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). leading to an increase in solute concentration in the growing medium. This subsequently generates ionic stress owing to the excessive accumulation of sodium (Na) and chloride (Cl). Salinity-induced oxidative and ionic stresses damage proteins, membranes, and DNA, resulting in poor plant growth [9].

Salinity stress also damages thylakoid membranes, disrupts chlorophyll synthesis, electron transport, and the efficiency of PS-II, and impairs the synthesis of ATP and NADPH, resulting in lower assimilate production and plant growth [10,11]. Furthermore, salinity stress causes nutritional imbalances in plants, for instance, an increase in Na<sup>+</sup> influx decreases the uptake and accumulation of K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, leading to a serious growth reduction [12–14]. Plants have developed diverse strategies to counteract salinity, for instance, they maintain osmotic potential through osmotic regulation and ionic balance by preventing the uptake of toxic ions (Cl and Na) [15]. Further, the antioxidant system also plays a crucial role in cell functioning counteracting ROS [16]. Plants also regulate ionic homeostasis, increase protein synthesis, enhance gene expression, and produce stress-responsive proteins and sugars to mitigate salinity stress [17].

Different strategies are being used globally to mitigate the toxicity of salinity. The application of micronutrients has shown promising results in mitigating the adversities of salinity [18,19]. For plants, zinc (Zn) is an essential nutrient, and it has shown significant potential in counteracting abiotic stresses [20,21]. Zinc plays a crucial role in the synthesis of auxin [22] and the production of secondary metabolites that help to counteract the lethal effects of abiotic stresses [23]. Germination is the most critical phase in a plant's life, playing a crucial role in seedling growth and stand establishment [24]. Salinity stress decreases seed germination and delays it by inducing osmotic and ionic toxicity [25]. Seed priming can assist in mitigating the toxicity of salinity via regulating defense mechanisms and neutralizing ionic toxicity [26]. Zinc seed priming increases germination rates, decreased germination time, and improved growth, development, and antioxidant activities under saline conditions [27–29]. Recent findings showed that nano-Zn seed priming mitigated the adversities of salinity and improved the seedling growth of Brassica by increasing chlorophyll and osmolyte synthesis, nutrient absorption, and antioxidant activities [30]. Zinc also maintains osmotic balance, enzyme activities, and gene expression, which help the plants to mitigate salinity toxicity [31,32].

Sorghum (*Sorghum bicolor* L.) is the fifth most imperative cereal crop, and it is well adapted globally due to its appreciable ability to tolerate abiotic stresses [33,34]. Many studies have witnessed a higher genetic variation in sorghum cultivars in response to salinity [35], and these variations can be used in identifying salt-tolerant cultivars. Therefore, salt tolerance should be monitored at critical and sensitive growth stages [36], with germination and emergence being important stages to evaluate the effects of salinity in sorghum [35].

In this context, we hypothesized that Zn seed priming can mitigate the toxic effects of salinity on sorghum cultivars by improving osmolyte accumulation, antioxidant activities, and ionic homeostasis. Therefore, the present study was performed with the following objective: to determine the impacts of Zn seed priming on germination, growth, nutrient homeostasis, osmolyte accumulation, and antioxidant activities of sorghum cultivars under salinity stress.

#### 2. Materials and Methods

#### 2.1. Experimental Details

This study was conducted to test the impact of Zn seed priming on the germination, growth, physiological, and biochemical functioning of sorghum cultivars to salinity stress. This study was conducted at Jiangxi Agricultural University Nanchang, China, in an open greenhouse. The soil for the experiment was collected from rice field and it had a silt loam texture with a pH of 5.39, available phosphorus and potassium of 26.33 and 108.13 mg kg<sup>-1</sup>, total nitrogen of 1.56 g kg<sup>-1</sup>, and organic carbon of 11.62 g kg<sup>-1</sup>. The sorghum cultivars used in the study were JS-2002 (salt-sensitive) and JS-263 (salt-tolerant) [37]. The salinity

stress level was 0 mM (control) and 120 mM [37], and control (no priming) and 4 mM Zn priming. The pots with a capacity of 5 kg were filled with 3 kg of dry soil, and 120 mM of salt stress solution was applied to the pots to impose salinity stress. NaCl solution (120 mM) was applied in 2-day intervals to replenish the evaporated water. Eight seeds of sorghum were sown in each pot and after germination five plants were kept in each pot. The weeds grown in pots were manually uprooted and coleoptile emergence above the soil was considered seedling emergence. Moreover, seedlings were harvested after 25 days to study different growth, physiological, and biochemical traits [38].

#### 2.2. Germination and Growth Parameters

The experiment was visited daily, and germination was counted until constant scores, and mean germination time (MGT) and time to 50% emergence and final emergence percentages were assessed following standard protocols [39]. The plants were carefully harvested; the roots and shoots were separated to measure their fresh and dry weights and their lengths.

## 2.3. Relative Water Contents, Oxidative Stress Markers, and Photosynthetic Pigments

For RWC, fresh leaf slices were sampled and weighed (FW) and then soaked in water for 24 h and weighed again (TW). Later, they were oven-dried (70 °C) for 24 h until constant weight was reached and weighed again (DW) to determine RWC [40]. To determine electrolyte leakage (EL), leaves were placed in water (10 mL) vials and incubated (25 °C) for 30 min to measure the first EC (EC<sub>1</sub>). The leaf sample was then placed in a water bath (90 °C) for 24 h and a second EC (EC<sub>2</sub>) was measured, and EL was assessed as follows: EL =  $EL_1/EC_2 \times 100$ .

To determine MDA and  $H_2O_2$  concentrations, 0.5 g of fresh samples was homogenized with trichloroacetic acid (TCA: 5 mL). Then, the samples were centrifuged (12,000 rpm) for 15 min at 4 °C and supernatant was mixed with 5 mL of thiobarbituric acid (0.1%; dimethylsulfoxide) and boiled for 30 min at 100 °C, cooled, and absorbance readings were measured at 532 nm and 600 nm to determine the MDA concentration [41]. To assess the  $H_2O_2$  concentration, the plant supernatant was mixed with potassium iodide (1 M) and potassium phosphate buffer (PPB: 100 µL; pH: 7.8), incubated at room temperature for 30 min, and  $H_2O_2$  was assessed by measuring absorbance at 390 nm [42]. For photosynthetic pigments, leaf sample were washed to remove contaminations. Thereafter, 1 g of the leaf sample was homogenized with 90% acetone and centrifuged to obtain the extract. Absorbance was measured at 663 nm (chlorophyll-a), 645 nm (chlorophyll-b), and 470 nm (carotenoid), respectively [43].

## 2.4. Antioxidant Enzymes

The fresh leaf samples (0.5 g) were collected and extracted using the 50 mM chilled potassium phosphate buffer (pH: 7.8). The extraction was carried out in a pre-cooled pestle and mortar and, thereafter, the homogenate was centrifuged (12,000 rpm) at 4 °C and supernatant was collected that was used to determine antioxidant activities. For the determination of APX contents, 100  $\mu$ L of enzyme extract was mixed with 100  $\mu$ L of ascorbate and H<sub>2</sub>O<sub>2</sub>, and absorbance (290 nm) was measured to determine APX activity by the procedures of Nakano and Asada [44]. The activity of CAT was determined by measuring H<sub>2</sub>O<sub>2</sub> degradation in a reaction mixture consisting of 50 mM of PPB (pH: 7.8), 10 mM of H<sub>2</sub>O<sub>2</sub>, and 2 mL of enzyme extract; thereafter, absorbance was measured at 240 nm [45]. To determine POD, the enzyme extract (100  $\mu$ L), PPB buffer (50 mM: pH: 7.8), and H<sub>2</sub>O<sub>2</sub> (300 mM) were mixed and absorbance was taken on a spectrophotometer at a wavelength of 470 nm [46]. For SOD activity, an enzyme mixture was prepared by adding 400  $\mu$ L of H<sub>2</sub>O<sub>2</sub>, 25 mL of PPB buffer, 100  $\mu$ L of Triton, 50  $\mu$ L of nitro-blue tetrazolium (NBT), and riboflavin, and absorbance was recorded at 560 nm [47].

#### 2.5. Determination of Potential Osmolytes and Ions Concentration

To assess total soluble proteins (TSPs), samples were ground using PPB and then centrifuged for 15 min at 15,000 rpm. Then, 1 mL of extract was collected and mixed with Bradford reagent (3 mL), and absorbance was taken at 595 nm. For proline, fresh leaf samples (0.5 g) were ground in 10 mL of 3% sulfosalicylic acid. The obtained extract was centrifuged (10,000 rpm) for 10 min; thereafter, it was incubated in a water bath (90 °C) for 30 min after mixing with 2 mL each of glacial acetic acid and acid ninhydrin. Thereafter, the prepared mixture was cooled down, toluene was added to the mixture, and absorbance was taken at 532 nm [48]. The roots and shoots of the sorghum plants were taken, dried at 65 °C, powdered, and digested using HCL and HNO<sub>3</sub> in a 1:2 concentration. Sodium (Na) and potassium (K) concentrations in the samples were analyzed using a flame photometer, while the chloride (Cl) concentration was determined by a chloride analyzer.

#### 2.6. Data Analysis

This study was carried out in a completely randomized design with a factorial arrangement and three replications. Data were collected from five randomly selected plants from each pot. The data collected on various traits were analyzed using a three-way ANOVA for the factors, including sorghum cultivars, seed priming, salinity stress, and their interactions. The significance among the means was compared by the least significant difference test (LSD) at  $p \le 0.05$  [49]. Moreover, the figures were prepared using SigmaPlot-10, and the PCA was drawn using R-studio.

# 3. Results

#### 3.1. Germination and Growth Traits

Salinity stress, and zinc seed priming (ZSP), showed a significant impact on germination and growth traits of sorghum cultivars (Table 1). Salinity stress delayed seed germination, and plants under salinity stress took more time for 50% emergence (20.19%) and final emergence (19.55%) compared to those under normal conditions. ZSP significantly improved germination, resulting in earlier germination compared to non-priming (Table 1). The cultivar JS-263 outperformed JS-2002 and took less time for 50% emergence and final emergence (Table 1). The maximum root (RL: 23.39 cm) and shoot length (SL: 34.73 cm) were observed in cultivar JS-263 with ZSP under control conditions, while the lowest SL (12.81 cm) and RL (17.73 cm) were observed in JS-2002 with non-primed seeds under salinity stress (Table 1). Furthermore, maximum root and shoot fresh and dry biomass was observed in JS-263 with ZSP growing under normal conditions, while the lowest was noted in JS-2002 with non-priming under salinity stress (Table 1).

Treatment	Cultivars	Priming	T50 (days)	MET (days)	FEP (%)	RL (cm)	SL (cm)	RFW (g)	RDW (g)	SFW (g)	SDW (g)
СК	JS-2002	NP	$3.18~\mathrm{de}\pm0.18$	$4.28~\mathrm{c}\pm0.21$	87	$18.02~\mathrm{c}\pm0.98$	$27.80 \text{ bc} \pm 1.15$	$6.55bc\pm0.09$	$4.44~\mathrm{de}\pm0.14$	$14.99~b\pm0.78$	$6.91~\mathrm{c}\pm0.29$
CK	JS-2002	ZSP	$2.95~\mathrm{ef}\pm0.22$	$3.84~\mathrm{e}\pm0.18$	93	$22.03~\mathrm{a}\pm1.23$	$31.43~\mathrm{ab}\pm0.92$	7.19 ab $\pm$ 0.19	$4.93b\pm0.21$	$16.42~\mathrm{a}\pm1.12$	$7.57~b\pm0.41$
CK	JS-263	NP	$3.11~\text{ef}\pm0.09$	$4.05~d\pm0.33$	93	$20.31~b\pm1.76$	$29.10bc\pm2.23$	$6.89b\pm0.065$	$4.75~bc\pm0.34$	$15.36b\pm0.87$	$7.20~\mathrm{c}\pm0.49$
CK	JS-263	ZSP	$2.89~\mathrm{f}\pm0.21$	$3.59~\mathrm{f}\pm0.22$	100	$23.39~\mathrm{a}\pm2.82$	$34.73~\mathrm{a}\pm0.79$	7.71 a $\pm$ 0.41	$5.29~\mathrm{a}\pm0.33$	17.31 a $\pm$ 0.56	$8.34~\mathrm{a}\pm0.55$
SS	JS-2002	NP	$3.89~\mathrm{a}\pm0.14$	$4.99~\mathrm{a}\pm0.17$	73	$12.81~{ m f}\pm 2.22$	$17.73~\mathrm{e}\pm0.98$	$5.09~\mathrm{f}\pm0.45$	$3.50 \text{ g} \pm 0.19$	$10.66~\mathrm{f}\pm0.55$	$5.19~\mathrm{f}\pm0.32$
SS	JS-2002	ZSP	$3.59~\mathrm{bc}\pm0.19$	$4.64b\pm0.29$	87	14.43 de $\pm$ 1.14	$21.87~\mathrm{de}\pm1.01$	$5.81~\mathrm{de}\pm0.52$	$3.91 \text{ ef} \pm 0.14$	$12.66 \text{ d} \pm 0.48$	$5.98~\mathrm{d}\pm0.29$
SS	JS-263	NP	$3.75~\mathrm{ab}\pm0.26$	$4.77b\pm0.32$	80	13.67 ef $\pm$ 1.76	$20.49~\mathrm{e}\pm0.67$	5.37 ef $\pm$ 0.13	$3.77 \text{ fg} \pm 0.29$	$11.72~\mathrm{e}\pm0.78$	$5.64~\mathrm{e}\pm0.24$
SS	JS-263	ZSP	$3.35 \text{ cd} \pm 0.17$	$4.43~\mathrm{c}\pm0.18$	87	$15.34 \text{ d} \pm 1.92$	$25.22 \text{ cd} \pm 1.17$	$6.16 \text{ cd} \pm 0.21$	$4.17  \mathrm{de} \pm 0.32$	$13.84b\pm0.82$	$6.24~\mathrm{d}\pm0.41$

Table 1. The effects of zinc seed priming on germination and growth characteristics of sorghum cultivars growing under salinity stress.

CK: control, SS: salinity stress, NP: non-priming, ZSP: zinc seed priming, T50: time to 50% emergence, FEP: final emergence percentage, RL: root length, SL: shoot length, RFW and RDW: root fresh and dry weight, SFW and SDW: shoot fresh and dry weight. The data are mean (n = 3) with ±SE, and different letters indicate significance among means ( $p \le 0.05$ ).

# 3.2. Photosynthetic Pigments

A significant difference in chlorophyll and carotenoid contents was observed under different treatments. Salinity stress reduced chlorophyll and carotenoid synthesis; nonetheless, ZSP increased chlorophyll and carotenoid synthesis (Figure 1). The maximum Chl-a (1.87 mg g<sup>-1</sup> FW) and Chl-b (1.10 mg g<sup>-1</sup> FW) were observed in JS-263 with ZSP under non-saline conditions, and the lowest Chl-a (1.24 mg g<sup>-1</sup> FW) and Chl-b (0.62 mg g<sup>-1</sup> FW) were noted in JS-2002 with non-priming under saline conditions (Figure 1). Additionally, the highest carotenoid content and relative water content (RWC) were found in JS-263 with ZSP under non-saline conditions, while the lowest carotenoid content and RWC were reported in JS-2002 grown under salinity stress (Figure 1).



**Figure 1.** The effects of zinc seed priming on photosynthetic pigments and relative water contents of sorghum cultivars growing under salinity stress. The data are mean (n = 3) with ±SE, and different letters indicate significant differences among means ( $p \le 0.05$ ). ZSP: zinc seed priming, Chl: chlorophyll, RWCs: relative water contents.

# 3.3. Oxidative Stress Markers and Potential Osmolyte

Salt stress augmented the synthesis of oxidative stress markers, including EL, MDA, and  $H_2O_2$  (Figure 2). However, ZSP reversed the toxic effects of salinity, as evidenced by a substantial decrease in all the tested oxidative markers (Figure 2). Sorghum cultivar JS-263 showed better performance and had the lowest EL, MDA, and  $H_2O_2$  production compared to JS-2002 (Figure 2). Overall, maximum EL (55%), MDA (8.24 µmol g<sup>-1</sup> FW), and  $H_2O_2$  production (12.70 µmol g<sup>-1</sup> FW) were observed in cultivar JS-2002 with non-priming seeds under saline conditions, while lowest EL (27.33%), MDA (2.93 µmol g<sup>-1</sup> FW), and  $H_2O_2$  production (4.21 µmol g<sup>-1</sup> FW) were observed in JS-263 with ZSP under normal conditions (Figure 2). Salinity stress also reduced the synthesis of total soluble proteins (TSPs), while ZSP increased TSP synthesis (Figure 3). Salinity stress increased the synthesis of proline in both sorghum cultivars, which reversed the toxic impacts of salinity (Figure 3).



**Figure 2.** The effects of zinc seed priming on oxidative stress markers of sorghum cultivars growing under salinity stress. The data are mean (n = 3) with ±SE, and different letters indicate significant differences among means ( $p \le 0.05$ ). ZSP: zinc seed priming, EL: electrolyte leakage, MDA: malondialdehyde, H<sub>2</sub>O<sub>2</sub>: hydrogen peroxide.



**Figure 3.** The effects of zinc seed priming on osmolyte synthesis of sorghum cultivars growing under salinity stress. The data are mean (n = 3) with ±SE, and different letters indicate significant differences among means ( $p \le 0.05$ ). ZSP: zinc seed priming.

## 3.4. Antioxidant Activities

Antioxidants play a crucial role in plant defense against abiotic stress conditions. In this study, antioxidant (APX, CAT, POD, and SOD) activity was evidently augmented under salinity conditions compared to non-saline conditions (Figure 4).

ZSP also caused a marked increase in antioxidant activities, and it significantly increased APX, CAT, POD, and SOD activities by 7.25%, 12.33%, 16.33%, and 15.12%, respectively (Figure 4). Furthermore, significant differences in antioxidant activities were observed among cultivars, with JS-263 exhibiting higher antioxidant activities compared to JS-2002 (Figure 4).

# 3.5. Nutrient Accumulation in Plants

Salinity stress, ZSP, and sorghum cultivars significantly impacted the concentrations of Na, Cl, and K in sorghum plant parts (Table 2). Salinity stress enhanced Na and Cl accumulation, with the highest concentrations were observed in roots compared to shoots. Conversely, ZSP reduced Na and Cl accumulation in plant parts compared to the control. Moreover, JS-263 showed a better ability for less accumulation of Na and Cl in roots than JS-2002 (Table 2). Conversely, salinity stress decreased K accumulation in roots and shoots, while ZSP considerably increased K accumulation in these plant parts (Table 2). Sorghum cultivar JS-2002 exposed to salinity showed a lower root and shoot K concentration than JS-263 (Table 2).



**Figure 4.** The effects of zinc seed priming on antioxidant activities of sorghum cultivars growing under salinity stress. The data are mean (n = 3) with ±SE, and different letters indicate significant differences among means ( $p \le 0.05$ ). ZSP: zinc seed priming, APX: ascorbate peroxidase, CAT: catalase, POD: peroxidase, SOD: superoxide dismutase.

Treatment	Cultivars	Priming	Root-Na	Shoot-Na	Root-Cl	Shoot-Cl	Root-K	Shoot-K
СК	JS-2002	NP	$5.95~\mathrm{c}\pm0.78$	$3.66 \text{ cd} \pm 0.26$	$6.80  ext{ cd} \pm 0.41$	$5.66~\mathrm{c}\pm0.44$	$9.70 \text{ d} \pm 0.82$	$7.55~\mathrm{c}\pm0.44$
СК	JS-2002	ZSP	$6.12~\mathrm{bc}\pm0.82$	$3.89\mathrm{bc}\pm0.29$	$7.02 \text{ bc} \pm 0.73$	$5.96\mathrm{bc}\pm0.19$	$10.18~{ m bc}\pm0.66$	$7.85\mathrm{bc}\pm0.62$
СК	JS-263	NP	$6.35~\mathrm{ab}\pm0.56$	$4.09~\mathrm{ab}\pm0.33$	7.24 ab $\pm$ 0.39	$6.18~\mathrm{ab}\pm0.28$	10.76 ab $\pm$ 0.78	$8.08~\mathrm{ab}\pm0.54$
СК	JS-263	ZSP	$6.56~\mathrm{a}\pm0.43$	$4.30~\mathrm{a}\pm0.41$	$7.45~\mathrm{a}\pm0.48$	$6.29~\mathrm{a}\pm0.35$	$10.95~\mathrm{a}\pm0.81$	$8.26~\mathrm{a}\pm0.39$
SS	JS-2002	NP	$4.72~\mathrm{e}\pm0.66$	$2.89~{ m g}\pm 0.25$	$5.28~\mathrm{f}\pm0.67$	$4.25~\mathrm{e}\pm0.56$	$7.22~\mathrm{f}\pm0.59$	$5.95~\mathrm{e}\pm0.33$
SS	JS-2002	ZSP	$4.93~\mathrm{e}\pm0.44$	$3.13 \text{ fg} \pm 0.22$	$5.86~\mathrm{e}\pm0.44$	$4.53~\mathrm{e}\pm0.61$	7.82 ef $\pm$ 0.67	$6.22~\mathrm{e}\pm0.49$
SS	JS-263	NP	$5.04~\mathrm{de}\pm0.29$	$3.28 \text{ ef} \pm 0.41$	$6.15~\mathrm{e}\pm0.56$	$4.98~\mathrm{d}\pm0.49$	$8.23~\mathrm{de}\pm0.49$	$6.73 \text{ d} \pm 0.39$
SS	JS-263	ZSP	$5.29~\mathrm{d}\pm0.33$	$3.51~\mathrm{de}\pm0.20$	$6.50~\mathrm{d}\pm0.88$	$5.24~\mathrm{d}\pm0.32$	$8.59~\mathrm{d}\pm0.55$	$6.95~d\pm0.34$

Table 2. The effects of zinc seed priming on root and shoot sodium and chloride and potassium concentration of sorghum cultivars growing under salinity stress.

Na: sodium, Cl: chloride, K: potassium. NP: non-priming, ZSP: zinc seed priming. The data are mean (n = 3) with  $\pm$ SE, and different letters indicate significance among means ( $p \le 0.05$ ).

## 3.6. Principal Component Analysis

The results indicated that two PCA components showed a total variance of 93.6%, and PC1 had a share of 85.7% while PC2 had a share of 7.6% (Figure 5). These results indicate that ZSP significantly mitigated the toxic effects of salinity and improved the performance of sorghum plants (Figure 5). The results also indicate a negative relationship between  $H_2O_2$ ,  $T_{50}$ , and MET and a positive association between APX, CAT, POD, SOD, MDA, EL, FEP, RL, SL, RFW, RDW, root-Na, shoot-Na, root-Cl, shoot-Cl, root-K, and shoot-K (Figure 5).



**Figure 5.** The scores (left) and loading plots (right) of PCA, indicating the impact of different treatments on the studied parameters.

#### 4. Discussion

Salinity stress caused a significant reduction in sorghum growth by disturbing water and nutrient uptake (Table 2) [50]. The excessive concentrations of Na decrease cell turgid pressure, causing cells to become rigid and preventing them from reaching their maximum size, leading to a reduction in plant growth [51,52]. Apart from this, salinity also increased the production of oxidative stress markers (MDA and EL) and ROS that damage cellular structures, thereby leading to growth losses. We found that ZSP significantly improved seedling growth. Zinc is crucial for metabolic activities and osmoregulation, protecting plants from the damaging effects of salinity by increasing antioxidant activities, thus ensuring better plant growth [53]. Zinc also plays a supportive role in auxin biosynthesis, and optimum auxin production accelerates cell division and elongation, thereby enhancing plant growth and biomass production. Furthermore, Zn regulates membrane stability by binding with phospholipids and sulfhydryl groups that protect plants from the damaging effects of abiotic stresses [54,55]. Therefore, exogenous Zn application enhances plant growth by mitigating oxidative and ionic toxicity and protecting cellular structures [56].

Salinity stress also induces ROS production, which causes the oxidation of DNA and cellular membranes, leading to a substantial increase in EL (Figure 2), which affects the

cell osmotic potential and subsequent plant growth [57]. In the present study, salinity also inhibited K uptake (Table 2), which likely disrupted stomata movements and impaired photosynthesis and plant growth [58]. ZSP substantially reduced the ROS and oxidative stress markers therefore, ensured better plant growth, which is consistent with earlier studies [59,60]. Besides this, Zn also promoted the synthesis of photosynthetic pigments, which ensured the better growth of sorghum plants under saline conditions [30]. In the present study, ZSP increased chlorophyll synthesis, which helps to capture light and drive photosynthesis. Better light capture leads to better assimilates production and subsequent plant growth; however, a larger amount of chlorophyll might cause photo-oxidative damages. Nonetheless, ZPS also increased the synthesis of carotenoids that counteract photo-oxidative damages by dissipating excessive light energy. Therefore, ZPS might maintain balanced chlorophyll and carotenoid production, which helped to maintain better photosynthetic efficiency and subsequent plant growth under saline conditions.

Salinity stress significantly decreased chlorophyll synthesis, which could be due to the enhanced activity of chlorophyll-degrading enzymes [61]. Soil salinity decreases magnesium uptake, which plays a critical role in chlorophyll synthesis [55]. Soil salinity damages the structure of chloroplasts by causing oxidative damage, thereby leading to a substantial reduction in chlorophyll synthesis [62]. Zinc seed priming improved chlorophyll and carotenoid synthesis by protecting photosynthetic apparatus owing to the decrease in salinity-induced oxidative damages [63]. ZSP increased antioxidant activities (Figure 4), which protect photosynthetic apparatus and ensure better chlorophyll synthesis [64,65]. Zinc plays a critical role in protochlorophyllide formation and repairs PS-II, protecting the chloroplast and increasing photosynthetic pigments [28]. The stabilization of the photosynthetic apparatus in response to ZSP improves net photosynthesis, ensuring better plant growth under stress conditions [66].

We found that salinity increased MDA production, causing substantial membrane damage, as indicated by higher EL (Figure 2). The excessive concentration of Na in the growth medium damages cellular membranes by increasing MDA production. Seed priming with Zn mitigated membrane damage by enhancing antioxidant activities (Figure 4), as witnessed by lower MDA and EL levels [67]. Leaf water contents (RWCs) were markedly decreased under saline conditions (Figure 1); nonetheless, ZSP reversed this reduction. Salinity causes osmotic stress, which decreases water uptake thereby, reduces leaf RWC [55]. The enhanced availability of Zn ensures better root growth (Table 1) and membrane stability, enzyme activity, and maintains stomata functioning. All these changes improved water uptake by plants and ensured better plant water status under stress conditions.

The results indicated that salinity reduced the concentration of soluble proteins while it increased proline synthesis. The present decrease in TSP synthesis under salinity stress was linked with reduced nitrogen uptake and protein degradation. Proline is an important osmolyte, and its synthesis was enhanced by both salinity and ZSP. Zinc is an important co-factor for different enzymes involved in proline biosynthesis, and it also increases the gene expression involved in proline metabolism [68]. Therefore, in the present study, ZSP might increase enzyme activity and gene expression, thereby leading to increased proline synthesis, which protected sorghum plants against salinity stress.

The activities of all antioxidants were regulated under saline stress, indicating that sorghum plants up-regulated their antioxidants to counteract salinity stress (Figure 4). These findings align with the results of Beyaz and Kır [69] and Beyaz [70], who also found that salinity stress increases antioxidant activities. The activities of all the antioxidants enhanced under saline conditions; however,  $H_2O_2$  production was also higher under saline conditions. This indicates that salinity-induced  $H_2O_2$  production overwhelms the capacity of antioxidants to scavenge  $H_2O_2$  or antioxidant enzymes become less efficient under salt stress. Nonetheless, ZPS enhanced the efficiency of antioxidants and led to a significant decrease in  $H_2O_2$  production. SOD is involved in the decomposition of  $O_2$ -radical into  $H_2O_2$  and serves as the first defense line against SS. The observed increase in antioxidants under salinity aligns with the outcomes El-Esawi et al. [71], who also documented a

substantial increase in antioxidant activities under saline conditions. Seed primed with Zn significantly boosted antioxidant levels, and it is consistent with the earlier findings of Rizwan et al. [72]. Zinc application increases gene expression associated with antioxidants, thereby maintaining a better activity of antioxidants under stress conditions [73]. Zinc deficiency can lead to a reduction in Zn-SOD activities, but its supplementation restores SOD activities because Zn is a crucial structural component of Zn-SOD [74]. Zinc also maintains higher CAT activities, which play a crucial part in  $H_2O_2$  detoxification [75]. Seed priming with Zn also increases both APX and POD activities, owing to Zn's capacity to facilitate the synthesis of these antioxidants [76].

Salinity also negatively affects plant growth by imposing ionic toxicity and disturbing ionic homeostasis. We found that salinity stress enhanced Na and Cl in plant parts (Table 2) and reduced K accumulation. Under salinity stress, plants absorb salts along with water, which are then transported to aerial plants, leading to the excessive accumulation of Na [77]. The excessive Na causes ionic imbalance which reduces K uptake [77,78]. Additionally, soil salinity also diminishes the effectiveness of Na/K antiporters in excluding excessive Na, leading to increased Na accumulation [77]. Potassium plays a crucial role in cell turgidity and enzyme activity linked to metabolic activities, and salinity stress reduces K uptake, limiting plant growth and development [79,80]. Nonetheless, Zn priming maintained a better K concentration while reducing Na accumulation. This could also be linked to the fact that Zn maintains the stability of root cell membranes, thereby maintaining higher K levels while reducing Na accumulation. Potassium, being an important nutrient, ensures membrane stability by maintaining osmotic balance and membrane potential. It also reduces the accumulation of Na by causing Na efflux, thus leading to higher K levels. Additionally, a higher level of K maintains a better stability of root cell membranes under salinity stress by protecting it from the damaging impacts of Na.

#### 5. Conclusions

Salinity stress significantly reduced the germination and growth of sorghum plants by decreasing photosynthetic pigments, and leaf water status, while increasing the uptake and accumulation of toxic ions. Zinc seed priming has shown profound impacts in terms of mitigating these issues, resulting in improved germination and growth. This improvement was linked with enhanced photosynthetic pigments, better leaf water status, osmolyte accumulation, antioxidant activities, and higher potassium accumulation. Therefore, zinc seed priming can be an effective and economical approach to improve salinity tolerance in sorghum. Nonetheless, future studies are required to test the molecular basis of these findings, which are lacking in the current research. Further, field studies are also necessary to optimize zinc seed priming rates and understand their mechanisms in inducing salt tolerance.

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