agronomy

MDPI

Article

Nitrogen and Phosphorus Counteracted the Adverse Effects of Salt on Sorghum by Improving ROS Scavenging and Osmotic Regulation

Xiaoqian Guo [1,2], Qidi Wu [1,2], Zhe Zhang [1,2], Guanglong Zhu [1,3] and Guisheng Zhou [1,2,4,*]

1 Joint International Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou 225000, China
2 China-Sudan Joint Laboratory of Crop Salinity and Drought Stress Physiology, The Ministry of Science and Technology of China, Yangzhou 225000, China
3 Jiangsu Provincial Key Lab of Crop Genetics and Physiology, Yangzhou University, Yangzhou 225000, China
4 College for Overseas Education, Yangzhou University, Yangzhou 225000, China
* Correspondence: gszhou@yzu.edu.cn

Abstract: Fertilizer management is one of the easiest and most practical ways of combating salt stress. This study was done to evaluate the alleviative effects of nitrogen and phosphorus on the growth and salt tolerance of salt-affected sorghum. A controlled study organized in a randomized block design with three replications was conducted, testing three nitrogen rates (N0: 0 kg ha^{-1}, N1: 180 kg ha^{-1}, N2: 360 N kg ha^{-1}) and phosphorus rates (P0: 0 P_2O_5 kg ha^{-1}, P1: 60 P_2O_5 kg ha^{-1}, P2: 120 P_2O_5 kg ha^{-1}). Nitrogen and phosphorus application had positive effects on morphological indexes (plant height, stem diameter), some physiological and biochemical attributes (the content of proline and soluble protein, and the activities of superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase), and aerial biomass (fresh and dry weight) of sorghum grown in saline soils. Reactive oxygen species accumulation and cell membrane damage were decreased with the application of nitrogen and phosphorus. Compared with sole fertilizer, the combined application of nitrogen and phosphorus showed better performance in alleviating salt damage on sorghum. Despite the fact that the maximum of most of the measured parameters and the minimum of reactive oxygen species accumulation and cell membrane damage were generally obtained at N1P1 and N2P2 treatment, N1P1 was recommended to be the suitable treatment considering economic benefits and environmental protection.

Keywords: fertilizer management; biomass; cell membrane damage; antioxidant defense; salt tolerance

Citation: Guo, X.; Wu, Q.; Zhang, Z.; Zhu, G.; Zhou, G. Nitrogen and Phosphorus Counteracted the Adverse Effects of Salt on Sorghum by Improving ROS Scavenging and Osmotic Regulation. *Agronomy* **2023**, *13*, 1020. https://doi.org/10.3390/agronomy13041020

Academic Editor: Mick Fuller

Received: 23 February 2023
Revised: 16 March 2023
Accepted: 25 March 2023
Published: 30 March 2023

1. Introduction

Sorghum is widely cultivated in Asia, Africa and North America [1,2]. At present, the sorghum planting area in the world is about 44.8 million hectares, and it ranks fifth among cereal crops [3]. Sorghum is used for poultry feed, sugar extraction, biofuels, and fiber extraction [4,5]. It is estimated that the annual demand for sorghum exceeds 20 million tons [6]. However, environmental changes bring about huge losses to agricultural production around the world [7]. Sorghum growth and production are usually influenced by various abiotic stresses such as salinity, temperature, drought, and heavy metal toxicity [8].

Salinity stress is the second largest abiotic factor limiting crop yield, which poses a major threat to global sustainable agricultural production and hinders accomplishing a goal of “zero hunger” [9]. Previous studies showed that salinity leads to reductions in plant growth and crop production in many cultivated areas, resulting in a loss of 65% of crop yield [10,11]. Plants under salt stress undergo morphological changes, including decreased germination, seedling growth, and yield, as well as related physiological and molecular changes which hinder plant growth and development [12]. Salinity brings about the excessive production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), which damage cell membranes, nucleic acids, and proteins [13,14]. The peroxidation of cell membrane under salt stress results in leakage of essential cellular electrolytes such as Ca^{2+}, K^+, and Ma^{2+}, affecting plant growth and metabolism [15].

The phenotypes of sorghum seedlings changed significantly under salt stress. Plant growth was restricted seriously, and the leaves of sorghum gradually turned yellow and wilted with enhancing duration of salt stress, suggesting that the photosynthetic system of sorghum plant was damaged [16]. Kaur et al. also revealed that sorghum germination percentage and speed, shoot and root length, and seedling fresh and dry weight reduced remarkably with increasing salinity levels [17]. Decrease in germination percentage, emergence rate, fresh and dry biomass, seedling length, vigour I, and vigour II, as well as distribution of photosynthates to harvestable economic parts, declined both the grain and fodder yield productivity of sorghum [18]. Moreover, marked physiological changes of sorghum induced by salt stress were also observed. Wang and Wei found significant decreases in chlorophyll content, stomatal conductance (Gs), photosynthetic rate (Pn), actual photochemical efficiency (ΦPSII), and photosynthetic electron transport rate (ETR), and remarkable enhancements in O_2^- content under salt stress [19]. Salt stress also caused a significant reduction in the ratio of plastidic lipids in sorghum leaves, especially phosphatidylglycerol (PG) and monogalactosyldiacylglycerol (MGDG), which may greatly impair photosynthetic membrane stability and chloroplast function [16]. Therefore, it is essential to implement reasonable cultivation and management measures to improve sorghum growth and salt tolerance.

The adverse effects resulting from salt stress can be mitigated by the application of fertilizer [20]. Under saline conditions, the optimum application rate of fertilizer improves nutritional quality and crop yield by improving mineral balance and reducing Na^+ toxicity [21–23]. Nitrogen and phosphorus are essential macronutrients for plant growth and development. Ahanger et al. reported that the application of nitrogen increased antioxidant accumulation and osmotic substance metabolism of wheat plants and mitigated the growth inhibition resulting from salt stress [24]. Kaci et al. pointed out that phosphorus fertilizer played a role in alleviating salt stress and improving soil quality for higher yield and symbiosis efficiency of chickpea [25].

However, so far, the effects of nitrogen and phosphorus have been largely investigated in isolation while numerous works in the literature indicate that these elements interact at some levels of integration [26]. We hypothesized that the application of nitrogen and phosphorus at suitable rates can alleviate the adverse effects of salt on sorghum, and their interaction has better performance. Therefore, the aims of this study were to investigate the effects of nitrogen and phosphorus as well as their interaction on the growth and salt tolerance of sorghum grown in saline soils.

2. Materials and Methods

2.1. Plant Materials and Experimental Site

This research was carried out using sorghum variety 'Jitian 3′ provided by Hebei Academy of Agriculture and Forestry Sciences at Dafeng Coastal Forest Farm, Yancheng City, China (Latitude: 33°20′ N; Longitude: 120°47′ E) from May to November 2021. Soil sample was collected before seeding from the depth of 0–20 cm to analyze physical and chemical properties. The soil pH, electrical conductivity (EC), and organic matter content was 8.8, 10.87 mS cm^{-1}, and 19.75 g kg^{-1}, respectively. Total nitrogen, available phosphorus, and potassium were 0.72 g kg^{-1}, 1.45 mg kg^{-1}, and 279 mg kg^{-1}, respectively.

2.2. Experimental Design

Two experimental factors, nitrogen and phosphorus, with three levels, respectively, (N0: 0 kg ha^{-1}, N1: 180 kg ha^{-1}, N2: 360 N kg ha^{-1}; P0: 0 P_2O_5 kg ha^{-1}, P1: 60 P_2O_5 kg ha^{-1}, P2: 120 P_2O_5 kg ha^{-1}) were used in the study. Nitrogen used in this study was in the form of urea. The experiment was conducted in a randomized block design, using three replicate field plots of 30 m^2 (15 m × 2 m) per treatment. Three seeds were burrowed in each hole, maintaining 15 cm × 50 cm spacing between sorghum plants. All phosphorus fertilizer was applied as the base fertilizer. Nitrogen fertilizer was applied as base fertilizer (40%), jointing fertilizer (30%), and booting fertilizer (30%), respectively. Water management and controls of weeds, diseases, and pests were carried out in conformity with local recommendations.

2.3. Observations and Measurements

Five sorghum plants were selected randomly from each plot at seedling, jointing, and maturity stage, respectively. The plant height was measured with a tape meter, and the stem diameter was measured with a vernier caliper. Subsequently, fresh weight (FW) was measured. The sample was dried in an oven at 105 °C for 30 min to inactivate enzymes and at 80 °C until a constant weight for dry weight (DW) determination.

At the same sampling date, the top-third leaves of five random sorghum plants were collected. Part of these leaves were used for the determination of relative conductivity according to the method of Zhang et al. [27], and the others were immersed in liquid nitrogen for 15 min, and then stored in −80 °C refrigerator for determination of the content of malonaldehyde (MDA), hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), proline, and soluble protein, and the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX).

MDA content (μmol g^{-1} FW) and SOD activity (U g^{-1} FW h^{-1}) were measured followed the method of Gao [28]. Proline content (μg g^{-1}) was measured according to the method of Li [29]. Soluble protein content (mg g^{-1}) was determined as described by Liu and Zhang [30]. POD activity (U g^{-1} FW min^{-1}) was determined according to Zhang and Qu [31]. CAT activity (U g^{-1} FW min^{-1}) was measured as described by Zou [32]. APX activity (μmol g^{-1} FW min^{-1}) was measured following the method of Zhao and Cang [33]. The content of H_2O_2 (μmol g^{-1}) and O_2^- (μmol g^{-1}) were measured by using assay kits (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China).

2.4. Statistical Analysis

Data obtained in this study were processed by SPSS 22.0 for analysis of variance (ANOVA). Significant differences were tested using Duncan 0.05 method for multiple comparisons. All graphs were constructed with Origin 2022b.

3. Results

Nitrogen and phosphorus significantly affected plant height and stem diameter of sorghum plants, but their interactive effects on stem diameter at jointing and maturity stages were not remarkable (Table 1). With the increase in application rate of nitrogen and phosphorus, plant height and stem diameter were enhanced, but there were no significant differences between P1 and P2 treatment on these two parameters at most stages. Compared with sole fertilizer application, combined application of nitrogen and phosphorus showed better performance on plant height and stem diameter. The highest plant height was recorded at N1P1 treatment (increased by 46.6% at seedling stage, 43.0% at jointing stage, and 33.2% at maturity stage as compared with the N0P0 treatment). For the sole fertilizer application treatments, the largest value was observed at N0P1 (enhanced by 20.9%, 27.8% and 17.6% at seedling, jointing, and maturity stage, respectively, as compared with the N0P0 treatment). During the whole growth period, stem diameter showed a relatively stable increasing trend while the largest increase in plant height was observed from jointing stage to maturity stage.

Table 1. Effects of nitrogen and phosphorus on plant height and stem diameter of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity.

Nitrogen	Phosphate	Height (cm)			Stem Diameter (mm)		
		Seedling	Jointing	Maturity	Seedling	Jointing	Maturity
N0	P0	53.5 ^{e}	80.7 ^{f}	258.5 ^{f}	5.31 ^{d}	9.6 ^{d}	16.8 ^{d}
	P1	64.6 ^{cd}	103.1 ^{cd}	304.0 ^{c}	5.98 ^{cd}	11.3 ^{bc}	17.1 ^{d}
	P2	61.2 ^{d}	95.6 ^{e}	288.3 ^{de}	5.76 ^{d}	10.7 ^{cd}	19.8 ^{bc}
N1	P0	65.0 ^{cd}	101.9 ^{cd}	299.1 ^{cd}	5.52 ^{d}	12.1 ^{ab}	19.2 ^{bc}
	P1	78.4 ^{a}	115.3 ^{a}	344.3 ^{a}	7.83 ^{a}	12.2 ^{ab}	21.1 ^{ab}
	P2	73.3 ^{b}	109.1 ^{b}	331.9 ^{b}	6.72 ^{bc}	12.4 ^{ab}	22.1 ^{a}
N2	P0	62.4 ^{cd}	97.8 ^{de}	277.1 ^{e}	5.42 ^{d}	10.4 ^{cd}	18.5 ^{cd}
	P1	66.5 ^{c}	105.3 ^{bc}	321.3 ^{b}	7.17 ^{ab}	12.4 ^{ab}	20.2 ^{bc}
	P2	71.0 ^{b}	107.2 ^{bc}	329.9 ^{b}	8.03 ^{a}	12.8 ^{a}	19.5 ^{bc}

Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

Aerial fresh and dry weight were significantly affected by nitrogen and phosphorus application (Table 2). These two parameters were enhanced by increasing nitrogen rate. Phosphorus had similar effects on fresh and dry weight but the values were higher at P1 than those at P2. Among the four sole fertilizer treatments, N0P1 ranked first over the growth period, with fresh and dry weight increased by 17.8% and 11.6% at maturity stage, respectively. However, better improvements were recorded with combined fertilizer applications and the largest increase percentages were generally observed at N2P2 and N1P1 (29.6% and 26.9% on aerial fresh weight, and 22.9% and 21.0% on aerial dry weight). Throughout the growth stages, aerial biomass increased rapidly from jointing to maturity stage while the growth rate was low before jointing stage.

Table 2. Effects of nitrogen and phosphorus on aerial fresh and dry weight of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity.

Nitrogen	Phosphate	Aerial Fresh Weight (g)			Aerial Dry Weight (g)		
		Seedling	Jointing	Maturity	Seedling	Jointing	Maturity
N0	P0	7.93 ^{e}	47.8 ^{e}	424.1 ^{d}	1.77 ^{e}	9.9 ^{e}	156.0 ^{e}
	P1	9.10 ^{d}	55.4 ^{cd}	499.7 ^{bc}	2.00 ^{cd}	11.1 ^{c}	174.1 ^{c}
	P2	9.06 ^{d}	53.4 ^{d}	472.0 ^{c}	1.88 ^{de}	10.3 ^{d}	164.1 ^{d}
N1	P0	8.89 ^{d}	54.7 ^{cd}	475.1 ^{c}	1.94 ^{d}	10.7 ^{c}	166.5 ^{d}
	P1	10.26 ^{ab}	59.3 ^{b}	538.2 ^{a}	2.23 ^{a}	12.0 ^{a}	188.7 ^{ab}
	P2	9.70 ^{c}	57.0 ^{bc}	509.5 ^{b}	2.08 ^{bc}	11.6 ^{b}	183.2 ^{b}
N2	P0	8.74 ^{d}	53.4 ^{d}	475.8 ^{c}	1.91 ^{d}	10.9 ^{c}	170.3 ^{cd}
	P1	9.98 ^{bc}	59.2 ^{b}	523.9 ^{ab}	2.13 ^{ab}	12.0 ^{a}	186.8 ^{ab}
	P2	10.54 ^{a}	62.1 ^{a}	549.5 ^{a}	2.26 ^{a}	12.3 ^{a}	191.7 ^{a}

Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

The application of nitrogen and phosphorus had remarkable effects on relative conductivity and MDA content of sorghum grown in saline soils (Table 3). Relative conductivity with no fertilizer application was higher than that treated with nitrogen or phosphorus, whereas the differences between N1 and N2 and between P1 and P2 were not significant. Similar results were observed on MDA content, but N2 had higher MDA content than N1 despite still being lower than the control. Combined fertilizer application reduced the MDA content of sorghum to a larger extent than sole fertilizer treatment. For relative conductivity, N1P0 had the lowest values among four sole fertilizer treatments, while N2P2 produced the lowest values among four combined fertilizer applications, decreasing by 25.1%, 21.3%, and 18.5% at seedling stage, jointing, and maturity stage, respectively. For MDA content, the minimum values were obtained at N1P1 treatment (8.4 μmol g^{-1} at seedling stage, 10.9 μmol g^{-1} at jointing stage, and 22.4 μmol g^{-1} at maturity stage). With the growth of sorghum, both relative conductivity and MDA content enhanced, reaching their maximum at maturity stage.

Table 3. Effects of nitrogen and phosphorus on relative conductivity and malonaldehyde (MDA) content of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity.

Nitrogen	Phosphate	Relative Conductivity (%)			MDA (μmol g^{-1} FW)		
		Seedling	Jointing	Maturity	Seedling	Jointing	Maturity
N0	P0	48.9 ^{a}	56.4 ^{a}	74.3 ^{a}	19.5 ^{a}	20.1 ^{a}	38.3 ^{a}
	P1	42.4 ^{b}	50.2 ^{cd}	68.7 ^{bc}	15.2 ^{cd}	16.9 ^{bcd}	30.9 ^{c}
	P2	43.0 ^{b}	52.0 ^{bc}	72.5 ^{a}	16.2 ^{bc}	17.3 ^{bc}	33.3 ^{b}
N1	P0	41.9 ^{bc}	49.4 ^{cd}	67.9 ^{bc}	14.6 ^{de}	16.1 ^{cd}	34.1 ^{b}
	P1	37.9 ^{de}	45.0 ^{f}	62.3 ^{de}	8.4 ^{h}	10.9 ^{f}	22.4 ^{f}
	P2	39.2 ^{d}	46.3 ^{ef}	63.9 ^{de}	11.9 ^{g}	14.3 ^{e}	27.3 ^{d}
N2	P0	43.7 ^{b}	53.0 ^{b}	70.6 ^{ab}	16.7 ^{b}	18.5 ^{ab}	34.9 ^{b}
	P1	39.9 ^{cd}	48.2 ^{de}	65.3 ^{cd}	12.3 ^{fg}	12.1 ^{f}	25.2 ^{e}
	P2	36.6 ^{e}	44.4 ^{f}	60.5 ^{e}	13.4 ^{ef}	15.3 ^{de}	29.6 ^{c}

Within each sampling date, the data followed with different letters are statistically different at the 0.05 probability level.

O_2^- and H_2O_2 contents of sorghum grown in saline soils were significantly affected by the addition of nitrogen and phosphorus (Table 4). With the increase in nitrogen and phosphorus rate, these two characteristics decreased despite the reduction at high fertilizer levels (N2 and P2) not being statistically significant compared with low fertilizer levels (N1 and P1). At seedling and jointing stages, O_2^- and H_2O_2 reduced with increasing phosphorus rate at N0 and N2 while at N1, P2 had larger O_2^- and H_2O_2 contents. At maturity stage, these two attributes decreased first and then increased with the application of phosphorus at N0 and N1, whereas they reduced with the increase of phosphorus rate at N2 and the smallest values were recorded at N2P2 (decreased O_2^- by 37.9% and H_2O_2 by 47.1%, respectively). Among three sampling stages, the greatest decrease percentages in O_2^- caused by the addition of nitrogen and phosphorus were observed at jointing stage. H_2O_2 experienced a trend of enhancement before reduction during the growth period.

Table 4. Effects of nitrogen and phosphorus on superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) content of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity.

Nitrogen	Phosphate	O_2^- (μ mol g^{-1})			H_2O_2 (μ mol g^{-1})		
		Seedling	Jointing	Maturity	Seedling	Jointing	Maturity
N0	P0	0.75 ^{a}	0.84 ^{a}	0.95 ^{a}	12.6 ^{a}	23.1 ^{a}	15.7 ^{a}
	P1	0.72 ^{ab}	0.62 ^{b}	0.80 ^{bc}	10.2 ^{bc}	19.1 ^{bc}	11.6 ^{cde}
	P2	0.65 ^{bc}	0.53 ^{bcd}	0.85 ^{ab}	9.0 ^{cd}	17.2 ^{bc}	13.2 ^{bc}
N1	P0	0.69 ^{abc}	0.52 ^{bcd}	0.86 ^{ab}	11.6 ^{ab}	20.0 ^{ab}	12.6 ^{bcd}
	P1	0.55 ^{de}	0.50 ^{bcd}	0.64 ^{de}	5.7 ^{e}	9.9 ^{d}	9.8 ^{ef}
	P2	0.61 ^{cd}	0.52 ^{bcd}	0.70 ^{cd}	7.5 ^{d}	16.5 ^{bc}	11.1 ^{de}
N2	P0	0.67 ^{abc}	0.60 ^{bc}	0.75 ^{bc}	10.9 ^{ab}	18.5 ^{bc}	13.6 ^{b}
	P1	0.60 ^{cd}	0.48 ^{cd}	0.62 ^{de}	8.4 ^{cd}	15.6 ^{c}	10.2 ^{ef}
	P2	0.48 ^{e}	0.46 ^{d}	0.59 ^{e}	5.0 ^{e}	9.1 ^{d}	8.3 ^{f}

Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

There were remarkable effects of nitrogen and phosphorus on proline and soluble protein content of sorghum grown in saline soils (Table 5). These two indexes with nitrogen and phosphorus treatments were higher than the control. Among sole fertilizer applications, N0P2 produced the highest proline content, followed by N2P0, while the largest soluble protein was recorded at N2P0. Combined fertilizer application had greater improvement on both indexes than the application of sole fertilizer. At the same nitrogen level, proline and soluble protein generally rose with the increase in phosphorus rate. N2P2, among all treatments, promoted both indexes to the highest level (increased by 24.3% and 29.8% at maturity stage, respectively). Proline content decreased slightly from seedling stage to jointing stage and then experienced a sharp reduction, while soluble protein content decreased to the minimum after the first increase over the growth period.

Table 5. Effects of nitrogen and phosphorus on proline and soluble protein content of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity.

Nitrogen	Phosphate	Proline (μg g^{-1})			Soluble Protein (mg g^{-1})		
		Seedling	Jointing	Maturity	Seedling	Jointing	Maturity
N0	P0	35.3 ^{e}	32.1 ^{e}	22.3 ^{f}	11.0 ^{e}	13.4 ^{g}	8.5 ^{e}
	P1	38.8 ^{d}	35.8 ^{d}	23.4 ^{de}	11.9 ^{d}	15.0 ^{de}	10.0 ^{d}
	P2	43.2 ^{c}	39.5 ^{bc}	24.6 ^{c}	12.4 ^{d}	14.3 ^{f}	9.6 ^{d}
N1	P0	39.2 ^{d}	37.2 ^{cd}	23.1 ^{ef}	12.1 ^{d}	14.7 ^{ef}	9.9 ^{d}
	P1	46.3 ^{b}	41.5 ^{ab}	26.2 ^{b}	14.0 ^{b}	15.8 ^{bc}	10.6 ^{bc}
	P2	47.8 ^{ab}	41.9 ^{a}	27.1 ^{ab}	14.5 ^{b}	16.4 ^{ab}	10.9 ^{ab}
N2	P0	42.4 ^{c}	38.9 ^{c}	24.2 ^{cd}	13.3 ^{c}	15.0 ^{de}	10.4 ^{c}
	P1	48.8 ^{a}	44.0 ^{a}	26.5 ^{b}	14.2 ^{b}	15.6 ^{cd}	10.7 ^{abc}
	P2	50.0 ^{a}	42.5 ^{a}	27.7 ^{a}	15.2 ^{a}	16.5 ^{a}	11.1 ^{a}

Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

Nitrogen and phosphorus significantly influenced the activities of SOD, CAT, POD, and APX of sorghum grown in saline soils (Figures 1–4). SOD and APX activities enhanced with increasing nitrogen and phosphorus rate, while high fertilizer rates (N2 and P2) produced lower POD and CAT activities than low fertilizer rates (N1 and P1). Except for CAT activity, three other antioxidant enzymes showed higher activities with phosphorus application compared to applying nitrogen only. The largest increase percentages of POD and CAT activities were recorded at N1P1 (68.9% and 92.0% at maturity stage, respectively), and for SOD and APX activities, the maximum values were generally obtained at N2P2 (increased by 41.3% and 91.9% at maturity stage, respectively). Among four antioxidant enzymes, the improvement of fertilizer application on APX activities ranked first, followed by CAT and POD activities.

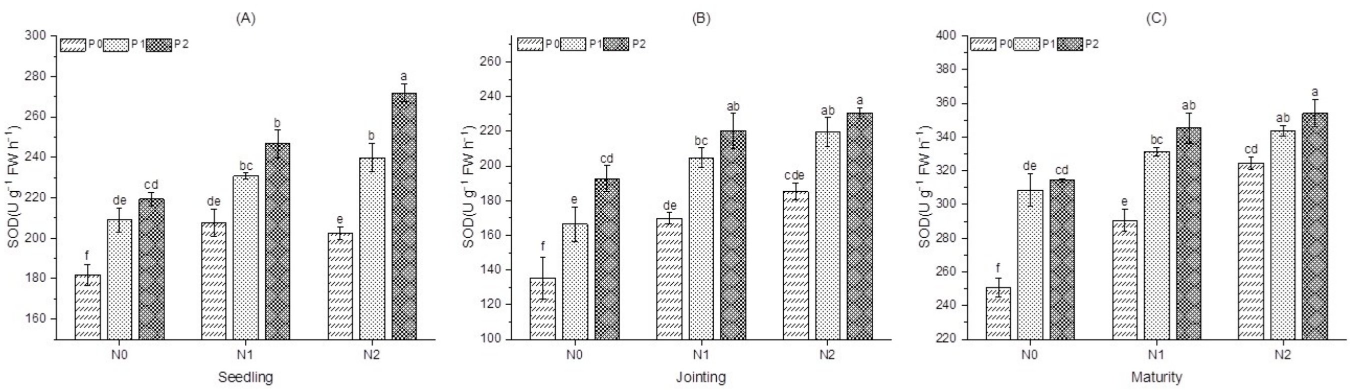

Figure 1. Effects of nitrogen and phosphorus on superoxide dismutase (SOD) activity of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity. (**A**) Seedling stage; (**B**) Jointing stage; (**C**) Maturity stage. Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

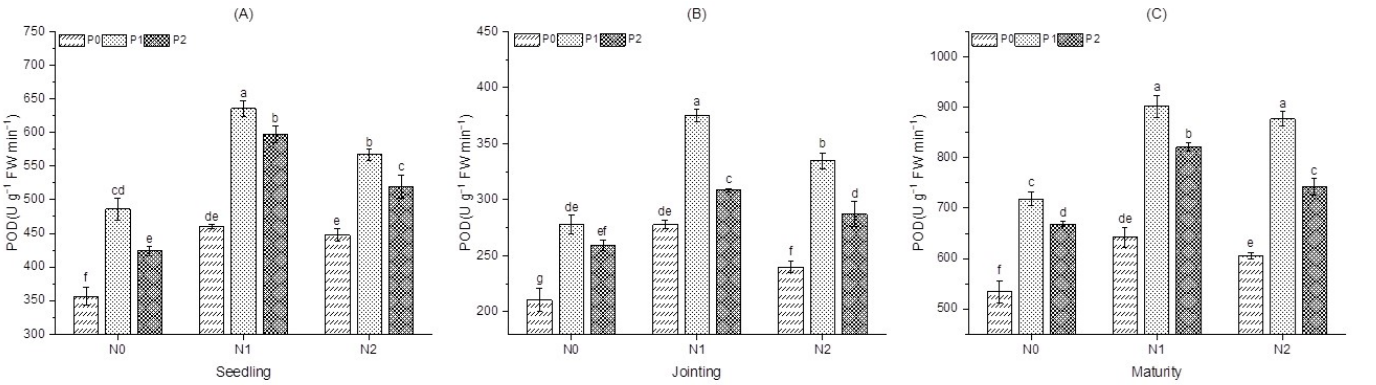

Figure 2. Effects of nitrogen and phosphorus on peroxidase (POD) activity of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity. (**A**) Seedling stage; (**B**) Jointing stage; (**C**) Maturity stage. Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

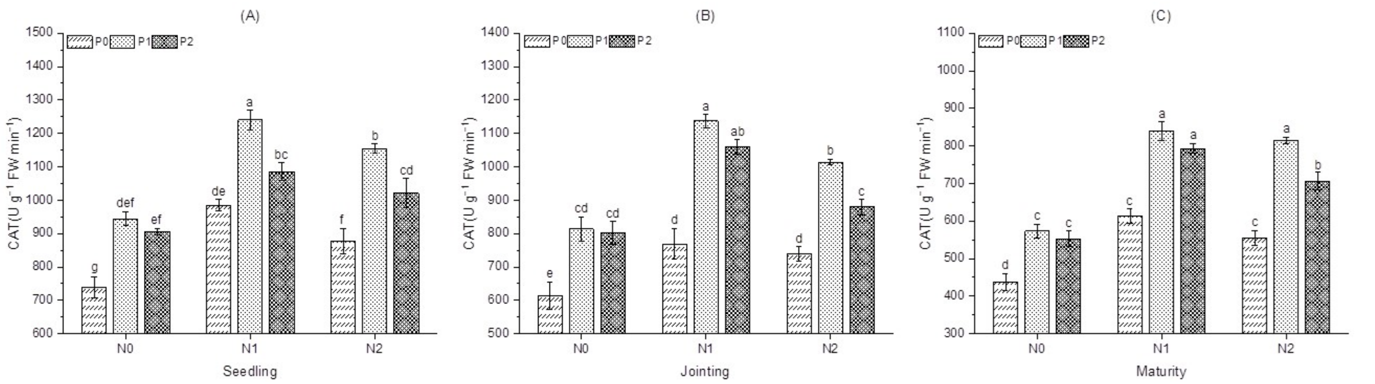

Figure 3. Effects of nitrogen and phosphorus on catalase (CAT) activity of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity. (**A**) Seedling stage; (**B**) Jointing stage; (**C**) Maturity stage. Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

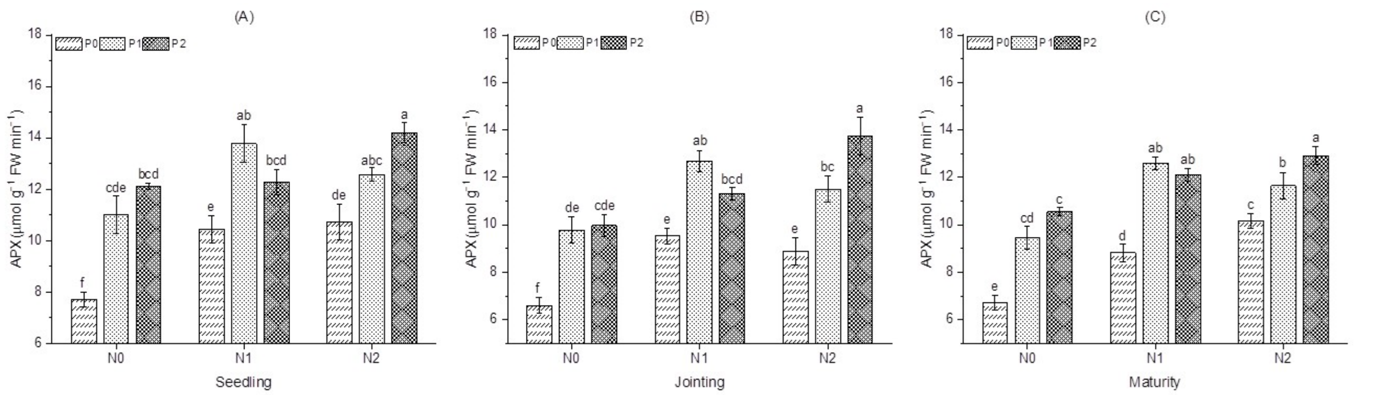

Figure 4. Effects of nitrogen and phosphorus on ascorbate peroxidase (APX) activity of sorghum plants grown in saline soils at the three growing stages of seedling, jointing, and maturity. (**A**) Seedling stage; (**B**) Jointing stage; (**C**) Maturity stage. Within each sampling date, the data followed by different letters are statistically different at the 0.05 probability level.

According to Pearson correlation analysis, significant and positive correlations were observed within aerial biomass, height, stem diameter, proline and soluble protein content, SOD, POD, CAT, and APX activities, as well as within MDA content, relative conductivity,

O_2^-, and H_2O_2 (Figure 5). Reversely, MDA, relative conductivity, O_2^-, and H_2O_2 were negatively correlated with other characteristics. Among all correlations, stem diameter had the smallest correlation coefficient with other parameters. In terms of aerial biomass of sorghum grown in saline soils and all the measured physiological and biochemical properties, the strongest positive correlation (0.86) was found between aerial biomass and soluble protein, followed by proline and APX, while the strongest negative correlation was recorded between aerial biomass and O_2^-, with a correlation coefficient reaching −0.91.

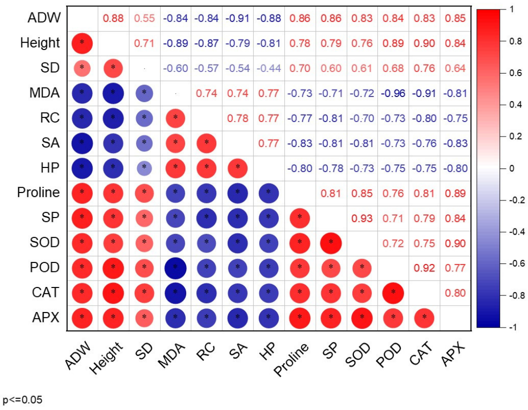

Figure 5. Correlation between aerial dry weight (ADW), height, stem diameter (SD), MDA, relative conductivity (RC), O_2^- (SA), H_2O_2 (HP), proline, soluble protein (SP), SOD, POD, CAT, and APX of sorghum at maturity stage grown in saline soils. *: significant difference at $p \leq 0.05$.

4. Discussion

Soil salinization is a main environmental salt stressor, which enhances the osmotic stress and ionic toxicity of plants, leading to the reduction of plant growth and function [34]. Previous studies showed that rational utilization of fertilizer could improve plant growth under salt stress [22,35]. In this study, we investigated the effects of nitrogen and phosphorus fertilizer as well as their combined effects on the growth of salt-affected sorghum.

One of the major consequences of salt stress is stunted growth and biomass reduction in planta, which usually decreases the yield of most plants [36]. In our study, the utilization of nitrogen and phosphorus promoted plant height, stem diameter, and aerial fresh and dry weight of sorghum grown in saline soils. Our results were in agreement with those found by Abdelkhalik et al., who reported that the application of high nitrogen levels alleviated the salt-induced damages to the growth and yield of hot pepper [37]. Kaci et al. also found that phosphorus fertilizer played a role in mitigating salt stress and improving soil quality to increase chickpea yield and symbiotic efficiency [25]. Over the whole growth period, stem diameter increased steadily, while plant height and aerial biomass both experienced a sharp increase from jointing to maturity, indicating that increased plant height is one of the main reasons for the increase in sorghum biomass. This was also confirmed by the correlation coefficients between aerial biomass and other measured indexes.

The photo-oxidation reactivity induced by salinity enhanced the biosynthesis of reactive oxide species (ROS), including H_2O_2 and O_2^-, which hinder the shoot growth and root growth, as well as biomass production of plants [38]. Excessive ROS can also destroy intracellular redox homeostasis and bring about damage to carbohydrates, lipids, proteins, and DNA, eventually resulting in oxidative stress [39–41]. Pearson correlation analysis indicated that the correlations between sorghum biomass and ROS accumulation were the strongest (Figure 5). Wang and Wei also found that salt stress remarkably increased O_2^- and H_2O_2 content of sorghum leaves and caused damage to the seedlings [19]. In this study, salt-affected sorghum plants treated with fertilizers showed lower content of H_2O_2 and O_2^-, indicating lesser oxidative damage and higher tolerance to salt stress in comparison to the control.

Excessive ROS have many negative effects on plants, of which membrane lipid peroxidation and cell membrane integrity damage are two of the most common [42,43]. MDA caused by lipid peroxidation of cell membrane is usually used as an indicator of oxidative and salt damage [44]. Relative conductivity is a vital harbinger of cell membrane permeability. Our results showed that the application of nitrogen and phosphorus decreased MDA content and relative conductivity of sorghum plants grown in saline soils. This may indicate that nitrogen and phosphorus could alleviate the damage of salt on sorghum growth by maintaining the membrane permeability and cell integrity. In the present study, both MDA content and relative conductivity are positively correlated with O_2^- and H_2O_2 content, suggesting that the reduction in cell membrane damage might be caused by down-regulating the accumulation of ROS in the tissues. These results were in accordance with previous findings reported by Kaya et al. and Tian et al. [45,46].

Enzymatic antioxidant systems, including SOD, POD, APX, CAT, and other scavengers, are key enzymes for plants to scavenge ROS [47]. SOD is considered the first enzymatic defense line against oxidative stress in plants, which exists in every cell. The main function of this enzyme is to disassociate or convert toxic O_2^- into molecular oxygen and H_2O_2 [48]. POD is mainly located in the vacuoles and apoplastic space and plays a significant role in catalyzing H_2O_2 to O_2^- and H_2O [49]. CAT and APX are other important scavengers of H_2O_2. In our research, the supplement of nitrogen and phosphorus-mediated improved antioxidant potential led to the alleviation of salt-induced oxidative damage to a remarkable level. This was also supported by the decrease in ROS content. Similar results were obtained in Brassica napus [46]. However, Kaci et al. found that phosphorus application decreased CAT activity of *Cicer arietinum* L. growing under salinity [25]. This difference between our results might be due to the varied species and growth conditions. In this study, the optimal mitigation effect was not always observed at the largest application rate of nitrogen and phosphorus. The fertilizer treatments for obtaining the best performance for different antioxidant enzymes were different.

The changes in content of proline and soluble protein are also interesting to note in the present study, especially when those are treated with combined fertilizers. Osmotic regulation is an essential mechanism allowing plants to tolerate salt stress [50]. Proline and soluble protein are known effective osmotic protectants which accumulate in some species under stress. They have the essential function that can maintain the integrity of cell membrane [51,52]. Our results indicated that the content of those two osmotic regulators increased with the application of nitrogen and phosphorus. The accumulation of soluble protein and proline in cytoplasm can rapidly reach a high level and play a more efficient role in balancing the vacuolar osmotic potential. Furthermore, since protein is not only osmotic, but is also a storage mechanism for nitrogen that is required for plant growth [53], our results indicated a possible limitation of high application rates of phosphorus without nitrogen for further biomass increase.

5. Conclusions

Nitrogen and phosphorus application significantly counteracted the adverse effects of salt on sorghum plants. On the one hand, sorghum grown in saline soils enhanced their tolerance to salt by accumulating osmoregulation substances (proline and soluble protein). On the other hand, the activities of antioxidant enzymes including SOD, CAT, POD, and APX were improved to alleviate the toxic effects produced by ROS by scavenging O_2^- and H_2O_2 (Figure 6). The combined application of nitrogen and phosphorus had better performance on alleviating salt damage on sorghum than applying nitrogen or phosphorus alone. Among all measured traits, the largest values of growth, osmotic regulation, and antioxidant defense, and the smallest damage were generally obtained in the N1P1 and N2P2 treatments.

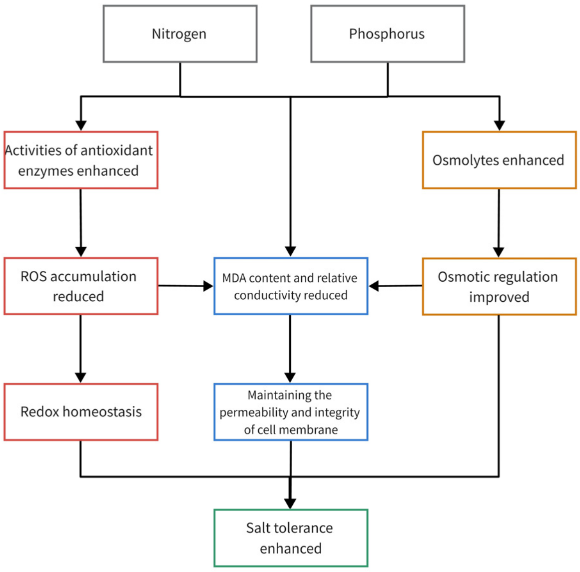

Figure 6. A schematic model depicting the alleviation induced by nitrogen and phosphorus on salt-affected sorghum.

Author Contributions: Conceptualization, X.G. and G.Z. (Guisheng Zhou); Data curation, G.Z. (Guanglong Zhu) and G.Z. (Guisheng Zhou); Formal analysis, X.G. and Q.W.; Funding acquisition, G.Z. (Guisheng Zhou); Investigation, X.G. and Q.W.; Methodology, X.G. and Q.W.; Project administration, G.Z. (Guisheng Zhou); Supervision, G.Z. (Guanglong Zhu) and G.Z. (Guisheng Zhou); Writing—original draft, X.G.; Writing—review and editing, X.G., Z.Z., G.Z. (Guanglong Zhu) and G.Z. (Guisheng Zhou). All authors have read and agreed to the published version of the manuscript.

Funding: This study is financially supported by China National Key R & D Program (2022YFE0113400) and Jiangsu Provincial Fund for Realizing Carbon Emission Peaking and Neutralization (BE2022305-1).

Data Availability Statement: Data used in this article are present in the tables and figures.

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

References

1. Velmurugan, B.; Narra, M.; Rudakiya, D.M.; Madamwar, D. Sweet sorghum: A potential resource for bioenergy production. In *Refining Biomass Residues for Sustainable Energy and Bioproducts*; Kumar, R.P., Raman, J.K., Gnansounou, E., Baskar, G., Eds.; Academic Press: London, UK, 2020; pp. 215–242.
2. Goshadrou, A.; Karimi, K.; Taherzadeh, M.J. Bioethanol production from sweet sorghum bagasse by *Mucor hiemalis*. *Ind. Crop. Prod.* **2011**, *34*, 1219–1225. [CrossRef]
3. FAO. Food and Agriculture Organization of the United Nations, Statistics. 2018. Available online: http://www.fao.org/faostat/en/#data/Q (accessed on 20 June 2018).
4. Nokerbekova, N.K.; Zavalin, A.A.; Suleimenov, Y.T.; Zhapayev, R.K. The Nutrition Influence of Nitrogen Fertilizers on the Sugar Content of Sweet Sorghum Plants in the Southeast of Kazakhstan. *Russ. Agric. Sci.* **2018**, *44*, 25–30. [CrossRef]
5. Sher, A.; Barbanti, L.; Ansar, M.; Malik, M.A. Growth response and plant water status in forage sorghum [*Sorghum bicolor* (L.) Moench] cultivars subjected to decreasing levels of soil moisture. *Aust. J. Crop Sci.* **2013**, *7*, 801–808.
6. Bai, Z.Z.; Hu, X.J.; Tian, J.P.; Chen, P.; Luo, H.B.; Huang, D. Rapid and nondestructive detection of sorghum adulteration using optimization algorithms and hyperspectral imaging. *Food Chem.* **2020**, *331*, 127290. [CrossRef]
7. Kumar, P.; Tokas, J.; Kumar, N.; Lal, M.; Singal, H.R. Climate change consequences and its impact on agriculture and food security. *Int. J. Chem. Stud.* **2018**, *6*, 124–133.
8. Kumar, P.; Tokas, J.; Singal, H.R. Amelioration of Chromium VI Toxicity in Sorghum (*Sorghum bicolor* L.) using Glycine Betaine. *Sci. Rep.* **2019**, *9*, 16020. [CrossRef]
9. Raza, A.; Tabassum, J.; Fakhar, A.Z.; Sharif, R.; Chen, H.; Zhang, C.; Ju, L.; Fotopoulos, V.; Siddique, K.H.M.; Singh, R.K.; et al. Smart reprograming of plants against salinity stress using modern biotechnological tools. *Crit. Rev. Biotechnol.* **2022**. [CrossRef]
10. Farahmand, N.; Sadeghi, V. Estimating soil salinity in the dried lake bed of Urmia Lake using optical Sentinel-2 images and nonlinear regression models. *J. Indian Soc. Remote* **2020**, *48*, 675–687. [CrossRef]
11. Ansari, F.A.; Ahmad, I. Plant growth promoting attributes and alleviation of salinity stress to wheat by biofilm forming *Brevibacterium* sp. FAB3 isolated from rhizospheric soil. *Saudi J. Biol. Sci.* 2018, *in press*. [CrossRef]

12. Khan, M.A.; Hamayun, M.; Asaf, S.; Khan, M.; Yun, B.-W.; Kang, S.-M.; Lee, I.-J. *Rhizospheric bacillus* spp. Rescues Plant Growth Under Salinity Stress via Regulating Gene Expression, Endogenous Hormones, and Antioxidant System of *Oryza sativa* L. *Front. Plant Sci.* **2021**, *12*, 665590. [CrossRef]
13. Parida, A.K.; Das, A.B. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf.* **2005**, *60*, 324–349. [CrossRef] [PubMed]
14. Jiang, D.; Lu, B.; Liu, L.; Duan, W.; Chen, L.; Li, J.; Zhang, K.; Sun, H.; Zhang, Y.; Dong, H.; et al. Exogenous melatonin improves salt stress adaptation of cotton seedlings by regulating active oxygen metabolism. *PeerJ* **2020**, *8*, e10486. [CrossRef] [PubMed]
15. Kamran, M.; Parveen, A.; Ahmar, S.; Malik, Z.; Hussain, S.; Chattha, M.S.; Chen, J.T. An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. *Int. J. Mol. Sci.* **2020**, *21*, 148–276. [CrossRef] [PubMed]
16. Ge, S.; Liu, D.; Chu, M.; Liu, X.; Wei, Y.; Che, X.; Xu, J. Dynamic and adaptive membrane lipid remodeling in leaves of sorghum under salt stress. *Crop J.* **2022**, *10*, 1557–1569. [CrossRef]
17. Kaur, M.; Gupta, N.; Kaur, N.; Sohu, R.S.; Mahal, A.K.; Choudhary, A. Preliminary screening of sorghum (*Sorghum bicolor* L.) germplasm for salinity stress tolerance at the early seedling stage. *Cereal Res. Commun.* **2022**. [CrossRef]
18. Saberi, A.R.; Siti, A.H. Growth analysis of forage sorghum (*Sorghum bicolor* L.) varieties under varying salinity and irrigation frequency. *Int. J. Biotechnol.* **2013**, *2*, 130–140.
19. Wang, Z.; Wei, Y. Physiological and transcriptomic analysis of antioxidant mechanisms in sweet sorghum seedling leaves in response to single and combined drought and salinity stress. *J. Plant Interact.* **2022**, *17*, 1006–1016. [CrossRef]
20. Chen, W.; Hou, Z.; Wu, L.; Liang, Y.; Wei, C. Effects of salinity and nitrogen on cotton growth in arid environment. *Plant Soil* **2009**, *326*, 61–73. [CrossRef]
21. Bouras, H.; Choukr-Allah, R.; Amouaouch, Y.; Bouaziz, A.; Devkota, K.P.; Mouttaqi, A.E.; Bouazzama, B.; Hirich, A. How Does Quinoa (*Chenopodium quinoa* Willd.) Respond to Phosphorus Fertilization and Irrigation Water Salinity? *Plants* **2022**, *11*, 216. [CrossRef]
22. Bouras, H.; Bouaziz, A.; Bouazzama, B.; Hirich, A.; Choukr-Allah, R. How phosphorus fertilization alleviates the effect of salinity on sugar beet (*Beta vulgaris* L.) productivity and quality. *Agronomy* **2021**, *11*, 1491. [CrossRef]
23. Bouras, H.; Bouaziz, A.; Choukr-Allah, R.; Hirich, A.; Devkota, K.P.; Bouazzama, B. Phosphorus fertilization enhances productivity of forage corn (*Zea mays* L.) irrigated with saline water. *Plants* **2021**, *10*, 2608. [CrossRef]
24. Ahanger, M.A.; Qin, C.; Begum, N.; Maodong, Q.; Dong, X.X.; El-Esawi, M.; El-Sheikh, M.A.; Alatar, A.A.; Zhang, L. Nitrogen availability prevents oxidative effects of salinity on wheat growth and photosynthesis by up-regulating the antioxidants and osmolytes metabolism, and secondary metabolite accumulation. *BMC Plant Biol.* **2019**, *19*, 479. [CrossRef] [PubMed]
25. Kaci, H.S.-A.; Chaker-Haddadj, A.; Aid, F. Interactive effects of salinity and two phosphorus fertilizers on growth and grain yield of *Cicer arietinum* L. *Acta Agric. Scand. Sect. B—Soil Plant Sci.* **2016**, *67*, 208–216.
26. Krouk, G.; Kiba, T. Nitrogen and Phosphorus interactions in plants: From agronomic to physiological and molecular insights. *Curr. Opin. Plant Biol.* **2020**, *57*, 104–109. [CrossRef] [PubMed]
27. Zhang, Z.L.; Qu, W.J.; Li, X.F. *Plant Physiology Experiment Instruction*; Higher Education Press: Beijing, China, 2009. (In Chinese)
28. Gao, J.F. *Plant Physiology Experiment Instruction*; Higher Education Press: Beijing, China, 2006; pp. 210–211. (In Chinese)
29. Li, H.S. *Principles and Techniques of Plant Physiological and Biochemical Experiments*; Higher Education Press: Beijing, China, 2001; pp. 258–260. (In Chinese)
30. Liu, Z.Q.; Zhang, S.C. *Physiology of Plant Resistance*; Agriculture Press: Beijing, China, 1994; pp. 371–372. (In Chinese)
31. Zhang, Z.L.; Qu, W.J. *Plant Physiology Experiment Instruction*; Higher Education Press: Beijing, China, 2005; pp. 123–124. (In Chinese)
32. Zou, Q. *Plant Physiology Experiment Instruction*; China Agriculture Press: Beijing, China, 2000; pp. 167–169. (In Chinese)
33. Zhao, S.J.; Cang, J. *Plant Physiology Experiment Instruction*; Agriculture Press: Beijing, China, 2015. (In Chinese)
34. Li, J.; Fan, X.L.; Zhu, Y.L.; Rao, G.S.; Chen, R.S.; Duan, T.T. Effects of irrigation and nitrogen fertilization on mitigating salt-induced Na^+ toxicity and sustaining sea rice growth. *Open Life Sci.* **2022**, *17*, 1165–1173.
35. Du, K.X.; Zhang, Y.Q.; Qin, S.S.; Wang, L.J.; Zhang, B.; Wang, S.J. Effects of nitrogen fertilization on physiological response of maize to soil salinity. *Agriculture* **2022**, *12*, 877. [CrossRef]
36. Khalifa, G.S.; Abdelrassoul, M.; Hegazi, A.M.; Elsherif, M.H. Attenuation of negative effects of saline stress in two lettuce cultivars by salicylic acid and glycine betaine. *Gesunde Pflanz.* **2016**, *68*, 177–189. [CrossRef]
37. Abdelkhalik, A.; El-Mageed, T.A.A.; Mohamed, I.A.A.; Semida, W.M.; Al-Elwany, O.A.A.I.; Ibrahim, I.M.; Hemida, K.A.; El-Saadony, M.T.; AbuQamar, S.F.; El-Tarabily, K.A.; et al. Soil application of effective microorganisms and nitrogen alleviates salt stress in hot pepper (*Capsicum annum* L.) plants. *Front. Plant Sci.* **2023**, *13*, 1079260. [CrossRef]
38. Hussain, S.; Ahmed, S.; Akram, W.; Li, G.; Yasin, N.A. Selenium seed priming enhanced the growth of salt-stressed *Brassica rapa* L. through improving plant nutrition and the antioxidant system. *Front. Plant Sci.* **2023**, *13*, 1050359. [CrossRef]
39. Mittler, R. ROS are good. *Trends Plant Sci.* **2017**, *22*, 11–19. [CrossRef]
40. Soares, C.; Carvalho, M.E.A.; Azevedo, R.A.; Fidalgo, F. Plants facing oxidative challenges—A little help from the antioxidant networks. *Environ. Exp. Bot.* **2019**, *161*, 4–25. [CrossRef]

41. Zhang, H.H.; Li, X.; Guang, Y.P.; Li, M.B.; Wang, Y.; An, M.J.; Zhang, Y.; Liu, G.; Xu, N.; Sun, G. Physiological and proteomic responses of reactive oxygen species metabolism and antioxidant machinery in mulberry (*Morus alba* L.) seedling leaves to NaCl and $NaHCO_3$ stress. *Ecotoxicol. Environ. Saf.* **2020**, *193*, 110259.
42. Sun, S.; Wen, D.; Yang, W.; Meng, Q.; Shi, Q.; Gong, B. Overexpression of caffeic acid O-methyltransferase 1 (COMT1) increases melatonin level and salt stress tolerance in tomato plant. *J. Plant Growth Regul.* **2020**, *39*, 1221–1235. [CrossRef]
43. Al Hinai, M.S.; Ullah, A.; Al-Rajhi, R.S.; Farooq, M. Proline accumulation, ion homeostasis and antioxidant defence system alleviate salt stress and protect carbon assimilation in bread wheat genotypes of Omani origin. *Environ. Exp. Bot.* **2022**, *193*, 104687. [CrossRef]
44. Mandhania, S.; Madan, S.; Sawhney, V. Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol. Plant.* **2006**, *50*, 227–231. [CrossRef]
45. Kaya, C.; Higgs, D.; Sakar, E. Response of two leafy vegetables grown at high salinity to supplementary potassium and phosphorus during different growth stages. *J. Plant Nutr.* **2002**, *25*, 2663–2676. [CrossRef]
46. Tian, T.; Wang, J.; Wang, H.; Cui, J.; Shi, X.; Song, J.; Li, W.; Zhong, M.; Qiu, Y.; Xu, T. Nitrogen application alleviates salt stress by enhancing osmotic balance, ROS scavenging, and photosynthesis of rapeseed seedlings (*Brassica napus*). *Plant Signal. Behav.* **2022**, *17*, e2081419. [CrossRef] [PubMed]
47. Farhangi-Abriz, S.; Torabian, S. Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. *Ecotoxicol. Environ. Saf.* **2017**, *137*, 64–70. [CrossRef] [PubMed]
48. García-Caparrós, P.; De Filippis, L.; Gul, A.; Hasanuzzaman, M.; Ozturk, M.; Altay, V.; Lao, M.T. Oxidative Stress and Antioxidant Metabolism under Adverse Environmental Conditions: A Review. *Bot. Rev.* **2020**, *87*, 421–466. [CrossRef]
49. Gratão, P.L.; Polle, A.; Lea, P.J.; Azevedo, R.A. Making the life of heavy metal-stressed plants a little easier. *Funct. Plant Biol.* **2005**, *32*, 481–494. [CrossRef]
50. Kiani, S.P.; Grieu, P.; Maury, P.; Hewezi, T.; Gentzbittel, L.; Sarrafi, A. Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L.). *Theor. Appl. Genet.* **2006**, *114*, 193–207. [CrossRef] [PubMed]
51. Sakamoto, A.; Murata, N. Genetic engineering of glycinebetaine synthesis in plants: Current status and implications for enhancement of stress tolerance. *J. Exp. Bot.* **2000**, *51*, 81–88. [CrossRef] [PubMed]
52. Sisay, T.A.; Nurbekova, Z.; Oshanova, D.; Dubey, A.K.; Khatri, K.; Mudgal, V.; Mudgal, A.; Neori, A.; Shpigel, M.; Srivastava, R.K.; et al. Effect of Salinity and Nitrogen Fertilization Levels on Growth Parameters of *Sarcocornia fruticosa*, *Salicornia brachiata*, and *Arthrocnemum macrostachyum*. *Agronomy* **2022**, *12*, 1749. [CrossRef]
53. Singh, N.K.; Bracken, C.A.; Hasegawa, P.M.; Handa, A.K.; Buckel, S.; Hermodson, M.A.; Pfankoch, E.; Regnier, F.E.; Bressan, R.A. Characterization of osmotin: A thaumatin-like protein associated with osmotic adaptation in plant cells. *Plant Physiol.* **1987**, *85*, 529–536. [CrossRef]