

## Article

# Exogenous Sodium Nitroprusside Mitigates Salt Stress in Lentil (*Lens culinaris* Medik.) by Affecting the Growth, Yield, and Biochemical Properties

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**Citation:** Yasir, T.A.; Khan, A.; Skalicky, M.; Wasaya, A.; Rehmani, M.I.A.; Sarwar, N.; Mubeen, K.; Aziz, M.; Hassan, M.M.; Hassan, F.A.S.; et al. Exogenous Sodium Nitroprusside Mitigates Salt Stress in Lentil (*Lens culinaris* Medik.) by Affecting the Growth, Yield, and Biochemical Properties. *Molecules* **2021**, *26*, 2576. <https://doi.org/10.3390/molecules26092576>

Academic Editors: Agnieszka Saeid, Witold Kwapinski and Marcello Iriti

Received: 30 January 2021

Accepted: 26 April 2021

Published: 28 April 2021

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**Abstract:** Soil salinity disrupts the physiological and biochemical processes of crop plants and ultimately leads to compromising future food security. Sodium nitroprusside (SNP), a contributor to nitric oxide (NO), holds the potential to alleviate abiotic stress effects and boost tolerance in plants, whereas less information is available on its role in salt-stressed lentils. We examined the effect of exogenously applied SNP on salt-stressed lentil plants by monitoring plant growth and yield-related attributes, biochemistry of enzymes (superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)) amassing of leaf malondialdehyde (MDA) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Salinity stress was induced by NaCl application at concentrations of 50 mM (moderate salinity) and 100 mM (severe salinity), while it was alleviated by SNP application at concentrations of 50 μM and 100 μM. Salinity stress severely inhibited the length of roots and shoots, the relative water content, and the chlorophyll content of the leaves, the number of branches, pods, seeds, seed yield, and biomass per plant. In addition, MDA, H<sub>2</sub>O<sub>2</sub> as well as SOD, CAT, and POD activities were increased with increasing salinity levels. Plants supplemented with SNP (100 μM) showed a significant improvement in the growth- and yield-contributing parameters, especially in plants grown under moderate salinity (50 mM NaCl). Essentially, the application of 100 μM SNP remained effective to rescue lentil plants under moderate salinity by regulating plant growth and biochemical pathways. Thus, the exogenous application of SNP could be developed as a useful strategy for improving the performance of lentil plants in salinity-prone environments.

**Keywords:** antioxidant enzymes; abiotic stress tolerance; mitigation; legumes; reactive oxygen species

## 1. Introduction

The emerging scenario of climate change and increasing soil salinity (SS) have become serious bottlenecks in attaining high yields of cereals and legume crops [1]. Among abiotic stresses, SS is gradually increasing and establishing rapidly to adversely affect the arable lands and ultimately slicing the available agricultural land and production of crops [1,2]. Salt-stressed plants usually confront toxic ions accumulation and disrupted plant water relations [3]. In addition, SS causes oxidative impairment in crop plants owing to the overabundance of reactive oxygen species (ROS), which are the toxic byproducts of aerobic metabolism [4]. Furthermore, SS suppresses plant development and growth by unbalancing the osmotic adjustments and disturbing hormonal interactions [5]. It disrupts the metabolism in plant cells by inducing water inadequacy, oxygen insufficiency, and nutrients unbalance [6,7]. Under salinity, plants become less dynamic to draw nutrients from the soil and, resultantly, exhibit nutrients deficiency symptoms [8]. If this situation remains unaddressed in the long run, a reduced photosynthetic rate, chlorosis, and necrosis [9] eventually reduce the yield traits, grain yield, and quality [10–12]. In such circumstances, the plant life cycle depends upon its antioxidant ability to detoxify the ROS. The plants respond to salinity by stimulating their antioxidant self-defense mechanisms [13], which defend them against abiotic stresses [14]. These defense mechanisms include enzymes like ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD), which are biosynthesized in plants, specifically in the chloroplast, and mitochondria [15,16].

Legumes belong to the family of Fabaceae, are an important source of proteins, vegetable oil, vitamins, and minerals [17], and can meet the future food demand in a prevailing climate change scenario. Legumes are very sensitive to salinity stress, which disrupts the nutritional balance, hormonal interactions, and osmotic adjustments that, eventually, affect the overall plant progression from seedling growth to maturity [18]. Among legumes, lentils (*Lens culinaris* Medik.) occupy a pivotal position and serve as an inexpensive source of protein. Similar to other legume crops, lentils are also much vulnerable to salt stress, which may reduce its yield up to 50% [19]. Under increasing soil salinity, it is of the utmost pertinence to find biologically viable ways, which ameliorate the adverse effects of SS and assist lentils to attain optimal vegetative growth along with robust reproductive growth for producing grain yield as per the varietal potential.

Numerous natural and synthetic compounds tend to regulate the biochemical attributes of crop plants under various environmental stresses. Under SS, sodium nitroprusside (SNP), which donates nitric oxide (NO), was found to be effective in promoting wound healing and reducing disease severity in muskmelons [20], inducing resistance against the fungal plant pathogen *Glomerella cingulate* in tea plants [21], against *Monilinia fructicola* in peach [22], against *Botrytis cinerea* in tomato [23], and against the rice black-streaked dwarf virus (RBSDV) [24]. Similarly, the application of SNP enables plants to mitigate abiotic stresses such as moisture stress [25], salinity [26], heat stress [27], chilling stress [28], and heavy metal stress [29]. More explicitly, the use of SNP, under salinity stress, improved the performance of many crop plants like soybean [30], lettuce [31], wheat [4,32], chickpea [33], maize [34], tomato [35], rice [36], and cotton [37]. It can moderate biological, physiological, and chemical reactions occurring in plants under unfavorable environmental conditions [38].

The supplemented SNP holds the potential to mitigate salt stress through the regularization of physiological (chlorophyll content, relative water content, electrolyte leakage, and stomatal behavior) [39] and biochemical (MDA, proline, phenolics, and antioxidant enzymes) attributes of plants [4]. Its role in coping with salinity stress has been well documented; however, its application in lentil crops for salt stress tolerance has not been studied previously. Thus, our research hypothesis was that lentils might respond differently to varying concentrations of SNP, and its dose optimization might alleviate SS effects. Hence, to fill the research gap, the current work was carried out to examine the adverse effects

of SS on the growth, yield attributes, and biochemical pathways of lentils along, with assessing the shielding effect of an exogenous application of SNP in different doses.

## 2. Materials and Methods

### 2.1. Experimental Arrangements

The experiment was accomplished in a wire-house during the winter season of 2017–2018. Lentil (cv. Masoor 2009) seeds were obtained from the Cereals Section of the National Agricultural Research Centre (NARC), Islamabad, Pakistan. Earthen pots, having 27 cm height and 24 cm diameter with a filling capacity of 7 kg, were used in this experiment. Peatmoss, pure sand, and sandy loam soil were used in a proportion of 1:3:3 to fill the pots. Prior to sowing, the lentil seeds were surface-sterilized with sodium hypochlorite. Afterwards, ten seeds were initially sown in each pot, and after successful germination, thinned to five seedlings per pot.

### 2.2. NaCl and SNP Treatments

Five-day-old seedlings were exposed to different levels of NaCl (0 mM, 50 mM, and 100 mM) and different concentrations of SNP (0  $\mu$ M, 50  $\mu$ M, and 100  $\mu$ M). The experiment was planned according to a completely randomized design (CRD), having four repeats for each treatment. Both the salinity and SNP treatments were applied weekly from 25 to 70 days after sowing (DAS) to the pots after adding a half-strength Hoagland's nutrient solution [33]. After 45 days of treatment application, the leaf samples were obtained from all the treatments at 70 DAS and evaluated for biochemical activities of enzymes.

### 2.3. Determination of Growth and Yield Parameters

Out of five seedlings, two seedlings from each pot (at 70DAS) were picked for assessing the biochemical dynamics, and the remaining three seedlings were retained up to maturity for determination of growth, yield-contributing parameters, and grain yield.

The root and shoot lengths of lentil plants were measured with a chrome glass measuring ruler, and their averages were used for further analysis. Similarly, the number of seeds, pods, and branches  $\text{plant}^{-1}$  were counted for the sampled plants, and the averages were recorded. Plant biomass and seed yield were also determined from three plants; subsequently, their average weight  $\text{plant}^{-1}$  was used for statistical analysis.

### 2.4. Estimation of Leaf Relative Water Content

The leaf relative water content (LRWC) was assessed according to the methodology proposed by [40]. Leaves were weighed after sampling and then immersed in distilled water at room temperature for four hours. After that, leaves were blotted dry and then oven-dried at 80 °C for twenty-four hours. The following formula was used for LRWC determination.

$$LRWC (\%) = \frac{FW - DW}{TW - DW} \times 100$$

where *FW* is the fresh weight of the leaves, *DW* is the dry weight of the leaves, and *TW* is the turgid weight of the leaves.

### 2.5. Determination of Leaf Greenness Index

Leaf greenness index was observed by using a portable chlorophyll meter (SPAD-502). For this purpose, five leaves per plant were taken, and their average was used for further analysis.

### 2.6. Estimation of H<sub>2</sub>O<sub>2</sub> and MDA Content

For measuring H<sub>2</sub>O<sub>2</sub> content, 500 mg fresh leaf samples were homogenized in trichloroacetic acid (5 mL, 0.1%) and centrifuged for 15 min at 12,000  $\times$  *g*. After that, supernatant (0.5 mL) was collected, then mixed with a potassium phosphate buffer (0.5 mL of 10 mM having pH 7.0) and potassium iodide (1 mL of 1 M). Finally, the absorbance was measured at 390 nm [41]. For determination of MDA content, fresh leaf samples weighing 500 mg

were homogenized with trichloroacetic acid (2.5 mL, 0.1%) and centrifuged for 10 min at  $10,000\times g$ .

The aliquot was added with trichloroacetic acid (4 mL, 20%) along with thiobarbituric acid (TBA, 0.5%). It was heated for 30 min at  $95\text{ }^{\circ}\text{C}$ , then cooled down, and again centrifuged for 15 min at  $10,000\times g$ . The absorbance was estimated at 532 nm, and the adjustments were taken for undefined turbidity by deducting the absorbance at 600 nm [42].

### 2.7. Antioxidant Enzyme Assays

Fresh leaf samples (500 mg) were mashed in a chilled phosphate buffer (0.1 M, pH 7.5) and EDTA (0.5 mM), followed by centrifuge for 10 min at  $12,000\times g$ . After that, the obtained supernatant was utilized for the estimation of antioxidant enzymes.

The activity of SOD was estimated by quantifying the ability of the enzyme, which inhibits the photochemical reduction of nitro-blue tetrazolium chloride [43]. The absorbance was read at 560 nm, whereas one unit of SOD activity (EU) was defined as the quantity of enzyme needed for 50% inhibition of the nitroblue tetrazolium (NBT) photoreduction rate. Catalase activity was measured by using the method of [44], whereas the change in absorbance was read out at 240 nm. The activity of peroxidase was estimated according to [45]. The absorbance was determined at 420 nm.

### 2.8. Statistical Analysis

The data were analyzed using the computer software Statistix 8.1 (Analytical Software, Tallahassee, FL, USA), and means were compared by employing the least significant difference (LSD) test at  $p \leq 0.05$ . Graphic outputs were made in Microsoft Office 2016 by using MS Excel.

## 3. Results

Our results exhibited that imposed salinity caused considerable damage to the lentil plants by reducing their growth traits like shoot and root length, relative water content, and chlorophyll content of leaf (Table 1). The adverse effects of salinity were increased with an increasing level of salinity as in the severe salinity-stressed treatment (NaCl-100 mM + SNP-0  $\mu\text{M}$ ), which was followed by moderate salinity-stressed treatment (NaCl-50 mM + SNP-0  $\mu\text{M}$ ). The severe salinity stress imparted a significant influence on shoot and root length, which were decreased by 41 and 63%, respectively. When comparing  $T_2$  (moderate salinity treatment) with  $T_4$  and  $T_6$  (moderate salinity treated with different levels of SNP), it was observed that all the parameters showed gradual improvement as the concentration of SNP was raised from 50  $\mu\text{M}$  to 100  $\mu\text{M}$ . Similarly, when comparing  $T_3$  (severe salinity) with  $T_5$  and  $T_7$  (severe salinity treated with different levels of SNP), it was found that the performance of lentil plants in terms of studied parameters was enhanced by increasing the level of SNP from 50  $\mu\text{M}$  to 100  $\mu\text{M}$ . In comparison, the relative water content and leaf greenness index were decreased by 47% and 42%, respectively (Table 1).

The salinity alleviating effects of SNP were evident in the treatments where moderate salinity stress (50 mM NaCl) was treated with the exogenous application of SNP (Table 1). Considerable improvement was observed in treatment the  $T_6$ , where all the growth parameters were improved significantly by applying 100  $\mu\text{M}$  SNP, as compared to the severe salinity-stressed treatment ( $T_3$ ), where all studied parameters like the shoot length, root length, leaf relative water content, and leaf greenness index were increased by 65, 142, 97, and 103%, respectively, as compared to  $T_3$  treatment (Table 1).

**Table 1.** Effect of SNP on shoot, root, and leaf attributes of lentils grown under different NaCl levels.

Treatments	Shoot Length (cm)	Root Length (cm)	Leaf Relative Water Content (%)	Leaf Greenness Index
T <sub>1</sub> = 0 mM of NaCl + 0 μM of SNP (Control)	40.12 ± 1.18 a	20.82 ± 0.62 a	81.58 ± 1.88 a	12.51 ± 0.97 ab
T <sub>2</sub> = 50 mM of NaCl + 0 μM of SNP	32.18 ± 0.83 c	12.55 ± 0.95 de	64.48 ± 2.05 d	9.42 ± 1.22 cd
T <sub>3</sub> = 100 mM of NaCl + 0 μM of SNP	23.41 ± 1.24 e	7.62 ± 0.76 f	42.86 ± 1.06 e	7.28 ± 1.05 d
T <sub>4</sub> = 50 mM of NaCl + 50 μM of SNP	36.66 ± 1.14 b	16.23 ± 0.96 bc	74.27 ± 1.71 b	12.27 ± 1.07 ab
T <sub>5</sub> = 100 mM of NaCl + 50 μM of SNP	30.55 ± 0.85 c	13.71 ± 0.83 cd	69.11 ± 1.68 c	10.24 ± 0.87 bc
T <sub>6</sub> = 50 mM of NaCl + 100 μM of SNP	38.72 ± 1.02 ab	18.42 ± 0.73 ab	84.32 ± 2.46 a	14.80 ± 0.97 a
T <sub>7</sub> = 100 mM of NaCl + 100 μM of SNP	27.18 ± 0.82 d	10.22 ± 0.87 ef	65.33 ± 1.99 d	11.71 ± 0.99 bc

The data presented are the mean values ± standard deviation ( $n = 4$ ). Different letters next to the mean value indicate significant differences at  $p \leq 0.05$ .

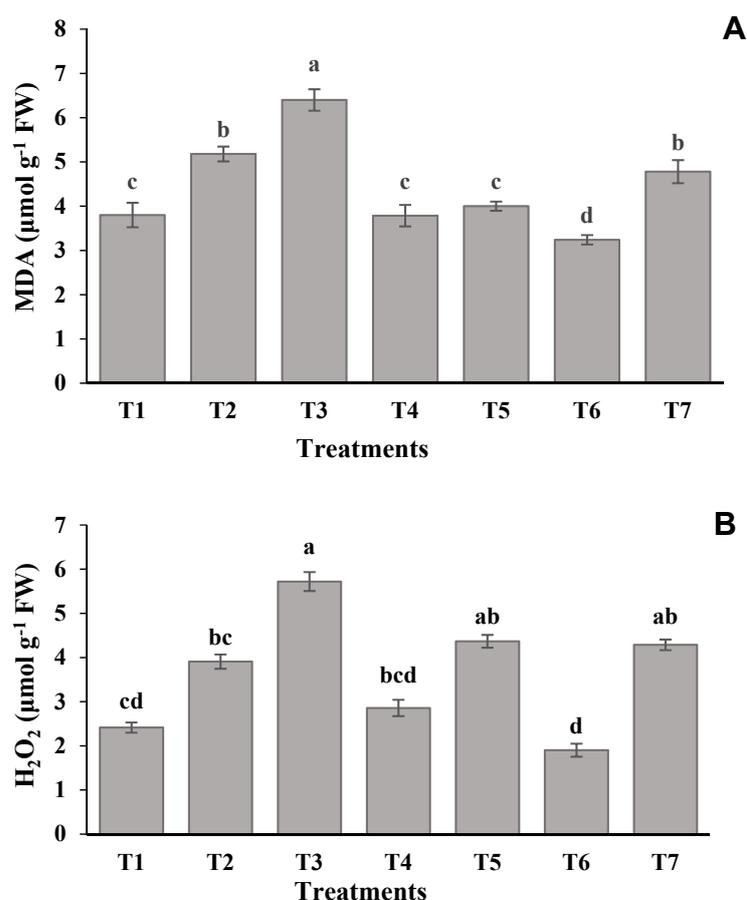
Significant reduction in yield-contributing parameters took place with the increase in the intensity of salinity (Table 2). As compared to the control treatment (T<sub>1</sub>), the decline was at the maximum in the severe salinity-stressed treatment (T<sub>3</sub>), where the number of branches, pods, and seed per plant was reduced by 53, 45, and 60%, respectively, while the plant seed yield and biomass were reduced by 60 and 54%, respectively. Plants supplemented with different levels of SNP showed significant tolerance to the induced salinity levels. Plants subjected to the highest dose of SNP possessed the maximum tolerance against the moderate level of salinity (50 mM). In this treatment, plant seed yield and biomass were increased by 211 and 235%, respectively, as compared to the severe salinity-stressed treatment. Similarly, the application of SNP increased the number of branches, pods, and seed per plant, compared to the severe salinity-stressed treatment by 196, 144, and 208%, respectively (Table 2).

**Table 2.** Effect of SNP on the yield attributes of lentils grown under different levels of NaCl.

Treatments	Number of Branches Plant <sup>-1</sup>	Number of Pods Plant <sup>-1</sup>	Number of Seeds Plant <sup>-1</sup>	Seed Yield (g Plant <sup>-1</sup> )	Biomass (g Plant <sup>-1</sup> )
T <sub>1</sub> = 0 mM of NaCl + 0 μM of SNP	10.50 ± 0.53 b	36.52 ± 2.50 b	54.50 ± 3.18 b	1.32 ± 0.18 ab	9.17 ± 0.73 b
T <sub>2</sub> = 50 mM of NaCl + 0 μM of SNP	7.25 ± 0.80 c	27.51 ± 2.11 d	34.52 ± 1.97 e	0.86 ± 0.04 ab	7.82 ± 0.58 b
T <sub>3</sub> = 100 mM of NaCl + 0 μM of SNP	4.91 ± 0.69 d	20.04 ± 1.73 e	21.54 ± 1.06 f	0.53 ± 0.06 b	4.22 ± 0.71 c
T <sub>4</sub> = 50 mM of NaCl + 50 μM of SNP	8.66 ± 0.82 c	34.54 ± 1.52 bc	49.48 ± 2.71 c	1.23 ± 0.08 ab	8.95 ± 0.84 b
T <sub>5</sub> = 100 mM of NaCl + 50 μM of SNP	7.33 ± 0.35 c	32.20 ± 2.09 c	39.50 ± 2.44 d	0.93 ± 0.12 ab	5.42 ± 0.62 c
T <sub>6</sub> = 50 mM of NaCl + 100 μM of SNP	14.58 ± 0.72 a	48.75 ± 2.81 a	66.25 ± 2.70 a	1.65 ± 0.21 a	14.15 ± 1.06 a
T <sub>7</sub> = 100 mM of NaCl + 100 μM of SNP	8.16 ± 1.01 c	33.75 ± 2.13 bc	48.52 ± 2.32 c	1.21 ± 0.13 ab	8.38 ± 0.74 b

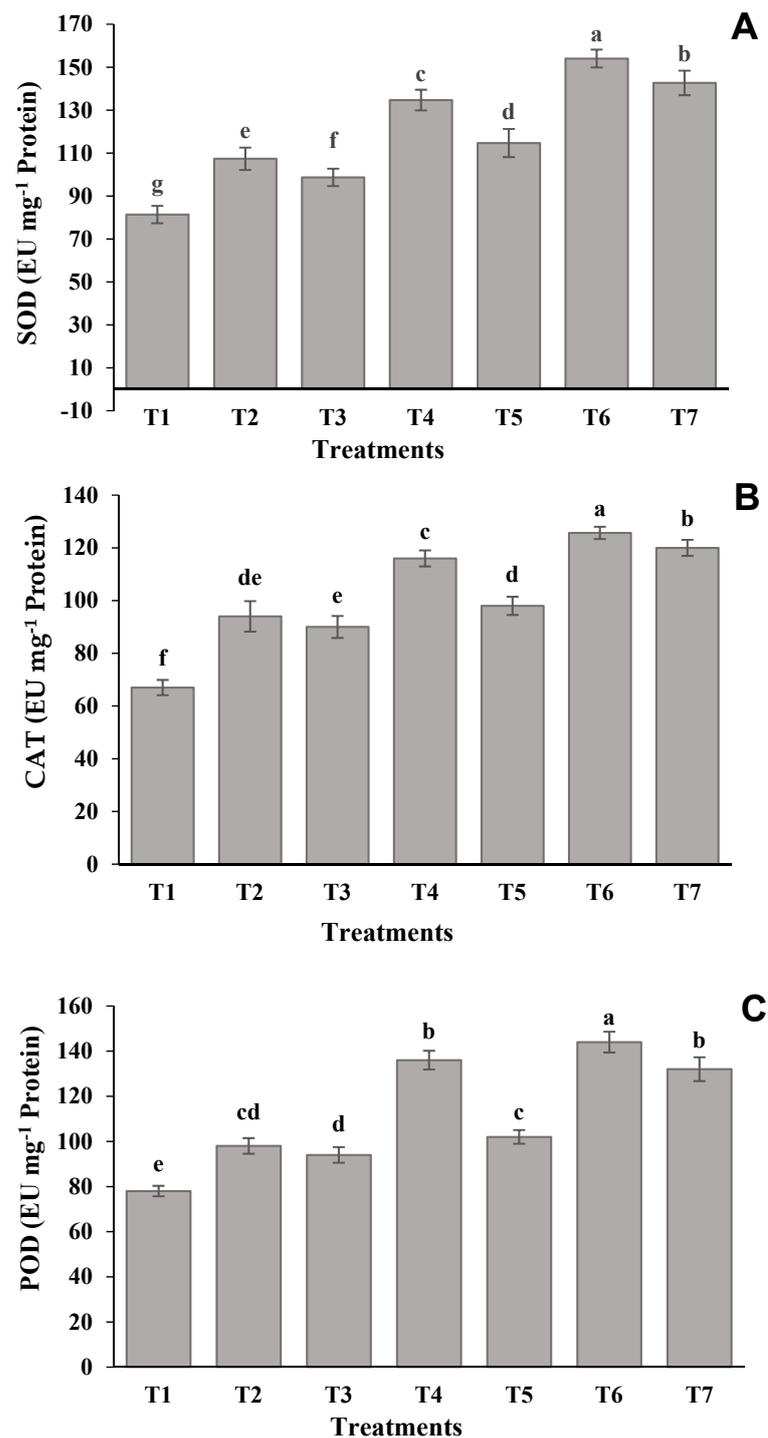
Data presented are the mean values ± standard deviation ( $n = 4$ ). Different letters next to the mean value indicate significant differences at  $p \leq 0.05$ .

The biochemical performances of lentils in terms of MDA, H<sub>2</sub>O<sub>2</sub>, SOD, CAT, and POD biosynthesis under different levels of salinity and SNP are presented in Figures 1 and 2. Both the figures revealed that the concentration of these compounds was higher in plants exposed to severe salinity-stressed treatment. However, the highest increment of MDA content (68%) over the control treatment (T<sub>1</sub>) was found for 100 Mm salinity levels, where SNP was not applied as a foliage spray.



**Figure 1.** Effect of SNP on the (A) MDA content and (B) H<sub>2</sub>O<sub>2</sub> content in leaves of lentil plants under salinity stress. Data presented are the means  $\pm$  SE ( $n = 4$ ). Different letters on the bars indicate significant differences among the treatments at  $p \geq 0.05$  for MDA and H<sub>2</sub>O<sub>2</sub> content. T1 = 0 mM of NaCl + 0  $\mu$ M of SNP (control); T2 = 50 mM of NaCl + 0  $\mu$ M of SNP; T3 = 100 mM of NaCl + 0  $\mu$ M of SNP; T4 = 50 mM of NaCl + 50  $\mu$ M of SNP; T5 = 100 mM of NaCl + 50  $\mu$ M of SNP; T6 = 50 mM of NaCl + 100  $\mu$ M of SNP; T7 = 100 mM of NaCl + 100  $\mu$ M of SNP.

Similarly, in comparison with the control treatment, the H<sub>2</sub>O<sub>2</sub> content was increased by 62 and 137% in plants exposed to 50 and 100 mM salinity levels, respectively, with no foliar application of SNP. The increment of SNP in the salinity-stressed treatments gradually reduced the building up of MDA and H<sub>2</sub>O<sub>2</sub>. The treatment, involving 50 mM salinity level and foliage-applied SNP (100  $\mu$ M), showed the maximum reduction of MDA (49%) and H<sub>2</sub>O<sub>2</sub> (65%), as compared to the severe salinity-stressed treatment (Figure 1). Correspondingly, a rapid increase in the activities of enzymes SOD, CAT, and POD was noted in treatments involving 50 and 100 mM of salinity levels with the absence of SNP application. When compared with the control treatment, the SOD, CAT, and POD were increased by 88, 84, and 86%, respectively, by the 100 mM salinity treatment. Contrariwise, the amount of SOD, CAT, and POD were reduced by 33, 29, and 34%, respectively, in T<sub>6</sub>, which was supplemented with a higher amount of SNP (Figure 2).



**Figure 2.** Effect of SNP on the antioxidant enzymes activity (A) SOD, (B) CAT, and (C) POD in leaves of lentil plants under salinity stress. Data presented are the means  $\pm$  SE ( $n = 4$ ). Different letters on the bars indicate significant differences among the treatments at  $p \geq 0.05$  for individual antioxidant enzymes. T1 = 0 mM of NaCl + 0  $\mu$ M of SNP (control); T2 = 50 mM of NaCl + 0  $\mu$ M of SNP; T3 = 100 mM of NaCl + 0  $\mu$ M of SNP; T4 = 50 mM of NaCl + 50  $\mu$ M of SNP; T5 = 100 mM of NaCl + 50  $\mu$ M of SNP; T6 = 50 mM of NaCl + 100  $\mu$ M of SNP; T7 = 100 mM of NaCl + 100  $\mu$ M of SNP.

#### 4. Discussion

In most crop plants, the seedling phase is the most vulnerable to harsh ecological conditions. The legume crops, especially lentils, is very sensitive to saline conditions at seedling growth stage [46]. The results of our research trial were in accordance with

the postulated hypothesis as salinity stress severely hampered the growth, biochemical processes as well as yield attributes of lentil plants by disrupting the normal functioning and performance of plants [47]. The SS negatively affected the water retention and turgidity of the plant cells [48], modulation of stomata, photosynthetic capacity, and ultimately declined in the growth and yield of plants became evident [49].

Under salinity stress, vegetative and reproductive growth of plants got adversely affected and revamping of biochemical pathways through exogenous application of salinity alleviating chemicals might enable plants to cope with adverse effects of abiotic stresses. In this study, plants under different intensities of NaCl stress produced significantly shorter roots and shoots, which was in line with the results of [4,32] in wheat [50] and rice. The addition of SNP to the plants under different intensities of salt stress markedly amended their shoot and root length (Table 1). Similar results were also obtained by [51,52] while working on wheat and safflower, respectively. It was found that nitric oxide reacted with phospholipids and modified the cell wall, which ultimately recovered the plant growth in stressful environments [53].

The photosynthetic capacity depends upon the amount of chlorophyll present in the plant leaves, while SS significantly degrades chlorophyll [54]. Similarly, our results highlighted a significant reduction in the greenness index of lentil leaves under severe salinity-stressed treatment (100 Mm NaCl), which led to a noticeable decline in the seed yield. The relative water content of the leaf was lowest in severe salinity-stressed treatment (Table 1), and it might be attributed to the disrupted plant root system by choking water absorption under higher NaCl concentration [55]. In addition to this, the remedial effects of nitric oxide were vibrant in the treatments where SNP was supplemented with NaCl, and lentil plants performed better to improve their leaf relative water and greenness index. These results also confirmed the early findings of [55] and [56] in mustard and rice, respectively, who observed that the water content and chlorophyll content of leaves were increased with the supplementation of nitric oxide under a saline environment. The application of nitric oxide was found effective in enhancing the formation of chlorophyll pigments, which were vital for triggering the photosynthesis rate through shielding the membranes of the cell organelles comprising chlorophyll [57].

Yield losses in different legume crops were recorded from 12 to 100% [58]. The reduction in yield-contributing parameters were significant under higher NaCl concentrations. The effects of salinity were more pronounced for 100 Mm NaCl stress, which caused a significant decline in the yield attributes, while this treatment was followed by a moderate level of salinity (Table 2). These findings were in agreement with [59], who also reported a noticeable drop in yield-related attributes in soybeans exposed to a severe salt-stress environment. Salinity caused a 20% reduction in the seed yield of chickpeas due to wrinkled seeds [60] and lower seed weight due to lesser grains per pod in mung bean [61].

Salinity stress interrupts metabolic pathways, including several enzymes and antioxidants, by accretion of unwanted reactive oxygen species, which damage plant cells and ultimately plant growth and development [13]. The current study revealed that MDA and H<sub>2</sub>O<sub>2</sub> concentrations were increased in severe a salinity-stressed treatment compared to control treatment. Contrarily, these parameters showed the minimum corresponding values, where the highest dose of SNP (100 mM) was added to cope with moderate salinity (Figure 1). Similar results were also obtained in chickpeas [33] and wheat [62]. It might be inferred that the SNP application as foliar spray might potentially impart tolerance against salinity by enabling the plants to make metabolic adjustments for reducing the levels of MDA and H<sub>2</sub>O<sub>2</sub> [63]. Optimizing the production of MDA and H<sub>2</sub>O<sub>2</sub> to normal limits (as in T<sub>1</sub> Figure 1) endorsed the shielding role of SNP to confrontation salinity.

Plants also have a high tendency to activate their defensive system by overproducing proline, APX, CAT, SOD, and GR as their self-defense mechanism under environmental severities [33]. The amount of SOD, CAT, and POD was found to be increased as the intensity of salinity was increased from 0 to 100 mM (Figure 2), while their corresponding values were declined significantly with an increasing concentration of SNP, which was

applied as a foliar spray (Figure 2). Moreover, it was noticed that the SNP application (100  $\mu$ M) on plants exposed to 50 mM salinity level exhibited the lowest values for SOD, CAT, and POD, and these values were closer to the normal ranges as observed in the control treatment. These results proved the ameliorating effects of SNP under different levels of salinity stress in lentils, which supported the earlier studies in different crops, as reported by [64] in mustard, [65] in cotton, and [35] in tomato.

## 5. Conclusions

The research trial was executed to determine the effects of varying levels of salinity on lentil growth, yield attributes, and biochemical functioning, along with exploiting the role of SNP in alleviating the harmful effects of salinity. The research findings were in line with the postulated hypothesis as salinity reduced the physiological functions, growth, yield traits, and yield of lentils to a great extent. The highest dose of SNP (100  $\mu$ M) remained effective in accelerating and modifying plant growth and yield by optimizing their biochemical and metabolic functioning in a salinity-prone environment. Therefore, it was recommended to use exogenous applications of SNP to obtain a better yield of lentils in moderate saline areas. However, it was also suggested to evaluate other concentrations of SNP for mitigating the harmful influence of salinity on lentils.

**Author Contributions:** Conceptualization, T.A.Y. and A.W.; methodology, T.A.Y. and M.A.; data curation, T.A.Y., N.S., A.W., and N.S.; investigation, A.K. and T.A.Y.; analysis, M.I.A.R. and K.M.; draft preparation, A.K., T.A.Y., M.A.I., and A.W.; review and editing, M.S.I., M.S., M.B., F.A.S.H., S.D., and A.E.S.; funding acquisition, F.A.S.H., M.S., M.B., M.M.H., and A.E.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** The current work was funded by Taif University Researchers Supporting Project number (TURSP-2020/143), Taif university, Taif, Saudi Arabia.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** The authors extend their appreciation to Taif University for funding current work by Taif University Researchers Supporting Project number (TURSP-2020/143), Taif University, Taif, Saudi Arabia.

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

**Sample Availability:** Samples of the compounds are not available from the authors.

## References

1. Abdelhamid, M.T.; El-Masry, R.R.; Darwish, D.S.; Abdalla, M.M.; Oba, S.; Ragab, R.; EL-Sabagh, A.; EL-Kholy, M.A.; Omer, E. Mechanisms of seed priming involved in salt stress amelioration. In *Priming and Pretreatment of Seeds and Seedlings*; Springer: Singapore, 2019; pp. 219–251.
2. EL-Sabagh, A.; Hossain, A.; Barutçular, C.; Iqbal, M.A.; Islam, M.S.; Fahad, S.; Erman, M. Consequences of salinity stress on the quality of crops and its mitigation strategies for sustainable crop production: An outlook of arid and semi-arid regions. In *Environment, Climate, Plant and Vegetation Growth*; Springer: Cham, Switzerland, 2020; pp. 503–533.
3. Islam, M.S.; Akhter, M.M.; El-Sabagh, A.; Liu, L.Y.; Nguyen, N.T.; Ueda, A.; Masaoka, Y.; Saneoka, H. Comparative studies on growth and physiological responses to saline and alkaline stresses of Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.). *Aust. J. Crop Sci.* **2011**, *5*, 1269–1277.
4. Ali, Q.; Daud, M.K.; Haider, M.Z.; Ali, S.; Rizwan, M.; Aslam, N.; Noman, A.; Iqbal, N.; Shahzad, F.; Deeba, F.; et al. Seed priming by sodium nitroprusside improves salt tolerance in wheat (*Triticum aestivum* L.) by enhancing physiological and biochemical parameters. *Plant Physiol. Biochem.* **2017**, *119*, 50–59. [[CrossRef](#)]
5. Yang, Y.; Guo, Y. Unravelling salt signaling in plants. *J. Integr. Plant Biol.* **2018**, *60*, 796–804.
6. Morari, F.; Meggio, F.; Lunardon, A.; Scudiero, E.; Forestan, C.; Farinati, S.; Varotto, S. Time course of biochemical, physiological, and molecular responses to field-mimicked conditions of drought, salinity, and recovery in two maize lines. *Front. Plant Sci.* **2015**, *6*, 314. [[CrossRef](#)] [[PubMed](#)]

7. Monsur, M.B.; Ivy, N.A.; Haque, M.M.; Hasanuzzaman, M.; EL-Sabagh, A.; Rohman, M.M. Oxidative stress tolerance mechanism in rice under salinity. *Phyton Int. J. Exp. Bot.* **2020**, *89*, 497–517.
8. Ashraf, M.; Shahzad, S.M.; Imtiaz, M.; Rizwan, M.S. Salinity effects on nitrogen metabolism in plants—Focusing on the activities of nitrogen metabolizing enzymes: A review. *J. Plant Nutr.* **2018**, *41*, 1065–1081. [[CrossRef](#)]
9. Khan, H.A.; Siddique, K.H.M.; Munir, R.; Colmer, T.D. Salt sensitivity in chickpea: Growth, photosynthesis, seed yield components and tissue ion regulation in contrasting genotypes. *J. Plant Physiol.* **2015**, *182*, 1–12. [[CrossRef](#)]
10. Manchanda, G.; Garg, N. Salinity and its effects on the functional biology of legumes. *Acta Physiol. Plant.* **2008**, *30*, 595–618. [[CrossRef](#)]
11. Hasan, M.K.; Islam, M.S.; Islam, M.R.; Ismaan, H.N.; EL-Sabagh, A.; Barutçular, C.; Saneoka, H. Water relations and dry matter accumulation of black gram and mungbean as affected by salinity. *Thai J. Agric. Sci.* **2019**, *52*, 54–67.
12. EL-Sabagh, A.; Hossain, A.; Islam, M.S.; Barutçular, C.; Ratnasekera, D.; Kumar, N.; Meena, R.S.; Gharib, H.S.; Saneoka, H.; Teixeira da Silva, J.A. Sustainable soybean production and abiotic stress management in saline environments: A critical review. *Aust. J. Crop Sci.* **2019**, *13*, 228–236. [[CrossRef](#)]
13. Farooq, M.; Hussain, M.; Wakeel, A.; Siddique, K.H.M. Salt stress in maize effects resistance mechanisms and management: A review. *Agron. Sustain. Dev.* **2015**, *35*, 461–481. [[CrossRef](#)]
14. Rasool, S.; Ahmad, A.; Siddiqi, T.O.; Ahmad, P. Changes in growth, lipid per oxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta. Physiol. Plant.* **2013**, *35*, 1039–1050. [[CrossRef](#)]
15. Ahmad, P. Growth and antioxidant responses in mustard (*Brassica juncea* L.) plants subjected to combined effect of gibberellic acid and salinity. *Arch. Agron. Soil Sci.* **2010**, *56*, 575–588. [[CrossRef](#)]
16. Liu, L.; Nakamura, Y.; Taliman, N.A.; Sabagh, A.E.; Moghaieb, R.E.; Saneoka, H. Differences in the growth and physiological responses of the leaves of *Peucedanum japonicum* and *Hordeum vulgare* exposed to salinity. *Agriculture* **2020**, *10*, 317. [[CrossRef](#)]
17. Islam, M.S.; Hasan, M.K.; Islam, B.; Renu, N.A.; Hakim, M.A.; Islam, M.R.; Chowdhury, M.K.; Ueda, A.; Saneoka, H.; Raza, M.A.; et al. Responses of water and pigments status, dry matter partitioning, seed production, and traits of yield and quality to foliar application of GA<sub>3</sub> in Mungbean (*Vigna radiata* L.). *Front. Agron.* **2021**, *2*, 596850. [[CrossRef](#)]
18. Patil, G.; Do, T.; Vuong, T.D.; Valliyodan, B.; Lee, J.D.; Chaudhary, J.; Shannon, J.G.; Nguyen, H.T. Genomic-assisted haplotype analysis and the development of high-throughput SNP markers for salinity tolerance in soybean. *Sci. Rep.* **2016**, *6*, 19199. [[CrossRef](#)]
19. Golezani, K.G.; Yengabad, F.M. Physiological responses of lentil (*Lens culinaris* Medik.) to salinity. *Int. J. Agric. Crop Sci.* **2012**, *4*, 531–535.
20. Wang, B.; Jiang, H.; Bi, Y.; He, X.; Wang, Y.; Zheng, X.; Prusky, D. Preharvest multiple sprays with sodium nitroprusside promote wound healing of harvested muskmelons by activation of phenylpropanoid metabolism. *Postharvest Biol. Technol.* **2019**, *158*, 110988. [[CrossRef](#)]
21. Som, P.R. Nitric oxide induced resistance in tea plants against *Glomerella cingulata* (stoneman) spauld & schrenk. *BIOINFOLET* **2020**, *17*, 336–342.
22. Li, G.; Zhu, S.; Wu, W.; Zhang, C.; Peng, Y.; Wang, Q.; Shi, J. Exogenous nitric oxide (NO) induces disease against *Monilinia fructicola* through activating the phenylpropanoid pathway in peach fruit. *J. Sci. Food Agric.* **2017**, *97*, 3030–3038. [[CrossRef](#)]
23. Lai, T.F.; Wang, Y.Y.; Li, B.Q.; Qin, G.Z.; Tian, S.P. Defensive responses of tomato fruit to exogenous nitric oxide during postharvest storage. *Postharvest Biol. Technol.* **2011**, *62*, 127–132. [[CrossRef](#)]
24. Lu, R.; Liu, Z.; Shao, Y.; Su, J.; Li, X.; Sun, F.; Zhang, Y.; Li, S.; Zhang, Y.; Cui, J.; et al. Nitric oxide enhances rice resistance to rice black-streaked dwarf virus infection. *Rice* **2020**, *13*, 24. [[CrossRef](#)]
25. Hameed, A.; Farooq, T.; Hameed, A.; Sheikh, M.A. Sodium nitroprusside mediated priming memory invokes water-deficit stress acclimation in wheat plants through physio-biochemical alterations. *Plant Physiol. Biochem.* **2021**, *160*, 329–340. [[CrossRef](#)]
26. Kram, N.A.; Hafeez, N.; Farid-ul-Haq, M.; Ahmad, A.; Sadiq, M.; Ashraf, M. Foliage application and seed priming with nitric oxide causes mitigation of salinity-induced metabolic adversaries in broccoli (*Brassica oleracea* L.) plants. *Acta Physiol. Plant.* **2020**, *42*, 155.
27. Lee, H.J.; Lee, J.H.; Lee, S.G.; An, S.; Lee, H.S.; Choi, C.K.; Kim, S.K. Foliar application of biostimulants affects physiological responses and improves heat stress tolerance in Kimchi cabbage. *Hortic. Environ. Biotechnol.* **2019**, *60*, 841–851. [[CrossRef](#)]
28. Diao, Q.; Cao, Y.; Fan, H.; Zhang, Y. Transcriptome analysis deciphers the mechanisms of exogenous nitric oxide action on the response of melon leaves to chilling stress. *Biol. Plant.* **2020**, *64*, 465–472. [[CrossRef](#)]
29. Ahmad, P.; Alam, P.; Balawi, T.H.; Atalayan, F.H.; Ahanger, M.A.; Ashraf, M. Sodium nitroprusside (SNP) improves tolerance to arsenic (As) toxicity in *Vicia faba* through the modifications of biochemical attributes, antioxidants, ascorbate-glutathione cycle and glyoxalase cycle. *Chemosphere* **2020**, *244*, 125480. [[CrossRef](#)] [[PubMed](#)]
30. Karthik, S.; Pavan, G.; Krishnan, V.; Sathish, S.; Manickavasagam, M. Sodium nitroprusside enhances regeneration and alleviates salinity stress in soybean (*Glycine max* Merrill). *Biocatal. Agric. Biotechnol.* **2019**. [[CrossRef](#)]
31. Campos, F.V.; Oliveria, J.A.; Pereira, M.G.; Farnese, F.S. Nitric oxide and phytohormone interactions in the response of *Lactuca sativa* to salinity stress. *Planta* **2019**, *250*, 1475–1479. [[CrossRef](#)]
32. Kausar, F.; Shahbaz, M. Interactive effect of foliar application of nitric oxide (NO) and salinity on wheat (*Triticum aestivum* L.). *Pak. J. Bot.* **2013**, *45*, 67–73.

33. Ahmad, P.; Abdel-Latif, A.A.; Hashem, A.; Abd-Allah, E.F.; Gucel, S.; Tran, L.S.P. Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. *Front. Plant Sci.* **2016**, *7*, 347. [[CrossRef](#)]
34. Zhang, Y.Y.; Liu, J.; Liu, Y.L. Nitric oxide alleviates growth inhibition of maize seedlings under salt stress. *J. Plant Physiol. Mol. Biol.* **2004**, *30*, 455–459.
35. Manai, J.; Kalai, T.; Gouia, H.; Corpas, F.J. Exogenous nitric oxide (NO) ameliorates salinity-induced oxidative stress in tomato (*Solanum lycopersicum*) plants. *J. Soil Sci. Plant Nutr.* **2014**, *14*, 433–446. [[CrossRef](#)]
36. Habib, N.; Ashraf, M.; Shahbaz, M. Effect of exogenously applied nitric oxide on some key physiological attributes of rice (*Oryza sativa* L.) plants under salt stress. *Pak. J. Bot.* **2013**, *45*, 1563–1569.
37. Liu, S.; Dong, Y.; Xu, L.; Kong, J. Effects of foliar applications of nitric oxide and salicylic acid on salt-induced changes in photosynthesis and antioxidative metabolism of cotton seedlings. *Plant Growth Regul.* **2013**, *73*, 67–78. [[CrossRef](#)]
38. Sahay, S.; Gupta, M. An update on nitric oxide and its benign role in plant responses under metal stress. *Nitric Oxide* **2017**, *67*, 39–52. [[CrossRef](#)] [[PubMed](#)]
39. Mohasseli, V.; Sadeghi, S. Exogenously applied sodium nitroprusside improves physiological attributes and essential oil yield of two drought susceptible and resistant specie of *Thymus* under reduced irrigation. *Ind. Crop. Prod.* **2019**, *130*, 130–136. [[CrossRef](#)]
40. Yamasaki, S.; Dillenburg, L.C. Measurements of leaf relative water content in *Araucaria angustifolia*. *Rev. Bras. Fisiol. Veg.* **1999**, *11*, 69–75.
41. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [[CrossRef](#)]
42. Rao, K.V.M.; Sresty, T.V.S. Antioxidative parameters in the seedlings of pigeon pea (*Cajanus cajan* L. Millspaugh) in response to Zn and Ni stresses. *Plant Sci.* **2000**, *157*, 113–128.
43. Giannopolitis, C.N.; Ries, S.K. Superoxide dismutase I. Occurrence in higher plants. *J. Plant Physiol.* **1977**, *59*, 309–314. [[CrossRef](#)] [[PubMed](#)]
44. Aebi, H. Catalase in vitro. *Method Enzymol.* **1984**, *105*, 121–126.
45. Chance, B.; Maehly, A.C. Assay of catalases and peroxidases. *Method Enzymol.* **1995**, *2*, 764–775.
46. Bandeoglu, E.; Eyidogan, F.; Yucel, M.; Oktem, H.A. Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. *Plant Growth Regul.* **2004**, *42*, 69–77. [[CrossRef](#)]
47. Ibrahim, E.A. Seed priming to alleviate salinity stress in germinating seeds. *J. Plant Physiol.* **2016**, *192*, 38–46. [[CrossRef](#)] [[PubMed](#)]
48. Garg, N.; Bharti, A. Salicylic acid improves arbuscular mycorrhizal symbiosis, and chickpea growth and yield by modulating carbohydrate metabolism under salt stress. *Mycorrhiza* **2018**, *28*, 727–746. [[CrossRef](#)] [[PubMed](#)]
49. Garg, N.; Manchanda, G. ROS generation in plants: Boon or bane? *Plant Biosyst.* **2009**, *143*, 81–96. [[CrossRef](#)]
50. Mostofa, M.G.; Fujita, M.; Tran, L.S.P. Nitric oxide mediates hydrogen peroxide- and salicylic acid-induced salt tolerance in rice (*Oryza sativa* L.) seedlings. *Plant Growth Regul.* **2015**, *77*, 265–277. [[CrossRef](#)]
51. Hasanuzzaman, M.; Hossain, M.A.; Fujita, M. Nitric oxide modulates antioxidant defense and the methylglyoxal detoxification system and reduces salinity-induced damage of wheat seedlings. *Plant Biotechnol. Rep.* **2011**, *5*, 353–365. [[CrossRef](#)]
52. Demir, M.; Arif, I. Effects of different soil salinity levels on germination and seedling growth of safflower (*Carthamus tinctorius* L.). *Turk. J. Agric.* **2003**, *27*, 221–227.
53. Leshem, Y.Y.; Haramaty, E. The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. foliage. *J. Plant Physiol.* **1996**, *148*, 258–263. [[CrossRef](#)]
54. Li, Y.; He, N.; Hou, J.; Xu, L.; Liu, C.; Zhang, J.; Wang, Q.; Zhang, X.; Wu, X. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Front. Ecol. Evol.* **2018**, *6*, 64. [[CrossRef](#)]
55. Zeng, C.L.; Liu, L.; Wang, B.R.; Wu, X.M.; Zhou, Y. Physiological effects of exogenous nitric oxide on *Brassica juncea* seedlings under NaCl stress. *Biol. Plant.* **2011**, *55*, 345–348. [[CrossRef](#)]
56. Habib, N.; Ashraf, M. Effect of exogenously applied nitric oxide on water relations and ionic composition of rice (*Oryza sativa* L.) plants under salt stress. *Pak. J. Bot.* **2014**, *46*, 111–116.
57. Farag, M.; Najeeb, U.; Yang, J.; Hu, Z.; Fang, Z.M. Nitric oxide protects carbon assimilation process of watermelon from boron-induced oxidative injury. *Plant Physiol. Biochem.* **2017**, *111*, 166–173. [[CrossRef](#)]
58. Farooq, M.; Gogoi, N.; Barthakur, S.; Baroowa, B.; Bharadwaj, N.; Alghamdi, S.S.; Siddique, K.H.M. Drought stress in grain legumes during reproduction and grain filling. *J. Agron. Crop Sci.* **2017**, *203*, 81–102. [[CrossRef](#)]
59. Khan, H.A.; Siddique, K.H.M.; Colmer, T.D. Salt sensitivity in chickpea is determined by sodium toxicity. *Planta* **2016**, *244*, 623–637. [[CrossRef](#)]
60. Vadez, V.; Krishnamurthy, L.; Serraj, R.; Gaur, P.M.; Upadhyaya, H.D.; Hoisington, D.A.; Varshney, R.K.; Turner, N.C.; Siddique, K.H.M. Large variation in salinity tolerance in chickpea is explained by differences in sensitivity at the reproductive stage. *Field Crop. Res.* **2007**, *104*, 123–129. [[CrossRef](#)]
61. Ahmed, S. Effect of soil salinity on the yield and yield components of mungbean. *Pak. J. Bot.* **2009**, *41*, 63–68.
62. Zheng, C.F.; Dong, J.G.; Liu, F.L.; Dai, T.B.; Liu, W.C.; Jing, Q.; Cao, W. Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Environ. Exp. Bot.* **2009**, *67*, 222–227. [[CrossRef](#)]
63. Begara-Morales, J.C.; Sanchez-Calvo, B.; Chaki, M.; Valderrama, R.; MataPerez, C.; Lopez-Jaramillo, J.; Padilla, M.N.; Carreras, A.; Corpas, F.J.; Barroso, J.B. Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and S-nitrosylation. *J. Exp. Bot.* **2014**, *65*, 527–538. [[CrossRef](#)] [[PubMed](#)]

- 
64. Khan, M.N.; Siddiqui, M.H.; Mohammad, F.; Naeem, M. Interactive role of nitric oxide and calcium chloride in enhancing tolerance to salt stress. *Nitric Oxide* **2012**, *27*, 210–218. [[CrossRef](#)] [[PubMed](#)]
  65. Dong, Y.J.; Jinc, S.S.; Liu, S.; Xu, L.L.; Kong, J. Effects of exogenous nitric oxide on growth of cotton seedlings under NaCl stress. *J. Soil Sci. Plant Nutr.* **2014**, *14*, 1–13. [[CrossRef](#)]