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RESEARCH ARTICLE

Nitrogen fertilizer promoting salt tolerance of two sorghum varieties under different salt compositions

Aboagla Mohammed Ibrahim Elsiddig[1], Guisheng Zhou[1*], Guanglong Zhu[1], Nimir Eltyb Ahmed Nimir[1, 2], Mohamed Suliman Eltyeb Suliman[1, 2], Muhi Eldeen Hussien Ibrahim[1, 3], and Adam Yousif Adam Ali[1, 4]

[1]Yangzhou University, Joint International Research Laboratory of Agriculture & Agri-Product Safety, Yangzhou 225009, China.
[2]University of Khartoum, Faculty of Agriculture, 11115 Khartoum, Sudan.
[3]Sudan University of Science and Technology, College of Agricultural Studies, Khartoum 13311, Sudan.
[4]University of Gadarif, College of Agricultural and Environmental Science, Al Qadarif 32214, Sudan.
[*]Corresponding author (gszhou@yzu.edu.cn).

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ABSTRACT

Soil salinization is more aggravating than ever before and techniques are needed to mitigate this problem; understanding salinity-fertilizer relationship is of considerable economic importance for promoting crop growth and productivity in saline soil. A pot experiment was done in a greenhouse to determine whether the N could alleviate the negative effect of different salts compositions on the growth, and physiological attributes of the two most used Sudan sorghum (*Sorghum bicolor* (L.) Moench) cultivars (Wadahmed and Tabat). The plant was subjected to the salt stress at 0, 0.3419 M NaCl, and 0.1408 M Na_2SO_4, and four levels of N application 0, 4, 8, and 12 g N kg^{-1} soil, designated as N0, N1, N2, and N3, respectively. Results showed that Na_2SO_4 was reduced plant height, leaf area index, number of leaves, dry weight (DW), chlorophyll *a*, total chlorophyll content, and increased soluble protein content, activities of peroxidase (POD) and catalase (CAT) activities by 16.4%, 29.7%, 61.5%, 28.7%, 39.6%, 12.7%, 99.7%, 34.3%, 51.8%, respectively, when compared with the non-saline treatment. The Na_2SO_4 salt had a more harmful effect than NaCl. This study revealed that N fertilizer was successful for alleviating the adverse impacts of both types of salt. In this study, 12 g N kg^{-1} soil was most effective on most of the measured parameters of two sorghum cultivars. These findings demonstrated that the N soil amendment application could alleviate the harmful impact of salinity. 'Wadahmed' was more tolerant to salinity stress than 'Tabat' during the boot stage of sorghum plant.

Key words: Boot stage, nitrogen application, NaCl, Na_2SO_4, *Sorghum bicolor*.

INTRODUCTION

Salinity is one of the most important environmental factors limiting crop production in arid and semi-arid (Majeed and Muhammad, 2019). Currently, about 20% of the total land area are affected by salinity (stress caused by various salts like excess sodium chloride and sodium sulphate) (Elsiddig et al., 2022). Worldwide, more than 45 million hectares of irrigation land have been damaged by salt, and 1.5 million hectares are taken out of production each year as a result of high salinity level in the soil (Ibrahim et al., 2018a). It is believed that 30% of agricultural land could be lost within the next 25 yr due to salt stress, a total loss that could reach 50% by 2050 (Hu et al., 2019).

Mineral salts are found in all irrigation water, but the composition and concentration of dissolved salts differs according to the water source and time of year (Nimir et al., 2020). Since salts can damage plant growth, it is necessary for water managers to identify the concentration and composition of irrigation water at the different times of the year (Grattan, 2006). Saline soils contain multiple types of soluble salt components, and the compositions of soluble salts in saline soils are quite different among locations (Nimir et al., 2020). Sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) salts are the most abundant in the area cultivated with sorghum in Sudan (Elsiddig et al., 2022).

Salinity stress affects all plant growth stages significantly inhibiting plant growth and decreasing crop productivity by causing osmotic pressure and ion toxicity such as Na^+ and Cl^-, as well as by reducing the absorption of essential nutrients (Elsiddig et al., 2022). However, salt stress in some areas leads to reduced sorghum growth (Nimir et al., 2020). The reduction in growth observed in many plants subjected to salinity stress often correlated with salt-induced osmotic effect, nutrient deficiency, specific ion toxicity or their combinations (Ali et al., 2021). When plants are subjected to environmental stresses, reactive oxygen species (ROS) are generated. Plants can develop various mechanisms to combat salt stress-induced oxidative stresses. One approach involves regulating enzymatic antioxidants such as peroxidase (POD), and catalase (CAT), which play important roles in eliminating ROS (Ali et al., 2019).

World agriculture is facing many challenges such as producing 70% more food for the growing population; however, crop productivity is not increasing at the same rate as the demand for food. The production of salt-tolerant variety is essential to sustain crop production in salt-prone areas. Efforts have been made to improve the salinity tolerance of a variety of crops by selective breeding techniques, but the effective development of such salt-resistant plants is time-consuming and commercial success is limited (Nimir et al., 2020).

Nitrogen is an essential nutrient in agricultural production, and its effective use to improve crop production is more than any other chemical fertilizer (Ahmad et al., 2022). Consequently, N fertilizer is an essential nutrient that has an important impact on crop growth, yield, and product quality. The proper application of N fertilizer can increase the soil's N content for better plant growth. It is understood that the adverse effects induced by saline stress can be mitigated by application of fertilizer (Ibrahim et al., 2016). Plants growing in saline soils are often N deficient. The lack of N is particularly common in crops and causes inhibition of plant growth whether plants are growing under salinity stress or natural conditions. Addition of N to N deficient soils at moderate salinity enhanced growth and yield of crops. In most cases, total N uptake decreases, but N concentration rises under optimal N conditions (Ibrahim et al., 2016).

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the five most important cereal crops grown worldwide, which is currently grown in 116 countries (Nimir et al., 2020). It is considered one of the most important cereal crops grown for human food, animal feed (grain and biomass), fermentation (methane production), fertilizer (utilization of organic), and biofuel (ethanol) (Elsiddig et al., 2022). It is more planted in arid and semi-arid regions where salt stress is often a factor restricting its productivity. In Sudan, sorghum is the most important cereal crop, which has become an important staple food (the main food for most of the Sudanese population, especially in rural areas). The majority of sorghum areas in Sudan are cultivated with the Wadahmed and Tabat cultivars (Elsiddig et al., 2022). Salinization of soil is one of the major factors limiting crop production in Sudan and these soils are subjected to salts under different concentrations and compositions (Nimir et al., 2020).

Most of the related studies on salt stress were focused on Na^+ accumulation, with relatively little research on chloride ion (Cl^-), and little attention has been paid to the accompanying anions. However, very limited information is available to ensure which salt composition (NaCl and Na_2SO_4) has the most impact on the sorghum plant during the boot stage period; this stage has proven to be most important to salt stress other growth stages. With these in mind, we designed a greenhouse experiment and used two sorghum cultivars (Wadahmed and Tabat) to investigate the effects of NaCl and Na_2SO_4 on the growth and physiological attributes. Hopefully, mitigating methods should be developed to alleviate the salt stress that sorghum plants suffer during the boot stage.

Therefore, we hypothesized that adverse effects of different salt stress could be alleviated by N fertilizer and will help to promote the physiological performance. The objective of this study was to examine the possibility of alleviating the impact of the different types of salt-on-salt tolerance of two sorghum genotypes by applying N soil amendment. We also tried to reduce the effects of both salts by using the optimum rate of N fertilizer (0-12 g N kg^{-1} soil) that can alleviate salt stress.

MATERIALS AND METHODS

A pot experiment was conducted in a greenhouse at the Experimental Farm of Yangzhou University, Yangzhou (32°39′ N, 119°41′ E), Jiangsu Province, China. In this study, the two sorghum (*Sorghum bicolor* (L.) Moench) 'Wadahmed' and 'Tabat' were provided by Agricultural Research Corporation, Sudan, routinely grown on saline soils in Sudan were selected as model cultivars (Nimir et al., 2020; Elsiddig et al., 2022). The seeds were less than 8-mo-old and had been stored in paper bags under laboratory conditions (RH 40%-60% at 15-20 °C). Before sowing, seeds were surface-sterilized with 2.5% NaClO for 3 min, and then thoroughly rinsed three times with distilled water and air-dried near to their original weight for seeding. The seeds of sorghum in this study were sown on a seedling bed containing the soil. For this study was selected the uniform seedlings at the age of 15 d after seeding. During the study, average temperature, relative humidity (RH), and cloud coverage were 31 °C, 76%, and 40%, respectively.

Soil and seedling preparation
The soil (typic Fluvaquents, Entisols) used in this study was collected from the surface of sandy loam soil (0-20 cm) of the Experimental Farm of Yangzhou University (32°39′ N, 119°41′ E), Jiangsu Province, China, in the sorghum growing season (summer 2021). The soil was air-dried and passed through a 5 mm mesh screen. Then the soil was separately spread at a thickness of about 50 mm over a piece of polyethylene sheet. Soil suspension was prepared in deionized water at a ratio of 1:2 (w/w) soil:water. The suspension was shaken and allowed to stand overnight. Thereafter, the electrical conductivity of the supernatant solution was evaluated as 0.26 dS m^{-1} with a conductivity meter (TZS-EC-I, Zhejiang Top Instrument, Hangzhou, China). The soil parameters were tested containing 1.0 g kg^{-1} total N, 12.2 g kg^{-1} organic matter, 14.1 mg kg^{-1} Bray P, and 77.3 mg kg^{-1} soil test K. The pH reading of the soil was 7.1.

Experimental design and pot and seedling preparation
The study designed as a factorial design with three factors, was arranged in a randomized completely block design with three replicates. The study consisted of three experimental factors. Main plots included two sorghums 'Wadahmed' and 'Tabat'; subplots included four different levels of N fertilizer as urea (46% N) 0, 4, 8, and 12 g N kg^{-1} soil, designated as N0, N1, N2, and N3, respectively. The sub-sub-plot included two different types of salt: 0, 0.3419 M NaCl, and 0.1408 M Na_2SO_4, designated as S0, S1, and S2, respectively. The saline soils at different types of salt were made before seedlings transfer by adding NaCl and Na_2SO_4 solutions to the non-saline soil. The control treatment of soil was made by adding tap water (0.26 dS m^{-1}). There were 72 pots in total in this study. There were 24 treatments with three replicates for each treatment. Each pot (32 cm in diameter × 28 cm in-depth, without holes at the bottom) filled with 15 kg dry soil. All pots were placed in a greenhouse according to the experimental design. The seeding date was 10 June 2021. On the 15^{th} day after seeding, four uniform seedlings were transplanted to each pot. Phosphorus fertilizer was applied twice to all treatments at an equal rate of 120 kg ha^{-1} at seedling and plant growth stages. The N and P fertilizers were applied to the soil in each pot before transplanting. Pots were weighed every 2 or 3 d to maintain soil water content at 80% field capacity. To avoid moisture stress, each pot was carefully watered by hand with 2 L water every 2 d.

Plant height, leaf area index, and antioxidant enzymes measurements
At boot stages, three plants from every pot were randomly selected and tagged, plant height (cm) were measure from a point immediately above the soil surface to the top of the plant, and then the means of height per plant were obtain. For the leaf area index (LAI), four leaves were randomly selected from the same plants, two plants were selected from each pot to measure LAI using a LAI meter (LI-3100C Area Meter; LI-COR, Lincoln, Nebraska, USA). The leaves of each treatment were sampled and immersed in liquid nitrogen for 15 min and then kept at -75 ºC to determine soluble protein content, chlorophyll content, and activity of peroxidase (POD) and catalase (CAT). Plant dry weight was measured after drying the samples in a forced-air drier at 80 °C for 72 h to constant weight by calculating water content.

Determination of biochemical parameters

The content of soluble protein was measured according to (Bradford, 1976) using bovine serum albumin as the protein standard. The content of chlorophyll *a*, *b*, and total chlorophyll was assayed according to the method of Lichtenthaler and Wellburn (1983). The activity of CAT was determined following the method of Janmohammadi et al. (2012), and the activity of POD was assayed according to the method of Xu and Ye (1989).

Statistical analysis

The experimental design was a factorial experiment with three factors and arranged as a split-plot in randomized complete block design with three replicates for each treatment. The data of each variable were subjected to ANOVA with the statistical package of MSTAT-C (version 2.00; Michigan State University, East Lansing, Michigan, USA) according to this design. When F values were significant, means were separated by the LSD test ($P \leq 0.05$).

RESULTS

Plant height, LAI, and number of leaves

The results showed that salinity, N, and variety, significantly affected the measured parameters of two sorghum cultivars on most occasions (Table 1).

Plant height declined significantly with different types of salt. At salinity level of 0.3419 M NaCl (S1) and 0.1408 M Na_2SO_4 (S2) plant height was reduced by 11.0% and 16.4%, respectively, compared with S0 (Table 2). In the interaction salinity and N treatment (S×N), plant height was improved by all N application levels as compared with control (N and S). For example, at S1 and S2 salinity levels, 4 (N1), 8 (N2), and 12 (N3) g N kg^{-1} soil increased plant height by 16.8%, 14.8%, and 13.1% and 4.3%, 5.9%, and 8.5%, respectively as compared with the control (S1 and S2) (Table 2). In Variety × Salinity interaction (V×S), both salts decreased plant height. However, there had nonsignificant difference between 'Wadahmed' at S1 and S2 and 'Tabat' for S0 and S1 treatments. Moreover, 'Wadahmed' was taller than 'Tabat' at all the salinity treatments (Figure 1a).

Leaf area index (LAI) was decreased with S1 and S2 levels by 19.3% and 29.7%, respectively, as compared with control (S0) (Table 2). At S1 and S2, all N levels had positive effect on LAI as relative with control (S1 and S2) (Table 2). At S1 and S2, N3 and N2 increased LAI by 25.2% and 18.2%, respectively, as compared with control (S1 and S2) (Table 2). In the interaction N×V, N2 and N3 increased LAI in both sorghum cultivars as relative to the control (Table 3). For N×V interaction at N2 level as compared with control (N0), 'Wadahmed' had 60.5% higher LAI than 'Tabat' (Table 3). In the V×S interaction at S2, 'Wadahmed' was 10.2% higher in LAI than 'Tabat' (Figure 1b).

Number of leaves were significantly affected by all the experimental factors and their combinations except for salinity (S), S×N, N×V, and the interaction among the three experimental factors (Table 1). In the V×S interaction, at S2 'Wadahmed' had 61.5% more leaves than 'Tabat' as relative to the control (S2) (Figure 2c). Number of leaves was decreased by both salt types (S1 and S2) (Figure 2c).

Dry weight and chlorophyll content

Dry weight (DW) was negatively affected by both types of salt. At S1 and S2, DW was decreased by 16.0% and 28.7%, respectively, as relative to control (S0). In the S×N interaction, at S1 and S2 levels, N3 level increased DW by 12.8% and 30.5%, respectively, relative to the control (S1 or S2) (Table 2). In the N×V interactions, at N1 as compared with control (N0), 'Wadahmed' was 59.8% higher in DW than 'Tabat' (Table 2). Moreover, 'Wadahmed' was improved by all N levels, however, 'Tabat' was enhanced by N1 and N3 treatments. In the S×V interaction at S2, 'Wadahmed' decreased DW by 27.3% than 'Tabat' as relative to the control (S2) (Figure 2d). At all salt levels, 'Wadahmed' was higher than 'Tabat' in DW (Figure 2d).

Chlorophyll *a*, *b*, and total chlorophyll content improved with N application. Both types of salt reduced chlorophyll a, *b*, and total chlorophyll content. As compared with the control (S0), S1 and S2 decreased chlorophyll *a*, *b*, and total chlorophyll by 18.0% and 39.6%, 17.5% and 10.6%, 7.8% and 12.7%, respectively (Tables 2 and 4). In the S×N, at S1 and S2 level, N1 and N3 increased chlorophyll *a* by 50.8% and 43.3%,

Table 1. ANOVA table for plant height (PH), leaf area index (LAI), number of leaf (NL), dry weight (DW), chlorophyll *a* and *b*, total chlorophyll, soluble protein content, activities of peroxidase (POD) and catalase (CAT) as influenced by salt type and N application of two sorghum cultivars (Wadahmed and Tabat) at boot stage. *, **, ***Significant difference at 0.05, 0.01 and 0.001 probability levels, respectively; ns: nonsignificant.

Dependent	PH		LAI		NL		DW		Chlorophyll *a*		Chlorophyll *b*		Total chlorophyll		Protein content		POD		CAT	
variables	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value
N	7720.6	87.70***	9076.0	13.66**	34.6	0.69ns	3953.9	1.72ns	23.2	0.34ns	71.30	19.36**	155.6	2.49ns	17.01	9.01*	347.7	3.87*	0.090	10.92**
Salinity (S)	1185.8	4.10*	3663.9	14.34***	208.1	7.70**	6875.9	3.39*	117.9	2.69*	61.20	6.04*	9.8	0.20ns	0.23	0.29ns	175.3	11.62***	0.007	0.43ns
N×S	1077.2	3.70*	15081.3	59.03***	38.8	1.43ns	4538.6	2.24*	194.5	4.40**	42.67	4.20**	301.9	6.23**	3.30	4.22**	235.9	15.64***	0.084	5.24**
Variety (V)	75.5	0.15ns	9145.9	20.85***	255.4	15.10***	2180.2	2.31ns	12.4	0.35ns	20.50	1.79ns	64.9	1.62ns	0.05	0.07ns	27.2	1.33ns	0.034	2.06ns
N×V	209.1	0.42ns	12942.1	29.50***	13.2	0.77ns	8612.1	9.12***	106.1	2.99ns	55.30	4.82**	213.9	5.34**	0.39	0.61ns	57.2	2.79*	0.087	5.26**
S×V	1358.1	2.69*	2381.5	5.42*	56.2	3.32*	4897.3	5.19*	211.5	5.95**	19.60	1.71ns	283.1	7.07**	0.52	0.82ns	32.3	1.57ns	0.072	4.37*
N×S×V	256.6	0.51ns	86.7	0.19ns	6.6	0.38ns	1917.0	2.03ns	38.3	1.07ns	10.90	0.95ns	67.9	1.69ns	0.66	1.04ns	41.4	2.02ns	0.034	2.03ns

Table 2. Effects of interactions between N application and salt type (control, NaCl, and Na_2SO_4) on plant height, leaf area index (LAI), dry weight (DW), chlorophyll *a*, chlorophyll *b* of two sorghum cultivars (Wadahmed and Tabat) at boot stage. Control: Unsalted treatment; NaCl: 0.3419 M; Na_2SO_4: 0.1408 M. Within the same column means followed by different letters are significantly different at the 0.05 probability level.

	Plant height (PH)			Leaf area index (LAI)			Dry weight (DW)			Chlorophyll *a*			Chlorophyll *b*		
N levels	Control	NaCl	Na_2SO_4	Control	NaCl	Na_2SO_4	Control	NaCl	Na_2SO_4	Control	NaCl	Na_2SO_4	Control	NaCl	Na_2SO_4
g kg^{-1} soil		cm						g plant^{-1}			mg g^{-1} FW			mg g^{-1} FW	
0	199.7d	177.8ef	167.0g	244.1de	197.1h	171.5j	172.4cd	144.8ef	123.0g	18.80c	15.41d	11.35e	11.74ef	9.69h	10.94fg
4	229.6a	207.6c	174.1f	268.5b	214.5f	188.4i	203.1b	146.6ef	135.7fg	25.78a	23.24b	15.45d	18.30a	13.17cd	10.97fg
8	219.8b	204.2cd	176.9ef	257.5c	238.6e	202.7g	179.5c	160.2de	157.5de	24.18ab	22.43b	17.72cd	16.99b	14.05c	10.17gh
12	230.5a	201.1d	181.2e	329.5a	246.8d	199.7gh	225.2a	163.4d	160.5de	26.48a	19.61c	16.27d	17.66ab	11.45ef	12.09de

Table 3. Effects of interaction between variety and N application on leaf area index (LAI), and dry weight (FW), chlorophyll *b* and total chlorophyll, peroxidase (POD), and catalase (CAT) of two sorghum cultivars (Wadahmed and Tabat) at boot stage. Within the same column means followed by different letters are significantly different at the 0.05 probability level.

	Leaf area index		Dry weight		Chlorophyll *b*		Total chlorophyll		POD		CAT	
N levels	Wadahmed	Tabat	Wadahmed	Tabat	Wadahmed	Tabat	Wadahmed	Tabat	Wadahmed	Tabat	Wadahmed	Tabat
g kg^{-1} soil			g plant^{-1}		mg g^{-1} FW		mg g^{-1} FW		U g^{-1} min^{-1}		U g^{-1} min^{-1}	
0	168.1e	195.4d	139.0d	147.9cd	8.42e	10.27d	25.55f	27.92e	5.95de	7.04d	0.30d	0.38c
4	241.7b	243.5b	222.1a	165.5b	12.92c	14.13b	35.26bc	33.11cc	9.68c	5.29e	0.44b	0.43b
8	269.8a	244.1b	153.0c	157.3bc	14.23b	14.09b	35.56b	31.14d	5.91de	9.62c	0.55a	0.55a
12	244.4b	232.2c	165.2b	164.7b	16.05a	14.7b	37.89a	36.19at	17.56a	14.39b	0.30d	0.39bc

Table 4. Effects of interactions between N application and salt type (control, NaCl, and Na_2SO_4) on total chlorophyll, protein content, peroxidase (POD), and catalase (CAT) of two sorghum cultivars (Wadahmed and Tabat) at boot stage. Control: Unsalted treatment; NaCl: 0.3419 M; Na2SO4: 0.1408 M. Within the same column means followed by different letters are significantly different at the 0.05 probability level.

	Total chlorophyll			Protein content			POD			CAT		
N levels	Control	NaCl	Na_2SO_4	Control	NaCl	Na_2SO_4	Control	NaCl	Na_2SO_4	Control	NaCl	Na_2SO_4
g kg^{-1} soil		mg g^{-1} FW			mg^{-1} g^{-1} FW			U g^{-1} min^{-1}			U g^{-1} min^{-1}	
0	29.74d	27.15e	25.97e	2.98e	3.68d	3.25e	5.95d	6.76d	9.06c	0.27g	0.44d	0.56b
4	34.69c	35.60bc	26.42e	4.96c	5.56b	5.97a	6.11d	9.43c	6.76d	0.41de	0.32f	0.34f
8	42.47a	37.28b	27.89de	5.60b	3.74d	5.04c	6.77d	11.70b	6.67d	0.36ef	0.50c	0.41d
12	28.34de	37.87b	40.53a	5.03c	5.86ab	5.045c	7.20d	7.29d	17.56a	0.32f	0.45d	0.65a

respectively, as relative to control (S1 and S2). Moreover, at the same levels of both salts, N2 and N3 increased chlorophyll *b* by 28.4% and 24.8%, respectively, as compared with control (S1 and S1). At S1 and S2, N3 increased total chlorophyll content by 39.5% and 56.1%, respectively as relative to control (S1 and S2) (Table 4). In the N×V interactions, at N3 level, compared with control (0), 'Wadahmed' was 90.6% and 48.3% higher in chlorophyll *b* and total chlorophyll content than 'Tabat' (Table 2). Nitrogen application improved both cultivars at all N levels. In comparison, the S×V interaction at S1 and S2, 'Tabat' was higher in chlorophyll *a* by 22.4% and 26.6% and lower in total chlorophyll content by 16.1% and 19.3% compared with 'Wadahmed' as relative to the control (S1 and S2), respectively (Table 5).

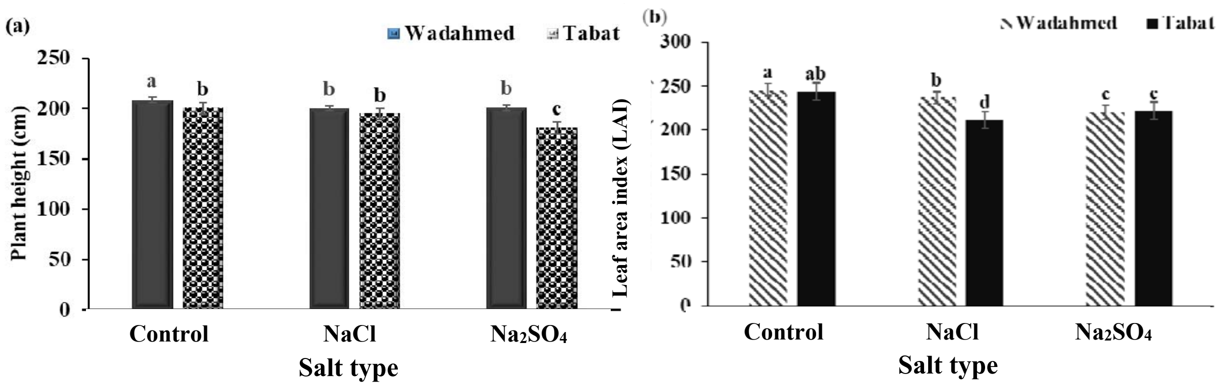

Figure 1. Effect on plant height (a) and leaf area index (b) of interaction between salt type (S) and two sorghum cultivars (Wadahmed and Tabat). Columns marked with different letters are significantly different at the 0.05 probability level. Control: Unsalted treatment; NaCl: 0.3419 M; Na_2SO_4: 0.1408 M.

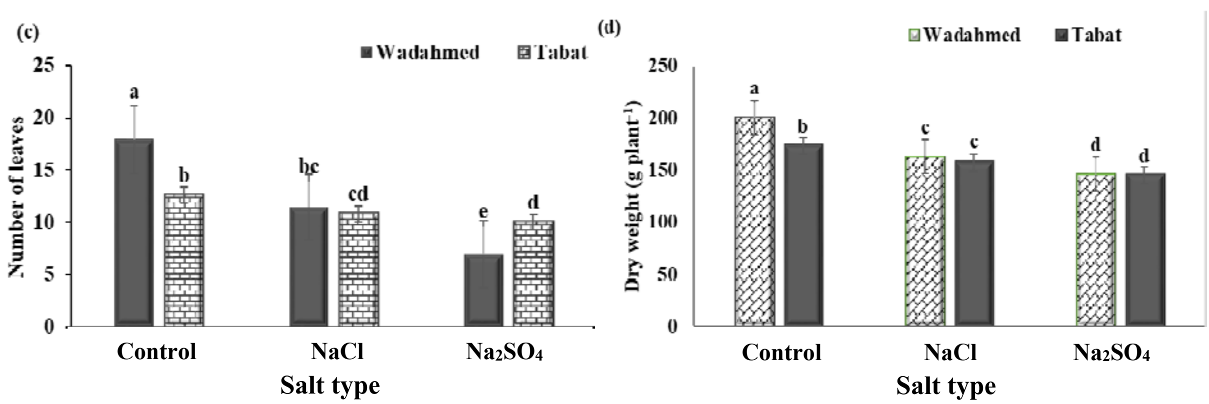

Figure 2. Effect on number of leaves (c) and dry weight (d) of interaction between salt type (S) and two sorghum cultivars (Wadahmed and Tabat). Columns marked with different letters are significantly different at the 0.05 probability level. Control: Unsalted treatment; NaCl: 0.3419 M; Na_2SO_4: 0.1408 M.

Soluble protein content, POD, and CAT activities

Protein content was significantly increased with both types of salt treatments. At S1 and S2, protein content was increased by 23.5% and 99.7%, respectively, as relative to control (S0). In the S×N, at S1 and S2 salinity level as compared with control (S1 and S2), N3 and N1 levels increased soluble protein by 56.5% and 83.7%, respectively (Table 5). In the S×N, at S1 and S2 level, N2, and N3 was increased POD and CAT by 73.1% and 93.8% and 13.6% and 16.07%, respectively, as relative to control (S1 and S2) (Table 2). Activities of POD and CAT increased significantly with different types of salt. For example, at S1 and S2, POD and CAT increased

Table 5. Effects of interaction between salt type (control, NaCl, and Na_2SO_4) and variety on chlorophyll *a*, total chlorophyll, and catalase (CAT) activity of two sorghum cultivars (Wadahmed and Tabat) at boot stage. Control: Unsalted treatment; NaCl: 0.3419 M; Na_2SO_4: 0.1408 M. Within the same column means followed by different letters are significantly different at the 0.05 probability level.

	Chlorophyll *a*		Total chlorophyll		CAT activity	
Salt type	Wadahmed	Tabat	Wadahmed	Tabat	Wadahmed	Tabat
	——— mg g^{-1} FW ———		——— mg g^{-1} FW ———		——— U g^{-1} min^{-1} ———	
Control	23.56a	24.07a	36.23a	36.00a	0.43b	0.52a
NaCl	18.64b	18.67b	35.13a	30.20b	0.41b	0.42b
Na_2SO_4	15.73c	17.68bc	30.35b	29.05b	0.36c	0.38bc

by 12.0% and 34.3% and 38.6% and 51.8%, respectively, as compared with control (S0) (Table 3). In the N×V interactions, N3 level, as compared with control (N0), 'Wadahmed' was 195.1% and 83.8% higher in POD and CAT than 'Tabat' (Table 5). In the interaction V×S, at S1 and S2, 'Tabat' was 19.2% and 26.9%, respectively, higher in CAT than 'Wadahmed' as relative to the control (S1 and S2) (Table 5).

DISCUSSION

Among abiotic stresses, salinity stress is especially critical in agriculture because it can significantly decrease crop productivity, which is one of the most measurable impacts on crop cultivation. Understanding interactions between salinity and fertilizer are of high economic value. Various studies have been carried out to determine the effect of fertilizer on plant growth under saline conditions (Ibrahim et al., 2018a). However, there is insufficient information about the effect of different salts compositions (NaCl and Na_2SO_4) treated with N during the boot stage of sorghum. Plant growth and physiological response disruptions caused by salinity have been widely studied (Ibrahim et al., 2016; Nimir et al., 2020). Our study has explained that growth parameters were negatively affected by both salt stress (Tarchoune et al., 2012).

In the present study, we found that plant height, LAI, number of leaves, and dry weight (DW) were more decreased with Na_2SO_4 than NaCl. The inhibiting effect of Na_2SO_4 in the initial increase of growth parameters is 20% stronger than for NaCl (Hasanuzzaman et al., 2020). *Prosopis strombulifera* plants were treated with Na_2SO_4 underwent structural cell and tissue alterations, resulting in changed growth patterns at different levels of the organization. Anatomical and histological differences in leaf stem and roots were observed in plants treated with Na_2SO_4 when compared with non-salinized plants (Nimir et al., 2020). The reductions in plant growth by salt may be due to the harmful impact of Na and Cl ions or due to the lower water absorption by the root system due to osmotic pressure (Ibrahim et al., 2019). These results concur with the studies by Ibrahim et al. (2018a; 2018b), who noticed a negative correlation between salinity and seedling growth.

On the other hand, in this investigation, growth parameters, plant height, LAI, number of leaves, and DW were positively affected by N treatments, which suggested that N could mitigate the adverse effect of both type of salt stress on plants. In the present study, results showed that the impact of N treatments on growth and physiological responses was positive.

The increase in plant growth under salinity stress by N was previously mentioned (Ali et al., 2021). These findings were in agreement with those of Ibrahim et al. (2019), who indicated that N application in salty soils is indicated as improving plant growth parameters. Also, similar result was reported by Amigo et al. (2021), who noticed that N fertilization increased DM production of lentils and reduce Fe and Mn concentrations on leaves to a level that is possibly under the threshold that causes toxicity in plant tissues. Furthermore, saline soil can change the role of N in plants metabolism. There is a possibility that the application of N fertilizers can alleviate harmful impacts of salinity and assist in increasing the plant growth (Murtaza et al., 2013).

In this study, different types of salt inhibited chlorophyll content in both sorghum cultivars. Moreover, chlorophyll content was reduced more with SO_4 than chloride (Cl^-). The reduction level of the chlorophyll content under salinity stress has previously been reported by Ali et al. (2020) and Mastrogiannidou et al. (2016), according to which chloride and sulfate types of salinity differ in their effect on growth, development, water balance, and a wide array of metabolic pathways, and this lead to a decrease in the chlorophyll content (Elsiddig et al., 2022). Moreover, the decrease in photosynthetic pigments under salinity stress may be due to the reduction in the uptake of minerals such as Mg and Fe, suppression of specific, the reduction of C use efficiency, increased the ethanol and lactate production and reduces the synthesis of chlorophyll (Ali et al., 2020). These results were in accordance with Jamil and Rha (2004). One possible explanation for sulfate toxicity in Na_2SO_4-treated plants is that SO_4^- anion interferes in the uptake of several essential macronutrients, causing decreased uptake and transport of Ca, Mg, K, and Fe at high salinity (Reginato et al., 2021). Another possibility is that the free sulfide could bind to cytochromes and consequently inhibit mitochondrial respiration with deleterious effects on growth. In this sense, the inhibition of cytb559 in the photosystem II (PSII) could also be possible thereby inactivating PSII resulting in a formation of ROS (Reginato et al., 2021). Besides in this study, the chlorophyll content of sorghum in N treatments was higher than other without N application. These results were in conformity with those of Zhang et al. (2013), who found that increasing levels of N increased chlorophyll content in soya bean (*Glycine max*). Similar results in wheat was observed by Vafadar et al. (2014), who founded that there was a positive relationship between N supply and the leaf chlorophyll content and biomass production (Ibrahim et al., 2018a).

The damaging effect of salt stress on plants can be recognized at all plant growth stages resulting in plant death and reduced production. Different types of salt, affect plant growth by changing the physiological processes (Ibrahim et al., 2018a; Elsiddig et al., 2022). In this research, results showed that different types of salt inhibited physiological parameter in term of soluble protein and antioxidant enzymes compared with non-saline conditions (Elsiddig et al., 2022).

In this work, soluble protein content was significantly affected by both types of salt stresses. These findings disagree with results reported by Mahboobeh and Akbar (2013); furthermore, protein content of the non-transgenic plants decreased when salinity was increased but, in 200 and 250 mM were much lower than the other salt concentrations. In contrast, the protein content of transgenic plants did not change up to 200 mM. In the latter case, the differences were nonsignificant. Also, in agreement with those who stated that, the soluble protein was considerably higher than those of plants under non-saline conditions (Ibrahim et al., 2018a), and disagreed with those who reported that salinity stress significantly reduced protein contents in plant (Irakoze et al., 2020), who suggested that soluble protein content decreased significantly by Na_2SO_4 treatments. Morales and Munné-Bosch (2019) attributed the less accumulation of protein to the decrease in roots absorbing zones due to the effect of the salinity on fresh and dry weighted plant. Moreover, protein content was increased when plants were treated with N. These findings are in agreement with Ibrahim et al. (2018a; 2018b).

Plants can develop an antioxidant defense system to cope with oxidative harm under salinity stress (Ibrahim et al., 2019). Osmotic stress leads to physiological changes in plants, including nutrient imbalance, reduced photosynthetic activity and differences in the production of antioxidant enzymes because of stomatal closure (Qamer et al., 2021). However, the specific mechanisms that cause differential tolerance in response to chloride and sulfate salts, are not yet completely understood (Reginato et al., 2021). In this study, we observed a significant increase in POD and CAT activities in leaves with both types of salt and N level. An increase in the antioxidative enzymes under salt stress could suggest an increased effect of reactive oxygen species (ROS) and improvement of a protective mechanism to decrease oxidative harm triggered by stress in plants (Ali et al., 2019). The result of ROS and improvement of a protective mechanism to decrease oxidative harm triggered by stress in plants. Catalase (CAT) in peroxisomes breaks down H_2O_2. Peroxidase in cytosol and chloroplast can correctly scavenge H_2O_2. The increase of POD activity by salt treatment in plants has also been reported by Kahrizi et al. (2012). The highest activity of CAT was observed in 'Tabat' and the lowest was in 'Wadahmed' under salt stress. Numerous researchers have also reported the increased activity of POD and CAT under salt stress in canola (*Brassica napus*) (Ebrahimian and Bybordi, 2012) and wheat (Kahrizi et al., 2012). Contrasting result was reported by Talat et al. (2017), who observed that Na_2SO_4 stress caused the reduction in CAT and POD activities in two wheat varieties. The difference results in activity of CAT and POD were probably lies on the differences in crop species (Ali et al., 2021).

In the present research, the highest POD and CAT activity was shown at the control and low (S0) salinity level with the N3 level (12 g N kg^{-1} soil). Our finding revealed that antioxidant enzymes (POD and CAT) activity increased with N treatments (Ali et al., 2021). A related result was observed by Rios-Gonzalez et al. (2002), and CAT initially increased with increasing N rate. Associated increases in the CAT has been followed by Ali et al. (2021). Fertilization with N may contribute to soil salinization and increase the negative effects of soil salinity on plant production (Ibrahim et al., 2018b). Also, the potential for NO_3 leaching may increase where moderate to high quantities of salts are present in the soils because plants under salt stress cannot absorb and use the applied N efficiently (Chen et al., 2010). Nevertheless, the interactions between N fertilization and salinity and N metabolism in plant is a highly complicated system.

CONCLUSIONS

The current study revealed that two types of salt (NaCl and Na_2SO_4) stress had various effects on the physiology and antioxidant enzymes of sorghum plants, which can improve our understanding of optimized N application to promote plant growth under salinity stress. However, the effects of two types of salt differed significantly among different measurements. The use of N fertilizer under both types of salt was determined as useful to mitigate salinity induced damages. Results showed that NaCl and Na_2SO_4 affected different growth and physiological responses in sorghum. The Na_2SO_4 salt appeared more toxic than NaCl on most of the measured parameters for both studied cultivars ('Wadahmed' and 'Tabat'). Based on these results, it is concluded that soils treated with high N (12 g N kg^{-1} soil) could have increased antioxidant enzymes and chlorophyll content under Na_2SO_4 salt type suitable rate. 'Wadahmed' was more tolerant to both types of salt compared to 'Tabat', therefore suggesting the possibility for its cultivation and suitability for the breeding program under different salt stress conditions. More investigation is needed to optimize the effectiveness of N treatments on more cultivars of sorghum.

Author contributions

Conceptualization, A.M.I.E., G-H.Z., G-G. Z.; methodology, N.E.A.E., M.S.E.S., M.E.H.I., A.Y.A.A.; software, A.M.I.E., G-H.Z.; validation, G-G. Z., N.E.A.E.; formal analysis, M.S.E.S., M.E.H.I.; investigation, A.Y.A.A.; resources, A.M.I.E.; data curation, G-H.Z.; writing-original draft preparation, A.M.I.E., G-H.Z., G-G. Z., N.E.A.E., M.S.E.S., M.E.H.I., A.Y.A.A.; writing-review and editing, A.M.I.E., G-H.Z., G-G. Z.; visualization, N.E.A.E., M.S.E.S.; supervision, M.E.H.I., A.Y.A.A.; project administration, A.M.I.E.; funding acquisition, G-G. Z. All authors have read and agreed to the published version of the manuscript.

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