RESEARCH

Effect of exogenous ascorbic acid on two sorghum varieties under different types of salt stress

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Received: 22 June 2021; Accepted: 5 November 2021; doi:10.4067/S0718-58392022000100010

ABSTRACT

Salinity is a severe environmental factor that has limited the growth and productivity of crops. Ascorbic acid (ASA) is one of the most important plant growth regulators for mitigating salt stress. A controlled experiment was conducted to evaluate the impact of different salt compositions (0, 50 mM NaCl, 50 mM Na_2SO_4, and 50 mM NaCl+50 mM Na_2SO_4, designed as S0, S1, S2, and S3, respectively) and different ASA concentrations (0, 284, and 850 µM) on the seedling growth and physiological attributes of two sorghum varieties (Wadahmed and Tabat). The present study proved significant differences between sorghum (*Sorghum bicolor* (L.) Moench) varieties under saline conditions at seedling stage and 'Wadahmed' was more tolerant to salinity stress than 'Tabat'. Both types of salt induced a strong decrease in all the parameter measured, including emergence percentage (48.4%), shoot length (42.1%), root length (39.6%), total dry weight (g) (23.5%), chlorophyll (SPAD) (43.5%), and recorded the highest value of soluble protein content, and malondialdehyde (MDA) content when compared with the non-saline treatment. At the S3 salinity level, emergence percentage, shoot and root length, total dry weight, soluble protein content, and MDA content were increased by 850 µM ASA levels. Our results found that the combination of both salts showed more damage than a single salt treatment. This study indicated that exogenous addition of ASA at an appropriate level can be a useful strategy to promote early seedling growth and improve antioxidant enzymes of sorghum plants grown under different saline types.

Key words: Ascorbic acid, NaCl, Na_2SO_4, seedling growth, sorghum, *Sorghum bicolor*.

INTRODUCTION

World agriculture is facing many challenges and 70% more food is needed to feed the increasing population on this planet. However, crop productivity is not increasing at the same rate as the demand for food. Lower productivity in most cases is attributed to various abiotic stresses (Nimir et al., 2020). Salinity is becoming an increasingly serious problem limiting crop production worldwide (Zhu et al., 2019a). Salt stress is one of the critical abiotic stresses that limiting agricultural productivity (Nimir et al., 2020). Currently, salt stress (stress caused by various salts like excess sodium chloride and sodium sulphate) affects 20% of the total land area and more than 50% of the irrigated agricultural area. 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 (Ali et al., 2021). Most saline water and saline soils are dominated by chloride or sulfate salt, but the composition and concentration of dissolved salts differs according to water source and time of a 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 a year (Ali et al., 2020). Saline soils contain multiple types of soluble salt and each one has a different effect on the initial growth of plants (Tobe et al., 2004). In addition, the composition of soluble salts in saline soils differs greatly among locations (Provin and Pitt, 2001). NaCl and Na_2SO_4 salts are the most abundant in the area cultivated with sorghum in Sudan (Nimir et al., 2020).

Salinity stress can significantly inhibit germination and seedling growth, decrease many physiological processes and ultimately reduce crop productivity by causing osmotic stress and/or ions toxicity as well as reducing the uptake of important ions such as Ca and K (Ali et al., 2021). In addition, salinity stress affects membrane damage, altered levels of growth regulators, inhibition the enzymatic and metabolic dysfunction, and decreased photosynthesis rate, which ultimately leads to plant death (Ibrahim et al., 2016). Crops can suffer salinity stress at all growth stages from germination to maturity stage, but germination and early plant growth stage are known to be more sensitive for most plant species (Ali et al., 2021).

Different strategies and techniques should be developed to mitigate the harmful effects of salinity on the growth and development of sorghum plants. Using the salt-tolerant crops is one of the most important strategies to solve the salinity problem. However, it is time-consuming, laborious, and highly dependent on existing genetic variability (Nimir et al., 2020). Seed soaking with exogenous plant growth regulators is a possible strategy that can increase the performance of emergence and physiological parameters of crops under stress conditions (Nimir et al., 2020). It is a process in which seeds are treated with one or several growth regulators at an appropriate level before they are sown. Seed priming is an alternative, low-cost, and feasible technique as compared with other agronomical practices to mitigate salt stress. By seed soaking some pre-germination metabolic processes are activated in the seed with the initiation of the imbibition process such as ATP synthesis, de novo synthesis of nucleic acids and proteins, activation of sterols and phospholipids, and repairing of DNA, induces antioxidant activity and storage protein solubilization and minimizes lipid peroxidation, which facilitate germination, seedling emergence, and early seedling growth (Hameed et al., 2014). Several plant regulators, such ascorbic acid (ASA), have been used for seed soaking under different abiotic stresses (Miceli et al., 2020). However, the effectiveness of these different soaking agents varies between crop species under different stresses (Nimir et al., 2020).

Ascorbic acid (ASA) is one of the essential plant growth regulators thought to be an important tool for mitigating damage in plants by salt stress (Akram et al., 2017). The ASA plays an important role in cell division and expansion, photosynthesis, hormone biosynthesis, regeneration of antioxidants and acts as a cofactor of many enzymes (Lisko et al., 2014); also regulates stress responses as a result of a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, and induction of stress responsive proteins synthesis (Akram et al., 2017). Several studies have shown that ASA can reduce salt stress (Saeidi-Sar et al., 2013).

Sorghum (*Sorghum bicolor* (L.) Moench) is 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) (Ibrahim et al., 2020). It is currently grown in 116 countries (Nimir et al., 2020). In Sudan, sorghum is the first grain crop cultivated in the large areas of irrigated and rain-fed schemes (Nimir et al., 2020). It is more planted in arid and semi-arid regions where salt stress is often a factor restricting its productivity. Seed priming is an alternative, low-cost, and feasible technique as compared with other agronomical practices to mitigate salt stress. However, little information is available on the effects of seed priming with ASA on sorghum during germination and early seedling growth under saline conditions.

In most studies on salt stress, was focus on Na^+ accumulation and little attention has been paid to the accompanying anions. So far, little information is available regarding the differential impacts of the two salts (NaCl and Na_2SO_4) and their combinations. In most cases, NaCl and Na_2SO_4 are mixed and pose combined stress at the early seedling stage of sorghum.

With these in mind, we designed a controlled experiment and used two sorghum varieties (Wadahmed and Tabat) that are widely planted in Sudan to investigate the effects of individual NaCl or Na_2SO_4, and their combination on the growth and physiological attributes. Hopefully, a new strategy can be developed to alleviate the salt stress that sorghum plants suffer at seedling stage. Therefore, we hypothesized that the adverse impact of different salt stress could be alleviated by ascorbic acid application. The aim of the present study was to explore the probability of alleviating different salt stress on salt tolerance of two sorghum genotypes at seedling stage by applying ascorbic acid.

MATERIALS AND METHODS

Plant materials and experimental design

The sorghum (*Sorghum bicolor* (L.) Moench) seeds (Wadahmed and Tabat) used in this study were provided by the Sudan Agricultural Research Cooperation. The seeds of two varieties were less than 1 yr old and had been stored in brown paper bags under laboratory conditions (RH 40% to 60% at 15 to 20 °C) to maintain good germination. Moreover, within each variety, the seeds were selected for the same shape, color, and size and sterilized with 2.5% NaClO solution for 3 min for disinfection, then flushed with purified water three times, and air-dried. Seed germination was tested according to the International Seed Testing Association (ISTA) rules before the experiment was started.

The study was conducted in the Joint International Research Laboratory of Agriculture and Agri-Product Safety, Yangzhou University, Yangzhou, China. It was a pot study filled with fine sands and was conducted twice. Before the start of the study, fine sands were washed and dried at 100 °C for sterilization.

The study was arranged in a factorial design with three factors: variety (Wadahmed and Tabat), salt (distilled water as control, NaCl, Na_2SO_4, NaCl+Na_2SO_4 designed as S0, S1, S2, and S3), and ascorbic acid (ASA) levels (control without ASA (CK), two ASA levels). It was arranged in a randomized complete block with three replicates for each treatment.

The salt levels included 0, 50 mM NaCl, 50 mM Na_2SO_4, and 50 mM NaCl+ 50 mM Na_2SO_4. The ASA levels included seed priming with no seed priming (CK), 284, and 850 µM ASA. The salt solutions at different levels prepared by dissolving individual NaCl, Na_2SO_4 or their mixture (NaCl+Na_2SO_4) in deionized water were added to the sand (deionized water was added to the sand as control). Before seed sowing, 50 g sorghum seeds were soaked with ASA for 12 h at room temperature in 500 mL of one of the exogenous ASA solutions. The solution was decanted off at the end of seed priming and the seeds were re-dried near to their original weight for 48 h. Unprimed seeds were kept without ASA as control (CK).

Before seeding, plastic pots (9.5 cm in diameter by 8.5 cm in depth, without holes at the bottom) were prepared and each pot was filled with 400 g dry sand. Saline sand in the salted treatments was made by incorporating NaCl, Na_2SO_4, and NaCl+Na_2SO_4 solution at different levels to the pots. The seeds in the control treatment without salt received the same treatment using deionized water.

In total, 72 pots were used in this study. Ten seeds were sown in each pot at a seeding depth of 1.5 cm. After seeding, all the pots were placed in growth chambers (Model PYX-300G-B, Yangzhou Yiwei Automatic Instrument Co. Ltd., Jiangsu, China) for 21 d at 25 °C with a photoperiod of 16:8 h and 500 mmol m^{-2} s^{-1} illumination through supplemented fluorescent light. The relative humidity was maintained at 70% and the evaporation rate was 7.6 mm d^{-1}. Full-strength Hoagland solution (25 mL) was applied twice when the sand began to dry but without visible excess water. The same amount of deionized water was added to each plastic pot every 2 d.

Observations and measurements

Emergence percentage (EP). The number of emerged seedlings of each replicate of each treatment was recorded on a daily basis. Seedlings were considered emerged when coleoptiles were visible above the substratum surface. After 10 d of seeding, EP was calculated with the following equation (Ellis and Roberts, 1981):

$$\text{Emergence percentage} = (\text{Number of emerged seeds}/\text{Number of total seeds}) \times 100$$

Seedling growth measurements. Growth parameters, including seedling shoot length, root length, and total fresh weight, were determined 3 wk after seed sowing, using five seedlings selected from each replicate of each treatment randomly. Total dry weight was determined for five plants from each pot after oven drying at 80 °C for 2 d to constant weight. Sample of leaves of seedlings (5 g) from each replicate of each treatment was sampled and carefully washed with tap water, immersed in liquid nitrogen for 15 min, and stored in a low-temperature freezer (-80 °C) for the determination of biochemical parameters.

Determination of biochemical parameters

The leaves of each seedling plant in each pot were used for SPAD determination with a chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan). The SPAD reading was recorded at the tip, middle, and base of each leaf. The average SPAD reading of the leaves of each pot was calculated. The content of soluble protein was measured according to Bradford (1976) using bovine serum albumin as the protein standard. The malondialdehyde (MDA) content was determined following the method of Zhang et al. (2007).

Data analysis

The study was repeated twice and provided similar trends. The mean of the two runs were used for statistical analysis using a statistical package of MSTATC (Abdelgadir et al., 2010) according to a 3-factorial experiment arranged in a completely randomized design. According to the design, data of each variable were subject to ANOVA table and means were separated by LSD test ($P \leq 0.05$) when 'F' values were significant.

RESULTS

Emergence percentage

Emergence percentage (EP) was significantly affected by all the experimental factors and their interactions except for ascorbic acid by variety (ASA×V) interaction and the interaction among the three experimental factors (Table 1). The EP was negatively affected by both types of salt and their combination. Compared with the unsalted treatment (S0), EP decreased gradually by 48.4%, 22.8%, and 6.8% in the treatments S3 (NaCl + Na_2SO_4), S2 (NaCl), and S1 (Na_2SO_4), respectively (Table 2). With salinity by ASA (S×ASA) interaction at S3, 850 and 284 µM ASA treatment significantly increased EP by 50.1% and 39.2%, respectively, as compared to the control (CK with S3) (Table 2). With S×V interaction, at S3 'Wadahmed' was 44.4% higher in EP than 'Tabat' (Figure 1).

Shoot and root lengths

Shoot and root lengths were significantly affected by all the experimental factors and their combinations except for the interaction among the three experimental factors (Table 1). In the interaction between S and seed priming with ASA, at S3, 850 and 284 µM ASA increased shoot and root lengths by 97.8% and 64.2%, respectively, relative to control (CK with S3). Shoot and root lengths decreased because the two types of salt and their interaction. Shoot and root lengths decreased more, by 42.1% and 39.6%, 27.9% and 22.3%, and 17.8% and 9.7% at S3, S2, and S1, respectively, as relative to control (S0) (Table 2). In the S×V interaction at S3, 'Wadahmed' had higher shoot and root lengths, 22.8% and 74.5% higher, respectively, than 'Tabat' (Figures 2 and 3). For ASA×V interaction at 850 µM ASA as compared with CK, 'Wadahmed' had 29.4% and 31.0% higher shoot and root lengths than 'Tabat' (Table 3).

Table 1. ANOVA for investigated traits of sorghum 'Wadahmed' and 'Tabat.

	Emergence percentage		Shoot length		Root length		Total dry weight		Chlorophyll content (SPAD reading)		Soluble protein content		Malondialdehyde (MDA)	
Source of variation	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value	MS	F value
Salinity (S)	3639.5	47.0***	225.9	45.74****	75.4	55.7***	0.8	101.1***	0.25	83.8***	225.6	7.9*	6.4	460.1***
Ascorbic acid (ASA)	207.3	3.0*	66.02	53.57***	55.8	43.8***	0.5	97.7***	0.20	30.2***	53.3	9.7**	0.75	53.6***
S×ASA	448.9	6.5**	6.3	5.07**	5.1	7.8***	0.1	17.9***	0.16	25.19***	93.0	16.8***	1.98	142.7***
Variety (V)	1939.6	35.1***	30.5	12.6**	3.5	3.9*	0.001	0.10ns	0.00	0.038ns	4.8	0.37ns	2.86	5.7*
S×V	458.7	8.3***	15.1	6.2**	8.3	9.4***	0.014	2.3ns	0.07	15.7***	56.3	4.27*	0.73	1.5ns
ASA×V	135.3	2.4ns	22.2	9.2**	13.8	15.6***	0.015	2.5ns	0.011	2.48ns	80.3	6.1**	2.37	4.7*
S×ASA×V	88.8	1.6ns	4.7	1.97ns	1.5	1.8ns	0.011	1.9ns	0.004	0.97ns	25.7	1.95ns	0.97	2.0ns

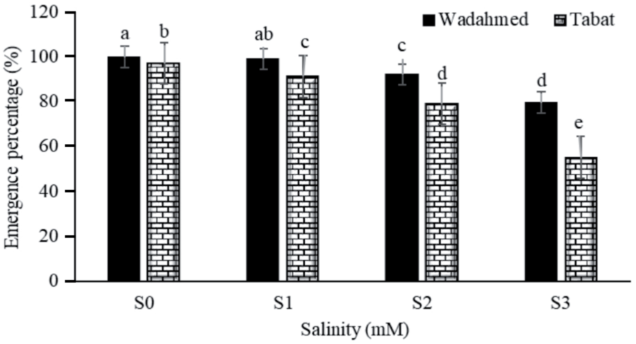
*, **, ***Significant difference at $P \leq 0.05$, 0.01 and 0.001 probability levels, respectively. Ns: Nonsignificant.

Table 2. Effect of interaction between salinity (S) and ascorbic acid (ASA) concentrations on emergence percentage, shoot length, root length, and total dry weight of sorghum 'Wadahmed' and 'Tabat'.

	Emergence percentage			Shoot length			Root length			Total dry weight		
Salinity level	CK	284	850	CK	284	850	CK	284	850	CK	284	850
mM		%			cm			cm			g	
S0	98.50ab	100.00a	95.85bc	23.26b	24.00b	18.54ef	10.13d	10.97c	14.10a	0.81e	1.15b	1.40a
S1	91.85d	95.07c	93.00cd	19.13e	20.23d	21.67c	9.15e	7.71g	11.81b	0.71f	0.94d	0.64gh
S2	76.07f	88.60e	100.00a	16.78h	17.26gh	19.07e	7.87g	9.08ef	8.77f	0.66g	0.81e	0.82e
S3	51.82h	72.13g	77.80f	13.47i	18.12fg	26.64a	6.12i	10.05d	7.29h	0.62h	0.65h	1.12c

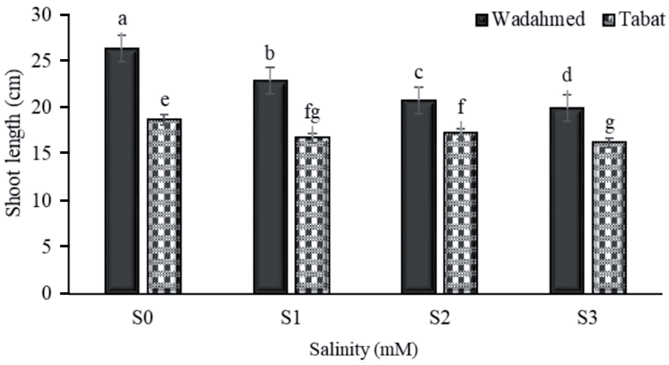
Within the same column means followed by different letters are significantly different at the 0.05 probability level.
S0: Unsalted treatment (control); S1: 50 mM Na_2SO_4; S2: 50 mM NaCl; S3: 50 mM Na_2SO_4 + 50 mM NaCl.

Figure 1. Effect on emergence percentage of interaction between salinity (S) and varieties of sorghum (‘Wadahmed’ and ‘Tabat’).



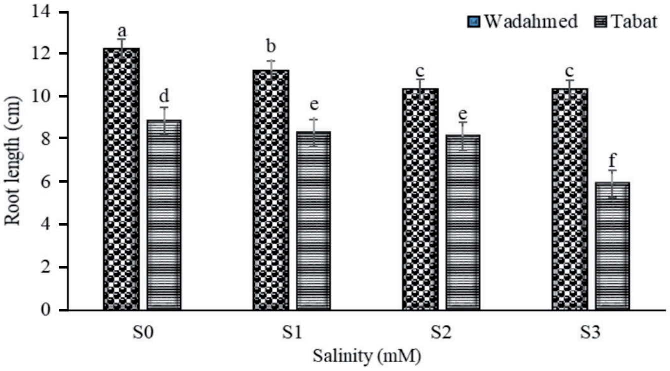
Columns marked with different letters are significantly different at the 0.05 probability level.
S0: Unsalted treatment (control); S1: 50 mM Na_2SO_4; S2: 50 mM NaCl; S3: 50 mM Na_2SO_4 + 50 mM NaCl.

Figure 2. Effect on shoot length of interaction between salinity (S) and varieties of sorghum (‘Wadahmed’ and ‘Tabat’).



Columns marked with different letters are significantly different at the 0.05 probability level.
S0: Unsalted treatment (control); S1: 50 mM Na_2SO_4; S2: 50 mM NaCl; S3: 50 mM Na_2SO_4 + 50 mM NaCl.

Figure 3. Effect on root length of interaction between salinity (S) and varieties of sorghum (‘Wadahmed’ and ‘Tabat’).



Columns marked with different letters are significantly different at the 0.05 probability level.
S0: Unsalted treatment (control); S1: 50 mM Na_2SO_4; S2: 50 mM NaCl; S3: 50 mM Na_2SO_4 + 50 mM NaCl.

Table 3. Effect on shoot length, root length, soluble protein content, and malondialdehyde (MDA) content of interaction between ascorbic acid (ASA) and varieties of sorghum (‘Wadahmed’ and ‘Tabat’).

	Shoot length		Root length		Soluble protein content		Malondialdehyde (MDA)	
ASA	Wadahmed	Tabat	Wadahmed	Tabat	Wadahmed	Tabat	Wadahmed	Tabat
µM	cm		cm		mg^{-1} g^{-1} FW		µmol g^{-1} FW	
CK	16.41c	19.93b	7.49d	9.91b	41.78c	40.60d	4.60cd	4.66bcd
284	19.91b	21.71a	9.15c	11.12a	44.70b	42.90c	4.43d	4.90b
850	21.24a	19.87b	9.86b	8.99c	46.59a	42.92c	5.54a	4.75bc

Within the same column means followed by different letters are significantly different at the 0.05 probability level.

Total dry weight

Total dry weight (TDW) was significantly affected by S, ASA, and S×ASA interaction (Table 1). Total dry weight declined more by S3 than a single of two S2 and S1 salt type. The TDW significantly decreased at S3, S2, and S1 with a reduction by 23.5%, 18.5%, and 12.3%, respectively, as compared to control at S0 (Table 2). In the S×ASA interaction at S3, 850 µM ASA, compared with control (CK with S3), TDW increased by 80.6% (Table 2).

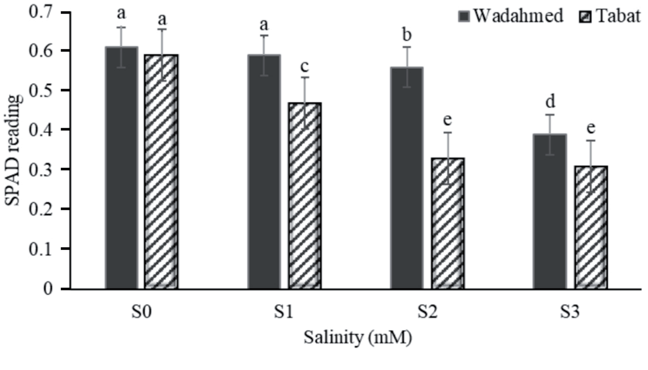
Chlorophyll content (SPAD reading)

Chlorophyll content was significantly affected by S, ASA, S×ASA interaction, and the S×V interaction (Table 1). Chlorophyll content was decreased by both salt types (S1 and S2) and their combination (S3). As relative to unsalted control (S0), chlorophyll content decreased significantly by 43.5%, 17.4%, and 15.2% at S3, S1, and S2, respectively (Table 2). In the S×ASA interaction, ASA greatly improved chlorophyll content at all salinity levels. For example, at S3 salinity level with 850 µM, ASA increased chlorophyll content by 53.8% as compared with control (CK with S3) (Table 2). Moreover, at S2 and S1, 284 µM ASA increased chlorophyll content by 107.7% and 55.3%, respectively, as relative with the non-seed soaking (CK with S2 and S1). In the S×V interaction at S3, ‘Wadahmed’ was 25.8% higher in chlorophyll content than ‘Tabat’ (Figure 4).

Soluble protein content

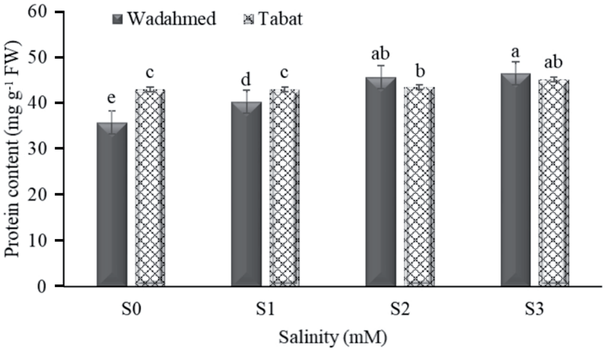
Protein content was significantly and differently affected by all the trial factors and their combinations except for variety and the interaction among the three experimental factors (Table 1). The S1, S2, and their mixture (S3) increased protein content. But it was less increased in S2 as compared with S1 and S3 (Table 2). In the S×ASA interactions, 850 µM ASA at S3 significantly increased protein content by 10.9% as relative with control (CK with S3). In the S×V interaction, at S2 and S3 there were nonsignificant differences in soluble protein content between both varieties. While at S1 salt level, ‘Tabat’ was 7.9% higher in soluble protein content as compared with ‘Wadahmed’ (Figure 5). In the ASA×V interactions at 850 µM level, compared with control (CK), ‘Wadahmed’ was 11.5% higher in protein content than ‘Tabat’ (Table 3).

Figure 4. Effect on chlorophyll (SPAD reading) of interaction between salinity (S) and varieties of sorghum (‘Wadahmed’ and ‘Tabat’).



Columns marked with different letters are significantly different at the 0.05 probability level.
S0: Unsalted treatment (control); S1: 50 mM Na_2SO_4; S2: 50 mM NaCl; S3: 50 mM Na_2SO_4 + 50 mM NaCl.

Figure 5. Effect on soluble protein content of interaction between salinity (S) and varieties of sorghum (‘Wadahmed’ and ‘Tabat’).



Columns marked with different letters are significantly different at the 0.05 probability level.
S0: Unsalted treatment (control); S1: 50 mM Na_2SO_4; S2: 50 mM NaCl; S3: 50 mM Na_2SO_4 + 50 mM NaCl.

Malondialdehyde content

Malondialdehyde (MDA) content was significantly affected by S, ASA, V, and their interactions except for the S×V and the interaction among the three experimental factors (Table 1). In the S×ASA interaction at S3, 850 μM ASA, MDA content increased by 24.0% as relative to control (S0) (Table 2). The MDA content increased gradually from S2, S1, and S3 as compared with unsalted treatment (S0) (Table 2). In the ASA×V interaction, compared with non-ASA treatment (CK), 850 μM ASA significantly increased MDA content by 20.4% in ‘Wadahmed’ than ‘Tabat’ (Table 3).

DISCUSSION

Emergence percentage

Our results showed that the EP of both varieties decreased more with a combination of two types of salt than a single salt treatment (Table 2, Figure 1). However, there were a few reports on the combined effect of NaCl and Na_2SO_4 on the growth and physiological responses of sorghum. These results agree with the report that indicated early seedling growth of two different rice cultivars were inhibited by increased NaCl concentrations (Ibrahim et al., 2016). Roy et al. (2017) pointed out that the absorption of toxic ions has been shown to affect embryo viability and cause biochemical toxic influences, including disruption of the structure of enzymes and other macromolecules, damage to cell organelles and plasma membrane, disruption of respiration, photosynthesis, and protein synthesis. Hasanuzzaman et al. (2020) indicated that the germination of three bean (*Phaseolus vulgaris* L.) and two sorghum cultivars were inhibited by increased Na_2SO_4 concentration. The decreased germination percentage could be due to the fact that Na_2SO_4 increased osmotic pressure, which slowed the seed imbibition process of sorghum; thus, seed metabolism activation and seed germination were delayed (Xie et al., 2019).

The EP of sorghum seedlings under salt stress was improved with ASA application. This treatment can reduce the deleterious effects of salt stress. Similar results were noticed by Irfan et al. (2021) in eggplant (*Solanum melongena* L.) They discovered that elevated EP in seeds soaked with ASA under salinity could be due to better oxygen absorption, enhanced α-amylase activity, and improved transfer of nutrients from cotyledons to embryos.

Seedling growth parameters

The seedlings growth characteristics, such as shoot and root length, are the most important parameters for salinity stress, because roots are the plant part that contact with the soil and absorb water from the soil and provide it to other plant parts (Ibrahim et al., 2016). Shoot and root length provide important evidence for plant response to salinity stress (Ibrahim et al., 2016), nevertheless, the reduction of root length was more noticeable as associated with shoot length. We found that shoot length, root length, and total dry weight decreased more with a combination of both salts than NaCl and Na_2SO_4 alone. The inhibiting effect of Na_2SO_4 in the initial growth of seedlings is 20% stronger than for NaCl (Hasanuzzaman et al., 2020). Xie et al. (2019) found similar results, that shoot length, root length, and total dry weight decreased more with Na_2SO_4 than NaCl. *Prosopis strombulifera* (Lam.) Benth. plants 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). Ibrahim et al. (2020) noticed that a negative correlation between salinity and seedling growth (shoot length, root length, and total dry weight) may be due to the harmful effect of salinity stress that occurs when inducing physical dehydration by osmotic impact that blocks water movement in the plants and by ion toxicity.

In this investigation, exogenous ASA has significantly increased seedling growth (root length, shoot length, and total dry weight) and ameliorative effects of salinity (Table 3, Figure 3). This is in support of earlier studies by Mittal et al. (2018) in *Brassica rapa* L. plants, who mentioned that seeds treatment with ASA has significantly improved root length, shoot length, and total dry weight. The improved seedling growth may be attributed to the ascorbic acid's antioxidant action and increased cell division/cell enlargement/or increased content of indole-3-acetic acid within the apical meristem of seedlings (Wang et al., 2019).

Chlorophyll content (SPAD reading)

The chlorophyll content is sensitive to oxidative changes due to stress, and therefore changes in chlorophyll level are seen on plants exposed to a biotic stress condition. In this study, different types of salt decreased the chlorophyll content in both sorghum cultivars (Tables 2 and 4). Chlorophyll content was reduced more under combinations of NaCl and Na_2SO_4 dominated salinity than under chloride and sulphate salinity alone. Comparing the treatments with each other, 50 Mm NaCl was the least effective treatment in reducing chlorophyll. This negative effect of salinity on chlorophyll content has previously been described by Fatemi (2014) and Ibrahim et al. (2016) according to which chloride and sulphate types of salinity differ in their effect on growth, development, water balance and a wide array of metabolic pathways and this lead to decreased on the chlorophyll content.

In the present work, chlorophyll content at early seedling growth stage of sorghum plant was promoted by ASA treatment. Similar results were reported by Gaafar et al. (2020) who noted that ASA increase the chlorophyll content under salt stress. This improvement may be attributed to the fact that ASA is an essential cofactor in photosynthetic enzymatic reactions, allowing for the efficient synthesis of gibberellins and ethylene through unique ASA-dependent dioxygenase catalyzed reactions (Mittal et al., 2018). A dissimilar result observed that ascorbic acid had nonsignificant effect on chlorophyll content (Zonouri et al., 2014). It seems that reduction in chlorophyll contents is due to the action of chlorophyllase, peroxidase and compounds of phenolics and is the result of chlorophyll degradation.

Soluble protein content

Increasing soluble protein is an important mechanism that plants use to mitigate high salt concentrations (Ghaderi et al., 2018). The results of our study show that, soluble protein content of plants subjected to different types of salt stress were greater than those of plants under non-saline conditions. These findings disagreed with the results reported by Lo'Ay and El-Khateeb (2018) 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. Also, in agreement with Hameed et al. (2015) findings, the highest soluble protein content was observed in plants grown at higher NaCl levels. This higher protein content might be attributed to their property as osmolytes in maintaining the osmotic imbalance (Ayala-Astorga and Alcaraz-Meléndez, 2010).

Table 4. Effect of interaction between salinity (S) and ascorbic acid (ASA) concentrations on chlorophyll content (SPAD reading), soluble protein content, and malondialdehyde (MDA) of sorghum 'Wadahmed' and 'Tabat'.

	Chlorophyll			Soluble protein content			Malondialdehyde		
	0 µM ASA	284 µM ASA	850 µM ASA	0 µM ASA	284 µM ASA	850 µM ASA	0 µM ASA	284 µM ASA	850 µM ASA
	SPAD reading			mg^{-1} g^{-1} FW			µmol g^{-1} FW		
S0	0.46d	0.55c	0.83a	39.18h	33.33i	41.50f	3.87i	4.10h	4.70f
S1	0.38f	0.59b	0.41e	44.42d	43.80d	45.48c	5.00c	5.03c	4.80e
S2	0.39ef	0.81a	0.40ef	42.94e	46.97b	40.53g	4.50g	4.10h	4.90d
S3	0.26h	0.30g	0.40ef	46.42b	42.94e	51.46a	5.30b	4.90d	6.57a

Within the same column means followed by different letters are significantly different at the 0.05 probability level.
S0: Unsalted treatment (control); S1: 50 mM Na_2SO_4; S2: 50 mM NaCl; S3: 50 mM Na_2SO_4 + 50 mM NaCl.

The results of our study show that soluble protein content was increased by ASA application. An increase in protein content in response to ASA treatment on common bean plants was observed by Saeidi-Sar et al. (2013). The increased protein content by ASA can be related to a decline in reactive oxygen species (ROS) damaging the essential proteins and/or nucleic acids, as well as increased activity of antioxidant enzymes in a salt stress environment (Akram et al., 2017).

Malondialdehyde content

Malondialdehyde (MDA) is a natural and end product occurring in membrane lipid peroxidation and considered to be an indicator of oxidative damage under salt stress (Zhu et al., 2019b). In the present study, MDA content was increased with both types of salt. A similar result was observed by Xie et al. (2019) and Ali et al. (2021), who noted that MDA content was higher in sulfate-treated plants than in control. This observation could be related to the fact that Na_2SO_4-treated plants more efficiently trigger antioxidant response than plants exposed to chloride toxicity. When salt NaCl was applied, MDA content was substantially higher than the control in sorghum plants. Similar results were reported by Nimir et al. (2015), who demonstrated that MDA content was increased by an increase in salinity in sweet sorghum and sunflower respectively. The increased MDA content under saline stress is the response to the alterations in structure and composition of plasma membrane lipids. In this process, the degree of free fatty acids and free sterols are increased, which subsequently leads to a decrease in the fluidity of cell membrane (Krupa-Małkiewicz et al., 2019).

In this study, MDA content was stimulated with ASA treatment; ASA appears to play a key role against oxidative stress by decreasing accumulation of ROS and preventing lipid peroxidation, which was induced by salinity. These alleviating effects of ASA were highly correlated with the increasing activities of antioxidant enzymes (Hameed et al., 2015). The ASA may directly interact with the components of plasma membranes to protect membrane structure and properties. Therefore, it could be proposed that the inhibition of salt-induced increases in lipid peroxidation by ASA may stabilize plasma membrane structure and maintain its properties and functions (Conklin and Barth, 2004). Our result is in disagreement with Saeidi-Sar et al. (2013), who mentioned that MDA content had significantly decreased when ASA is applied on sorghum plant under salt stress. The different results between these two studies probably lies in the difference in crop species (Ali et al., 2021).

CONCLUSIONS

Ascorbic acid (ASA) has rarely been used for improving sorghum salt tolerance under different salinity types. These results reveal that ASA application at appropriate levels under different salinity has been determined to be useful to mitigate salinity induced damages. Based on these results, it is concluded that sorghum seed soaking with 850 μM ASA could be the most suitable concentration for promoting seedling growth of sorghum, improving stress resistance, and effectively alleviating the inhibition of NaCl and Na_2SO_4 salts. Results presented here show that NaCl, Na_2SO_4, and their combination salinity affected different growth and physiological response in sorghum. The Na_2SO_4 treatment was more detrimental than NaCl treatment. Also, combination $NaCl+Na_2SO_4$ at both concentrations appeared more toxic than a single salt treatment for seedling growth and physiological parameters. Thus, a combination of different salt stress indices helps in the selection and identification of stable sorghum varieties for similar salt-affected areas. Identifying and selecting the most salt-tolerant varieties of species is very important for agriculture. The results of this study indicate that 'Wadahmed' was more tolerant (better performance) to both types of salinity (50 mM NaCl + 50 mM Na_2SO_4) compared to 'Tabata', therefore suggesting the possibility for its cultivation and suitability for breeding program under different salt stress conditions. Furthermore, more investigations are needed to optimize the effectiveness of ASA and apply different concentrations of ASA on more varieties of sorghum under saline conditions.

ACKNOWLEDGEMENTS

This study was financially supported by China National Key R & D Program (2018YFE0108100) and Jiangsu Provincial Forestry Science and Technology Innovation and Extension Program (LYKJ (2019)47).

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