Humic Acid and Jasmonic Acid Improves the Growth and Antioxidant Defense System in Salt Stressed - Forage Sorghum Plants

Adam Yousif Adam Ali
Yangzhou University
Muhi Eldeen Hussien Ibrahim
Sudan University of Science and Technology
Guisheng Zhou (adamyousif537@gmail.com)
Yangzhou University
Aboagla Mohammed Ibrahim Elsiddig
Yangzhou University
Xiurong Jiao
Yangzhou University
Guanglong Zhu
Yangzhou University
Tian Yao meng
Yangzhou University
Irshad Ahmed
Yangzhou University
Ebtehal Gabralla
Yangzhou University

Research Article

Keywords: Abiotic stress, salinity, jasmonic acid, humic acid, forage sorghum

Posted Date: May 7th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-490134/v1

Abstract

Salinity is one of the primary abiotic stresses that cause several negative physiological and biochemical changes due to the oxidative stress caused by the generation of ROS. The effect of foliar application of jasmonic acid (JA) and humic acid (HA) as a fertilizer on growth and biochemical attributes exposed to salinity stress was investigated. Soil treated with NaCl at levels of 0 (S0), 2 (S1), and 4 g NaCl kg-1 dry soil (S2) and fertilized with 0 (HA0), 3 (HA1), and 6 g HA kg-1 dry soil (HA2). The plant spray with three JA levels (0, 5, and 10mM JA). Under salinity, JA and HA significantly improved all parameters tested. Salinity stress increased carotenoid, soluble protein content, SOD and MDA. In contrast, salinity stress reduced plant height, leaf area index, relative growth rate, proline content, POD, CAT, and APX. Under S2, HA2 rate increased plant high (9.69%), relative growth rate (70.79%) and CAT (45.47). While, HA1 increased leaf area index (12.45%), chlorophyll content (22.32%), carotenoid contents (38.05%), SOD (20.93), MDA (17.95%), POD (24.64%) and APX (21.67%). At S2, the highest plant height, chlorophyll content, soluble protein content and APX value recorded at 5mMJA, while, the highest value of leaf are index, carotenoid contents, proline, MDA, POD and CAT was achieved at 10mMJA. This study revealed that the level of 10 mM JA and HA1 had a positive effect on forage sorghum plants physiological responses. Furthermore, the results showed that jasmonic acid and humic acid successfully mitigated salinity stress's adverse effects.

Core Idea

- Plant growth and development associated physiochemical changes were studied in forage sorghum.
- Antioxidant enzyme demonstrated the importance of salts in plant growth and development.
- Activity of antioxidant enzymes combated the adverse effects of stress during plant growth.

1. Introduction

Salinity stress is one of the abiotic stress, accumulation of salt stress in plants when passes the different level, resulting in toxicity in plants and led to many changes of physiological and morphological [1,2]. Sodium chloride is essential for structural and functional parts of the vital machinery of the plant cell. The requirement of NaCl for the plant is very low for normal growth and development. Unfortunately, plants find an ample supply of NaCl through their roots from the soil and accumulated in the system, causing stress and triggering specific physiological responses [3]. Salinity stress affects all plant growth stages. The root zone is known to be more sensitive to salinity, caused significantly inhibit root elongation, and ultimately reduce crop yield by causing osmotic stress and ion toxicity such as Na^+ and Cl^-, as well as by reducing the absorption of essential nutrients such as Ca^{+2} and K^{+}[4,5].

Reactive oxygen species (ROS) are very harmful to the plant growth and development of most organisms as they affect the structure and function of biomolecules [6,7]. Anti-oxidative enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) remove H_2O_2 to hydrogen peroxide and dioxygen [6]. The activity of SOD, CAT, and POD increase during biotic and abiotic stresses to protect cells from the potentially hazardous effects of ROS [8]. CAT efficiently scavenges H_2O_2 and does not require a reducing substrate to perform the task [6,7]. CAT was localized in leaf tissue in peroxisomes to scavenge the H_2O_2 produced by glycolate oxidase. CAT isozymes differ in biochemical properties and developmental specificity, some related to germination, but their principal role involving probably fatty acid conversion. In contrast, others were related to lignification, photorespiration, or the aging process [9]. MDA was produced when polyunsaturated fatty acids in the membrane undergo peroxidation [10].

Jasmonic acid (JA) is a lipid-derived plant hormone that mediates diverse biological phenomena. It is a member of plant growth regulators named jasmonates, which are important cellular regulators involved in several developmental processes such as seed germination, root growth, fertility, fruit ripening, and senescence. Jasmonate (JAs), including methyl jasmonate (MeJA) and jasmonic acid (JA), stimulates MDA accumulation and inhibition of the Fe-induced release of chelators, intracellular chelators counteract the salt stress, and it suggests that MeJA trigger some other protective mechanisms [11]. [12] observed that high JA levels in wounded leaves caused significant changes in the protein pattern of rice plants. Since JAs are the mediator of cellular responses in defense or stress [13]. Ascorbic acid (ASA) is an essential antioxidant, which protects the plants under oxidative stress [6]. Exogenous JA application increases and may regulate ASA metabolism in different plants [13]. [14] reported that ASA increased due to wounding, which led to improved stress resistance. Likewise, [15] also demonstrated enhanced accumulation of AsA *Arabidopsis* by wounding. MeJA enhanced ASA in *Arabidopsis* and tobacco suspension cells and influenced ASA's metabolism [16]. Soares et al. (2010) have demonstrated gradual accumulation of H_2O_2 in *Ricinus communis* (L) and a sharp formation of ROS at the initial moment of MeJA and ascribe ditto the decrease in the enzymatic antioxidants activities. In another study with pomegranate (*Punicagranatum* L.), [18] observed an increase in antioxidant activity in the foliage treated with JAs. [16] found that in the treatment of plants with JAs application, APX activity was upregulated in tobacco plants, and less membrane damage of barley plants. In addition to its role in plant growth and development, jasmonate has been proposed as a critical regulator of plant responses to salinity [19].

Forage sorghum (*Sorghum bicolor* (L.) Moench) is an essential crop for fresh fodder, hay, and silage. And is becoming increasingly important in many regions of the world [20] because of its high productivity and high nutritive value, and it can adapt to different environmental conditions, especially in arid and semi-arid areas [21]. Sorghum is considered a moderate tolerance to soil salinity [22]. Due to the progressive salinization of the world arable lands, the exogenous hormone protectants to mitigate salt-induced damages has been more important than ever. However, to our knowledge, there are

no reports on the effects of exogenous application of jasmonic acid and humic acid application in forage sorghum on soluble protein, proline content, total chlorophyll content, carotenoid and ant oxidative enzymes parameters of forage sorghum plants subject to salinity. Here, we examine the possibility of mitigating salinity stress by using exogenous JA and HA applications. Therefore, the present study was done to assess the interactive effects of salinity, JA, and HA on chlorophyll *a* and *b*, carotenoid, protein, proline, and ant oxidative enzymes parameters of forage sorghum.

2. Materials And Methods

2.1 Location and climate of the experimental site

A two-year pot experiments were conducted in a greenhouse in two consecutive years (2018 and 2019) at the experimental farm that belongs to the Yangzhou University, Yangzhou, Jiangsu Province, located in west China (lat. 32°39'N ; long. 119°41'E). Data of monthly, the average temperature was 31°C, the minimum relative humidity was 76%, and cloud cover was 40%, day/night cycle of 16 h/8 h, at 35 1C/26 1C, respectively and a light intensity of 200 mmol photons m^2 s^1 were obtained from the weather station located at the experimental farm.

2.2 Soil characteristics

The soil collected from the surface of sandy loam soil (0-20 cm) of the Experimental Farm of Yangzhou University. The soil air-dried and passed through a 10 mm mesh screen. The soil was then separately spread at a thickness of about 70 mm over a piece of polyethylene sheet. The soil suspension was prepared in deionized water at a ratio of 1:2 (w/w) soil: water. The suspension shaken and allowed to stand overnight. After that, the electrical conductivity of the supernatant solution was determined at 0.26 dS/m using a conductivity meter. The soil presented a sandy loam texture with a pH of 7.1, 12.2 g/kg of organic matter, 1.0 g/kg of total N, and 14.1 mg/kg $Bray^{-1}$ and 77.3 mg/kg of P and K, respectively.

2.3 Plant material

Pot performance of the forage sorghum seeds variety Abu sabeein adapted to Sudan conditions, kindly donated from the Agricultural Research Corporation (Madani, Sudan), were evaluated in a randomized complete block design with three replications. Seeds were less than eight months old and had been stored in paper bags under laboratory conditions (RH 40–60% at 15-20°C). Seeds were surface-sterilized with 3% sodium hypochlorite solution for 1 min and then thoroughly rinsed three times with deionized water and air-dried near to their original weight for seeding.

Seeds were germinated on 10^{th} May in 2018, and on 15^{th} May in 2019, on seedbed for 15 days at the greenhouse. The strongest and uniform seedlings selected and transferred into pots (32 m × 45 m). The crop was fertilized at the rate of 150 kg ha^{-1}, as NH_4NO_3 (nitrogen), 100 kg ha^{-1} as P_2O_5, 100 kg ha^{-1}, as K_2O (phosphorus and potassium), and 0.5 kg ha^{-1}, as $ZnSO_4$ (zinc) were applied before planting. Another half dose used on the 45^{th} days of transplanting. Furthermore, the plants were watered regularly to maintain the water level every three day with a tap water. According to local recommendations, conducted the spray of pesticides and weed control. All required approvals were obtained for the study, which complied with all relevant regulations.

2.4 Experimental treatments

The study consisted of three experimental factors, three salinity levels, three humic acid rates, and 3 levels of jasmonic acid. Three different humic acid rates supplied as fertilizer, including 0, 373.21, and 746.42 kg HA ha^{-1} were combined with 3 different NaCl levels including 0. 2 and 4 g NaCl kg^{-1} dry soil (0.26, 2.3, and 4.7 dS m^{-1} respectively) making a 9 different treatments solutions was added to the non-saline soil before seedling transferred. The control treatment of soil (without salinity and humic acid) was created by adding tap water (0.26 dSm). The different humic acid rates and salinity levels were chosen based on previous seedlings. Before salinity and humic acid treatment, a 100 g soil sample was collected and oven-dried at $75^{o}C$ to constant weight, and the moisture content was calculated.

For the jasmonic acid, on the 20th day after seedling transferred, the plants at each NaCl level and humic acid rates were treated with exogenous jasmonic acid solutions (0, 5, and 10 mM) as a foliar application. The spraying was then repeated every 15th day. During jasmonic acid application, care was taken to avoid any drift of different levels using a plastic shelter to separate each treatment.

2.5. Observations and measurements

2.5.1. Plant height (cm) and leaf area index

Three plants from every plot will randomly select and tagged, plant height will measure from a point immediately above the soil surface to the top of the plant, and then the mean of height per plant will obtain in cm. Four leaves will randomly select the same plants, two plants selected from each plot

to measure leaf area using a leaf area meter.

2.5.2. Relative Growth Rate (RGR):

The RGR was calculated according to [23]:

$$\text{Relative Growth Rate} = \frac{\text{Plant height at time two} - \text{Plant height at time one}}{\text{Time two} - \text{Time one}}$$

2.5.3. Preparation of enzyme extracts

After 45 days of the seedling transfer, the leaves harvested and immersed in liquid nitrogen for 20 min and stored in a low-temperature freezer to determine enzyme activity, proline, and protein content. Leaf protein extract was made by sodium phosphate dibasic dehydrate and sodium phosphate monobasic dehydrate to makes a phosphate buffer solution. 0.2 g of fresh weight tissue with 2 ml of the phosphate buffer solution crushed, and the slurry centrifuged for 20 min at 4°C. The supernatant, which contained enzyme activity, used as the enzyme source.

2.5.4. Soluble proteins contents

Protein contents were estimated for each extract [24]. A dye stock solution added to the earlier centrifuged samples and incubation at room temperature for 25–30 min. The absorbance of the reaction mixture recorded at 595 nm.

2.5.5. Proline contents

Proline contents were estimated using the protocol of [25]. Fresh samples (0.5g) extracted with sulfosalicylic acid, and the extract filtered to separate the residue. All the filtrates mixed with acidic ninhydrin, orthophosphoric acid, and glacial acetic acid and incubated at 100°C for 30 min. The mixtures cooled, incorporated in toluene, and vortexed. Absorbance recorded at 520nm with a spectrophotometer.

2.5.6. Determination of physiological parameters

The activity of SOD and CAT was determined following the method of [26]. The POD activity was assayed according to the method of [27]. The MDA content was determined following the method of [28]. The APX content was measured according to [29].

2.5.7. Total chlorophyll content and carotenoid content

The determination of chlorophyll *a* and *b* and carotenoid content pigments were created according to the method reported by [30]. Each fresh leaf sample was soaked in acetone solution (80%) in the dark. The extracted samples centrifuged. The absorbance recorded at 453, 645, and 663 nm using a spectrophotometer.

2.6. Experimental design and statistical analysis

The study consisted of three experimental factors, three NaCl levels; 0.26 (S0), 2.3 (S1), and 4.7 dS m^{-1} (S2), three levels of humic acid (0, 373.21, and 746.42 kg HA ha^{-1}) designed as HA0, HA1 and HA2 respectively, and 3 levels of jasmonic acid at 0, 5, and 10 mM JA. The experiment designed as a factorial design with three and arranged in a randomized completely block design (RCBD) with three replications for each treatment. There were 81 pots in this study. All pots placed in a greenhouse according to the experimental design.

The data of each variable subjected to analysis of variance (ANOVA) for the factorial RCBD with the statistical package of MSTATC according to this design [31]. When *F* values were significant, means were separated by the least significant difference (LSD) test ($P \leq 0.05$) of probability as described by [32].

3. Results

The results revealed that jasmonic acid, humic acid, salinity, and their interaction significantly affected measured parameters on most occasions (Table 1).

Table 1

Analysis of variance for effects of jasmonic acid, humic acid, salinity and their combination on growth parameters total chlorophyll content carotenoid contents, protein, proline, and antioxidative enzymes activities of forage sorghum.

	F value											
	Plant Height	Leave area index	Relative growth rate	Total chlorophyll	Carotenoids contents	Protein Content	Proline	SOD	POD	CAT	MDA	APX
Salinity (S)	74.24***	66.87***	16.1***	20.10**	6.9*	7.97*	5.28*	1.56^{ns}	22.44**	0.29^{ns}	11.6*	25.9**
Humic Acid (HA)	22.6***	120.7**	0.1^{ns}	10.31**	12.3**	5.37*	12.56**	0.88^{ns}	2.4^{ns}	4.11*	48.0***	6.34*
S×HA	7.91**	10.95**	7.5***	5.99*	20.6***	2.36^{ns}	2.18^{ns}	4.06*	11.62**	6.44**	19.8**	2.6*
Jasmonic Acid (JA)	342.2**	17.09*	12.1*	25.09**	80.5***	3.30*	14.83**	8.11**	2.3^{ns}	40.96***	9.46*	23.8**
S×JA	246.0***	10.98***	4.6*	8.60*	41.3***	0.68^{ns}	6.41**	1.74^{ns}	3.32*	6.23***	13.1**	4.0**
HA×JA	5.37**	11.24^{ns}	2.5*	10.11*	14.2***	2.28*	0.88^{ns}	2.69*	2.21*	8.33***	2.44^{ns}	6.5^{ns}
S×HA×JA	0.85^{ns}	1.90^{ns}	0.9^{ns}	1.14^{ns}	3.2^{ns}	0.99^{ns}	0.37^{ns}	1.67^{ns}	0.95^{ns}	0.63^{ns}	8.21^{ns}	0.84^{ns}

ns = no significant difference. *, **, *** = Significant differences at $P \leq 0.05$, 0.01 and 0.001 probability level respectively.

3.1. Growth parameters as affected by the combination between salinity and humic acid

Growth traits such as plant height (PH), leaf area index (LAI), and relative growth rate (RGR) significantly decreased slightly with progression of soil salinity concentration. Without humic acid (HA0), high soil salinity concentration of S2 was reduced the plant height by 9.10% (Fig 1a), leaf area index by 20.01% (Fig. 1b), and relative growth rate by 44.66% (Table 2), in comparison to control (S0) at HA0. The growth parameters was improved by humic acid. At S2, high rate of humic acid of HA2 was increased a plant height by 9.69 (Fig 1a), and relative growth rate by 70.79 (Table 2), in comparison to HA0 (Fig. 1a). At the same salinity level, HA1 rate was increased the leaf area index by 12.45% (Fig. 1b), in comparison to HA0 (Fig. 1b). At the S1, HA1 rate was achieved the highest plant height value (Fig. 1a), leaf area index value (Fig. 1b), and relative growth rate value (Table 2).

3.2. Growth parameters as affected by the combination between salt and jasmonic acid

Regarding the combination between NaCl and JA. Jasmonic acid had the positively effect and improved the PH, LAI and RGR. Both jasmonic acid levels had increased growth parameters. According to the results, at high saline concertation of S2, both jasmonic acid levels of 5 mM and 10 mM, had significant increased on plant height by 23.63 and 14.20 % respectively(Fig. 2a), and leaf area index by 10.07 and 14.71% respectively (Fig. 2b), in comparison to salinity control. Under S1, 5mM was increased plant height from 125.55 to 156.16 cm (Fig 2a), and leaf area index from 231.25 to 251.88 cm^2 (Fig 2b).

Table 2

Relative growth rate and APX of forage sorghum [*Sorghum bicolor* (L.) Moench] variety Abu Sabeein as effected by the combination between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m^{-1}] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha^{-1}].

Salinity Levels	Humic Acid Rates	Relative Growth Rate	APX ($U g^{-1} min^{-1}$)
S0	HA0	3.65 ± 0.48cd	51.68 ± 18.53d
	HA1	4.45 ± 1.20ab	91.66 ± 38.52a
	HA2	4.65 ± 1.28a	77.81 ± 24.00b
S1	HA0	3.02 ± 0.81e	46.27 ± 8.87g
	HA1	4.27 ± 1.12b	67.96 ± 31.60e
	HA2	3.85 ± 0.81c	72.27 ± 19.70c
S3	HA0	2.02 ± 0.69f	39.72 ± 14.70h
	HA1	3.00 ± 1.19e	54.79 ± 12.13f
	HA2	3.45 ± 1.49d	33.35 ± 14.55i
Note: All analyzed data are expressed as mean ± SD values of 3 biological replicates per treatment. The means with the same letters indicate non-significant differences at $p \leq 0.05$ between parameters in the same column.			

3.3. Chlorophyll content, carotenoid content and soluble protein content and proline content total as affected by the combination between salinity with jasmonic acid

Total chlorophyll content significantly decreased with progression of soil salinity concentrations. However, increased salinity stress was significantly increased carotenoid content. Without humic acid (HA0), high soil salinity concentration of S2 was decreased the total chlorophyll content by 43.43% (Fig. 2a) in comparison to S0 at HA0. Moreover, at the same rate of humic acid (HA0), S2 showed the highest carotenoid content value (2.05 mg g^{-1} FW), while S0 showed the lowest value (1.65 mg g^{-1} FW) (Fig. 2b). The total chlorophyll content and carotenoid content was improved by humic acid. At S2, HA1 rate was increased the total chlorophyll content by 22.32 % (Fig. 3a), and carotenoid content by 38.05% (Fig. 3b), in comparison to control (Fig. 1b). At the S1, the highest value of total chlorophyll and carotenoid recorded at the HA2 (Fig2a and b respectively).

3.4. Soluble protein content, proline content chlorophyll content and carotenoid as affected by the combination between jasmonic and salinity

Soluble protein content and proline content significantly decreased slightly with increased soil salinity concentrations. Without jasmonic acid (0 mM), high salinity concentration of S2 was reduced the proline content by 54.64% (Fig. 4c), in comparison to control (S0) at HA0. Moreover, at the same rate of jasmonic acid (0 mM), S2 increased soluble protein content by 26.18% (Fig. 4d). Regarding the interaction between salinity and jasmonic acid. Jasmonic acid had the positively effect and improved the soluble protein content, proline content, total chlorophyll content and carotenoid content. Both jasmonic acid levels had increased on these parameters. According to the results, at high saline concertation of S2, 5 mM level had highly significant increased on chlorophyll content and soluble protein content by 48.21% (Fig. 4a) and 4.52% (Fig. 4c), in comparison to salinity control. At the same salinity level, 10 mM increased carotenoid content by 10.67% (Fig. 4b), and proline content by 58.50% (Fig. 4d).

3.5. Jasmonic acid and humic acid improvement the growth parameters and chlorophyll content

Plant height, relative growth rate, chlorophyll content and carotenoid were affected by interaction between jasmonic acid and humic acid rates. According to the results, forage sorghum plants treated with a HA2 and spray with 5 mM JA was achieved the highest value of plant height (263.56 cm) and relative growth rate (5.28). However, treatment of HA1 + 5 mM was recoded the highest an average value of chlorophyll content (3.53 mg g^{-1} FW) and carotenoid (1.77 mg g^{-1} FW) (Table 3).

Table 3

Plant height, relative growth rate, chlorophyll content and carotenoid of forage sorghum [*Sorghum bicolor* (L.) Moench] variety Abu Sabeein as effluence by the interaction between different jasmonic acid levels [0 mM JA, 5mM JA and 10 mM JA as a foliar application] with different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha^{-1}].

Humic acid	Jasmonic acid	Plant Height (cm)	Relative Growth Rate	Chlorophyll Content (mg g^{-1} FW)	Carotenoid (mg g-1 FW)
HA 0	0 mM	193.39 ± 18.92e	2.79 ± 0.43ed	2.33 ± 0.43e	0.96 ± 0.14ef
	5 mM	226.93 ± 20.47d	4.54 ± 0.78b	2.77 ± 0.56d	1.14 ± 24cd
	10mM	178.90 ± 27.46f	3.91 ± 0.54c	2.80 ± 0.38cd	1.13 ± 0.16cd
HA1	0 mM	231.38 ± 29.16d	2.63 ± 0.41d	2.81 ± 0.74cd	0.81 ± 0.14f
	5 mM	227.99 ± 32.64d	3.79 ± 0.98c	3.53 ± 0.23a	1.77 ± 1.25a
	10mM	192.41 ± 22.67e	2.86 ± 0.21d	3.03 ± 0.64bc	1.23 ± 0.24c
HA2	0 mM	254.30 ± 27.99b	3.94 ± 0.85c	2.74 ± 0.59d	1.00 ± 0.18de
	5 mM	263.56 ± 22.05a	5.28 ± 0.65a	2.99 ± 0.42bc	1.51 ± 0.33b
	10mM	240.37 ±27.68c	4.99 ± 0.65ab	3.21 ± 0.59b	1.20 ± 0.20c
Note: All analyzed data are expressed as mean ± SD values of 3 biological replications per treatment. The means with the same words indicate significant differences at $p \leq 0.05$ between parameters in the same column.					

3.6. Antioxidants enzyme as affected by the salinity and humic acid

Salinity stress was significantly reduced antioxidants enzyme except SOD and MDA. However, increased salinity stress was significantly increased SOD and MDA content. Without treated the plant with humic acid (HA0), high salinity stress of S2 was decreased the POD, CAT, and APX by 33.84% (Fig. 5c), 42.07% (Fig. 5d) and 36.44% (Table 2) respectively, over the control (S0). Moreover, at the same rate of humic acid (HA0), as compared with S0, S2 was increased SOD from 14.34 to 16.96 U g^{-1} min^{-1} (Fig. 5a) and MDA from 5.67 to 9.75 U g^{-1} min^{-1} (Fig. 5b). The antioxidants enzyme was enhanced and increased by humic acid application. At S2, the medium rate of humic acid of HA1 was increased a SOD by 20.93% (Fig. 5a), MDA by 17.95% (Fig. 5b), POD by 24.64% (Fig. 5c), and APX by 21.67 (Table 2), in comparison to control (HA0). At the same salinity level, HA2 rate was increased the CAT by 45.47% (Fig. 5d) over the control.

3.7. Antioxidants enzyme as affected by the combination between JA and salt stress

Regarding the combination between salinity and jasmonic acid. Jasmonic acid had the positively effect and improved the antioxidants enzyme. Both jasmonic acid levels had increased on antioxidants enzyme activities. According to the results, at S2, 5 mM and 10 mM jasmonic acid levels had significant increased on MDA by 20.74 and 43.17% respectively (Fig. 6a), POD by 29.67 and 47.54% respectively (Fig, 6b), CAT by 22.61 and 51.78% respectively (Fig. 6c), and APX by 67.84 and 22.57% respectively (Fig. 6d), in comparison to jasmonic acid control (0 mM JA). Under S1, 5mM JA was more effected on the antioxidants enzyme activities except APX. As compared with 0 mM JA, 5 mM JA increased MDA from 6.81 to 8.62 U g^{-1} min^{-1} (Fig 6a), POD from 52.91 to 58.75 U g^{-1} min^{-1} (Fig 6b), and CAT from 21.23 to 28.23 U g^{-1} min^{-1} (Fig. 6c). While 10 mM JA was increased the APX from 6.81 to 8.62 U g^{-1} min^{-1} (Fig. 6d).

Figure 6. Plant height (a) and leaf area index of forage sorghum [*Sorghum bicolor* (L.) Moench] variety Abu Sabeein as effluence by the interaction between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m^{-1}] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha^{-1}]. The values of each trait labeled by different letters indicate significant differences separated by the LSD test (P < 0.05

3.8. Jasmonic acid and humic acid improved the soluble protein content and antioxidants enzyme

Soluble protein content and most antioxidants enzyme activities were significantly affected by combination between jasmonic and humic acid rates. According to the results, forage sorghum plants treated with a HA1 and spray with medium level of jasmonic acid (5 mM JA) was achieved the highest value of soluble protein content and CAT activity (147.4 mg g^{-1} FW and 40.57 U g^{-1} min^{-1} respectively). However, forage sorghum plants treated with a high rate of humic acid of HA2 and spray with 10 mM JA reached a highest an average value of SOD (25.65 U g^{-1} min^{-1}) and POD (66.68 U g^{-1} min^{-1}). On the other hand, the treatment of JA0 with HA0 recorded the lowest value of soluble protein content and most antioxidants enzyme activities (Table 4).

Table 4

Soluble protein content and most antioxidants enzyme activities traits of forage sorghum [*Sorghum bicolor* (L.) Moench] variety Abu Sabeein as effluence by the interaction between different jasmonic acid levels [0 mM JA, 5mM JA and 10 mM JA as a foliar application] with different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha^{-1}] at the soft dough stage.

Humic acid Rates	Jasmonic acid Levels	Soluble Protein Content (mg g^{-1} FW)	SOD ($U\ g^{-1}\ min^{-1}$)	POD ($U\ g^{-1}\ min^{-1}$)	CAT ($U\ g^{-1}\ min^{-1}$)
HA 0	0 mM	103.0 ± 07.61 g	13.52 ± 0.97 g	34.93 ± 08.44 f	19.45 ± 1.32 g
	5 mM	110.9 ± 04.22 f	16.52 ± 1.74 f	43.43 ± 10.50 e	26.38 ± 1.91 de
	10mM	118.9 ± 05.87 e	17.38 ± 2.37 ef	58.70 ± 21.19 c	21.27 ± 3.53 fg
HA1	0 mM	125.8 ± 06.26 cd	18.58 ± 2.47 de	50.55 ± 09.90 cd	23.94 ± 3.90 ef
	5 mM	147.4 ± 14.88 a	20.59 ± 2.32 c	62.78 ± 20.85 ab	40.57 ± 10.79 a
	10mM	139.1 ± 22.39 b	19.13 ± 2.55 d	50.95 ± 13.77 cd	32.78 ± 5.92 c
HA2	0 mM	127.6 ± 11.04 cd	19.65 ± 3.40 c	62.78 ± 11.66 b	27.77 ± 3.30 d
	5 mM	132.0 ± 07.75 c	23.15 ± 2.49 b	65.53 ± 21.49 ab	35.57 ± 7.29 bc
	10mM	123.5 ± 07.26 de	25.65 ± 3.40 a	66.68 ± 23.92 a	36.90 ± 7.50 b

Note: All analyzed data are expressed as mean ± SD values of 3 biological replicates per treatment. The means with the same letters indicate non-significant differences at $p \leq 0.05$ between parameters in the same column.

4. Discussion

When plants are grown under saline conditions, as soon as the new cell starts its elongation process, the excess of salts modifies the cell wall's metabolic activities, causing the deposition of various materials that limit cell wall elasticity. Cell walls become rigid, and consequently, the turgor pressure efficiency in cell enlargement decreased.

4.1. Growth parameters

In this study, NaCl salinity stress significantly inhibited plant growth traits of forage sorghum, including plant height, leaf area index, and relative growth rate. Plant height gradually decreased with increased salinity. These results indicated that the inhibition in plant height might be due to ions' toxicity, decreased nutrient absorption and decreased elongation of the internodes. Also reduced internode length and reduced formation of new apical tissues [33,34]. The contrary results were reported by [35], who noted that the plant height significantly increased at the low salinity levels, sill, at the high salinity level, the plant height significantly decreased. However, similar findings were reported by [36] on bean (*Vigna aconitifolia* L.), [37] on cabbage plant (*Brassica oleracea capitata* L), [38] on cowpea (*Vigna unguiculata* L.).

The decrease in leaf area index under salinity stress has been attributed to suppressed cell division [39]. The reduction on the leaf area index under salinity stress might be due to shrinkage of the cell contents these lead to reduced development and differentiation of tissues, unbalanced nutrition, and disturbed avoidance mechanism [40]. Our findings agree with [41], who noticed a negative correlation between salinity and leaf area index.

Relative growth rate (RGR) depends on canopy photosynthesis per area unit of land. The RGR of plants in the high salinity treatment was lower than that of the other salinity treatments. Our results disagree with [39] they mentioned that the cheatgrass plants in the lowest and medium salt treatments experienced a reduction in RGR by reduced in plant growth and leaf elongation after salinity application. A similar finding with [39] at the final harvest reported that the RGR decreased in the high salinity level.

Plant hormonal play essential roles in stress responses and adaptation. It is clearly defined that jasmonic acid (JA) increased in response to salinity [42]. In this study, the foliar spraying of JA alleviated the adverse effects of salinity stress on plant height, leaf area index and relative growth rate. Alavi et al. (2015) reported that the JA application recorded the highest plant height and leaf area index at the high salinity stress. Similar results were also observed for pepper, wheat, soybean, and rice seedlings [43,44]. In contract, Alavi et al. (2015) reported that the JA application not significant effect on measured growth parameters. The increased in leaf area index in this study agrees with reports in wheat [45] and rice plants [46].

Humic acid (HA) can improvement the plant growth by increases the permeability of the cell membrane, this progresses increased water absorption and other nutrients uptake and increased absorbing capacity of roots in the presence of humic acid [47]. [48,49] Ali et al. (2019) noticed that the cells might continue their extension growth for more extended periods under the effect of humic acid. [50] stated that the HA increases plant growth through increased in nutrients uptake to overcome the lack of nutrients, this lead to beneficial effects on growth, production, and quality improvement of

agricultural products. In this study, under different salinity levels, the highest plant height, leaf area index, and relative growth rate was recorded at the different humic acid rates at the vegetative growth stage. The increase in the plant height in the HA amended treatments most probably was due to the root zone's improvement [51]. Our results showed that HA could relieve the growth inhibition induced by NaCl in plant height leaf area index, and relative growth rate. Similar impacts were shown by [52] for (*Borago officinalis* L.) and [53] for corn plants, who reported that HA increased the plant length. [54] they noted that HA has remarkable effects on the plant's vegetative growth and increases photosynthetic activity and leaf area index. The results were consistent with [55], who reported that, relative growth rate was enhanced by the treatment with the application of potassium fertilizer and humic acid than control.

4.2. Total chlorophyll content and carotenoid content

Salinity stress adversely affected antioxidant enzyme activity [49,56]. The chlorophyll content is widely used as an index to indicate the abiotic tolerance level in plants. Protection of chloroplast and photosynthetic machinery, including the chlorophyll content, is the first target of defense under stressful conditions [57]. It is well documented that the plants exposed to stressful environments such as salinity resulted in decreased chlorophyll concentration, thereby leading to overall retarded growth [58]. In this study, soil salinity caused a decrease in total chlorophyll content, but increased the carotenoid content on a forage sorghum plant), which is in agreement with some previous studies on different crops e.g., sunflower (Ashraf & Sultana, 2000), and wheat (El-Hendawy et al., 2005). Similar results were showed by [59] in common bean and [60] in the pepper plants. The reduction in chlorophyll content under salt stress may be due to the reduction in the carbon use efficiency, synthesis of chlorophyll, uptake of minerals such as Mg and Fe, and increased ethanol and lactate production [59].

Application of jasmonic acid and humic acid increased the total chlorophyll content and carotenoid content under saline conditions. Thus, higher leaf chlorophyll content is one of the additional factors that may have contributed to a higher photosynthetic of plant under saline conditions. The results presented here show that foliar application of JA led to a significant increase in total chlorophyll content and carotenoid content concentration under soil saline stress. [5], and [43] reported that exogenous JAs significantly improved the total leaf chlorophyll content exposed to salinity stress. These results suggested that exogenous JA treatment could alleviate salinity stress, allowing plants to increase their tolerance to unfavorable conditions. Humic acid caused stimulation in photosynthetic pigments may be due to the decrease of pH value and increase in the activity of soil organisms which release more nutrients from the soil such as Fe [59]. As the amount of HA increased in our study, the total chlorophyll content and carotenoid content also increased. These results confirm by [60] and [61]. The HA application can enhance photosynthesis activity like chlorophyll and increase tolerance in stress conditions by increasing the enzyme rubisco [59].

4.3. Soluble protein content and proline content

Improvement of the soluble protein and proline content are an important mechanism that alleviates and protection the plants from the harmful effect of salinity stress [62] [1]. In the present investigation, salinity stress increased soluble protein content. However, proline content was decreased with salinity increased. This different result was reported by the results of [63], who noted that under salinity stress, the soluble protein content was significantly reduced. The reduction in soluble protein content when the soil is subjected to salinity may be due to decreased potassium content. Consequently, increased sodium content, proline synthesis, protease enzyme activity, and hydrolysis of the rubisco enzyme [62]. Moreover, these results were similar to [64] and [65]. Similar results by increased proline content were reported by [65]. These findings were dissimilar with [66], who reported that salinity stress significantly increased the proline content in wheat and paulownia *(Paulownia imperialis* L) plants.

Jasmonic acid and humic acid application improved the soluble protein content and proline contents of the forage sorghum. A decreased in proline contents with the comparison with the controls was reported by Farahat et al. (2012) during an investigation of exogenous applications of humic acid on seedlings of *Khaya senegalensis*. The agreement results by increased protein content were showed by [67] and [68]. These results are same with [9], who reported that the improved accumulation of protein content might be due to the rapid accumulation of a specific protein set. In the present investigation, HA improved the protein content. Similar findings were reported by [69] and [62]. JA can protect the plant from toxicity ions in the different stages by managing the antioxidant machinery and synthesis of proteins [70]. Enhancement of proline content in the plant under salinity stress is associated with the increment of some enzymes such as Pyrroline-5- carboxylate synthase. In this study, JA improved proline content. An increased in proline contents in comparison with the control by jasmonic acid was reported by Ali. (2020). Our result disagrees with [5], who reported that jasmonic acid reduced proline content under salt stress in the wheat.

4.4. Superoxide dismutase and Malondialdehyde contents

Superoxide radicals ($O2^-$) generated by oxidative metabolisms were detoxified by superoxide dismutase (SOD) and converted into H_2O_2 and O_2. SOD is one of the enzymes responsible for eliminating O_2^- and is considered an essential antioxidant in cells. Improved SOD activity positively correlated with improved protection from damage and adverse effect associated with oxidative stress induced by salinity stress [57]. The malondialdehyde contents (MDA) a product of lipid peroxidation, is generally an indicator of free radical damage to cell membranes causing severe oxidative stress [57,58]. In our

study, high salinity stress increased the activity of SOD and MDA content. The increase in SOD activity coincided with an increase in the activities of Mn-SOD and Fe-SOD. The reduction in SOD activity could minify the plant's ability to scavenge O_2 radicals favoring an accumulation of ROS, which could cause membrane damage [48]. These results were contrary to [71], who reported that the SOD activity was decreased under salinity stress in *Gypsophila oblanceolate* plant. However, agree results were reported for sweet sorghum and sunflower by Nimir et al. (2015) and [72], who noted that salinity stress could increase the same antioxidant enzyme activity. This result similar with the findings of [73], and [65] in forage sorghum and sweet sorghum, who suggests that under soil saline, MDA content was substantially increased by an increase in soil salinity as compared with the non-soil salinity.

Our study showed that a significant increase in SOD activity and MDA content was observed in forage sorghum plants treated with JA, suggesting that JA had a good O_2^- scavenging ability to protect the plant from oxidative damage. Our study of the increase in SOD activity agree with the results of [57,74]who reported that exogenous JA application of soybean under salinity stress significantly improved the activities of SOD. However, our study of the an increase in MDA content under salt by jasmonic acid different from the findings of [75], and [66], who reported that JA and MeJA treatment could effectively alleviate NaCl stress-induced by oxidative stress as indicated by the decreased in the MDA, H_2O_2 and the production rate of O_2^-. Similar results were reported by Ali et al. (2019). Jasmonic acid might fulfill crucial roles in scavenging radicals, thus inhibiting lipid peroxidation by excess ROS produced under salinity conditions [66].

In our results, the application of HA was positively useful under saline conditions to increase the actions of the SOD and MDA content. Disagree result showed by [61] reported that the HA fertilizer reduced the activity of SOD in the Maize plant under NaCl salinity stress. Similar results were reported by [76], who found that HA fertilizer increased the antioxidant enzyme activity, including SOD activity in response to salinity stress. Similar results were reported by [77], who showed that MDA content under NaCl-stressed plants increased significantly by applying a humic acid.

4.5. Catalase and peroxidase activity

Enhancement of the anti-oxidative enzymes in plants under saline condition could increment ROS and improve a protecting mechanism to decrease adverse impacts by salt stress. In this study, soil saline stress caused a reduction in catalase (CAT) and peroxidase (POD) activities. Reduced CAT activity under salinity stress might have promoted H_2O_2 accumulation, which could result in a Haber–Weiss reaction from hydroxyl radicals, which are known to damage biological systems [78]. Our study of decrease in POD and CAT activities also confirmed with the results of [66] and [71]. The disconfirmed results has been shown by [79,80] for wheat plant treated with soil saline, who reported that POD activity increase under NaCl stress. Also, disconfirmed results were shown by [81] for maize and by [82] for wheat, who noted that salinity stress increased CAT activities.

In this study, foliar application of jasmonic acid could improve CAT and POD activities in the leaves of forage sorghum plants under soil saline condition. Moreover, the highest value of POD activity was shown in the 10mM at high salinity concentration and 5 mM JA under medium salinity concentration and the lowest value of POD have been registered at the control. Our result was similar to the findings of [57], who suggested that the wheat and soybean plants exposed to salinity and treatment with JA application significantly increase the antioxidant enzyme activities including CAT and POD and play an essential role for ant-oxidative defense required for salt tolerance. Also, [5] noted that the treatment of soybean plants under salt stress by salicylic acid and jasmonic acid promote the antioxidant enzyme activities.

Humic acid (HA) plays an essential role in the plant by improvement the biochemical and physiological processes, synthesize of protein and ability of roots absorption of the nutrients and water [83], these processes lead to an increase the activation, concentrations and stimulation of the antioxidant's enzymes [84]. In this study, the application of HA improved plant defense systems such as POD and CAT in the forage sorghum plants, and the both rates increased the POD and CAT activities. Many studies have also reported that the increased activities of POD and CAT by application of HA was efficient in improving tolerance of salt in cucumber [85], date plam (*Phoenix dactylifera* L), and hot pepper [83]. However, our result different from the findings of [61] found that the humic acid application decreased CAT and POD activities under salt stress.

4.6 Ascorbate peroxidase activity

The ascorbate peroxidase activity (APX) may help the plant against the H_2O_2 damage [43]. Under salt stress, the APX plays an essential role in protective the plant by reduce H_2O_2 [43,57]. Our study showed that increased salinity levels reduced APX activity. A similar result was reported by [66] and [71]. However, different results by [5,86] who reported that salinity stress reduced APX.

The capability of HA as a scavenger to reactivate oxygen species caused by regulating the direct water flow and solutes between the cytoplasm and vacuolar compartments, the ability to regulate turgor and osmotic pressure, membrane permeability, and cell osmotic balance [87]. In our results, HA was positive effective on APX activity. Increased APX activity under HA application has been reported in maize plants exposed to salinity [61]. [76,88] reported that HA improved APX activity response to salinity stress. Our results showed that exogenous JA was significantly increased APX activity. Our work agrees with [89] findings, who suggested that exogenous JA increased APX activity under salt stress of tobacco (*Nicotiana tabacum*) plants. Also,

[90] report that APX activity increased in plants treated with JA exposed to Pb stress. Moreover, exogenous JA induced the synthesis of antioxidant metabolites that provided additional resistance to neutralize the toxic effects of salt stress generated ROS [66].

5. Conclusions

Our study examines the effects of salinity stress on growth parameters, chlorophyll content, carotenoid content and plant defense system of forage sorghum exposed to different humic acid rate as fertilizer and jasmonic acid application as a foliar spry. Soil salinity stress at 4 g NaCl kg^{-1} soil decreased all study parameters, except protein content, SOD activity, and MDA. In this study, we found that forage sorghum spray with jasmonic acid at 10 mM JA level successfully improved POD and SOD activities. Among different humic acid rate, 3 g HA kg^{-1} dry soil successfully increased all parameters. These results proposed that 5 mM JA could efficiently protect the forage sorghum plant from salty stress damage by enhancing total chlorophyll content, carotenoid content, and antioxidant enzyme. The interaction between 3 g HA kg^{-1} soil and 5 mM JA was most effective in alleviating salinity stress. Therefore, JA and HA combined applications could enhance the chlorophyll content, carotenoid, and antioxidant enzyme in plants. Therefore, JA and HA application management are required in salt-affected soil to sustain forage growth and increase crop yield and productivity under salinity conditions.

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Figures

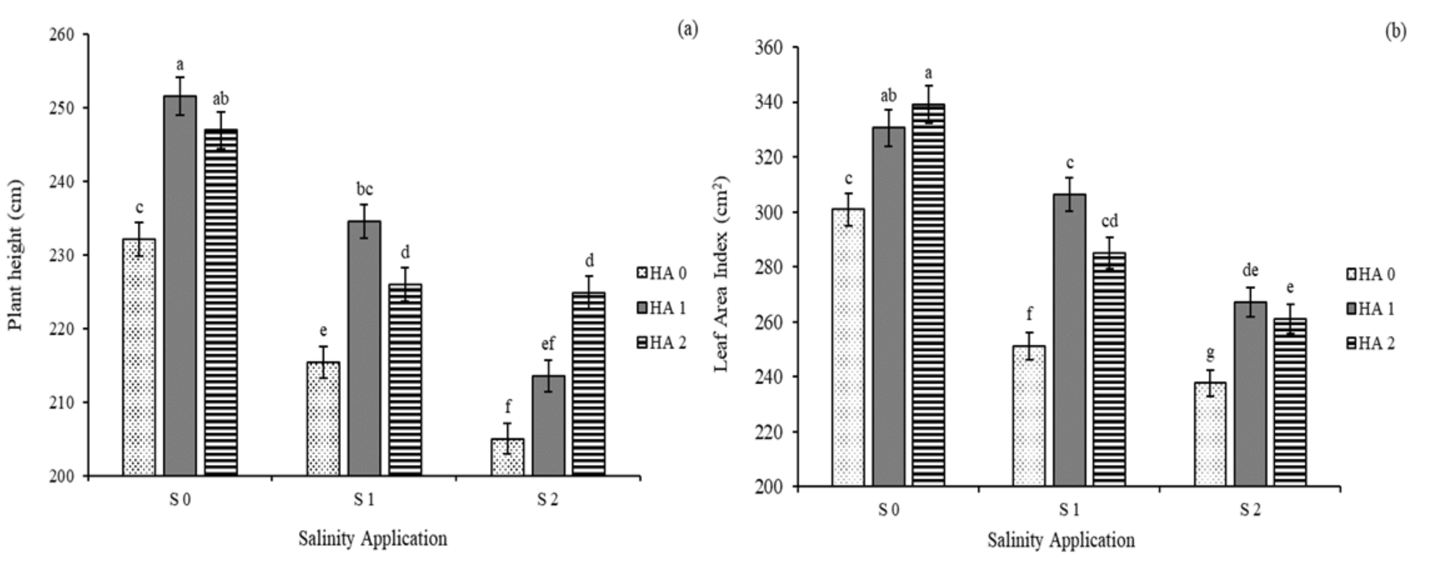

Figure 1

Plant height (a) and leaf area index (b) of forage sorghum [Sorghum bicolor (L.) Moench] variety Abu Sabeein as effluence by the combination between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m−1] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha-1]. The values of each trait labeled by different words show significant differences separated by the LSD test (P < 0.05.

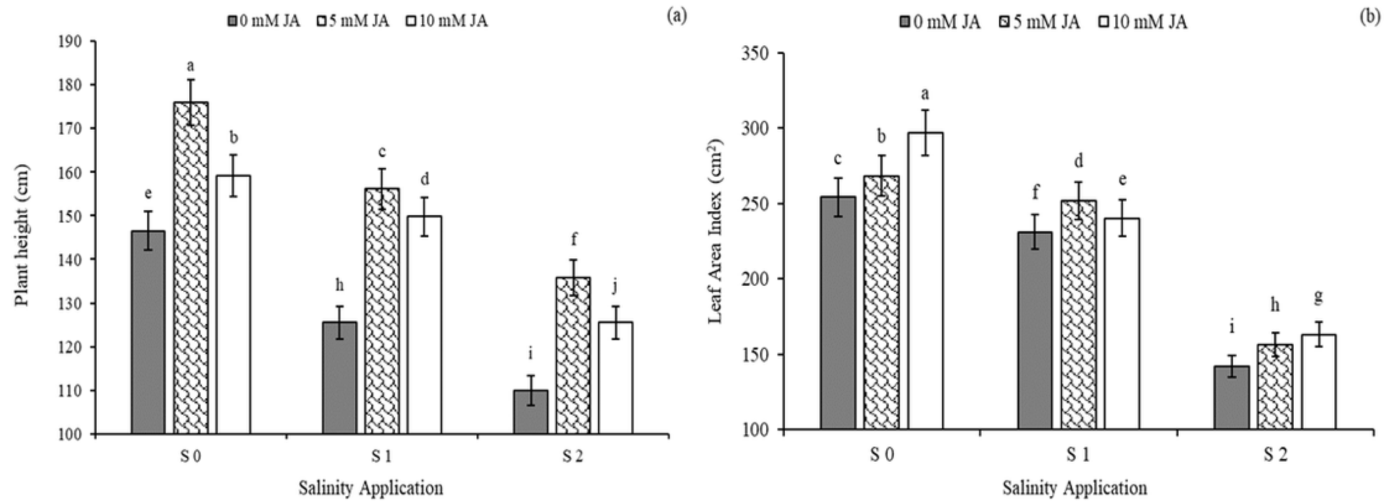

Figure 2

Plant height (a) and leaf area index (b) of forage sorghum [Sorghum bicolor (L.) Moench] variety Abu Sabeein as effluence by the combination between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m−1] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha-1]. The values of each trait labeled by different letters indicate significant differences separated by the LSD test (P < 0.05).

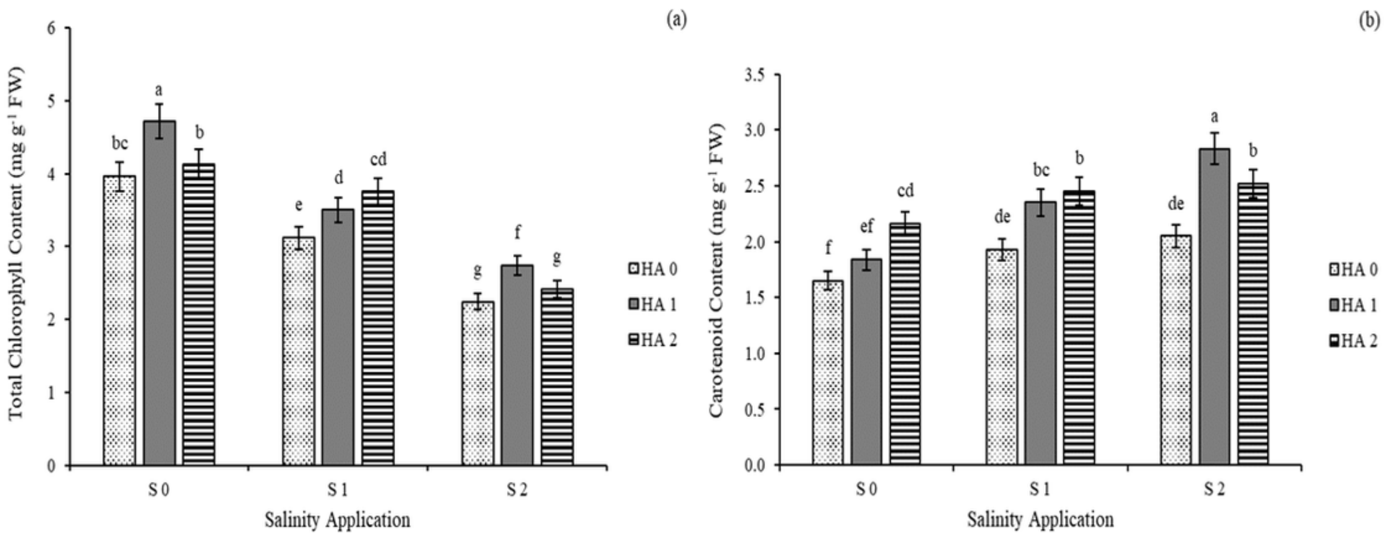

Figure 3

Total chlorophyll content (a) and carotenoid content (b) of forage sorghum [Sorghum bicolor (L.) Moench] variety Abu Sabeein as effluence by the combination between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m−1] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha-1]. The values of each trait labeled by different letters indicate significant differences separated by the LSD test (P < 0.05

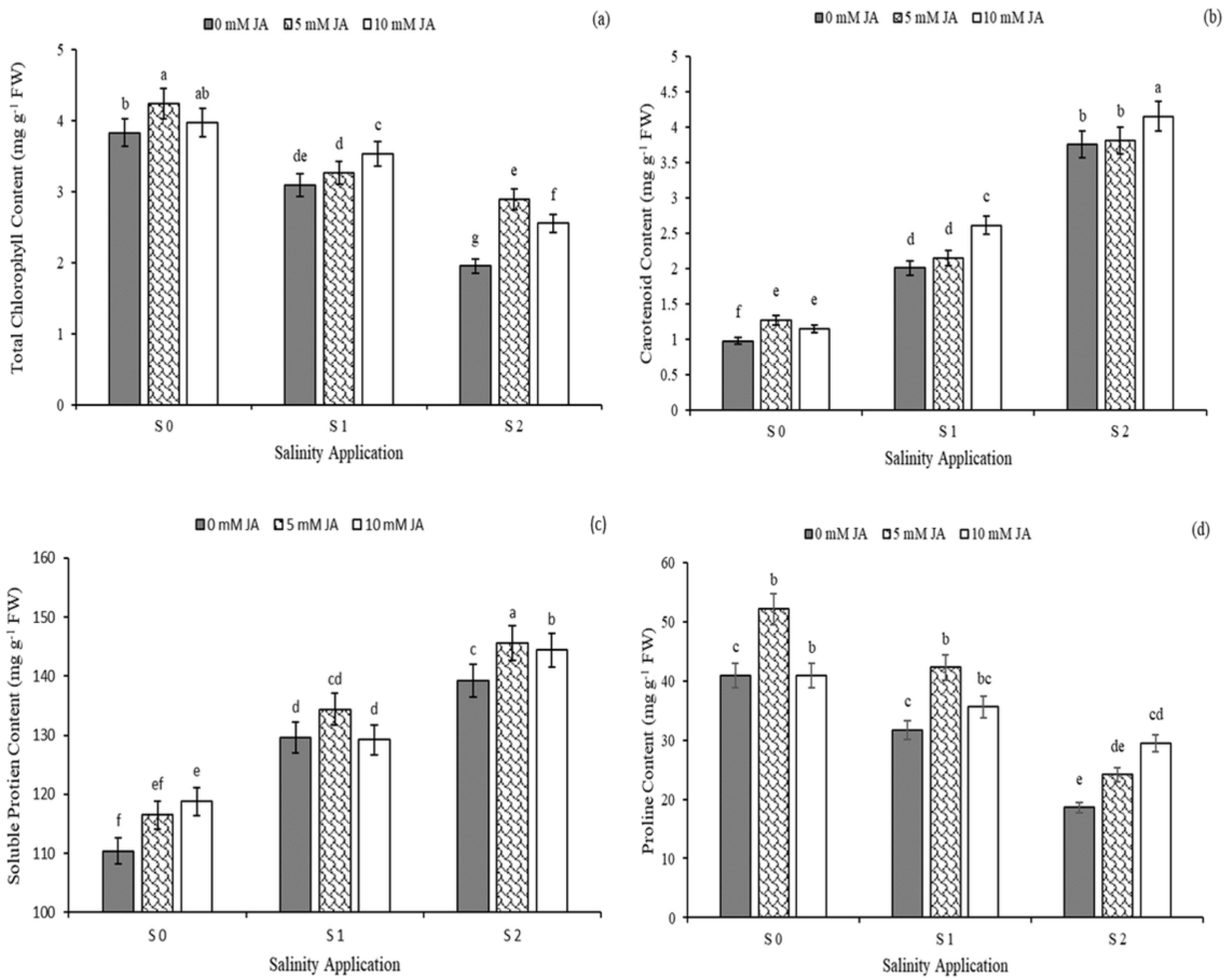

Figure 4

Total chlorophyll content (a), carotenoid content (b), soluble protein content (c) and proline content (d) of forage sorghum [Sorghum bicolor (L.) Moench] variety Abu Sabeein as effluence by the combination between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m−1] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha-1]. The values of each trait labeled by different letters indicate significant differences separated by the LSD test (P < 0.05).

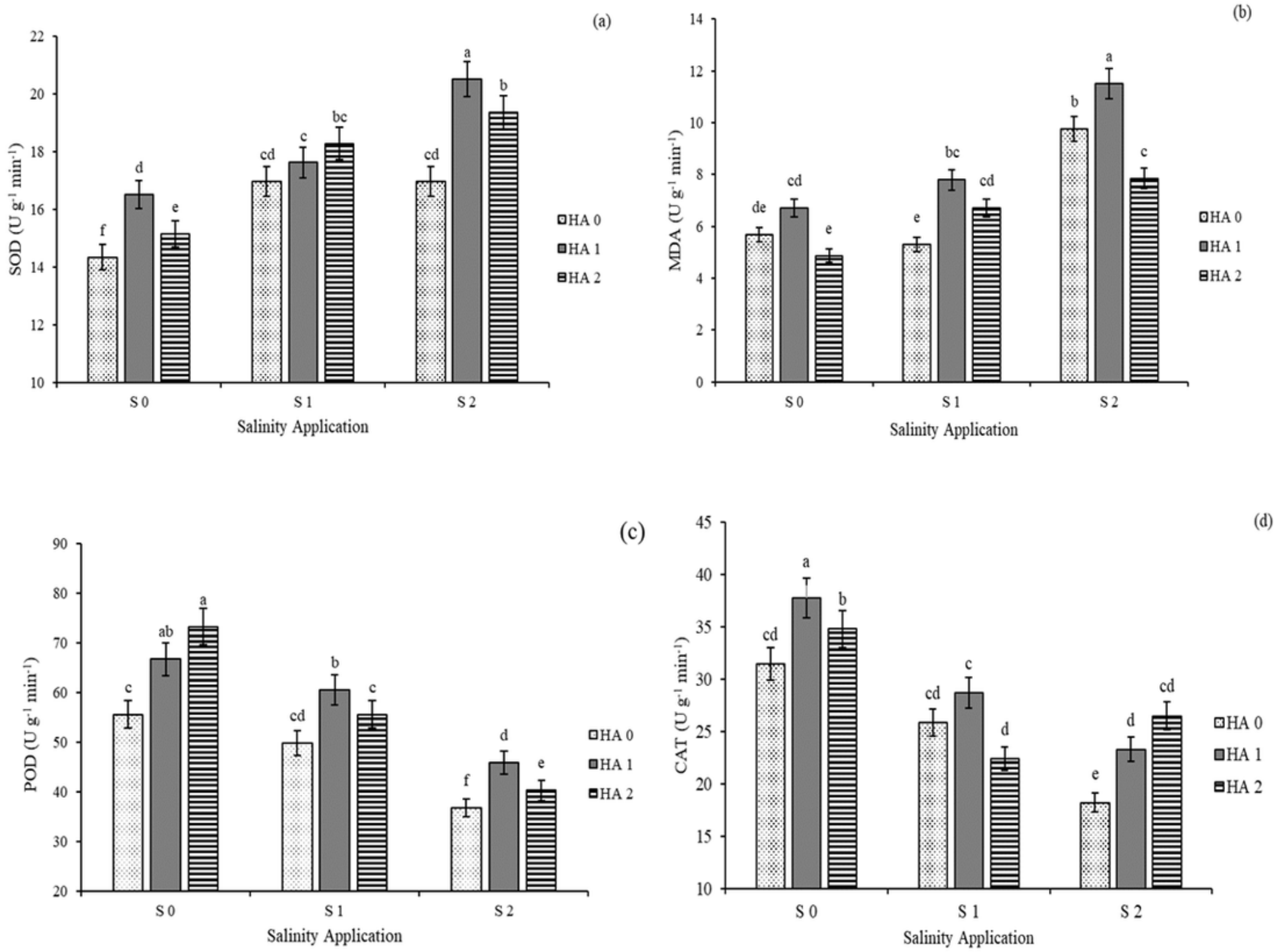

Figure 5

SOD (a), MDA (b), POD (c) and CAT (d) of forage sorghum [Sorghum bicolor (L.) Moench] variety Abu Sabeein as effluence by the interaction between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m−1] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha-1]. The values of each trait labeled by different letters indicate significant differences separated by the LSD test (P < 0.05).

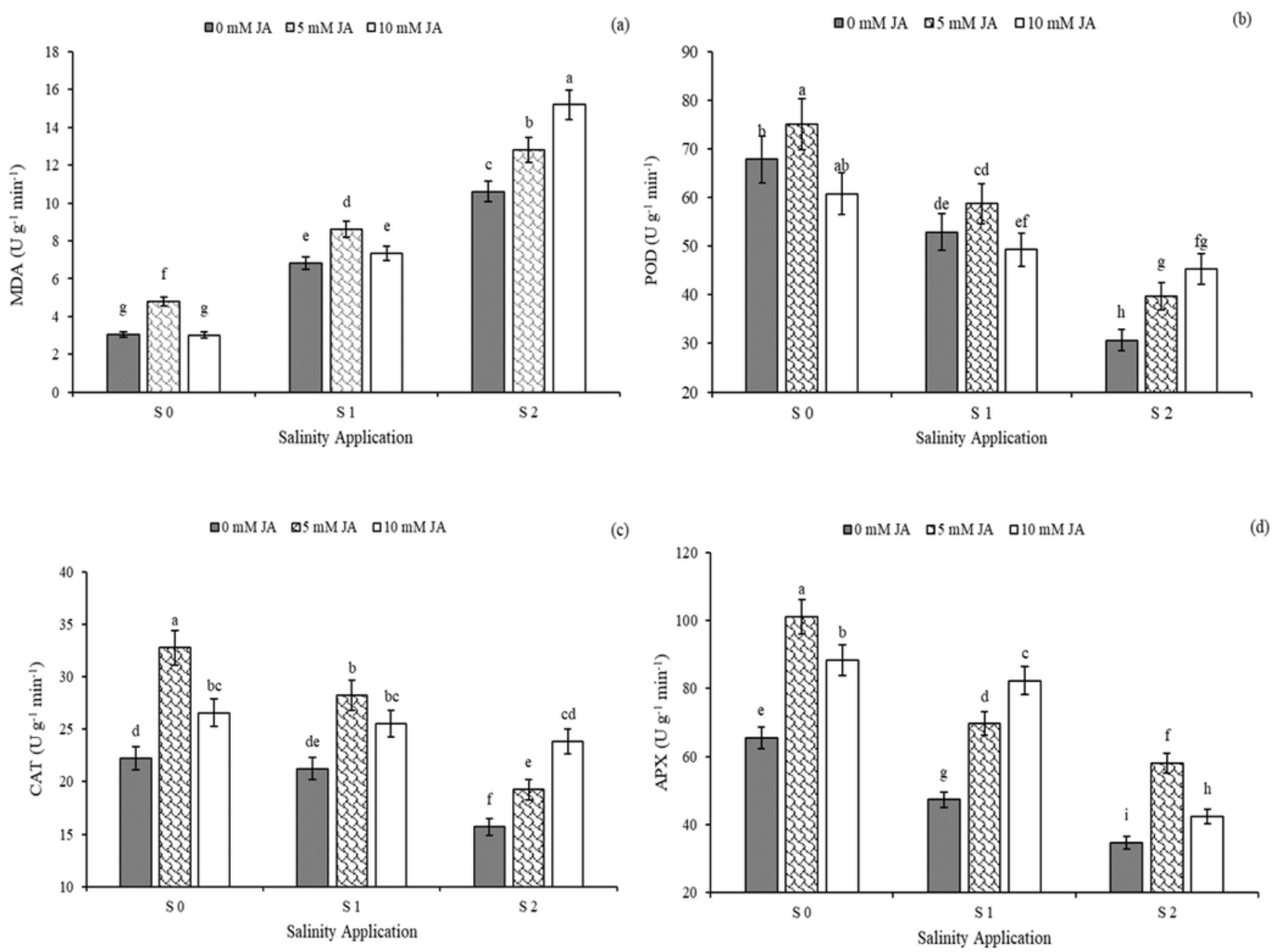

Figure 6

Plant height (a) and leaf area index of forage sorghum [Sorghum bicolor (L.) Moench] variety Abu Sabeein as effluence by the interaction between different salinity levels [S0 = 0.26, S1 = 2.3 and S2 = 4.7 dS m−1] and different humic acid rates [HA0 = 0, HA1 = 373.21 and HA2 = 746.42 kg HA ha-1]. The values of each trait labeled by different letters indicate significant differences separated by the LSD test (P < 0.05