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Ascorbic acid enhances growth and dualex parameters in *Arachis hypogaea* L. under salt stress

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Abstract
Salt stress limits crop productivity in arid and semi-arid regions, posing a significant challenge to agricultural sustainability. This study investigates the potential of ascorbic acid (AA) to alleviate the harmful effects of sodium chloride (NaCl) in *Arachis hypogaea* L. by applying AA concentrations of 0, 100, 200, and 300 ppm and NaCl levels of 100 and 200 mM under controlled conditions. Results showed that the application of 300 ppm AA under 200 mM NaCl notably mitigated salt-induced reductions, enhancing stem and root lengths by 33% and 14% and increasing stem fresh and dry weights by 67% and 39%, respectively. Comparative analysis showed a 3% decrease in plant temperature, a 56% increase in leaf area, and a 59% reduction in malondialdehyde, underscoring AA's protective effects. Although not all measured parameters responded significantly to AA under salt stress, these findings highlight the compound's potential to counteract growth reductions in A. hypogaea, demonstrating its value for sustainable agricultural practices under saline conditions.

Keywords Abiotic stress · Salinity mitigation · Antioxidant compounds · Biostimulation · Fertilization reducing

1 Introduction

Arachis hypogea, belonging to the leguminous family, is a significant oilseed plant for human health due to its oil, protein, carbohydrates, and various vitamins and minerals [1]. Varieties such as Virginia, Runner, Spanish, and Valencia peanuts are grown worldwide for different economic purposes. In Turkey, varieties like Virginia and Spanish are generally cultivated [2]. Globally, 37.8% of peanut production occurs in China, 15.6% in India, and 6.9% in Nigeria [3]. Although *A. hypogaea* accounts for 10.9% of total oilseed cultivation areas in Turkey, it is predominantly grown in the Mediterranean region, where climatic conditions are most favorable for production. From a biotechnological standpoint, *A. hypogaea* is an important raw material for the global oil industry, although its role in the Turkish oil industry remains limited [4]. Salinity has increasingly become a critical issue for *A. hypogaea* production, particularly in arid and semi-arid regions, where climate change and water scarcity exacerbate yield and quality losses [5–7]. One of the methods to counteract this negative trend involves using reactive oxygen species (ROS) to improve plant responses to stresses [8]. Under saline conditions, plants activate an antioxidant enzyme network that includes superoxide dismutase (SOD), catalase (CAT), and

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ascorbate peroxidase (APX), as well as non-enzymatic antioxidants [9]. Regarding *A. hypogea*, several research focused on the role of other antioxidant activities, like the role of anthocyanins and flavonoids, or investigated methods as ion regulation and osmotic adjustment to increase salt stress tolerance [10–12].

Among these non-enzymatic antioxidants, ascorbic acid (AA) plays a key role in ROS modulation [13]. Among them, ascorbic acid (AA) is a crucial antioxidant that protects against oxidative damage by inhibiting cell membrane peroxidation, in conjunction with other components of the defense system [14]. It also serves as a cofactor for various molecules in the plant's physiological metabolism and is a primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide [15–17]. The exogenous application of AA plays a fundamental role in protecting plants against multiple environmental stresses, including salinity stress [18, 19]. Additionally, numerous studies have shown the biostimulant potential of ascorbic acid, evidencing the acceleration of vegetative and reproductive development of a variety of crops, contingent upon the dosage, plant species, and growth stages [20, 21].

However, studies specifically investigating the effects of AA application on *A. hypogaea* under saline conditions are limited [22, 23]. However, previous research on bread wheat, corn, and flax plants under salt stress has demonstrated that AA application improves crop yields and qualities [24–27]. Our study aims to address this gap by examining the potential of AA to mitigate the effects of salt stress in *A. hypogaea*, focusing on physiological and biochemical responses under saline conditions. Given these considerations, we hypothesize that AA treatment can enhance *A. hypogaea* resilience to NaCl-induced stress, potentially contributing to improved crop performance under challenging environmental conditions. The implications of this research are significant for agricultural practices in regions facing soil salinity issues. By exploring the efficacy of AA in supporting *A. hypogaea* under stress, our findings could offer insights for more sustainable approaches to managing peanut production in saline environments, which is increasingly important for global food security.

2 Materials and methods

2.1 Plant material

The *A. hypogea* 'Halisbey' seeds were obtained from the Directorate of the Central Research Institute (Department of Field Crops, Faculty of Agriculture, Van Yüzüncü Yıl University). This variety was selected due to its known adaptability to the climatic conditions in Turkey and its agricultural importance in the region. The experiments were conducted under semi-controlled climate room conditions in the same department in July 2021. Seeds were planted in a mixture of peat, perlite, and soil (1:1:2) in 500 cc pots and grown in a room maintained at 25 °C, 65% relative humidity, with an 8/16 h dark/light photoperiod. Developmental stages were identified following the BBCH Scale [28].

2.2 Experimental design

After one month, the seedlings (BBCH101) were transferred to the soil. At the BBCH105 developmental stage (5th–6th genuine leaf unfolded), ascorbic acid was applied to the seedlings at different concentrations (0 ppm, 100 ppm, 200 ppm, and 300 ppm). The ascorbic acid was applied foliarly four times, with four-day intervals between applications. After two months (BBCH600) of ascorbic acid application, the plants were subjected to salinity stress using different salt doses (SD): 0 mM, 100 mM, and 200 mM NaCl, for five applications with one-week intervals. Each treatment group consisted of ten plants, and the study was designed with four replications, leading to a total of 40 plants per treatment. In all treatments, no further fertilisation was performed throughout the study period.

2.3 Dualex measurment

During plant development, chlorophyll, flavonoid, and anthocyanin contents, as well as the nitrogen balance index (NBI), were measured using the Dualex scientific + instrument (FORCE-A, France) [29]. At BBCH609, the number of flower buds per plant was recorded just before harvest.

2.4 Plant development parameters

Root and stem lengths were measured using a digital caliper (Digital Caliper ABS IP54, Yamer Co., Turkey). Samples were processed at 40 °C for 72 h to establish dry weights. Fresh and dry weights of roots and stems were measured using an analytical balance with 0.0001 g sensitivity (Shimadzu AUW-D series, Japan).

2.5 Leaf physiological parameters

At BBCH609 and the end of the study, various physiological parameters on leaf tissues were estimated as described below.

2.5.1 Leaf area and leaf surface temperature

Leaf area (cm^2) was measured using the Easy Leaf Area software [30], and leaf surface temperatures (°C) were measured using a portable infrared thermometer (Everest Interscience Inc., Model 510B, Germany).

2.5.2 Relative water content (RWC)

Relative water content (RWC) of leaves was measured at harvest. Three leaves were sampled from each plant, and four disks were cut from each leaf. Fresh weights of the disks were recorded with a precision balance, and the average fresh disk weight (YA) was used to determine water content. Disks were then immersed in ultrapure water at 25 °C for two hours, after which their turgid weights (TA) were measured. The disks were subsequently dried at 110 °C for 24 h to obtain the average dry weight (KA). RWC was calculated using the equation [31]:

$$\text{RWC } \% = \frac{(\text{YA} - \text{KA})}{(\text{TA} - \text{KA})} \times 100$$

2.5.3 Ion leakage (ILLT) and membrane resistance index (MRI)

Ion leakage (ILLT) and membrane resistance index (MRI) were determined using 0.1 g fresh leaf samples collected at the BBCH609 stage before harvest. Leaf samples were washed with tap water and then rinsed with distilled water. They were then immersed in 10 ml of distilled water at 40 °C for 30 min, and the electrical conductivity (EC) of the solution was measured (C1). Samples were subsequently placed in a hot water bath at 100 °C for 10 min, and EC was measured again (C2). ILLT and MRI were calculated using the following equations [32]:

$$\text{ILLT } \% = \frac{(\text{C1})}{(\text{C2})} \times 100$$

$$\text{MRI } \% = (1 - \frac{\text{C1}}{\text{C2}}) \times 100$$

2.5.4 Malondialdehyde (MDA) content

The enzymatic activity of malondialdehyde (MDA) in plants was expressed as MDA content. A 0.1 g leaf sample was homogenized with 10 ml of 0.1% trichloroacetic acid and centrifuged at 15,000 g for 5 min. One ml of the supernatant was resuspended in 0.5% thiobarbituric acid dissolved in 4 ml of 20% trichloroacetic acid. The samples were left in a hot water bath (95 °C) for 30 min, rapidly cooled in an ice bath, and centrifuged at 10,000 g for 10 min. The absorbance of the supernatant was determined at 532 nm and 600 nm. MDA content, expressed in nmol ml^{-1}, was calculated using the following equation [32]:

$$\text{MDA } = \left(\frac{(\text{A532} - \text{A600}}{155000} \right) \times 106$$

2.6 Statistical analysis

The study was conducted as a factorial experiment with four replications based on a completely randomized experimental design. The main factors were ascorbic acid concentration (0, 100, 200, and 300 ppm) and salt dose (0, 100, and 200 mM NaCl). All recorded data were subjected to ANOVA test using the COSTAT (version 6.3) software, after analyses of normality and homogeneity by Shapiro–Wilk and Levene's tests. The Duncan Multiple Range Test was used to compare the average data at $p < 0.05$.

3 Results

3.1 Plant development

The effect of ascorbic acid (AA) on the growth and development of *A. hypogea* was investigated in both the presence and absence of salt stress. Table 1 summarizes the results obtained using the Dualex probe. Salt stress at different doses did not significantly affect the nitrogen balance index (NBI), chlorophyll, flavonoid, or anthocyanin contents. Similarly, AA application, whether in the presence or absence of salt stress, did not show a significant impact on these parameters. The interaction between AA and salt dose (SD) was also not significant. The chlorophyll content ranged from 39.3 to 43.3 µg cm^{-2}, while flavonoid content varied between 0.46 and 0.52 units. Anthocyanin content ranged from 0.001 to 0.007 units, and NBI ranged from 82.7 to 93.1 mg g^{-1}. However, it is noteworthy that both chlorophyll

Table 1 The effects of ascorbic acid treatments on Dualex values of peanuts (*A. hypogaea*) under salt stress

AA dose (ppm)	NaCl (mM)	NBI (mg g^{-1})	Chlorophylls (µg cm^{-2})	Flavonoids (dx)	Anthocyanins (dx)
AAD-0	SD-0	98.8	43.8	0.46	0
	SD-100	95.1	41.9	0.41	0
	SD-200	69.5	32.3	0.5	0.003
AAD Mean		87.8	39.3	0.46	0.007
AAD-100	SD-0	78.9	42.1	0.54	0
	SD-100	94.4	44.5	0.48	0
	SD-200	96.2	44.1	0.51	0.003
AAD Mean		89.8	43.6	0.51	0.001
AAD-200	SD-0	78.2	40.9	0.53	0.01
	SD-100	85.4	43.6	0.52	0
	SD-200	84.3	42.3	0.5	0.003
AAD Mean		82.6	42.3	0.52	0.001
AAD-300	SD-0	93.6	42.6	0.48	0.01
	SD-100	97.6	42.8	0.44	0.013
	SD-200	80.7	46.2	0.57	0.003
AAD Mean		90.6	43.9	0.5	0.004
SD Mean	SD-0	87.4	42.3	0.5	0.005
	SD-100	93.1	43.2	0.46	0.001
	SD-200	82.7	43.3	0.52	0.008
CV (%)		19.7	14.6	16.6	17.5
Salt Dose		ns	ns	ns	ns
Ascorbic Acid Dose		ns	ns	ns	ns
SD x AAD		ns	ns	ns	ns

The values followed by the same case letter are not significantly different ($p > 0.05$), according to ANOVA with Duncan Multiple Range Test. **$p < 0.01$; *$p < 0.05$; ns: $p > 0.05$, *CV* coefficient of variation, *AAD* ascorbic acid dose, *SD* salt doses, *NBI* nitrogen balance index

and NBI exhibited limited responses to AA treatments under salt stress, underscoring that AA did not have a substantial impact on these parameters in saline conditions.

Figure 1a shows the root length recorded under different experimental conditions. In the absence of salt stress, the highest root length was observed with a 300 ppm dose of ascorbic acid (36.4 cm), while the control group recorded the lowest root length (24.4 cm). Under salt stress (SD100), root elongation was promoted by 100 ppm and 300 ppm doses of AA. However, at a higher salt dose (SD200), no significant differences in root length were observed between the treatments. Figure 1b illustrates the stem length results. Without salt stress, increasing doses of ascorbic acid resulted in longer stem lengths. The highest stem length was observed with a 300 ppm dose of AA (41.5 cm), and the control group had the shortest stems (33.7 cm). In the presence of salt stress (both SD100 and SD200), stem elongation was enhanced by ascorbic acid application, with the best results observed at SD100 with a 300 ppm dose of AA.

Table 2 presents the results of fresh and dry weights of stems and roots recorded under different experimental conditions. Significant effects were observed only for stem weights. Ascorbic acid (AA) applications did not significantly influence root weights, either in the presence or absence of salt stress. However, all levels of AA application significantly affected stem dry weights compared to the control. The AA300 application promoted the highest fresh stem weight (7.60 g; $p < 0.01$). Similarly, stem dry weights were positively influenced by AA application compared to the control. The interaction between AA and salt dose (SD) was significant for fresh stem weights.

3.2 Leaf development

Figure 2 illustrates the effects of ascorbic acid (AA) application on leaf relative water content (RWC), membrane stability index (MSI), and ion leakage (ILLT). The RWC in leaf tissues varied between 81 and 93%. These values decreased in the presence of NaCl, with the highest RWC observed in the control (92%) and the lowest in the SD200 treatment (80%). The interaction between salt dose (SD) and AA was not significant for this parameter ($p > 0.05$). Under both salt stress and control conditions, the effects of AA application on MSI and ILLT were not statistically significant. MSI values ranged from 42 to 53%, while ILLT values ranged from 47 to 57%. This lack of significant change in MSI and ILLT under salt stress suggests that AA may play a role in maintaining membrane stability even in stressful saline conditions.

Figure 3 shows the leaf area index (LAI) recorded under different experimental conditions. Significant differences were observed among the experimental conditions. Gradual increases in AA resulted in increased LAI. The highest LAI was measured in AA300 (10.5 cm^2), and the lowest was in the control (4.6 cm^2). In the presence of NaCl, LAI values decreased. However, AA application showed a protective effect. At SD200, all AA application rates recorded higher LAI values (6.2–8.4 cm^2) compared to AA0 (2.4 cm^2). At SD100, AA applications at 200 and 300 ppm resulted in significantly higher LAI values (9 cm^2 and 7.5 cm^2, respectively) than AA0 (3.6 cm^2).

Table 3 presents the leaf surface temperature (LST) recorded under various experimental conditions. Significant differences were observed only among ascorbic acid (AA) applications. The highest average LST was recorded for AA300 (19.5 °C), while the lowest was observed for AA0 (19.0 °C). No significant interaction between salt dose and AA was observed in relation to LST, suggesting that AA treatments did not notably impact LST across varying levels of salt stress.

Figure 4 shows the effect of ascorbic acid (AA) on malondialdehyde (MDA) content in leaf tissues under normal and saline conditions. Despite the presence of salt stress, the highest MDA concentration was observed with AA300 (8.9 nmol g^{-1}). No significant differences were noted among AA0-AA200 application rates, with average concentrations of 5.3 nmol g^{-1}. The presence of NaCl and the interaction between salt dose and AA application (SD x AA) did not have a statistically significant effect on MDA content. The elevated MDA concentration observed at the AA300 level under salt stress conditions suggests a potential marker of oxidative stress response to high AA doses.

4 Discussion

The application of various nutrients, including ascorbic acid (AA), has been shown to promote plant growth and development. Despite salt stress, AA application significantly enhanced most of the plant growth and development parameters measured in this study. This observation aligns with recent research indicating that plant growth regulators, such as salicylic acid (SA), polyamines (PA), abscisic acid (ABA), and jasmonic acid (JA), can improve salt tolerance in plants [33–35].

Salinity generally has a toxic effect on root growth and elongation [36], but the impact varies among plant species and cultivars [37]. For instance, rice varieties have shown improved root length and biomass under salt stress [38], while salinity adversely affects bean germination [39]. Similarly, Na_2SO_4 disrupts water absorption in sorghum, leading to

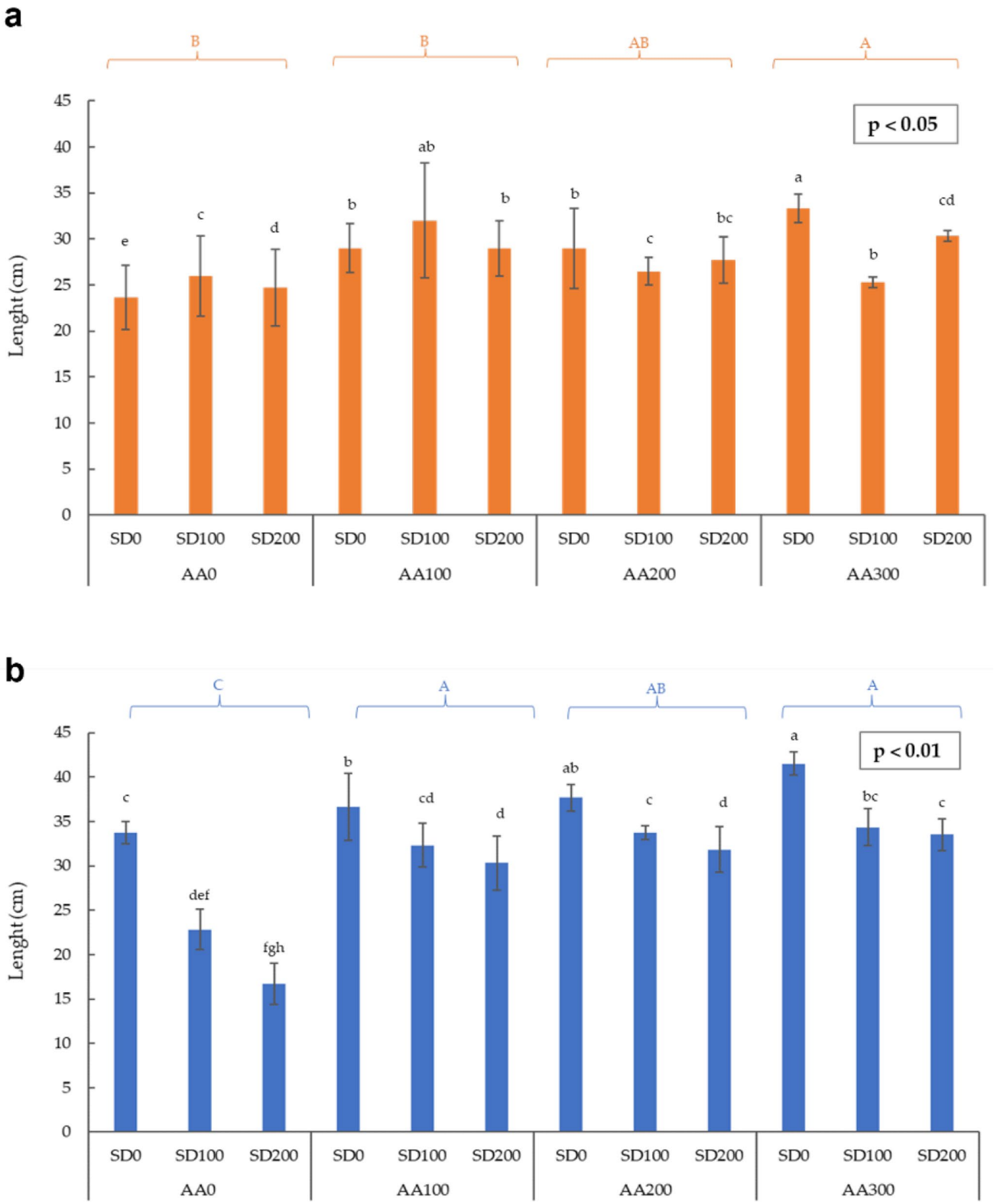


Fig. 1 *Arachis hypogea* root (**A**) and stem (**B**) lengths recorded under different salt doses (SD) and ascorbic acid (AA) application rates. The values followed by the same case letter are not significantly different ($p > 0.05$), according to ANOVA with Duncan Multiple Range Test

Table 2 The effects of ascorbic acid treatments on the fresh and dry weights of stems and roots of peanuts (*A. hypogaea*) under salt stress

AA dose (ppm)	NaCl dose (mM)	Stem weights (g)		Root weights (g)	
		Fresh	Dry	Fresh	Dry
AA0	SD0	5.14 bc	0.84	3.12	0.40
	SD100	4.90 c	1.02	3.02	0.42
	SD200	2.78 cde	0.60	3.51	0.52
Average		4.27 B	0.82 C	3.22	0.45
AA100	SD0	7.01 a	1.34	3.38	0.47
	SD100	5.74 b	1.25	3.99	0.56
	SD200	4.91 c	1.19	4.12	0.57
Average		5.89 AB	1.26 B	3.83	0.53
AA200	SD0	6.88 ab	1.40	4.25	0.62
	SD100	6.01 b	1.36	4.12	0.67
	SD200	5.08 c	1.27	3.04	0.56
Average		5.99 AB	1.30 AB	3.80	0.62
AA300	SD0	9.72 a	1.96	4.75	0.70
	SD100	6.54 ab	1.48	3.37	0.62
	SD200	6.53 ab	1.63	4.73	0.73
Average		7.60 A	1.34 A	4.28	0.68
SD Average	SD0	7.18 A	1.38	3.87	0.54
	SD100	5.79 AB	1.27	3.62	0.56
	SD200	4.82 B	1.17	3.85	0.59
CV (%)		19.2	19.6	19.3	18.2
SD		**	ns	ns	ns
AA		**	*	ns	ns
AA x SD		**	ns	ns	ns

The values followed by the same case letter are not significantly different ($p > 0.05$), according to ANOVA with Duncan Multiple Range Test. **$p < 0.01$; *$p < 0.05$; ns: $p > 0.05$, *CV* coefficient of variation, *AA* ascorbic acid dose, *SD* salt doses

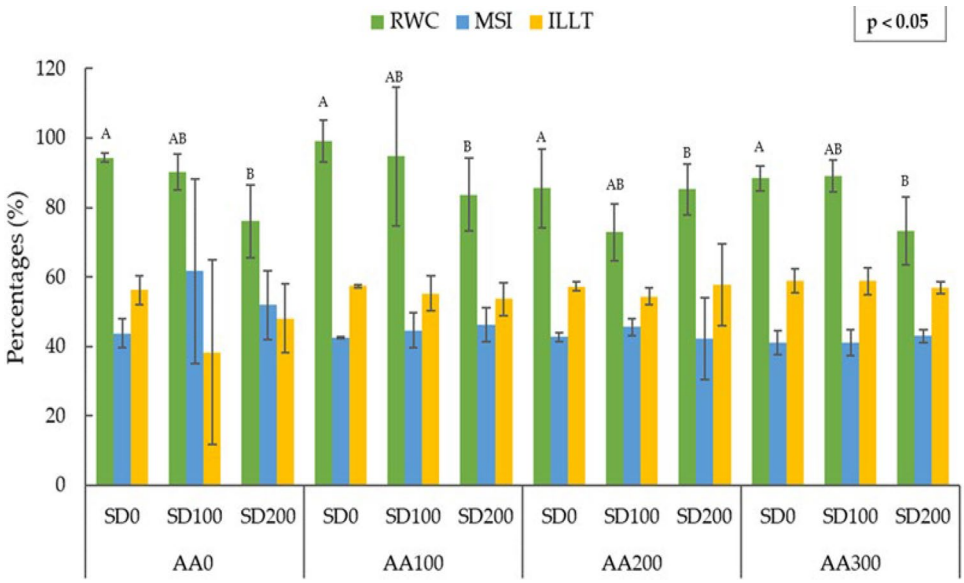


Fig. 2 The effects of ascorbic acid treatments on leaf relative water content (RWC), membrane stability index (MSI), and ion leakage (ILLT) of peanuts (*A. hypogaea*) under salt stress. The values followed by the same case letter are not significantly different ($p > 0.05$), according to ANOVA with Duncan Multiple Range Test

increased osmotic pressure and reduced growth parameters [40]. Numerous studies have investigated various nutrients' ability to mitigate plant salt stress. Among these, ascorbic acid (AA) has been identified as a potentially effective solution to reduce crop salinity damage. For instance, different rates of AA application have been shown to increase plant length

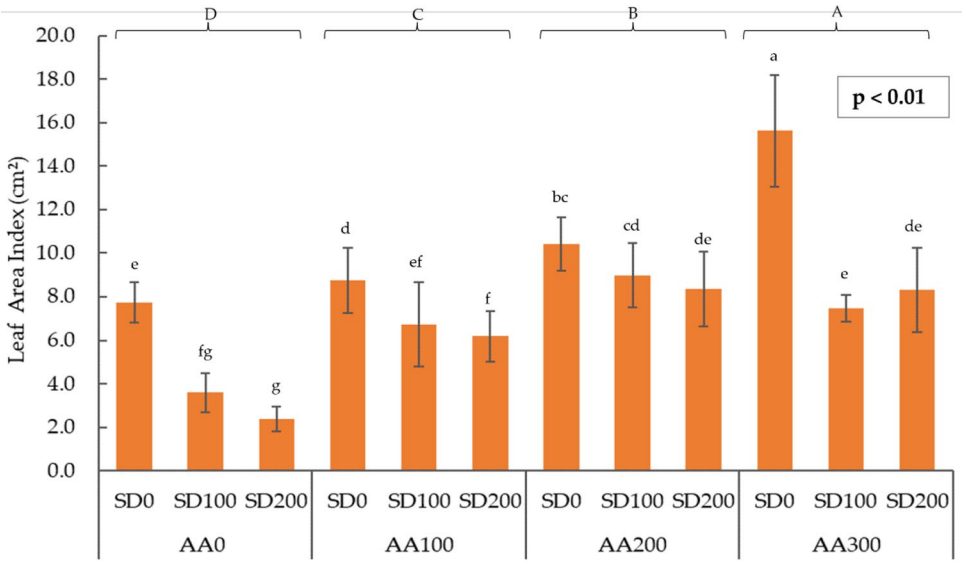


Fig. 3 The effects of ascorbic acid treatments on the leaf area index (LAI) of peanuts (*A. hypogaea*) under salt stress. The values followed by the same case letter are not significantly different ($p > 0.05$), according to ANOVA with Duncan Multiple Range Test

Table 3 The effects of ascorbic acid treatments on leaf surface temperature (LST) of peanuts (*A. hypogaea*) under salt stress

AA dose (ppm)	NaCl dose (mM)	LST (°C)
AAD-0	SD-0	18.80
	SD-100	18.90
	SD-200	19.30
AAD Mean		19.0 ^{B}
AAD-100	SD-0	19.00
	SD-100	19.10
	SD-200	19.20
AAD Mean		19.1 ^{AB}
AAD-200	SD-0	19.10
	SD-100	19.10
	SD-200	19.30
AAD Mean		19.2 ^{AB}
AAD-300	SD-0	19.50
	SD-100	19.50
	SD-200	19.50
AAD Mean		19.5 ^{A}
SD Mean	SD-0	19.10
	SD-100	19.10
	SD-200	19.30
CV (%)		1.36
Salt Dose		ns
Ascorbic Acid Dose		**
SD x AAD		ns

The values followed by the same case letter are not significantly different ($p > 0.05$), according to ANOVA with Duncan Multiple Range Test. **$p < 0.01$; *$p < 0.05$; ns: $p > 0.05$, *CV* coefficient of variation, *AAD* ascorbic acid dose, *SD* salt doses, *LST* leaf surface temperature

by up to 97.9% in sorghum varieties exposed to salt stress [38]. Similarly, the foliar application of AA has enhanced stem fresh weight in wheat [41]. The beneficial effects of AA in alleviating salt stress are evident in anatomical and histological studies. These studies reveal that AA application can result in notable differences in petiole stems and roots under salt stress conditions [42]. Moreover, research on turnips demonstrated that AA treatment applied to seeds significantly increased root and stem lengths [43, 44]. These findings are consistent with our results, highlighting the effectiveness

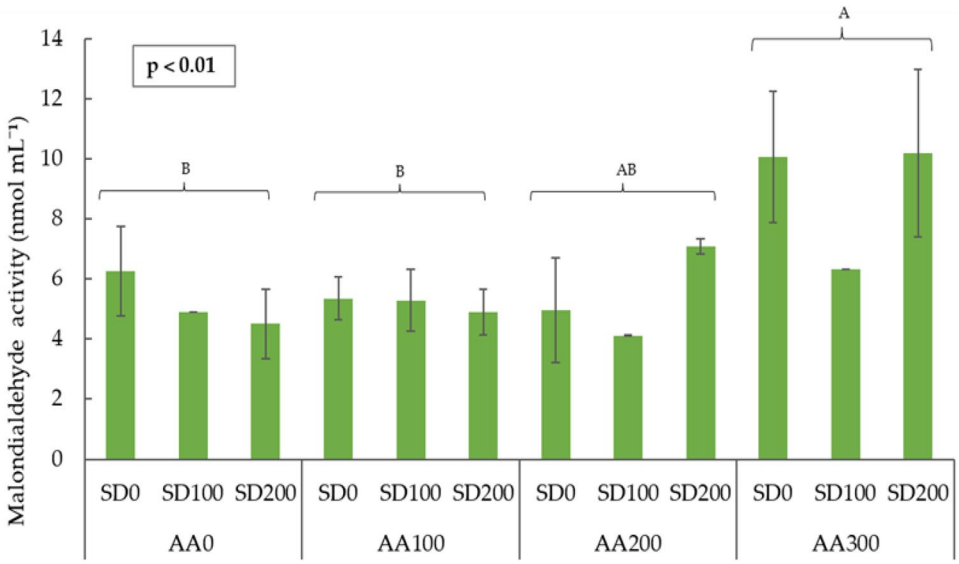


Fig. 4 The effects of ascorbic acid treatments on malondialdehyde (MDA) content in leaf tissues of peanuts (*A. hypogaea*) under salinity conditions. The values followed by the same case letter are not significantly different ($p > 0.05$), according to ANOVA with Duncan Multiple Range Test

of AA in promoting growth under salt stress conditions. The efficacy of ascorbic acid in enhancing plant development and alleviating stress may fluctuate according to several factors, such as genotypic variations, application methods, and concentration. In the first case, distinct plant species and even variants within a species may have differing sensitivities and reactions to stress and treatment. Furthermore, various application methods of AA (foliar or soil) demonstrate that the time spent on stress exposure and AA concentration can significantly influence its efficacy, as seen by divergent results in the scientific literature.

One study on wheat found that foliar application of AA significantly increased stem dry weight. However, in our research, the effect of salt doses on peanut hull dry weight was statistically insignificant. This discrepancy may be attributed to differences in plant species and varieties, as the effectiveness of AA applications can vary accordingly [43]. Ascorbic acid's antioxidant properties help mitigate plant stress factors, and its role in enhancing cell division and proliferation is linked to the accumulation of indole-3-acetic acid in the meristem cells at the apical points of seedlings [45].

The variety of plant, the intensity of stress, and the duration of exposure influence root weight, seedling development, and salinity response. Root health is essential for evaluating plant resilience, as roots directly engage with soil [37]. For example, in the case of *Prosopis strombulifera* (Lam.) Benth, root length, and total dry weight losses were more severe under sodium sulfate (Na_2SO_4) salt stress compared to sodium chloride (NaCl) salt stress [40]. Similar studies have shown that vegetation temperature can vary significantly among plant genotypes and species, especially under stress conditions such as drought and salinity. This variability is often due to differences in morphological characteristics, including leaf color, wax content, and leaf size, which can influence canopy temperatures [46]. Factors such as radiation absorption, thermal conductivity, and latent heat flux also contribute to these temperature differences [47]. To mitigate extreme temperatures, plants employ various protective mechanisms. For example, they may open their stomata to enhance cooling through gas exchange. However, excessive temperatures can increase enzyme activity in stomatal cells, leading to cellular damage and reduced yield [48]. This highlights the complex interplay between plant physiological responses and environmental stressors, which can significantly impact plant growth and productivity.

Salt stress significantly impacts plants' photosynthetic activity, primarily affecting leaves through stomata closure, reduced transpiration, and CO_2 fixation, and reducing leaf area, further exacerbated by salt stress [41]. Research has shown that exogenous ascorbic acid (AsA) applications can help mitigate these adverse effects. For example, increasing the leaf area index through AsA applications has been reported to counteract some of the detrimental impacts of salt stress on plant growth. This is achieved through improvements in chlorophyll and carotenoid content, increased proline accumulation, and decreased hydrogen peroxide (H_2O_2) levels, all of which contribute to enhanced stress tolerance and growth in plants like soybean (*Glycine max* L. Merr.) [49, 50].

A study investigating drought stress in grapes found that ascorbic acid applications significantly improved the relative water content of the plants compared to the control group. The relative water content in this study ranged between 84.9% and 89.9% [51]. This improvement is crucial because low relative water content and turgor pressure in leaf tissues often lead to growth and development retardation under stress conditions [47]. Enhanced relative water content helps plants maintain cell turgor and overall physiological functions, mitigating some of the adverse effects of drought stress.

The membrane durability index can vary based on plant species, stress duration, severity, and the type of antioxidant applied [52]. Studies show that humic acid and silicon, similar to ascorbic acid, can improve membrane durability under stress conditions like salt and drought, protecting plant cells by enhancing stability [53]. Results from various research may be affected by environmental conditions and plant genotypic traits, resulting in discrepancies in the efficacy of stress treatment interventions. In studies examining the impact of salt stress on plants, the increase in ion leakage with higher salt doses is commonly attributed to damage to the plant cell membrane and its stability [54]. This damage results in higher electrolyte leakage, which is a crucial indicator of membrane integrity and plant stress response. Various pre-treatments, including humic acid, ascorbic acid, silicon, gibberellic acid, and seaweed, have been shown to offer protective and preventive effects against stress conditions [14]. Researchers can evaluate membrane damage and determine a plant's stress resistance index by measuring electrolyt leakage from cells [55]. Furthemore, it was observed that ascorbic acid applications led to a 20.4% increase in MDA values in two sorghum cultivars subjected to salt stress [39]. MDA, a byproduct of membrane lipid peroxidation, serves as an indicator of oxidative damage within cells [56]. This increase in MDA levels reflects the extent of cellular damage caused by oxidative stress. By inhibiting salt-induced lipid peroxidation, AA may stabilize the plasma membrane, thereby preserving its integrity and reducing overall cellular damage [57]. The findings suggest that ascorbic acid applications can partially mitigate the stress-induced damage to plant cells, contributing to improved stress resilience. A two-year study on wheat found that the nitrogen content in leaves ranged from 3.0 to 10.93 mg g^{-1} [58]. This variability highlights that the nitrogen balance index (NBI) can differ significantly depending on plant parts, as well as between different plant genera and species [59]. Our investigation revealed no substantial alteration in chlorophyll levels attributable to the moderating influence of ascorbic acid (AA) treatments, in contrast to prior research on sorghum conducted by Gaafar and colleagues. Research indicates that AA can augment chlorophyll content by serving as a cofactor in the formation of gibberellin and ethylene [60]. A similar study found that ascorbic acid (AA) helps protect photosynthetic pigments in peanut plants under salt stress, enhancing their resilience compared to control groups [61]. AA is known for its role in synthesizing antioxidants such as flavonoids, anthocyanins, and α-tocopherol, which are crucial for maintaining photosynthesis, cell development, and cell wall expansion [62, 63]. While these findings align with the benefits observed in this study, it is noted that the impact of AA on flavonoid and anthocyanin levels was statistically insignificant. This suggests that the effectiveness of AA can vary based on stress severity and plant type, highlighting the need for further investigation into how different conditions affect its role in antioxidant synthesis and overall plant health.

5 Conclusion

Ascorbic acid has shown protective and curative effects on peanuts under salt stress. Under 200 mM salt stress, reductions in stem length, weight, leaf area index, and relative water content were observed. Increasing salt doses led to reduced growth and development parameters. However, 300 ppm ascorbic acid application resulted in significant improvements, including increased stem length, root length, stem fresh weight, stem dry weight, plant temperature, and leaf area index. Salt stress did not significantly affect root fresh and dry weight, membrane strength index, ion leakage, or certain morphological, physiological, and Dualex values. These findings highlight the efficacy of ascorbic acid as a biofertilizer and regulatory agent to alleviate salt stress in crops, emphasizing the necessity of developing and implementing sustainable strategies for farmers to uphold agricultural sustainability, taking into account various factors such as the timing of foliar application and optimal concentrations of ascorbic acid in conjunction with other agronomic practices. This research contributes valuable insights for future studies aimed at enhancing crop resilience and productivity under stress conditions.

Author contributions Conceptualization: LN and RT Methodology: EO, LN, MT, SN Software: YRD and LN Validation: RT, MT, and LN Formal analysis: LGP, BF, and YRD Investigation: LN, EO, RT, and SN Resources: MT and RT Data curation: YRD and LGP Writing—original draft preparation: RT, EO, LN and MT Writing—review and editing: YRD, LGP, and BF Supervision, MT, and YRD Funding acquisition, RT, and LN.

Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Competing interests The authors declare no competing interests.

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