Received: 8 December 2025 | Revised: 13 April 2026 | Accepted: 9 May 2026

https://doi.org/10.56946/jspae.v5i1.844
Volume 5 (1): 25-33

Journal of Soil, Plant and Environment

Research Article

Independent and Interactive Effects of Salinity and Proline on Physiological and Biochemical Responses of Agave Plants

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Abstract

Salinity is one of the most critical abiotic stresses limiting plant growth and productivity in arid and semi-arid regions. A pot experiment was conducted under greenhouse conditions at the National Research Centre, Giza, Egypt, during two successive seasons (2020–2021) to evaluate the effects of salinity stress and exogenous proline application on growth performance and biochemical attributes of *Agave angustifolia* var. *pacifica*. The experiment included four salinity levels (0, 20,000, 25,000, and 30,000 ppm NaCl) combined with three foliar proline concentrations (0, 100, and 200 ppm). Results showed that increasing salinity levels significantly reduced growth parameters, including plant height, leaf number, leaf area index, and root length. Similarly, photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll) were markedly decreased under salt stress. In contrast, salinity stress enhanced osmotic pressure, leaf electrical conductivity, and endogenous proline accumulation, indicating a strong stress response. Exogenous application of proline mitigated the adverse effects of salinity by improving chlorophyll stability, enhancing carbohydrate and protein content, and supporting osmotic adjustment under moderate stress conditions. However, its protective efficiency declined under severe salinity (30,000 ppm NaCl). Interaction analysis revealed that proline supplementation partially alleviated salinity-induced damage, improving the overall physiological performance of agave plants. These findings highlight the potential role of proline as a protective osmolyte and antioxidant in improving salinity tolerance. The study suggests that exogenous proline application can be an effective strategy to enhance the resilience and physiological stability of agave plants under saline environments.

Keywords: *Agave angustifolia*, Salinity stress, Proline, Osmotic adjustment, Biochemical traits, Sress physiology.

1. Introduction

Soil salinity is among the most pervasive abiotic stresses limiting plant growth and agricultural productivity worldwide. It currently affects more than 20% of irrigated lands, and this proportion is expected to increase due to climate change, improper irrigation practices, and rising sea levels [1]. Salinity imposes a dual constraint on plants: osmotic stress, which limits water uptake, and ionic toxicity, which disrupts nutrient balance and metabolic processes [2, 3]. In addition, salt stress induces oxidative damage through the excessive generation of reactive oxygen species (ROS), resulting in cellular injury, chlorophyll degradation, and reduced photosynthetic efficiency [4].

To cope with these adverse conditions, plants have evolved a range of adaptive mechanisms, including osmotic adjustment, maintenance of ion homeostasis, activation of antioxidant defense systems, and accumulation of compatible solutes such as proline, glycine betaine, and soluble sugars [5, 6]. Among these, proline has gained considerable attention as a multifunctional protective molecule. It acts not only as an osmoprotectant but also as a ROS scavenger, redox buffer, and stabilizer of proteins and cellular membranes under stress conditions [7, 8, 9].

Recent studies have highlighted the agronomic significance of exogenous proline application in enhancing plant tolerance to salinity stress. For example, foliar application of 200 ppm proline significantly improved yield and physiological performance in sugar beet under saline conditions [10]. Similarly, in guava, proline treatment enhanced chlorophyll content, gas exchange parameters, and overall plant growth under saline irrigation [11]. Furthermore, a meta-analysis of proline metabolism-related genes demonstrated that genetic regulation of proline biosynthesis can enhance tolerance to both drought and salinity stresses; however, these benefits are often stress-specific and may diminish under extreme conditions [12].

Species of the genus Agave, particularly *Agave angustifolia* var. *pacifica*, are perennial succulent plants well adapted to arid and semi-arid environments. These plants are increasingly valued not only for ornamental purposes but also for their roles in ecological restoration, fiber production,

bioenergy generation, and pharmaceutical applications [13, 14]. Despite their inherent tolerance to drought, Agave species remain susceptible to salinity stress, especially during early developmental stages. Previous studies have reported that salinity adversely affects growth, photosynthetic performance, and biochemical metabolism in Agave plants [15]. However, comprehensive investigations into the mechanisms of salinity tolerance in Agave—particularly the role of proline in stress mitigation—remain limited compared to other horticultural crops.

Moreover, Agave species are frequently utilized in coastal regions for sand dune stabilization and mitigation of seawater erosion, underscoring the practical importance of improving their salinity tolerance. Therefore, elucidating the potential of exogenous proline to alleviate salt stress in Agave is both scientifically and ecologically significant. Accordingly, the present study aimed to evaluate the individual and interactive effects of different salinity levels (0, 20,000, 25,000, and 30,000 ppm NaCl) and proline concentrations (0, 100, and 200 ppm) on growth parameters and biochemical attributes of *Agave angustifolia* var. *pacifica*, with physiological interpretations supported by updated literature up to 2025.

2. Materials and Methods

2.1 Experimental site

This study was conducted in a greenhouse at the National Research Centre (NRC) in Giza Governorate, Egypt, during two successive growing seasons (2020 and 2021). The site is located at approximately 30.00° N latitude and 31.21° E longitude. The region is characterized by a hot arid desert climate (BWh, Köppen–Geiger classification), with a mean annual air temperature of approximately 22°C, hot summers, mild winters, and very low annual rainfall averaging 15–20 mm, mainly occurring during winter months. Mean relative humidity ranges from 35% to 55% throughout the year [16]. These climatic conditions are representative of typical arid urban environments and are relevant for evaluating plant responses to salinity stress and proline applications.

2.2 Plant material and growing conditions

Uniform seedlings of *Agave angustifolia* var. *pacifica* were obtained from Al Noubaria farms (affiliated with the National Research Centre). Seedlings were transplanted into plastic pots of 30 cm diameter (one seedling per pot). Each pot contained 5 kg of homogenized sandy loam soil. Pots were placed under naturally lit greenhouse conditions with average day/night temperatures of 28/18°C and relative humidity of 45–50%. All agricultural practices (irrigation, weeding, pest control) other than the experimental treatments were applied uniformly according to the recommendations of the Egyptian Ministry of Agriculture.

2.3. Experimental design and treatments

The experiment was conducted using a completely randomized design (CRD) with a factorial arrangement of two factors: salinity at four levels and proline at three levels, resulting in a total of 12 treatment combinations. Each treatment was replicated three times, yielding 36 experimental units (pots) per growing season.

2.3.1. Salinity treatments (NaCl)

Salinity stress was imposed by irrigating plants with sodium chloride (NaCl) solutions at concentrations of 0 ppm (control, tap water), 20,000 ppm (2% NaCl), 25,000 ppm (2.5% NaCl), and 30,000 ppm (3% NaCl). To minimize osmotic shock, salinity levels were increased gradually over a one-week acclimation period. Thereafter, plants were irrigated with the respective saline solutions every 3–4 days, maintaining soil moisture at approximately 70% of water-holding capacity (field capacity). Control plants received equal volumes of tap water with an electrical conductivity (EC) of approximately 0.4 dS m^{-1}.

2.3.2. Proline treatments

Proline (L-proline, ≥99% purity, Sigma-Aldrich, USA) was prepared in distilled water and applied as a foliar spray at concentrations of 0, 100, and 200 ppm. Foliar applications were carried out three times during the growing season, specifically at 30, 60, and 90 days after transplanting. Sprays were applied in the early morning (07:00–08:00 h) until run-off, with an approximate volume of 50 mL per plant. Control plants were treated with distilled water only. A surfactant (Tween-20 at 0.1%) was added to all spray solutions to ensure uniform leaf coverage.

2.4. Sampling and measurements

At the end of each growing season (120 days after transplanting), plants were carefully uprooted and washed with distilled water to remove adhering soil particles. The plants were then separated into shoots and roots for subsequent growth and biochemical analyses.

2.4.1. Growth parameters

Vegetative growth characteristics were assessed by measuring plant height, leaf number, leaf area, and root length. Plant height (cm) was recorded from the soil surface to the tip of the longest leaf using a graduated ruler. Leaf number was determined by counting all fully expanded green leaves per plant. Leaf area (cm^2) was estimated using a non-destructive method based on the formula: leaf area = length × maximum width × 0.75, where 0.75 represents a calibration factor specific to Agave. Root length (cm) was measured from the root crown to the tip of the longest root. All measurements were obtained from three replicate plants per treatment and averaged.

2.4.2. Biochemical parameters

Fresh leaf samples were collected from the middle portion of fully expanded leaves, immediately frozen in liquid nitrogen, and stored at −80°C until further analysis. Photosynthetic pigments, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, were extracted using 80% acetone and quantified spectrophotometrically following the methods of Saric et al. [17] and Lichtenthaler [18], with absorbance measured at 663, 645, and 470 nm, respectively. Pigment contents were expressed as mg g^{-1} fresh weight (FW). Total

carbohydrates were determined colorimetrically using the phenol–sulfuric acid method described by Dubois et al. [19], using glucose as a standard, and expressed as mg g^{-1} dry weight. Total soluble proteins were extracted in phosphate buffer (pH 7.0) and quantified using the Lowry method as modified by Gupta et al. [20], with bovine serum albumin (BSA) as a standard. Free proline content was measured according to Bates et al. [21] using the acid-ninhydrin method, with absorbance recorded at 520 nm, and expressed on a dry weight basis. Total phenolic content was determined using the Folin–Ciocalteu reagent as described by A.O.A.C. [22], with gallic acid as the reference standard. Osmotic pressure of leaf sap was estimated based on freezing point depression using an osmometer (Model 3320, Advanced Instruments, USA) and expressed in atmospheres (atm). Leaf electrical conductivity (EC) was determined by incubating 0.5 g of fresh leaf discs in 10 mL of distilled water for 24 h at 25°C, followed by measurement using a conductivity meter according to Arvin [23].

2.5. Statistical analysis

Data from both growing seasons were subjected to analysis of variance (ANOVA) appropriate for a completely randomized design with a factorial treatment structure. The assumptions of normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. As the interaction between season and treatment was not significant ($p > 0.05$), data from both seasons were pooled for subsequent analysis. Treatment means were separated using the least significant difference (LSD) test at the 5% probability level, following the procedure of Snedecor and Cochran [24]. Combined analysis across seasons was conducted according to Steel and Torrie [25]. All statistical analyses were performed using IBM SPSS Statistics (version 25.0; IBM Corp., Armonk, NY, USA)..

3. Results

3.1. Independent effect of salinity on growth and biochemical traits

Salinity exerted a significant ($p \leq 0.05$) negative effect on the vegetative growth of *Agave angustifolia* var. *pacifica* (Figure. 1-3). Increasing NaCl concentration progressively reduced plant height, leaf number, leaf area index, and root length (Figure 1). The most pronounced reduction was observed at 30,000 ppm, where root length declined sharply from 125.33 cm in the control to 28.50 cm. Similarly, leaf number decreased from 13.72 to 7.78–9.44 leaves per plant under salinity stress. Photosynthetic pigments were also significantly affected by salinity (Figure 2). Although fluctuations were observed, overall trends indicated impairment of the photosynthetic apparatus under saline conditions. Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids showed variability but were generally associated with stress-induced physiological imbalance. Salinity markedly altered biochemical constituents (Figure 3). Total carbohydrates decreased from 142.91 mg g^{-1} DW in the control to 112.27 mg g^{-1} DW at 25,000 ppm. In contrast, protein content slightly increased with salinity, reaching 1.94 mg mL^{-1} FW at 30,000 ppm (Figure 3A-D). Electrical conductivity (EC), an indicator of membrane damage, increased substantially under salinity stress (73.45 ms μs^{-1} at 20,000 ppm compared to 42.02 in control) (Figure 3D). Similarly, endogenous proline accumulation increased under moderate salinity (17.76 mg mL^{-1} DW at 20,000 ppm), indicating activation of osmotic adjustment mechanisms.

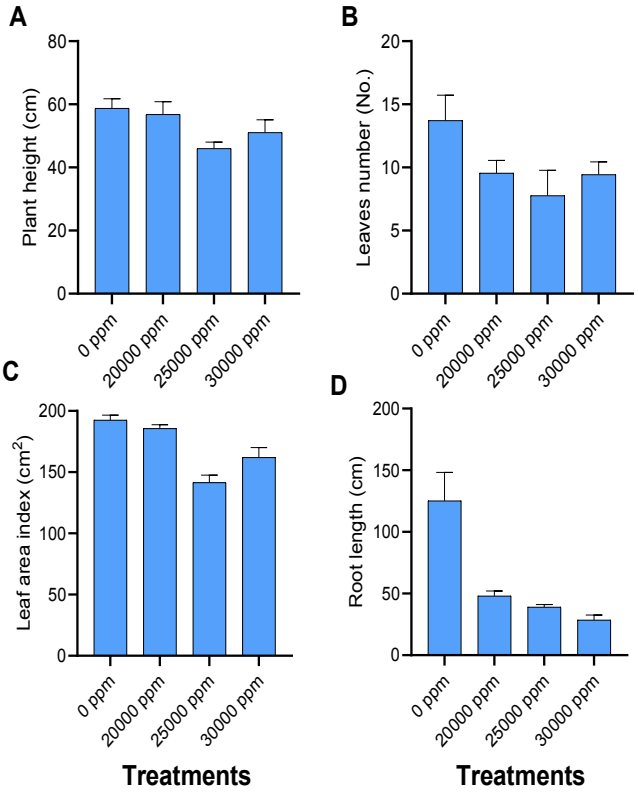

Figure 1. Effect of different salinity treatments on (A) plant height, (B) number of leaves, (C) leaf area index, and (D) root length. 0–30,000 ppm represents salinity levels.

3.2. Independent effect of proline on growth and biochemical traits

Exogenous application of proline significantly influenced growth and biochemical parameters of Agave plants (Table 1). Proline treatments exhibited a dose-dependent response, with 200 ppm generally improving physiological performance under stress conditions. Application of 200 ppm proline enhanced photosynthetic pigments, with total chlorophyll increasing to 0.97 mg g^{-1} FW compared to 0.75 mg g^{-1} FW in untreated plants. Carotenoid content also showed a slight increase, suggesting improved photoprotection(Table 2). Biochemical parameters responded positively to proline application. Total carbohydrates increased markedly at 200 ppm (139.03 mg g^{-1} DW), while protein content also improved compared to the control (Table 3). Osmotic pressure increased with proline application, indicating enhanced osmotic adjustment capacity. However, growth parameters showed mixed responses. While proline improved stress tolerance, excessive concentrations (200 ppm) slightly reduced plant height and leaf area compared to untreated plants, suggesting a trade-off between growth and stress adaptation.

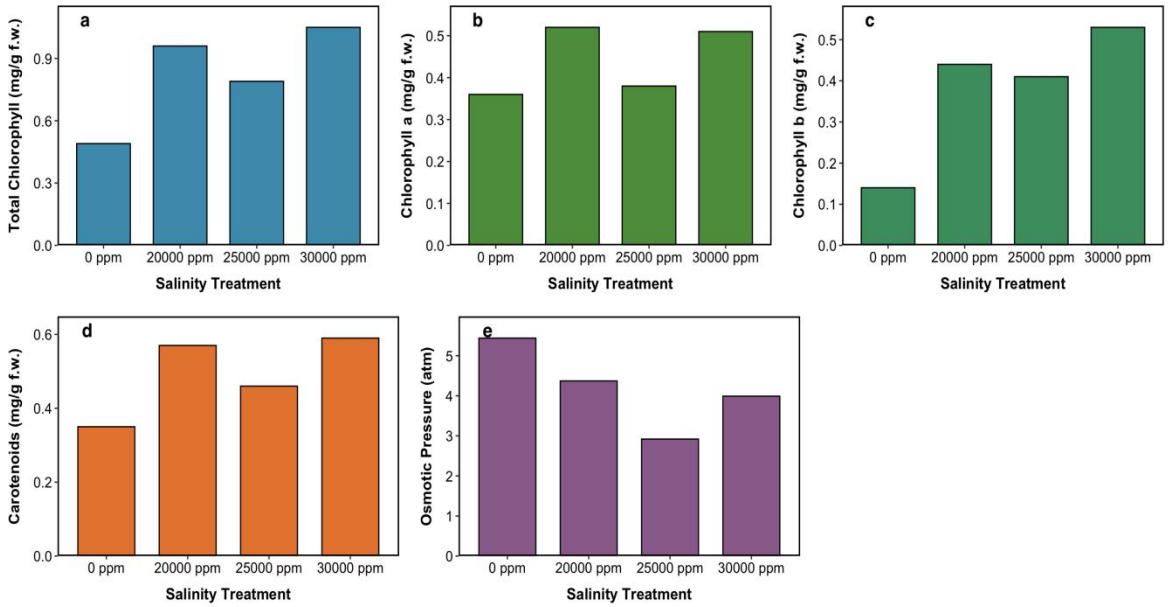

Figure 2. Effect of salinity concentration (0-30000 ppm) on (a) total chlorophyll, (b) chlorophyll a, (c) chlorophyll b, (d) carotenoids, and (e) leaf osmotic pressure of Agave plants. Values represent means from two growing seasons.

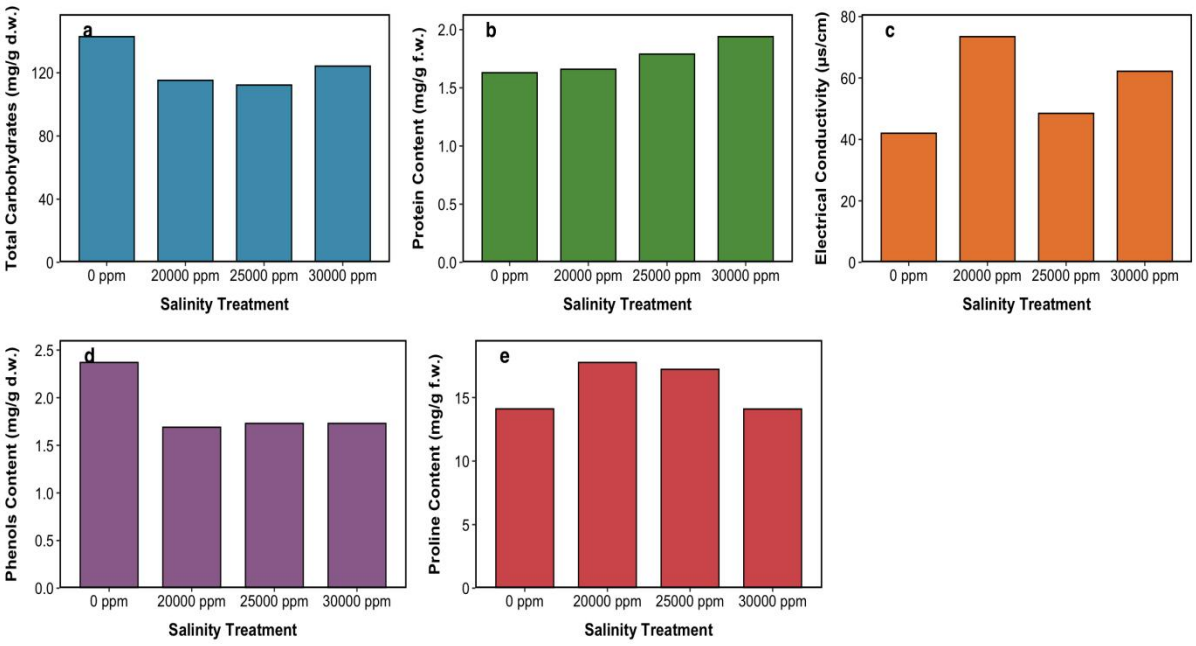

Figure 3. Effect of salinity on biochemical parameters of agave plants: (a) total carbohydrates, (b) protein content, (c) electrical conductivity, (d) phenols content, and (e) proline content.

Table 1. Effects of proline application on growth-related morphological traits of Agave plants (mean of two seasons)

Treatments	Plant height (cm)	Leaves number (No.)	leaf area index (cm^2)	Root length (cm)
0ppm	59.08	12.75	195.20	70.83
100 ppm	53.96	08.67	163.54	48.38
200 ppm	46.50	08.96	153.72	61.63
L.S.D. 0.05	04.20	01.46	012.24	13.92

Table 2. Effects of proline application on photosynthetic pigments and osmotic regulation in Agave plants (mean of two seasons)

Treatments	Total chlorophyll content (mgg^{-1}f.w.)	Chlorophyll a content (mgg^{-1}f.w.)	Chlorophyll b content (mgg^{-1}f.w.)	Carotenoid's content (mgg^{-1}f.w.)	Osmotic pressure of leaf (atm)
0ppm	0.750	0.40	0.35	0.47	3.890
100 ppm	0.750	0.42	0.33	0.48	3.870
200 ppm	0.970	0.51	0.46	0.52	4.780
L.S.D. 0.05	0.003	8.43	8.43	8.43	0.008

Table 3. Effects of proline application on biochemical and physiological attributes of Agave plants (mean of two seasons).

Treatments	Total carbohydrates (mgg^{-1}d.w.)	Protein content (mgml^{-1}f.w.)	Electrical conductivity of leaves (msμs^{-1})	Phenol content (mgg^{-1}d.w.)	Proline content (mgml^{-1}d.w.)
0ppm	115.780	1.590	40.210	2.200	16.010
100 ppm	116.230	1.880	68.830	1.740	16.160
200 ppm	139.030	1.810	60.570	1.660	15.230
L.S.D. 0.05	000.008	0.008	00.008	0.008	00.008

3.3. Interactive effects of salinity and proline

The interaction between salinity stress and exogenous proline application showed a statistically significant effect ($p \leq 0.05$) on most of the measured morphological and biochemical parameters of Agave plants (Table 4). Overall, the results clearly indicate that plant performance was strongly influenced by the combined effect of both factors, rather than by salinity or proline alone. Increasing salinity levels consistently reduced growth, physiological activity, and biochemical constituents, while proline application partially alleviated these adverse effects, particularly under moderate stress conditions. However, the magnitude of this mitigation varied depending on the severity of salinity stress, indicating a threshold-dependent response.

In terms of growth parameters, control plants exhibited the highest values for plant height (63.33 cm), leaf number (14.67), leaf area index (243.13 cm^2), and root length (168.00 cm), reflecting optimal growth under non-stress conditions. Even under low salinity levels (100–200 ppm), only slight reductions were observed, suggesting that Agave can tolerate mild salinity without major growth suppression. However, a progressive decline became evident as salinity increased to 20,000 ppm and beyond. At 20,000 ppm salinity alone, plant height slightly decreased to 61.00 cm, while more noticeable reductions were observed in leaf area (194.08 cm²) and especially root length (60.00 cm), indicating that root growth is more sensitive to salinity-induced osmotic stress than shoot growth.

The application of proline under this moderate salinity level helped maintain improve growth performance. For example, with 100 ppm proline under 20,000 ppm salinity, plant height remained stable at 61.00 cm and leaf number showed only a slight reduction (13.50 compared to 13.67 in salinity alone), suggesting that proline contributes to osmotic adjustment and helps sustain cell turgor under saline conditions. Similar trends were observed in leaf area, where proline-treated plants showed improved retention compared to untreated stressed plants, indicating partial protection of photosynthetic structures.

As salinity increased further to 25,000 ppm and 30,000 ppm, growth suppression became more pronounced. At 25,000 ppm salinity, plant height declined to 52.00 cm, leaf number reduced to 8.00, and root length dropped to 41.00 cm. This reduction became more severe at 30,000 ppm, where plant height reached only 48.00 cm and root length decreased sharply to 26.50 cm, indicating strong inhibition of both shoot and root development. When proline was applied at these higher salinity levels, only slight improvements were observed. For instance, at 25,000 ppm combined with 100 ppm proline, plant height decreased further to 49.00 cm and root length remained low (40.00 cm), while at 30,000 ppm with 200 ppm proline, plant height reached a minimum of 38.00 cm and root length declined drastically to 19.00 cm. These results clearly show that although proline provides some buffering capacity, its effectiveness diminishes significantly under extreme salinity stress.

A similar pattern was observed in physiological and biochemical traits (Table 5). Total chlorophyll content, chlorophyll a, chlorophyll b, and carotenoids all decreased progressively with increasing salinity. The highest chlorophyll content (1.560 mg g^{-1} fresh weight) was recorded in control plants, whereas the lowest value (0.340 mg g^{-1}) was observed under 30000 ppm salinity with proline application. This strong reduction indicates severe impairment of chloroplast structure and photosynthetic efficiency under high salinity conditions. Proline application helped maintain relatively higher pigment levels under moderate salinity, suggesting a protective role in stabilizing chloroplast membranes and reducing oxidative damage. However, under severe salinity stress, this protective effect was insufficient to prevent pigment degradation.

Total carbohydrates and protein content followed a similar decreasing trend with increasing salinity. Carbohydrates declined from 23.84 mg g^{-1} in control plants to nearly half under high salinity conditions, reflecting reduced

photosynthetic activity and carbon assimilation. Protein content also decreased steadily, indicating disruption in nitrogen metabolism and increased protein degradation under stress. Proline application slightly improved carbohydrate and protein retention under moderate salinity, suggesting that it may enhance metabolic stability and enzymatic function under stress conditions. However, this effect was not sustained at higher salinity levels.

Table 4. Interactive effects of salinity & proline on growth measurements of Agave plants. (Mean of two seasons)

Treatments	Plant height (cm)	Leaves number (No.)	Leaf area index (cm²)	Root length (cm)
0 ppm	63.33 ± 3.17	14.67 ± 0.73	243.13 ± 8.16	168.00 ± 8.40
100 ppm	63.00 ± 3.15	14.00 ± 0.70	198.67 ± 7.93	108.50 ± 5.43
200 ppm	61.50 ± 3.08	14.00 ± 0.70	198.00 ± 9.10	99.50 ± 4.98
20000 ppm	61.00 ± 3.05	13.67 ± 0.68	194.08 ± 9.70	60.00 ± 3.00
20000 ppm + 100 ppm	61.00 ± 3.21	13.50 ± 0.68	181.30 ± 9.07	59.00 ± 2.95
20000 ppm + 200 ppm	52.33 ± 2.62	8.67 ± 0.43	161.00 ± 8.05	43.33 ± 2.17
25000 ppm	52.00 ± 2.60	8.00 ± 0.50	157.50 ± 7.88	41.00 ± 2.05
25000 ppm + 100 ppm	49.00 ± 2.45	8.00 ± 0.47	153.00 ± 7.65	40.00 ± 2.00
25000 ppm + 200 ppm	48.00 ± 2.40	8.00 ± 0.40	145.60 ± 7.28	32.00 ± 1.60
30000 ppm	48.00 ± 2.30	6.67 ± 0.33	142.00 ± 7.10	26.50 ± 1.33
30000 ppm + 100 ppm	41.00 ± 2.05	6.33 ± 0.32	137.00 ± 6.85	26.50 ± 1.33
30000 ppm + 200 ppm	38.00 ± 1.90	6.00 ± 0.30	134.75 ± 6.74	19.00 ± 0.95
L.S.D. (0.05)	8.4	2.92	24.29	27.83

Table 5. Inertactive effects of salinity & proline on chemical constituents of Agave plants. (Mean of two seasons)

Treatments	Total chl	Chl a	Chlb	Carotenoids	Total carbohydrates (mgg^{-1}d.w.)	Protein content ($mgml^{-1}$f.w.)	Electrical conductivity of leaves (mS μs^{-1})	Phenols content (mgg^{-1}d.w.)	Proline content ($mgml^{-1}$d.w.)
	(mgg^{-1}f.w.)								
0ppm	1.560	0.74	0.830	0.850	23.84	2.32	96.06	9.18	154.11
100 ppm	1.550	0.72	0.830	0.770	22.92	1.97	68.71	6.41	144.71
200 ppm	1.490	0.71	0.750	0.770	18.71	1.92	66.91	5.96	142.73
20000 ppm	1.120	0.50	0.600	0.610	17.75	1.89	63.62	3.97	136.46
20000 ppm + 100 ppm	0.800	0.48	0.340	0.560	17.71	1.85	62.67	3.97	132.79
20000 ppm + 200 ppm	0.750	0.46	0.270	0.490	16.90	1.84	62.35	3.97	131.89
25000 ppm	0.590	0.37	0.220	0.430	14.12	1.81	61.61	3.58	120.84
25000 ppm + 100 ppm	0.490	0.32	0.220	0.400	13.80	1.80	50.89	3.58	119.18
25000 ppm + 200 ppm	0.480	0.31	0.110	0.310	11.66	1.66	50.12	3.12	110.74
30000 ppm	0.390	0.27	0.170	0.250	11.64	1.47	48.14	3.18	108.01
30000 ppm + 100 ppm	0.340	0.27	0.120	0.240	10.43	1.35	47.20	1.59	101.29
30000 ppm + 200 ppm	0.340	0.17	0.020	0.220	10.10	1.20	00.09	1.59	081.36
L.S.D. 0.05	0.005	0.002	0.002	0.002	00.02	0.02	00.02	0.02	000.01

Note: Chl = chlorophyll; Chl a = chlorophyll a; Chl b = chlorophyll b; Carotenoids = total carotenoid pigments. Total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids are expressed as mg g^{-1} fresh weight (f.w.). Total carbohydrates and phenols are expressed as mg g^{-1} dry weight (d.w.). Protein content and proline content are expressed as mg mL^{-1} fresh weight (f.w.) and mg mL^{-1} dry weight (d.w.), respectively. Electrical conductivity (EC) of leaves is expressed as mS μs^{-1} . L.S.D. refers to Least Significant Difference at the 5% probability level ($p \leq 0.05$). Values are means of two seasons. Treatments include salinity levels (ppm) alone and in combination with exogenous proline application. "+" indicates combined treatment of salinity and proline, and "0 ppm" indicates control (no salinity, no proline application).

Leaf osmotic pressure increased under salinity stress, indicating an adaptive response to maintain cellular water balance (Table 5). However, extreme salinity caused a disruption in this regulation, and proline application helped only partially in stabilizing osmotic adjustment. Electrical conductivity of leaf tissues increased significantly with salinity, reflecting ion accumulation and membrane damage. Proline slightly reduced conductivity under moderate stress, indicating improved membrane stability, but under severe stress, conductivity remained high regardless of treatment, suggesting extensive cellular injury.

Phenolic content generally declined with increasing salinity, although proline application helped maintain slightly higher levels under moderate stress conditions. This suggests that proline may support antioxidant defense mechanisms by promoting secondary metabolite accumulation. However, under severe salinity stress, phenolic synthesis was significantly inhibited, indicating that metabolic disruption outweighed protective responses.

4. Discussion

The present study demonstrated that salinity stress markedly inhibited the growth and altered the biochemical composition of Agave plants, confirming the sensitivity of early vegetative development to saline environments despite the inherent stress tolerance typically associated with succulent species.

4.1. Salinity Restricts Growth and Alters Metabolic Functions

Salinity stress significantly restricted Agave growth, consistent with the combined effects of osmotic stress and ionic toxicity previously reported in various plant species [2, 3, 26, 27,]. The observed reductions in chlorophyll and carotenoid contents indicate severe impairment of the photosynthetic apparatus, likely resulting from oxidative damage and chloroplast structural degradation, as similarly documented in wheat and turfgrass systems [28, 29, 4]. In the present study, salinity markedly reduced plant height, leaf number, leaf area index, and root length, reflecting a generalized inhibition of both cell expansion and division. Such growth suppression is a well-established consequence of elevated soil osmotic potential, which limits water uptake and disrupts physiological processes [27]. Comparable reductions in vegetative growth, ion homeostasis, and antioxidant activity have also been reported in maize genotypes exposed to NaCl stress [26]. Moreover, exogenous application of proline has been widely recognized for enhancing salt tolerance through improved osmolyte accumulation and regulation of ion balance across multiple plant systems [30-32], while recent studies further emphasize its role in strengthening antioxidant defenses and minimizing Na^+ toxicity under saline conditions [33-35].

4.2. Endogenous Proline Accumulation as an Adaptive but Limited Response

The accumulation of endogenous proline under salinity stress observed in this study confirms its role as a key adaptive metabolite involved in osmotic adjustment, protein stabilization, and reactive oxygen species (ROS) scavenging [7, 33, 36, 37]. However, the magnitude of this response was insufficient to fully counteract the detrimental effects of severe salinity, indicating that intrinsic proline accumulation alone does not ensure stress resilience under high ionic load. In parallel, salinity induced a pronounced decline in chlorophyll and carotenoid contents, a response commonly attributed to chloroplast degradation, inhibition of pigment biosynthesis, and enhanced chlorophyllase activity. These alterations ultimately reduce photosynthetic efficiency and accelerate oxidative stress, thereby further limiting plant growth and productivity [27, 39].

4.3. Exogenous Proline Enhances Tolerance Under Moderate Salinity Stress

Exogenous application of proline significantly alleviated the adverse effects of salinity, particularly under moderate stress conditions, by improving osmotic adjustment and maintaining cellular turgor [8, 9], stabilizing chloroplast structure and preserving pigment integrity [5], enhancing ROS detoxification capacity, and reducing lipid peroxidation [30, 38], as well as supporting carbohydrate and protein metabolism [10, 34]. Nevertheless, under severe salinity conditions (30,000 ppm), the protective effect of proline became negligible, suggesting that ionic toxicity and metabolic disruption exceeded the physiological buffering capacity conferred by osmoprotectants. Similar stress-intensity-dependent responses have been reported in sugar beet [10] and guava [11], where proline application improved performance only under moderate stress levels. Furthermore, a recent meta-analytic synthesis supports the view that the efficacy of exogenous proline is highly context-dependent, being limited under extreme environmental stress conditions [12]. These findings collectively suggest that proline functions primarily as a stress mitigator rather than a comprehensive protective agent, emphasizing the importance of integrated management strategies combining biostimulants, beneficial microorganisms, and genetic improvement approaches.

4.4. Stress-Intensity-Dependent Interaction Between Salinity and Proline

The significant interaction between salinity levels and proline treatments observed in this study underscores the critical role of stress intensity in determining the physiological effectiveness of osmop
rotectants. The pronounced beneficial effects of proline under moderate salinity stress indicate that its protective mechanisms are most efficient when stress remains within the adaptive threshold of the plant system. However, as stress severity increases, the capacity of proline to maintain cellular homeostasis declines, reflecting the overwhelming impact of ionic and osmotic imbalance. Similar interaction patterns have been reported in various horticultural and ornamental species, where exogenous proline application exhibits maximal efficacy under moderate but not extreme stress conditions [9, 15].

5. Conclusion

Salinity stress significantly reduced growth performance and altered biochemical constituents of Agave plants, mainly due to osmotic stress, ionic toxicity, and impaired nutrient uptake. Exogenous proline effectively mitigated the adverse effects of moderate salinity by improving growth, photosynthetic pigments, carbohydrates, and protein contents. However, under severe salinity, its protective effect was limited, indicating a stress-intensity-dependent response. Overall, proline serves as a partial ameliorative agent against salinity stress in Agave. Future research should focus on integrating proline with other mitigation strategies and elucidating its molecular mechanisms in salinity tolerance.

Author contributions

Conceptualization, Sami Ali Metwally Mohamed; methodology, Sami Ali Metwally Mohamed and Nermeen Mahdy Taha El-Sayed Badawy; formal analysis, Sami Ali Metwally Mohamed; investigation, Sami Ali Metwally Mohamed and Ibrahim El-Abasery Habba; data curation, Nermeen Mahdy Taha El-Sayed Badawy; writing—original draft preparation, Sami Ali Metwally Mohamed; writing—review and editing, Nermeen Mahdy Taha El-Sayed Badawy and Ibrahim El-Abasery Habba; supervision, Sami Ali Metwally Mohamed. All authors have read and agreed to the published version of the manuscript.

Ethical approval

Not applicable.

Conflicts of Interest

The authors report no conflicts of interest.

Acknowledgment

The authors thank the national research Centre (NRC), Giza, Egypt, for providing the facilities and support necessary to conduct this study.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Funding

This research was funded by the Bangladesh Jute Research Institute (BJRI).

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How to cite this article: Mohamed, S. A. M., Badawy, N. M. T. E.-S., & Habba, E. E.-A. (2026). Independent and Interactive Effects of Salinity and Proline on Physiological and Biochemical Responses of Agave Plants. Journal of Soil, Plant and Environment, 5(1), 25–33.