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Evaluation of Salt Tolerance Mechanisms in Common Bean (*Phaseolus vulgaris* L.) Genotypes Exposed to Sodium Chloride and Sodium Sulfate: Morphological and Biochemical Perspectives

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ABSTRACT

Background: Common bean is a nutritionally important crop, but increasing soil salinity threatens seedling establishment and productivity.

Methods: This greenhouse pot study compared early-stage morphological and biochemical responses of two common bean genotypes (G3 and G13) exposed to two salt types (NaCl and Na_2SO_4) at two concentrations (20 and 40 mM), alongside a non-saline control. Plant height, biomass traits, leaf area, SPAD chlorophyll index, photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids), chlorophyll a/b ratio and proline were assessed.

Result: Salt treatments influenced SPAD and pigment-related traits, whereas effects on growth traits and proline were smaller or less consistent under the tested conditions. Genotype-dependent differences were observed across multiple traits. Overall, pigment traits may be useful for early-stage screening of salt responses under controlled conditions; however, conclusions should be interpreted cautiously given the limited genotype set.

Key words: Na_2SO_4, NaCl, Osmotic adjustment, *Phaseolus vulgaris*, Photosynthetic pigments, Salinity, SPAD.

INTRODUCTION

Global climate change, together with increasing drought and salinity stress, poses a serious threat to agricultural production (Kumar *et al*., 2025). Soil salinity, driven in part by inadequate irrigation and drainage, is expanding in many regions and remains a major soil-degradation problem limiting crop performance and yield (Yavuzlar *et al*., 2021). It is considered one of the most critical environmental constraints to plant growth, particularly in arid and semi-arid ecosystems (Temizgül, 2025).

At the plant level, salinity suppresses photosynthesis, disrupts metabolic and enzymatic processes and induces oxidative stress, ultimately leading to growth and yield losses (Borromeo *et al*., 2024). These effects are reflected in measurable morphological, physiological and biochemical changes (Kıpçak *et al*., 2019). Plants respond through ion exclusion or vacuolar sequestration, osmotic adjustment and activation of antioxidant defense systems (Kumar *et al*., 2025; Zhang *et al*., 2024). Early developmental stages are particularly informative for detecting stress impacts within a short time frame (Zhu *et al*., 2020).

Common bean (*Phaseolus vulgaris* L.) is an important food legume and improving its performance under salinity is relevant for both production stability and breeding. However, salinity studies frequently rely on NaCl alone, although field salinity may be chloride-or sulfate-dominated. Comparing NaCl and Na_2SO_4 can therefore provide a more accurate understanding of salt-tolerance mechanisms (Zhou *et al*., 2023). Linking salt type and intensity to measurable traits and integrating morphological and biochemical indicators strengthens screening approaches, while leaf structural and micromorphological descriptors may provide additional support. Trait-based screening can complement molecular marker and genome-editing strategies for developing stress-tolerant cultivars and enhancing food security (Fu and Yang, 2023; Priyadharshini *et al*., 2019; Kouki *et al*., 2021; Chaitanya *et al*., 2014).

Few studies have compared chloride- and sulfate-dominated salinity at the early seedling stage using combined pigment profiling and multivariate integration. Therefore, this study compared early morphological and biochemical responses of two common bean genotypes under two concentrations of NaCl and Na_2SO_4. We hypothesized that (i) pigment traits would respond rapidly to increasing

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salinity and (ii) genotype-specific differences would be detectable in pigment and osmotic-adjustment traits under controlled greenhouse conditions.

MATERIALS AND METHODS

Plant material and experimental design

The study was conducted in 2025 under greenhouse conditions at the Faculty of Agriculture, Recep Tayyip Erdoğan University, to evaluate the responses of two common bean candidate lines (Genotype 3 and Genotype 13) provided and coded by the national seed registration authority. Codes were retained for traceability and no salt-tolerant or -sensitive reference cultivars were included; thus, results reflect comparative responses under experimental conditions. Genotype 13 is bush-type with ~13 cm flat yellow pods, whereas Genotype 3 has white seeds (~8 mm) and high adaptability.

Treatments included: control, NaCl 20 mM (1.75 g/2 L) and 40 mM (3.51 g/2 L) and Na_2SO_4 20 mM (4.26 g/2 L) and 40 mM (8.52 g/2 L). Plants were grown in 2-L pots filled with sterile peat (pH 6.0; 1.0 g L^{-1} fertilizer), with five seedlings per pot. The experiment followed a 2 (genotype) × 5 (salt) factorial design with three replications; three pots per combination were used and the pot was considered the experimental unit (Fig 1). Salt solutions were applied once at 10 DAS after a 1-day irrigation pause and respective solutions were maintained for 30 days (controls received water). Measurements were taken 30 days after treatment. Data were averaged at the pot level to avoid pseudo-replication. Measured traits included plant height, SPAD, green/dry weight, leaf area, chlorophyll a and b, total chlorophyll, total carotenoids, chlorophyll a/b ratio and proline.

Biochemical analysis

Photosynthetic pigments (total chlorophyll, chlorophyll a and b and total carotenoids) were quantified spectrophoto-metrically using the Arnon method (Arnon, 1949) and proline (µmol g^{-1} FW) was determined following Bates (1973) using the acid-ninhydrin assay with toluene extraction and a standard curve at 520 nm (Fig 2-3).

Statistical analysis

Statistical analyses were performed in R (lme4; Bates *et al*., 2015) using a two-factor mixed model with genotype, salt treatment and their interaction as fixed effects and replication as a random effect (pot = experimental unit); significant effects ($p<0.05$) were followed by Tukey's HSD for mean separation.

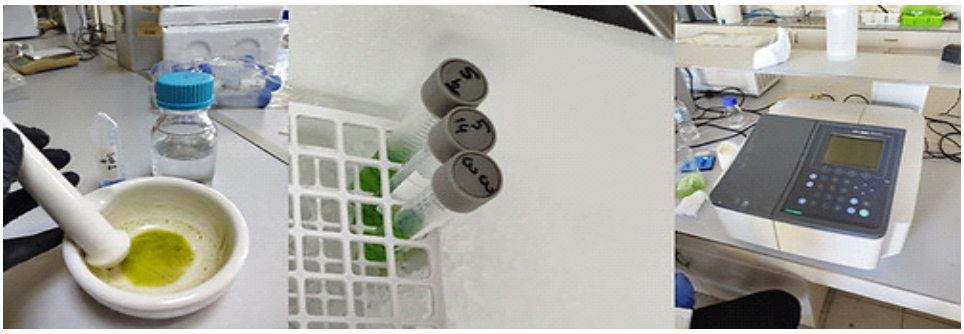
RESULTS AND DISCUSSION

Correlation analysis showed strong positive relationships among pigment-related traits (Fig 4). Total chlorophyll was strongly correlated with chlorophyll a ($r>0.90$, $p<0.001$), chlorophyll b ($r>0.95$, $p<0.001$) and total carotenoids



The image illustrates plant growth conditions, uniform pot arrangement and controlled environmental settings applied during the SET5 salinity stress experiment.

Fig 1: Greenhouse experimental setup showing common bean (*Phaseolus vulgaris* L.) plants subjected to five salinity treatments (Control, NaCl 20 mM, NaCl 40 mM, Na_2SO_4 20 mM and Na_2SO_4 40 mM).



The workflow includes (i) Grinding fresh leaf tissue, (ii) Transferring samples into pre-labeled cooled tubes for pigment extraction and (iii) Spectrophotometric measurement of absorbance values for chlorophyll a, chlorophyll b and total carotenoids.

Fig 2: Overview of the laboratory procedures used for chlorophyll and carotenoid extraction in common bean leaves.

(r = 0.98, p<0.001). Chlorophyll a and chlorophyll b were also positively correlated.

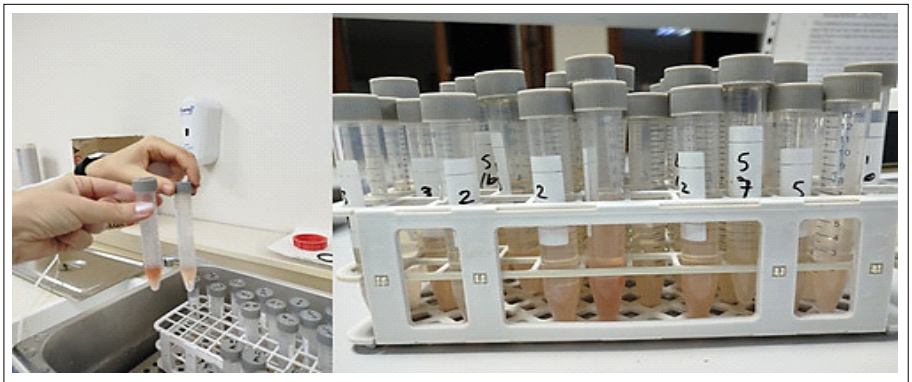
Proline showed positive correlations with green weight (r = 0.40) and leaf area (r = 0.66, p<0.05). Correlations between proline and chlorophyll-related parameters were weak (r<0.20). Plant height showed low negative correlations with other traits (ranging from -0.05 to -0.30). Leaf area and chlorophyll (SPAD) values were positively correlated (p<0.01).

Principal component analysis (PCA)

PCA separated treatments along PC1-PC2 based on combined morphological and biochemical variation (Fig 5). Growth/biomass traits loaded mainly on one axis, whereas pigment/biochemical variables loaded on the other. Pigment vectors were long and similarly oriented, while proline pointed in a distinct direction. Controls clustered near pigment variables; Na_2SO_4 40 mM separated and aligned with biomass traits and proline. NaCl and Na_2SO_4 treatments diverged and Na_2SO_4 20 mM was close to NaCl 40 mM in associations with leaf area and growth traits.

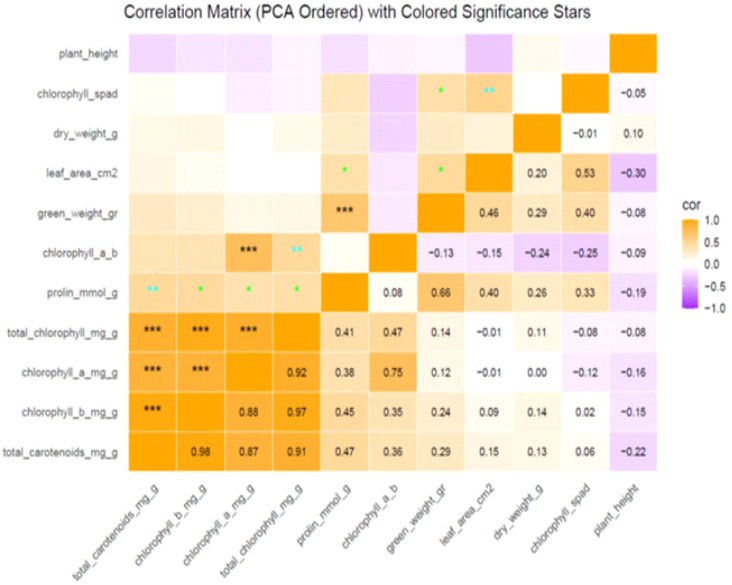
Pigment traits

Pigment traits differed among treatments (Fig 6; Table 2-3): controls showed the highest values and reductions increased with salinity, particularly at 40 mM. The chlorophyll



The figure shows (i) Color development of leaf extracts following ninhydrin reaction and heating and (ii) Labeled sample tubes arranged in racks prior to spectrophotometric measurement for quantification of free proline content.

Fig 3: Laboratory workflow for proline determination in common bean leaves.



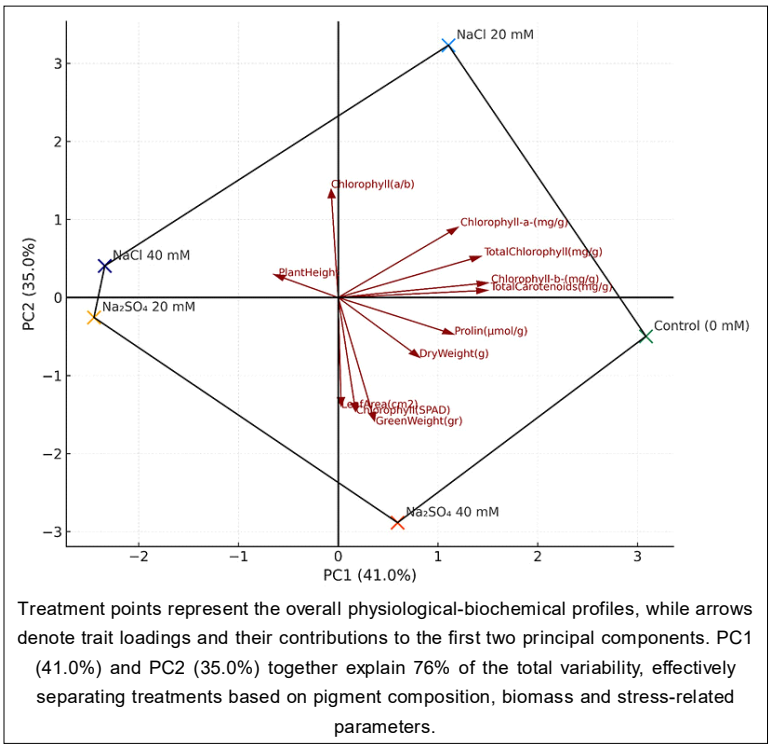
The matrix is ordered using PCA-based clustering to improve visual grouping of related traits. Color gradients represent correlation coefficients (-1 to +1) and significance levels are indicated with asterisks (p<0.05, p<0.01, p<0.001).

Fig 4: Correlation matrix illustrating pairwise relationships among morphological, physiological and biochemical traits of common bean plants exposed to different salinity treatments.

a/b ratio varied, with higher values under some salt treatments (notably Na_2SO_4 40 mM). Low-salt treatments occasionally matched or slightly exceeded control values, but both salts at 40 mM reduced pigments overall.

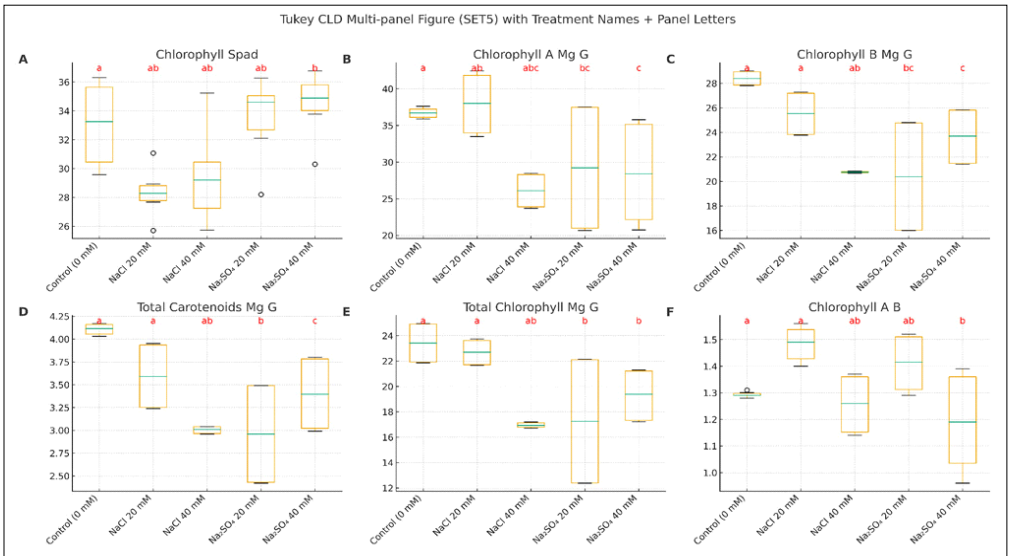
Hierarchical clustering

Clustering grouped treatments into high-salt (Na_2SO_4 40 mM, NaCl 40 mM) vs control/low-salt (control, Na_2SO_4 20 mM, NaCl 20 mM) clusters (Fig 7). Na_2SO_4



Treatment points represent the overall physiological-biochemical profiles, while arrows denote trait loadings and their contributions to the first two principal components. PC1 (41.0%) and PC2 (35.0%) together explain 76% of the total variability, effectively separating treatments based on pigment composition, biomass and stress-related parameters.

Fig 5: Principal component biplot (PC1 vs. PC2) illustrating the multivariate response of common bean plants to five salinity treatments: Control (0 mM), NaCl 20 mM, NaCl 40 mM, Na_2SO_4 20 mM and Na_2SO_4 40 mM.



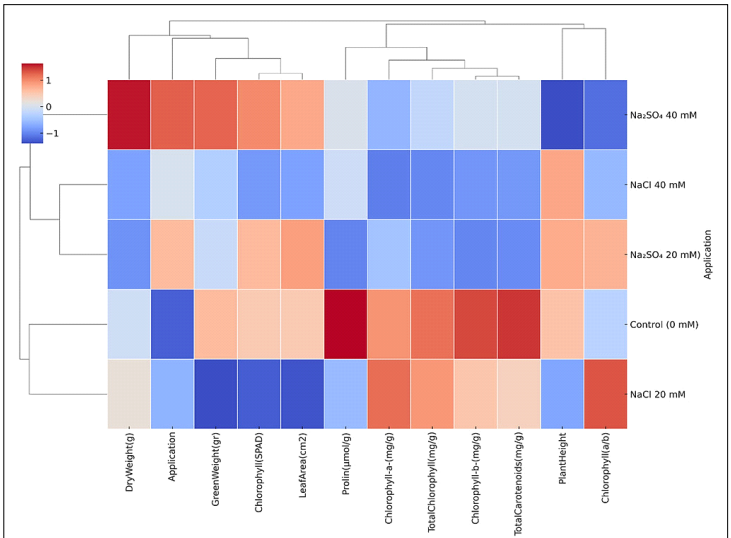
Boxplots depict the distribution of each trait and red letters indicate statistically homogeneous groups based on Tukey's HSD test ($p<0.05$). Panels show: (A) Chlorophyll SPAD, (B) Chlorophyll-a (mg g^{-1}), (C) Chlorophyll-b (mg g^{-1}), (D) Total carotenoids (mg g^{-1}), (E) Total chlorophyll (mg g^{-1}) and (F) Chlorophyll a/b ratio.

Fig 6: Tukey HSD compact letter display (CLD) for six pigment-related traits of common bean plants subjected to five salt treatments: Control (0 mM), NaCl 20 mM, NaCl 40 mM, Na_2SO_4 20 mM and Na_2SO_4 40 mM.

40 mM showed lower dry weight, green weight and SPAD with higher proline; NaCl 40 mM showed the lowest pigment traits. Pigments clustered together, whereas proline formed a separate cluster. Genotypes separated (Fig 8): G3 had higher pigments, proline and green/dry weight, while plant height was not higher; G13 showed lower values.

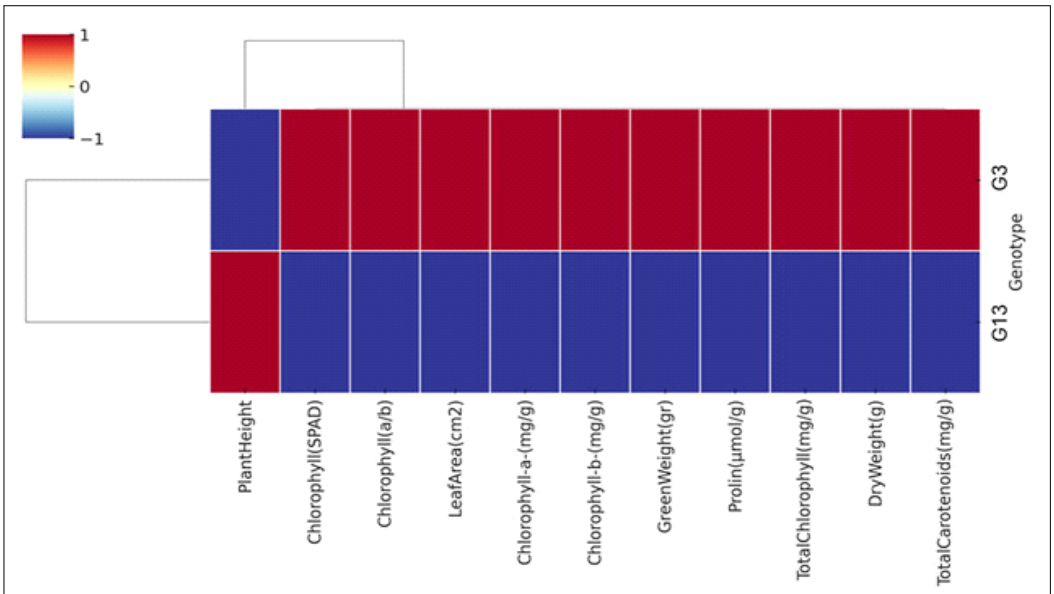
Agronomic traits

Plant height was not affected by treatments (p = 0.886; 35.35-39.97 cm) (Table 1). SPAD was significantly influenced (p = 0.002), with the highest value at 40 mM Na_2SO_4 (34.45) and the lowest at 20 mM NaCl (28.32). Green weight did not differ among treatments (p = 0.435;



Both treatments (rows) and traits (columns) were clustered hierarchically to visualize similarities and divergence in the physiological and pigment-related responses of common bean plants.

Fig 7: Z-score normalized hierarchical clustered heatmap of mean trait values across five salinity treatments: Control (0 mM), NaCl 20 mM, NaCl 40 mM, Na_2SO_4 20 mM and Na_2SO_4 40 mM.



Clustering was performed based on Euclidean distance to visualize trait-level contrasts between genotypes. Red tiles represent relatively higher trait values, whereas blue tiles represent relatively lower values for each genotype.

Fig 8: Z-score normalized hierarchical clustered heatmap comparing the physiological, biochemical and pigment-related traits of genotypes G3 and G13.

0.38-0.47 g). Genotype effects were significant for plant height (p = 0.001) and green weight (p = 0.005) (Table 1).

Dry weight was not treatment-significant (p = 0.603; 0.06-0.12 g; highest at 40 mM Na_2SO_4) (Table 2). Leaf area was not affected by treatments (p = 0.131; 20.50-27.79 cm2), but genotype effects were significant (p = 0.002).

Pigment traits (Table 2-3) generally declined at higher salinity; chlorophyll a was relatively higher at 20 mM NaCl

Table 1: Significance levels for plant height, chlorophyll (SPAD) and green weight in bean genotypes under different salt-stress treatments.

Application	Plant height (cm)	Chlorophyll (SPAD) values	Green weight_(gr)
Control (0 mM)	39.47	33.06ab	0.45
Sodium chloride (NaCl)-20 mM	36.71	28.32b	0.38
Sodium chloride (NaCl)-40 mM	39.97	29.47b	0.42
Sodium sulfate (Na_2SO_4)-20 mM	39.92	33.48ab	0.42
Sodium sulfate (Na_2SO_4)-40 mM	35.35	34.45a	0.47
Mean	38.28	31.76	0.43
		P-value	
Genotype	0.001	0.330	0.005
Application	0.886	0.002	0.435
CV (%)	23.97	8.63	19.56

Means followed by the same lowercase letters within rows and the same uppercase letters within columns indicate that, for the corresponding variable, no significant difference was detected according to the Tukey test at the 5% significance level (P<0.05). G = Effect of genotype. A = Effect of applications. CV = Coefficient of variation.

Table 2: Significance levels for dry weight, leaf area and chlorophyll a in bean genotypes under different salt-stress treatments.

Application	Dry weight (g)	Leaf area (cm^2)	Chlorophyll a (mg g^{-1})
Control (0 mM)	0.08	26.51	36.71ab
Sodium chloride (NaCl)-20 mM	0.09	20.50	37.94a
Sodium chloride (NaCl)-40 mM	0.07	22.32	26.09c
Sodium sulfate (Na_2SO_4)-20 mM	0.06	27.79	29.18bc
Sodium sulfate (Na_2SO_4)-40 mM	0.12	27.52	28.46c
Mean	0.08	24.93	31.68
		P-value	
Genotype	0.594	0.002	0.001
Application	0.603	0.131	0.000
CV (%)	76.09	14.11	14.44

Means followed by the same lowercase letters within rows and the same uppercase letters within columns indicate that, for the corresponding variable, no significant difference was detected according to the Tukey test at the 5% significance level (P<0.05). G = Effect of genotype. A = Effect of applications. CV = Coefficient of variation.

Table 3: Significance levels for chlorophyll b, total carotenoids and total chlorophyll in bean genotypes under different salt-stress treatments.

Application	Chlorophyll b (mg g^{-1})	Total carotenoids (mg g^{-1})	Total chlorophyll (mg g^{-1})
Control (0 mM)	28.40a	4.11ab	23.41a
Sodium chloride (NaCl)-20 mM	25.52a	3.59a	22.68a
Sodium chloride (NaCl)-40 mM	20.75ab	3.00ab	16.95ab
Sodium sulfate (Na_2SO_4)-20 mM	20.39bc	2.96b	17.25b
Sodium sulfate (Na_2SO_4)-40 mM	23.65c	3.40c	19.29b
Mean	23.74	3.41	19.91
		P-value	
Genotype	0.000	0.000	0.011
Application	0.000	0.000	0.000
CV (%)	8.26	6.97	12.11

Means followed by the same lowercase letters within rows and the same uppercase letters within columns indicate that, for the corresponding variable, no significant difference was detected according to the Tukey test at the 5% significance level (P<0.05). G = Effect of genotype. A = Effect of applications. CV = Coefficient of variation.

Table 4: Significance levels for chlorophyll a/b ratio and proline in bean genotypes under different salt-stress treatments.

Application	Chlorophyll a/b ratio	Proline (µmol g^{-1} FW)
Control (0 mM)	1.29 ab	44.76
Sodium chloride (NaCl)-20 mM	1.48 a	36.33
Sodium chloride (NaCl)-40 mM	1.26 ab	38.12
Sodium sulfate (Na_2SO_4)-20 mM	1.41 ab	34.60
Sodium sulfate (Na_2SO_4)-40 mM	1.19 b	38.59
Mean	1.33	38.48
	P-value	
Genotype	0.026	0.002
Application	0.001	0.738
CV (%)	8.16	34.76

Means followed by the same lowercase letters within rows and the same uppercase letters within columns indicate that, for the corresponding variable, no significant difference was detected according to the Tukey test at the 5% significance level (P<0.05). G = Effect of genotype. A = Effect of applications. CV = Coefficient of variation.

(Table 2), whereas chlorophyll b was lower at 20 mM Na_2SO_4 and 40 mM NaCl (Table 3). Total chlorophyll and carotenoids decreased at higher salt levels. Proline did not differ among treatments (Table 4), but genotype effects were significant for pigments and proline (Table 2-4).

Multivariate analyses indicate that salt stress modifies common bean performance through coordinated shifts in photosynthetic pigments and more variable responses in growth and osmotic traits. This pattern supports the view that salinity acts through partially independent physiological pathways, with photosynthesis, biomass accumulation and osmotic adjustment differing according to salt type, stress intensity and genotype (Hasan *et al*., 2016; Zhu *et al*., 2020; Mir *et al*., 2024).

The correlation matrix (Fig 4) showed strong clustering among pigment traits, with total chlorophyll strongly and positively associated with chlorophyll a, chlorophyll b and total carotenoids. This suggests that the pigment system functions as an integrated unit under salinity, consistent with the protective roles of chlorophylls and carotenoids in maintaining photosystem integrity (Mir *et al*., 2024). Previous studies report greater sensitivity of chlorophyll a and overall chlorophyll decline under increasing salinity (Aşcı *et al*., 2021; Kibar *et al*., 2020) and the present results indicate that pigment reductions occur in a coordinated rather than isolated manner.

Proline related positively to green weight and leaf area but weakly to pigment traits, indicating its accumulation isn't merely due to pigment decline and may be controlled by different pathways affected by genotype and stress duration (Mir *et al*., 2024). The linkage between leaf area and chlorophyll status, as well as the positive association between leaf area and SPAD values, supports coordinated variation in leaf development and chlorophyll density under stress (Zhang *et al*., 2024). Compensatory physiological adjustments may partly explain why morphological correlations were weaker than pigment correlations (Karakaş *et al*., 2019).

PCA (Fig 5) showed treatment responses driven by stress intensity and salt type. Separation along growth/biomass and pigment/biochemical axes indicates salinity disrupts photosynthesis and prompts adaptive changes. (Hasan *et al*., 2016; Zhu *et al*., 2020). The control group's proximity to pigment vectors reflects expected pigment decline under salinity (Zhang *et al*., 2024; Aşcı *et al*., 2021). Divergence between NaCl and Na_2SO_4 indicates partially distinct metabolic reorganization pathways (Levitt, 1985). The proximity of 20 mM Na_2SO_4 and 40 mM NaCl in certain morphological associations suggests that once stress thresholds are exceeded, different salts may impose comparable growth constraints while operating through different biochemical routes (Mi *et al*., 2024).

Na_2SO_4 (40 mM) aligned with proline and biomass variables, indicating disrupted homeostasis and potential water and nutrient uptake limitations (Farzana *et al*., 2021). In contrast, stronger pigment depletion under NaCl is consistent with higher photo-oxidative pressure and direct effects on chlorophyll stability (Zhang *et al*., 2024; Aşcı *et al*., 2021). The synchronized pigment vectors versus the proline direction support independent regulation of osmotic adjustment and pigment maintenance (Mir *et al*., 2024).

Controls showed the highest pigment values, while high salinity significantly reduced them, confirming chlorophylls and carotenoids are highly responsive to salt stress (Mir *et al*., 2024; Zong *et al*., 2021). More pronounced NaCl losses align with inhibited chlorophyll synthesis and faster pigment degradation (Zhang *et al*., 2024; Emirzeoğlu and Başak, 2020). Shifts in the chlorophyll a/b ratio suggest stress-driven restructuring of light-harvesting complexes (Kaymak and Acar, 2020; Tuna and Eroğlu, 2017; Elsiddig *et al*., 2023). Slight low-salinity increases may indicate hormesis (Mankar *et al*., 2021), while higher concentrations harm pigment biosynthesis and photosystem II stability (Fitzner *et al*., 2023; Zhang *et al*., 2024). Thus, pigment traits are particularly informative for early screening, although multiple pigment parameters should be integrated for robust evaluation (Mi *et al*., 2024).

Hierarchical clustering (Fig 7) separated high- from low-salt treatments, highlighting concentration as the dominant driver (Fidan and Ekincialp, 2017). Within the high-salt cluster, Na_2SO_4 (40 mM) showed lower biomass and SPAD but higher proline, while NaCl (40 mM) caused stronger pigment suppression, indicating different stress signatures of sulfate-and chloride-based salinity (Elsiddig *et al*., 2023; Farzana *et al*., 2021; Mir *et al*., 2024). Similar growth reductions under salinity have been widely reported (Fidan and Ekincialp, 2020; Kibar *et al*., 2020; Temizgül, 2025).

Although genotype-dependent responses to salinity are documented (Aini *et al*., 2014), this study included only two genotypes; findings should be seen as genotype-specific, not generalizable. Differences in pigment and biomass traits indicate genetic background influences photosynthetic stability and biomass partitioning under salt stress. Because salt tolerance is polyfactorial, integrating morphological and biochemical traits is essential, but broader conclusions need validation with more genotypes sets (Mi *et al*., 2024).

CONCLUSION

Under greenhouse pot conditions, salt treatments primarily influenced pigment-related traits and the SPAD index, indicating early sensitivity of the photosynthetic apparatus to both NaCl- and Na_2SO_4-dominated salinity. Growth traits showed smaller or less consistent treatment effects, while genotype differences were evident across multiple traits. Proline responses were genotype-dependent and did not present a consistent treatment-driven pattern under the exposure conditions used. Because only two genotypes were evaluated and the experiment was conducted under controlled greenhouse conditions, broader breeding conclusions should be interpreted cautiously. Future work should validate screening traits using larger genotype panels and sustained salinity regimes across environments.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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