

Mitigating Severe Salinity Stress in *Vicia faba* L. via Exogenous Proline: A Climate Resilience Intervention

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Mitigating Severe Salinity Stress in *Vicia faba* L. via Exogenous Proline: A Climate Resilience Intervention

Abstract

Climate change and its impacts on soil salinization threaten faba bean (*Vicia faba* L.) production in the Nile Delta. Although the osmotic benefits of exogenous osmoprotectants are well recognized, long-term, tissue-specific partitioning mechanisms of repeated foliar proline under unmitigated severe saline irrigation (12 dS m⁻¹) are poorly quantified. In the study, a holistic hydrochemical and agronomic approach was used to evaluate the efficacy of weekly foliar proline (500 mg L⁻¹) as an intervention for climate-resilience. The hydrochemical modeling confirmed severe irrigation constraints at 12 dS m⁻¹, with a Sodium Adsorption Ratio (SAR) of 32.41 and positive Chloro-Alkaline indices, indicating strong reverse base-exchange phenomena. Pod counts were declined to 30 per plant under unmitigated saline irrigation (12 dS m⁻¹), and reproductive output collapsed. Weekly proline applications reprogrammed the stress response, with pod counts returning to 33.67 and 91.75% reproductive potential preserved. Under moderate salinity (6 dS m⁻¹), proline application recovered 70.91% of the yield potential lost due to osmotic stress. Metabolic recovery was governed by a coordinated N–K–protein trait axis, as indicated by a multivariate principal component analysis (97.47 % of the variance explained). Critically, proline was not just allowing bulk sodium exclusion; it was altering tissue electrophysiology to preferentially maintain leaf potassium (2.01% vs. 1.56%) and protect nitrogen assimilation. Thus, the leaf crude protein increased to 21.84%, and the harvested seeds showed a high protein fraction of 23.90%. The study offers a robust data-driven framework to transform hazardous saline water into an agricultural input and a scalable climate-adaptation approach aligned with SDGs 2, 6, and 13.

Keywords: Climate resilience; Severe salinity; Faba bean; Proline; Multivariate analysis

1. Introduction

The Nile Delta in Egypt is suffering from an acute agricultural crisis due to soil salinization, accelerated by rising sea levels and the upwelling of brackish groundwater (Mabrouk et al., 2013). With the advent of climate change, water availability is reducing, and evaporative demand is increasing, compelling farmers to use saline irrigation water with salinity ranging from 6 to 12 dS m⁻¹ (Abdelrady et al., 2024; Gheisary et al., 2025). This practice inevitably leads to catastrophic losses in crop yields and widespread soil degradation (El-Ramady et al., 2013). Effective climate adaptation strategies are urgently needed to maintain agricultural productivity in vulnerable coastal regions globally.

The faba bean (*Vicia faba* L.), also known as the broad bean, is an important leguminous crop belonging to the Fabaceae family and one of the earliest domesticated crops. Archaeological evidence from the southern Levant shows that intensive cultivation started as early as 10,200 years ago (Caracuta et al., 2015). The crop gradually spread from its Near Eastern origins across Eurasia and North Africa, becoming a key component of early human agricultural systems (Abou-Khater et al., 2022). At present, it is appreciated

for its high nutritional density, particularly its protein content, and for its natural role in improving soil fertility through nitrogen fixation (Torabian et al., 2024). It is undergoing a dynamic global renaissance, driven by increasing consumer demand for plant-based proteins, and Egypt has emerged as one of the world's leading producers (Alharbi & Adhikari, 2020; Multari et al., 2015).

In Egypt and the wider Mediterranean basin, faba beans are a major protein source and have substantial cultural and economic importance. However, their cultivation is constrained by limited arable land and competition with other winter crops, such as wheat and clover. Beyond these agronomic challenges, the crop is moderately sensitive to salinity, a serious threat to regional food security and soil biological health. Salinity adversely affects legume physiology through osmotic stress and severe ion toxicity, primarily by sodium replacing potassium at key enzyme sites. This ionic disequilibrium impairs stomatal control, disrupts photosynthesis, and actively inhibits nodulation, causing a secondary nitrogen deficiency that critically exacerbates the primary stress.

Exogenous application of osmoprotectants is a promising approach for addressing the combined effects of salinity and osmotic stress (Ashraf & Foolad, 2007). Recent studies have provided evidence that the use of organic amendments (e.g. compost, fermented clover) and inorganic agents (e.g. agricultural gypsum) is a viable approach to alleviate salt stress, enhance soil structural stability, and improve the water use efficiency across diverse crop production systems (Abu-Elela et al., 2018; Farrag et al., 2021, 2025). Proline is a multifunctional amino acid that plays important roles as a compatible solute, a scavenger of reactive oxygen species, and a stabilizer of proteins under abiotic stress (Alvarez et al., 2022; Hayat et al., 2012). However, most of the available literature evaluates the effects of proline application in short-term hydroponic experiments or early seedling assays (Desouky et al., 2023). The persistence of efficacy of repeated foliar proline applications for a full growing season under realistic and severe saline irrigation conditions has been poorly quantified. More importantly, a fundamental mechanistic gap remains regarding whether exogenous proline confers tolerance by strictly limiting bulk sodium uptake or by reprogramming tissue-specific ion and nitrogen partitioning.

This study addresses a critical oversight inherent in whole-plant averages by examining leaf and seed responses in parallel. The theory was that supplemental proline mitigates harsh salt conditions by altering ion movement, leading to higher leaf potassium levels and preserved protein synthesis, rather than relying on sodium exclusion. This hypothesis was tested by irrigating faba beans with salinity levels of 0.33, 6, and 12 dS m⁻¹, and then applying proline at 500 mg L⁻¹ to their leaves weekly to evaluate their physiological, nutritional, and yield responses. The study results indicate that 90.8% of freshwater yield can be recovered with weekly proline application, even under severe salinity. Moreover, results show a selective enhancement of leaf potassium and maintenance of protein synthesis, providing an extremely practical, immediate climate-resilience intervention that directly contributes to global sustainability goals, namely, SDG 2 (Zero Hunger), SDG 6 (Clean Water), and SDG 13 (Climate Action).

2. Materials and Methods

2.1 Experimental Site and Plant Material

A pot experiment was conducted in the greenhouse at the National Water Research Center in Cairo, Egypt, during the winter season 2024-2025. The relative humidity was 65-75%, with average temperatures of 27-35°C during the day and night. Faba bean (*Vicia faba* L.) seeds were kindly supplied by the Egyptian Ministry of Agriculture and Land Reclamation. The seeds were sown in the last week of November 2024. Seedling emergence occurred about two weeks later, in the second week of December 2024. Seeds were sown in cylindrical pots (30 cm diameter x 25 cm height; 8 kg/pot) containing clay loam soil collected from an adjacent agricultural field, leaving 5 cm space below the rim of the pot. The baseline soil physical characteristics were 27% coarse sand, 41% fine sand, 12% silt, and 20% clay. Ten seeds per pot were initially sown and thinned to three uniform seedlings two weeks after emergence.

2.2 Experimental design and Salinity treatments

The experiment was laid out in a completely randomized design with five treatments and three replications, as seen in Figure 1. Irrigation treatments were:
T1 (Control): Irrigation with fresh water ($EC\ 0.33\ dS\ m^{-1}$) without proline application.
T2: Irrigation with moderate saline water ($EC\ 6\ dS\ m^{-1}$) without proline application.
T3: Irrigation with severe saline water ($EC\ 12\ dS\ m^{-1}$) without proline application.
T4: Irrigation with moderate saline water ($EC\ 6\ dS\ m^{-1}$) supplemented with a foliar proline spray ($500\ mg\ L^{-1}$).
T5: Irrigation with severe saline water ($EC\ 12\ dS\ m^{-1}$) supplemented with a foliar proline spray ($500\ mg\ L^{-1}$).

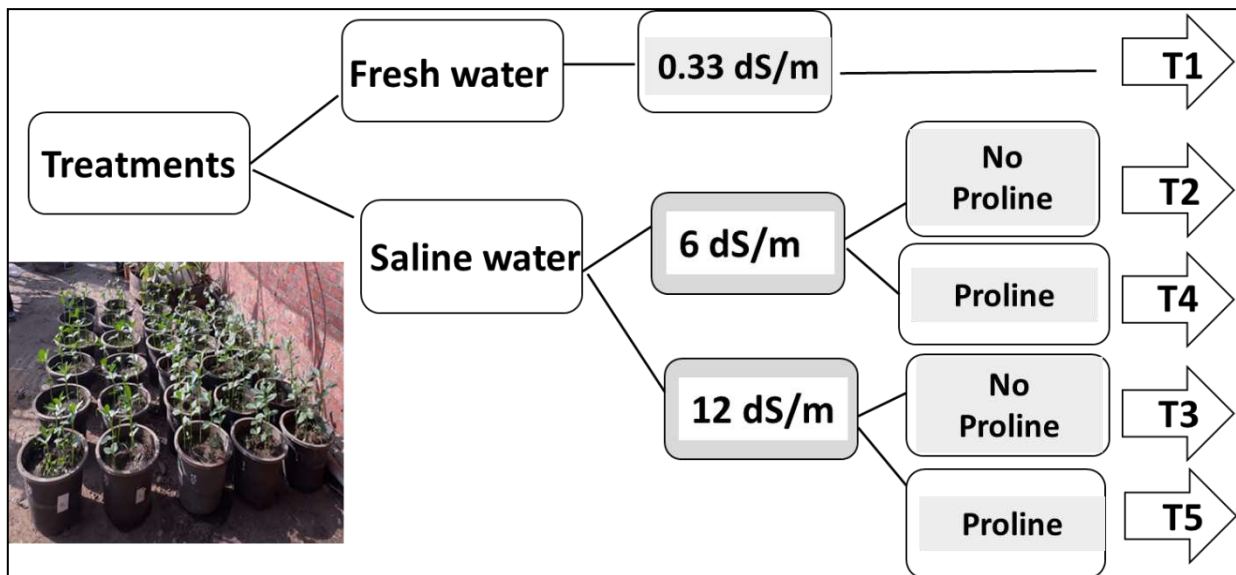


Figure 1. Layout representation of the different treatments used to test Fava bean growth under different treatments.

Note: T1: Fresh water ($0.33\ dS\ m^{-1}$); T2: ($6\ dS\ m^{-1}$) + without proline); T3: ($12\ dS\ m^{-1}$ + without proline); T4: ($6\ dS\ m^{-1}$ + foliar proline spray $500\ mg\ L^{-1}$); T5: ($12\ dS\ m^{-1}$ + foliar proline spray $500\ mg\ L^{-1}$).

The salinity levels of 6 and 12 dS m⁻¹ were prepared artificially by dissolving crude sea salt in tap water until the desired electrical conductivity was reached. Three types of irrigation water were used.

S1: Irrigation with fresh water (EC 0.33 dS m⁻¹) without proline application.

S2: Irrigation with moderate saline water (EC 6 dS m⁻¹)

S3: Irrigation with severe saline water (EC 12 dS m⁻¹)

All pots were irrigated and maintained at field capacity throughout the growing season. Irrigation scheduling was based on daily pot weight measurements, with the control treatment serving as the reference. After sowing, 3 liters of water were added to each experimental unit to reach field capacity. Irrigation was applied when 40% of the available water at field capacity had been depleted. Water demand was met by replenishing 40% of the water lost through evapotranspiration (1.2 liters). At the end of the experimental period (135 days), plants were equally irrigated, and each pot received a total of 40.5 liters of irrigation water.

2.3 Baseline Characteristics of Irrigation Water and Experimental Soil

The properties of the irrigation water and soil were analyzed before and during the experiment. Table 1 illustrates the broad chemical properties of the benchmark water classes. Fresh Water (FW), Moderate Saline Water (S1), and Severe Saline Water (S2). The chemical analysis details the precise distribution of pH, total salt loads, major cations, and major anions. At the same time, the properties of the base soil samples were observed for three conditions with respect to these water applications: Soil A: (original background soil), Soil B: (soil irrigated with moderate saline water of 6 dS m⁻¹), Soil C: (soil irrigated with severe saline water of 12 dS m⁻¹). The states are summarized in Table 2. The texture of all experimental pots was uniformly clay loam (CL). Continuous irrigation led to documented soil evaluations showing evident accumulation trends in electrical conductivity, organic matter, calcium carbonate, extractable cations, and soluble anions.

Table 1. Physiochemical characteristics of the irrigation water source.

Parameters	S1	S2	S3	Recommended values (1)
	Concentration			
pH	7.7	7.2	7.2	6.5–8.5
EC (dSm ⁻¹)	0.33	6	12	> 3 Severe
TDS (mgL ⁻¹)	211.2	3840	7680	> 2000 Severe
Major Cations				
Ca (mg L ⁻¹)	37.21	81.8	114.68	0–400
K (mg L ⁻¹)	4.99	46	120	0–70
Mg ((mg L ⁻¹)	7.39	129.76	234.25	0–60
Na (mg L ⁻¹)	20.6	1350	3000	0–900
Major Anions				
Cl (mg L ⁻¹)	4.55	1764	3739	0–1100
NO ₃ (mg L ⁻¹)	1.13	0.9	1.76	0–40

Parameters	S1	S2	S3	Recommended values (1)
	Concentration			
PO ₄ (mg L ⁻¹)	0.2	0.2	0.2	0–6
SO ₄ (mg L ⁻¹)	14.63	448	1125	0–900

Note: S1: Freshwater; S2: Saline Water (6 dS m⁻¹); S3: Saline Water (12 dS m⁻¹); EC: Electrical Conductivity; TDS: Total Dissolved Solids; Ca: Calcium; K: Potassium; Mg: Magnesium; Na: Sodium; Cl: Chloride; NO₃: Nitrate; PO₄: Phosphate; SO₄: Sulfate; (¹) (Ayers & Westcot, 1985)

Table 2. Main properties in soils studied

Parameters	Soils		
	A	B	C
Coarse sand (%)	2.20	4.42	4.14
Fine sand (%)	32.32	28.22	26.23
Silt (%)	28.75	29.26	32.51
Clay (%)	36.71	38.1	37.12
Texture	CL	CL	CL
S.P.(%)	44	45	45
F.C.(%)	33.48	34.48	34.49
W.P.(%)	20.36	21.13	20.61
A.W.(%)	13.12	13.35	13.88
pH (H ₂ O)	7.66	7.54	7.15
EC (dS m ⁻¹)	0.456	0.682	1.506
CaCO ₃ tot (%)	1.51	1.22	1.49
OM (%)	3.28	3.96	4.13
N _{tot} (mg kg ⁻¹)	10.28	13.2	14.8
P _{ava} (mg kg ⁻¹)	0.18	0.24	0.28
Major Cations			
Ca (mg.kg ⁻¹)	75	68.8	225
K (mg.kg ⁻¹)	37	78.3	138.22
Mg (mg.kg ⁻¹)	28	43	70.9
Na (mg.kg ⁻¹)	74.6	197.8	388
Major Anions			
Cl (mg.kg ⁻¹)	228.5	370.5	523.63
HCO ₃ (mg.kg ⁻¹)	175.37	208.05	228.6
NO ₃ (mg.kg ⁻¹)	13.7	16.3	19.2
PO ₄ (mg.kg ⁻¹)	0.33	0.48	0.49
SO ₄ (mg.kg ⁻¹)	46.8	78.3	99.8

Note: A: Reference soil; B: Saline Water (6 dS m⁻¹) Irrigated Soil; C: Saline Water (12 dS m⁻¹) Irrigated Soil; CL: Clay Loam; S.P: Saturation Percentage; F.C: Field Capacity; W. P: Wilting Point; A.V: Available Water; EC: Electrical Conductivity; CaCO₃: Calcium Carbonate; OM: Organic Matter; N_{tot}: Total Nitrogen; P_{ava}: Available Phosphorus

2.4 Proline Application and Growth Conditions

The exogenous proline concentration of 500 mg L⁻¹ was selected based on the established dose-response threshold for leguminous crops (Sadak et al., 2015). Commercial proline (99% purity, Loba Chemie Pvt. Ltd.) was dissolved in distilled water. Foliar proline application was initiated after seedling emergence, when the plants had reached the true-leaf stage, during the second week of December 2024. The prepared solution was applied once weekly for approximately 4 months, continuing until the second week of April 2025, for a total of approximately 16 applications. Control plants were sprayed with an equal volume of distilled water to control for any possible mechanical effects of the foliar application. Approximately one liter of the prepared spray solution was sufficient for three weekly applications under the experimental greenhouse conditions. About 5.3 liters of the solution were consumed per proline-treated experimental unit over the 16-week treatment period. The total cost of foliar proline material, including an estimated preparation cost of L.E 4 L⁻¹, amounted to approximately L.E 21 per treated experimental unit.

2.5 Harvest and Yield Metrics

Plants were harvested in April 2025 in accordance with standard agronomic practices (Devkota, 2025). Various growth and yield parameters were recorded at harvest, including total plant height (cm), number of pods per plant, and pod weight per pot (g). Total seed yield was extrapolated and expressed in kg/feddan (1 feddan = 4200 m²).

2.6 Biochemical and Tissue Nutrient Analysis

Mature leaves and green seeds were collected as plant samples, washed with tap water, and rinsed three times with distilled water to remove surface contaminants. Biological samples were oven-dried to constant weight at 70°C and then ground mechanically to a fine powder. 0.5 g of dried tissue from each replicate was digested with a mixture of nitric acid and hydrogen peroxide for elemental extraction (Tabatabai, 1998). Total nitrogen was determined by the standard Kjeldahl digestion method (Bremner & Mulvaney, 1982). The vanadomolybdophosphoric yellow colorimetric method was used to determine phosphorus concentrations. Potassium content was analyzed by flame photometer (410 Sherwood). Total crude protein content was calculated by multiplying the total nitrogen percentage by the standard conversion factor of 6.25.

2.7 Soil and Irrigation Water Characterization

The irrigation water samples were tested for pH, electrical conductivity (EC), total dissolved solids, and major cations. Ca^{2+} , Mg^{2+} , Na^+ , K^+ and major anions Cl^- , CO_3^{2-} , PO_4^{3-} , SO_4^{2-} . The EC of water was measured at 25°C using a WTW Multi 9620 IDS meter, and anions were quantified by ion chromatography using a DX-500 system (Pfaff, 1993; Rice et al., 2012). At harvest, soil samples were taken from each pot, air-dried, crushed, and sieved through a 2.0 mm sieve. A 1:5 soil-water extract was prepared, and the final soil pH, EC, and residual chemical properties were determined following standard methodologies (Loveland, 2011). Soil calcium and magnesium were analyzed using the EDTA titrimetric method (Hendershot et al., 2007).

2.8 Hydrochemical Indices and Predictive Models

A comprehensive set of established mathematical models was used to classify the suitability and the exact stress profile of the experimental saline irrigation water. Since faba bean's salinity sensitivity is primarily controlled by specific ion toxicity rather than osmotic stress (Hasanuzzaman et al., 2014; Maas & Grattan, 2015), it is important to quantify the exact ionic balance of the irrigation water to establish baseline stress conditions prior to proline intervention (Zaman et al., 2018). A complete water quality assessment was performed by converting all ionic concentrations to milliequivalents per liter (meq L⁻¹) and incorporating them into the predictive models presented in Tables 3 and 4. These indices are grouped into three major hazard categories.

Table 3. Mathematical models and classification ranges for hydrochemical water quality indices for irrigation

Parameter and Formula adopted	Range	Classification	Reference
$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}} \quad (1)$	<10	Excellent	(Richards, 1954)
	10-18	Good	
	18-26	Doubtful	
	>26	Unsuitable	
$SSP = \frac{Na^+}{Ca^{2+} + Mg^{2+} + Na^+ + K^+} \times 100 \quad (2)$	<20	Excellent	(Khodapanah et al., 2009; Todd & Mays, 1980)
	20-40	Good	
	40-60	Permissible	
	60-80	Doubtful	
$\%Na = \frac{Na^+ + K^+}{Ca^{2+} + Mg^{2+} + Na^+ + K^+} \times 100 \quad (3)$	<20	Excellent	(Eaton, 1950; Wilcox, 1955)
	20-40	Good	
	40-60	Permissible	
	60-80	Doubtful	
	>80	Unsuitable	
$KR = \frac{Na^+}{Mg^{2+} + Ca^{2+}} \quad (4)$	< 1	Suitable	(Kelley, 1941; Ramesh & Elango, 2012)
	> 1	Unsuitable	
$MH = \frac{Mg^{2+}}{Ca^{2+} + Mg^{2+}} \quad (5)$	< 50	Suitable	(Khodapanah et al., 2009; Raghunath, 1987)
	> 50	Unsuitable	
$PI = \frac{Na^+ + \sqrt{HCO_3^-}}{Ca^{2+} + Mg^{2+} + Na^+} \times 100 \quad (6)$	< 25	Unsuitable	(Doneen, 1964)
	25-75	Good	
	> 75	Suitable	
$RSC = (HCO_3^- - CO_3^{2-}) - (Ca^{2+} + Mg^{2+}) \quad (7)$	< 1.25	Safe	(Eaton, 1950; Raghunath, 1987; Wilcox, 1955)
	1.25–2.50	Marginal	
	> 2.5	Unsuitable	
$RSCB = HCO_3^- - Ca^{2+} \quad (8)$	< 5	Safe	(I. C. Gupta, 1983; S. K. Gupta & Gupta, 1987)
	5-10	Marginal	
	> 10	Unsuitable	
$PS = Cl^- + \frac{1}{2} SO_4^{2-} \quad (9)$	< 3	Suitable	(Doneen, 1964)
	> 3	Unsuitable	
$TH = 2.5 Ca^{2+} + 4.1 Mg^{2+} \quad (10)$	<75	Soft	(Allen & Goltermann, 1970; Vasanthavigar et al., 2013)
	57-150	Moderately Hard	
	150-300	Hard	
	> 300	Very Hard	

Note: **SAR:** Sodium Adsorption Ratio; **SSP:** Soluble Sodium Percentage; **Na%:** Sodium Percentage; **MH %:** Magnesium Hazard; **KR:** Kelly's Ratio; **RSC:** Residual Sodium Carbonate; **RSBC:** Residual Sodium Bicarbonate; **PS:** Potential Salinity; **PI:** Permeability Index; **TH:** Total Hardness

Table 4. Mathematical models and classification ranges for Chloro-Alkaline Indices of irrigation water applied.

Parameter and Formula adopted	Range	Classification	Reference
$CHI = \frac{Cl^-}{(Ca^{2+} + Mg^{2+})} \quad (11)$	< 0.1	Soft and non-corrosive	(Ryznar, 1944; Schoeller, 1965)
	> 0.5	Severe pitting on metal surfaces	
	> 1	Highly corrosive water	
$CHI - I = \frac{(Cl^- - HCO_3^-)}{(Cl^- + HCO_3^-)} \quad (12)$	Negative	Stable water (bicarbonate dominance) provides sufficient buffering)	
	Zero	Equilibrium	
	Positive	High corrosivity risk (chlorides dominate)	
$CHI - II = \frac{(Cl^- - HCO_3^-)}{(Ca^{2+} + Mg^{2+})} \quad (13)$	Negative	Stable water	
	Zero	Equilibrium	
	Positive	Higher risk of corrosion or infrastructure degradation	
$CAI - I = Cl^- - \frac{(Na^+ + K^+)}{Cl^-} \quad (14)$	Negative	Normal (Base) Ion Exchange	
	Zero	No base exchange	
	Positive	Reverse Ion Exchange	
$CAI - II = Cl^- - \frac{(Na^+ + K^+)}{(SO_4^{2-} + HCO_3^- + NO_3^- + CO_3^-)} \quad (15)$	Negative	Normal (Base) Ion Exchange	
	Zero	No base exchange	
	Positive	Reverse Ion Exchange	

Note: **CHI:** Chloride-Bicarbonate Ratio Index; **CHI-I:** Chloride-Bicarbonate Balance; **CHI-II:** Chloride-Hardness Index; **CAI-I, CAI-II:** Chloro-Alkaline Index

a) Sodicty and Salinity Hazards

The Sodium Adsorption Ratio (SAR), Soluble Sodium Percentage (SSP), and Percentage of Sodium (Na%) are the key diagnostic tools for predicting sodicity hazards. These indices indicate the relative concentrations of the active sodium ions (Na^+) and the stabilizing alkaline-earth metals (Ca^{2+} and Mg^{2+}). Higher values of these specific parameters indicate a high risk of sodium replacing calcium on the soil cation exchange complex (Qadir et al., 2014). This ionic substitution leads to clay dispersion, deterioration of soil macro-structure, and severe limitations in root oxygenation and water infiltration, all of which contribute to physiological stress in the legume (Minhas et al., 2020).

b) Alkalinity and Carbonate Hazards

The carbonate and bicarbonate risk assessment was done by determining Residual Sodium Carbonate (RSC) and Residual Sodium Bicarbonate (RSBC) (Eaton, 1950). Particularly, RSC is very important in determining the suitability of water. Water with an

RSC value $> 2.5 \text{ meq L}^{-1}$ is considered unsuitable for agricultural purposes (Richards, 1954). Under high RSC conditions, the available calcium and magnesium are precipitated as insoluble carbonates by excess carbonate (Minhas et al., 2021). This reaction artificially increases the sodium hazard in the soil solution, thereby severely aggravating ionic toxicity experienced by plant roots (Muyen et al., 2011).

c) Permeability and Specific Cation Hazards

The specific secondary cation imbalances were evaluated by Magnesium Hazard (MH) and Kelly's Ratio (KR). The MH value being over 50 suggests that magnesium ions are more influential than calcium ions in the environment. This condition can cause deterioration of soil structure and a decrease in infiltration rate. Physiologically, this condition is similar to the excess-sodium condition (Rengasamy, 2016). Simultaneously, a KR value above 1.0 indicates an excess of sodium relative to the combined calcium and magnesium, serving as an early indicator of potential permeability issues (Berhe, 2020).

d) Corrosivity Hazards and Chloro-Alkaline Exchange Dynamics

The Chloride-Bicarbonate Ratio (CHI) and its sub-indices (CHI-I, CHI-II) evaluate the potential for metal pitting and scaling in the hydraulic systems. Furthermore, the Chloro-Alkaline indices (CAI-I and CAI-II) were used to identify active ion-exchange processes in the aquifer matrix, following the methods of Ryznar (1944) and Schoeller (1965). The classification of these results provides a comprehensive evaluation of the geochemical evolution of irrigation water sustainability and the specific material needs of agricultural infrastructure over the long term.

2.9 Statistical analysis

One-way analysis of variance (ANOVA) was performed on the data generated to evaluate the effects of treatment. Mean separations were carried out by the Least Significant Difference (LSD) test at $p < 0.05$. The phenotypic responses were determined by performing multivariate analysis using growth and yield parameters. Principal Component Analysis (PCA) was used to identify major axes of variance that captured overall plant performance and morphological trade-offs. In parallel, the five treatments were classified by phenotypic similarity using Hierarchical Clustering Analysis (HCA) with Ward's linkage and Euclidean distances.

3. Results and Discussion

3.1 Hydrochemical Evolution and Salinity Constraints of the Irrigated Water

Table 5 presents the distinct hydrochemical properties of irrigation water across the experimental groups, revealing significant water-quality degradation due to saline stress. Potential Salinity (PS) and Total Hardness (TH) are important parameters for the total dissolved salt load and the concentrations of alkaline earth metals.

Table 5. Hydrochemical indices and hazard suitability classifications of the applied irrigation water.

Parameters	S1	S2	S3
	Concentration		
SAR	0.81	20.711	32.41
SSP	29.34	80.78	83.23
Na%	25.69	78.99	80.95
MH %	24.67	67.86	76.81
KR	0.36	4.11	4.82
PI	53.05	83.08	84.68
RSC	-1.68	-9.73	-16.69
RSBC	-1.070	-1.12	0.61
PS	0.28	52.34	115.67
TH	123.35	635.66	1127.20
CH	0.05	3.92	4.68
CHI-I	-0.72	0.89	0.90
CHI-II	-0.27	3.68	4.42
CAI-I	-7.85	48.69	104.41
CAI-II	-0.79	43.21	101.22

Note: S1: Irrigation with fresh water (EC 0.33 dS m⁻¹), S2: Irrigation with saline water (EC 6 dS m⁻¹) and S3: Irrigation with saline water (EC 12 dS m⁻¹)

Freshwater is characterized by low salinity (0.28 meq L⁻¹) and moderate hardness (123.35 mg L⁻¹). This showed that freshwater (S1) was of good quality for irrigation. However, irrigation with saline water at moderate (S2) and severe (S3) salinity resulted in rapid growth in both measurements. TH increased to 635.66 and 1127.20 mg L⁻¹ for moderate and severe salinity, respectively, thus classifying these waters as very hard. The large increase in solute concentration poses a major threat of osmotic stress and severely limits water uptake by the roots. In present-day hydrochemical assessments, total hardness values >300 mg L⁻¹ are considered a severe constraint for agricultural viability due to the combined effects of osmotic stress (Tejashvini et al., 2024). Moreover, the high PS values for severe salinity, S3 (>115 meq L⁻¹) corroborate the highly toxicological nature of untreated saline water, aligning with recent assessments indicating that extreme salinity indices significantly impair crop productivity and soil health (Ouhakki et al., 2024).

3.2 Sodict Hazard Profile of the Irrigated Water

The ratio of sodium to divalent cations (Ca^{2+} and Mg^{2+}) is an important factor in controlling soil structure stability. Sodium Adsorption Ratio (SAR), Soluble Sodium Percentage (SSP), and overall Sodium Percentage (Na%) collectively indicate the hazard of sodification. For the freshwater (S1), all the sodification hazard indices were well below the optimum agronomic limits (SAR: 0.81; SSP: 29.34%; Na%: 25.69%; Table 5). However, there was a strong shift in ionic dominance for moderate and severe salinity S2 and S3. The SAR values were increased to 20.71 and 32.41 for T2 and T3, respectively. The saline had SAR > 10, Na%, and SSP values of around 80%, which can pose an acute risk of soil deflocculation. Consequent ionic imbalance leads to disruption of clay flocculation and destruction of soil macro-aggregates, collapse of pore space, and severely impaired hydraulic conductivity. Furthermore, the Kelly's Ratio (KR), which assesses the relative concentrations of sodium, calcium, and magnesium, is used in this assessment. The

KR ratios for all irrigation water S1, S2, and S3 were 0.36, 4.11, and 4.82, respectively. Regarding the severe sodic hazard, the results for moderate and severe salinity S2 and S3 are consistent with the contemporary water quality model. Recent studies provide evidence that SAR values beyond the usual agricultural limits, with Na% over 60%, are likely to cause soil deflocculation and pose a significant threat to structural integrity (Methal et al., 2024). The results of the KR further validate these contemporary predictive models, indicating that a $KR > 1$ is a dangerous excess of sodium and affirming the high probability of long-term structural deterioration under continuous saline irrigation (Ouhakki et al., 2024; Tejashvini et al., 2024).

3.3 Permeability, Alkalinity, and Ion Exchange Dynamics of the Irrigated Water

The interaction between the applied irrigation water and the soil matrix was evaluated more comprehensively by using magnesium hazard (MH), permeability index (PI), chloride-bicarbonate ratio index (CHI), chloride hardness index (CH II), chloro-alkaline indices (CAI-I, CAI-II)

- **Magnesium and Permeability Hazards:** The MH increased from harmless 24.67% for freshwater (S1) to deleterious levels of 67.86% and 76.81% for moderate and severe salinity (S2 and S3), (see Table 5). Values above the 50% cut-off point indicate that high magnesium levels may cause soil alkalization, further reducing water infiltration rates. Meanwhile, the PI increased from 53.05 (freshwater, S1) to >83 for saline water. The increase of MH% over the 50% limit in saline treatments confirms recent models of structural degradation, pointing to magnesium dominance in the evolution of long-term permeability restrictions (Tejashvini et al., 2024). The positive correlation of PI values with the dominance of sodium and bicarbonate, however, is a functional one, and in the case of extreme SAR, it indicates a pathway to severe long-term restrictions of permeability
- **Carbonate Dynamics:** All three irrigation samples had negative residual sodium carbonate (RSC) and residual sodium bicarbonate (RSBC) values (e.g., RSC: -1.68 to -16.69). The negative values indicate that the sum of alkaline earth metals ($Ca^{2+} + Mg^{2+}$) is greater than that of carbonates and bicarbonates. This absence of residual carbonate hazard is consistent with the safe threshold limits recently reported in semi-arid irrigation assessments, suggesting that neither carbonate precipitation nor bicarbonate toxicity is a limiting factor for these irrigation waters (Methal et al., 2024; Ouhakki et al., 2024).
- **Base Exchange Mechanisms:** The Chloro-Alkaline Indices (CAI-I, CAI-II, CHI-I, CHI-II) provide valuable insights into the process of ion exchange occurring between irrigation water and the soil's exchange complex. The negative indices noted for freshwater, S1 (e.g., CAI-I: -7.85), are characteristic of a normal base exchange reaction, in that sodium and potassium fixed in the soil are replaced by calcium and magnesium from the water, thus maintaining the soil structure. In contrast, high positive indices of moderate and severe saline water S2 (48.69) and S3 (104.41), respectively, show strong reverse ion exchange. In this case, the excess water causes sodium to displace native calcium and magnesium from the soil's colloidal surfaces. This reverse exchange is confirmed in the recent hydrochemical

literature as the main physicochemical driver of rapid agricultural soil sodication (Tejashvini et al., 2024).

3.4 Sustainability and Management of the Irrigated Water

The hydrochemical indices clearly classify the S1-irrigated freshwater as optimal and highly suitable for sustainable crop production. In contrast, the S2 and S3, which are moderately to severely saline-irrigated waters, are classified as marginal to strictly unsuitable due to their extreme osmotic stress potential, high sodic hazard ($SAR > 10$), and propensity to induce reverse ion exchange. Using such hazardous irrigated water requires aggressive ameliorative management, such as applying exogenous osmoprotectants (e.g., proline), increasing leaching fractions, or using chemical amendments to neutralize the water's innate toxicity before application.

3.5 Agronomic Performance and Yield Recovery via Proline Application

The translation of the aforementioned severe saline constraints into actual plant growth penalties is quantified in Table 6. The data explicitly demonstrate the detrimental impact of unmitigated saline irrigation on the agronomic performance of faba beans, as well as the robust mitigative capacity of exogenous proline.

Table 6. Growth characteristics and yield of faba bean plants under varying salinity and proline treatments.

Treatments	Plant Height	No. of Pods	Weight of Pods /pot	Yield
	Cm	No	g	kg fed ⁻¹
T1	101.00 ± 3.00 a	34.00 ± 2.00 a	510.00 ± 30.00 a	1203.67 ± 70.53 a
T2	92.33 ± 4.93 b	30.33 ± 2.08 b	424.67 ± 29.14 c	1002.00 ± 68.70 c
T3	79.67 ± 4.73 c	30.00 ± 1.73 b	390.00 ± 22.52 d	920.33 ± 53.12 d
T4	100.67 ± 6.66 a	34.67 ± 0.58 a	485.33 ± 8.08 ab	1145.00 ± 19.05 ab
T5	90.33 ± 8.62 b	33.67 ± 0.58 a	437.67 ± 7.51 bc	1032.67 ± 17.90 bc
MSE	34.88	2.4	475.6	2638.73
LSD ($\alpha = 0.05$)	10.74	2.82	39.67	93.44
HSD ($\alpha = 0.05$)	15.87	4.16	58.6	138.03

Note: T1 (Control): Irrigation with fresh water ($EC\ 0.33\ dS\ m^{-1}$) with no proline application, T2: Irrigation with saline water ($EC\ 6\ dS\ m^{-1}$) with no proline application, T3: Irrigation with saline water ($EC\ 12\ dS\ m^{-1}$) with no proline application, T4: Irrigation with saline water ($EC\ 6\ dS\ m^{-1}$) supplemented with a foliar proline spray ($500\ mg\ L^{-1}$) and T5: Irrigation with saline water ($EC\ 12\ dS\ m^{-1}$) supplemented with a foliar proline spray ($500\ mg\ L^{-1}$).

3.5.1 Salinity-Induced Growth Inhibition

There was a clear dose-dependent reduction in plant growth and reproductive traits under unmitigated saline treatments (T2 and T3). Plant height was significantly reduced to

79.67 cm under severe salinity (T3, 12 dS m⁻¹; group c), compared with 101.00 cm in the unstressed control (T1; group a). This reduction of 21.33 cm, equivalent to 21.12%, exceeded the Least Significant Difference (LSD) threshold of 10.74 cm, confirming a significant salt-induced inhibition of vegetative growth.

Similarly, the number of pods per plant was significantly reduced to 30 (group b). This reduction of 4 pods compared to the control is greater than the established LSD margin of 2.82, which clearly shows a statistically significant penalty to reproduction. At the same time, total pod weight and final extrapolated yield were significantly reduced to 390.67 g pot⁻¹ (group d) and 920.33 kg fed⁻¹ (group d), respectively. The absolute reductions (119.33 g pot⁻¹ and 283.34 kg fed⁻¹) were significantly greater than their respective LSD thresholds of 39.67 and 93.44, which demonstrates that there is a severe penalty on mass-based agronomic output due to severe unmitigated salinity. Recent studies in legume agronomy indicate that high salinity rapidly inhibits vegetative growth and causes the loss of reproductive organs in *Vicia faba* L. due to severe osmotic stress that limits cellular elongation before any significant biomass reduction. These findings are consistent with the marked suppression of morphological traits (Ehtaiwesh & Abuiflayjah, 2024).

3.5.2 Mitigation and Trait Recovery via Proline

The weekly foliar spray of 500 mg L⁻¹ proline efficiently reprogrammed the plant's stress response, with strong rescue of critical reproductive traits and yield. Under severe salinity, the pod count per plant was restored to 33.67 with proline intervention (T5). The recovery (group a) is a statistically significant relative increase of 12.23%, 3.67 pods (Figure 2) over the untreated T3 plants (group b), exceeding the LSD margin of 2.82. Most importantly, the deficit recovery showed that proline restored 91.75% of the stress deficit in the reproductive organs (Figure 3), confirming their recovery to levels statistically comparable to those of the unstressed control. Furthermore, for the T5 treatment, plant height improved to 90.33 cm (group b). Relative growth increase is 13.38%, recovering 49.98% of the height loss in relation to the severe stress baseline of 79.67 cm (group c). The total yield for T5 (1032.67 kg fed⁻¹, group bc) showed a notable improvement compared with T3 (920.33 kg fed⁻¹, group d). The mass-based recovery of 112.34 kg fed⁻¹ (+12.21% relative increase) was above the LSD threshold of 93.44 and thus definitively protected the crop's yield capacity under stress by successfully restoring 39.65% of the total lost yield potential. Likewise, pod weight was restored to 437.67 g pot⁻¹ (group bc), representing a significant 12.22% (47.67 g) relative improvement over the untreated severe-salinity group (390 g pot⁻¹, group d). This clears the 39.67 LSD margin and achieves a deficit recovery of 39.73%.

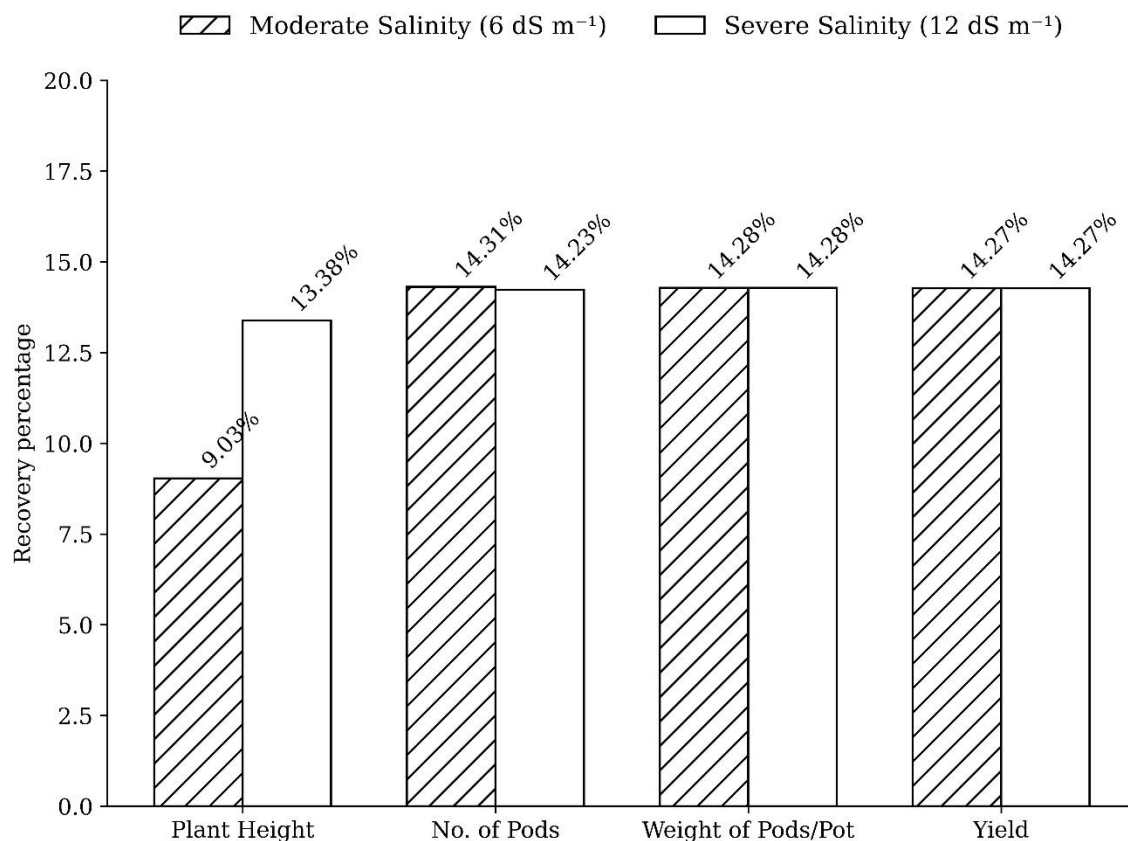


Figure 2. Relative percentage increase in Faba bean agronomic traits following foliar proline application.

These recovery parameters are in line with the results of state-of-the-art agricultural experiments showing that external osmoprotectants (such as proline) help maintain cell turgor, protect critical reproductive phases, thereby preventing pod abortion, and provide structural stability to the plant under critical stress conditions (El-Beltagi et al., 2025). Average data show that maintenance of reproductive organs during the early period of the stress cycle (almost complete recovery of pod number, 91.75%) ensures the preservation of overall agronomic viability and the final biomass yield of the crop.

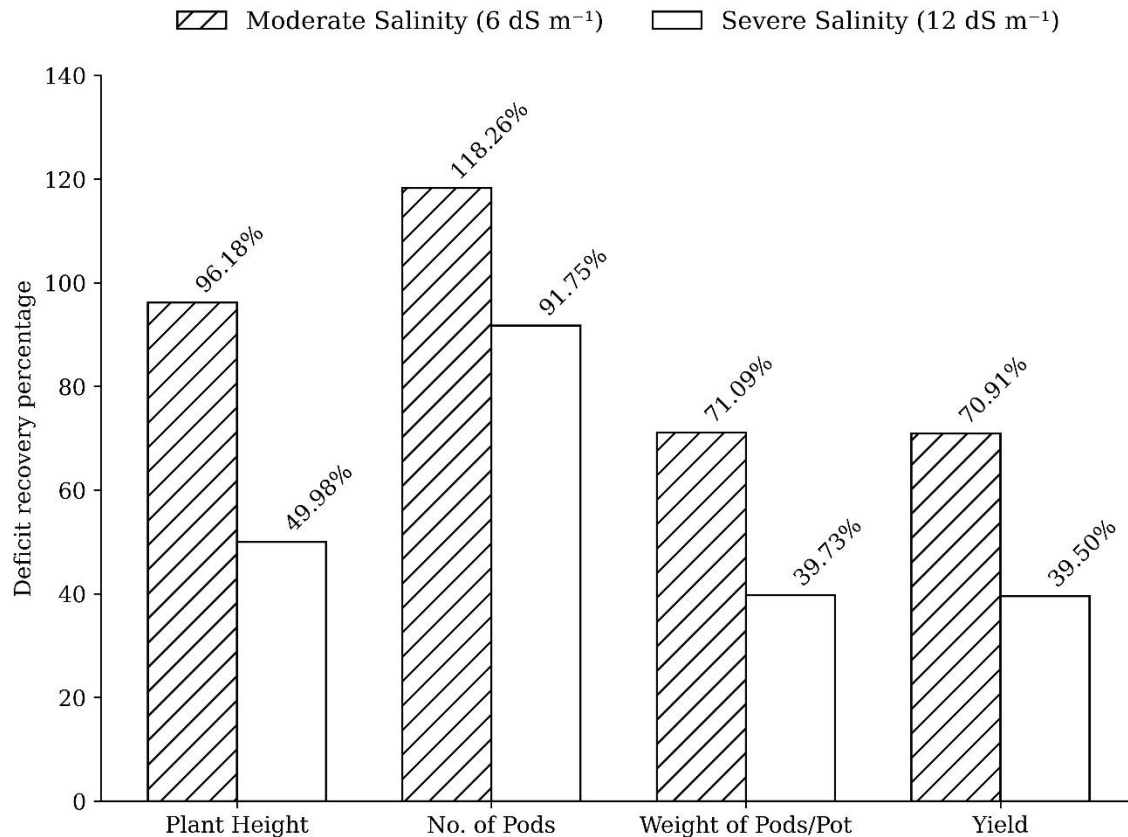


Figure 3. Deficit recovery percentage for moderate and severe saline treatments restored by proline

3.6 Tissue Nutrient Partitioning and Protein Metabolism

The systemic resilience observed in agronomic parameters is mechanistically underpinned by the biochemical partitioning of macronutrients between leaves and seeds (Table 7). Salinity is characterized by severe ion toxicity, in which sodium competitively excludes essential macronutrients at the root-soil interface. The data below quantify this nutritional lockout and explicitly demonstrate proline's statistical efficacy in restoring metabolic homeostasis.

3.6.1 Salinity-Induced Ionic Imbalance and Nutrient Lockout

A progressive and significant disruption in the balance of tissue nutrients occurred due to a lack of exogenous osmoprotectants, alongside rising salinity. Severe salinity (T3) without mitigation reduced leaf N to a critical 1.87% (Table 7). This absolute reduction of 1.32% over the control (T1) was strictly greater than the LSD value of 0.267, confirming a statistically significant collapse in nitrogen assimilation ($p < 0.05$). Likewise, leaf K was significantly depressed to 1.56% (a drop of 0.32%, well outside the LSD margin of 0.165). Furthermore, the reduction in leaf P under severe stress (from 0.47% in T1 to 0.30% in T3) was statistically significant and greater than the LSD (0.131).

Table 7. Mean macronutrient (N, P, K) and crude protein concentrations in faba bean leaves and seeds under varying salinity and proline treatments.

I	Leaves				Seeds			
	N (%)	P (%)	K (%)	Protein	N (%)	P (%)	K (%)	Protein
T1	3.19 ± 0.19 c	0.47 ± 0.16 a	1.88 ± 0.07 bc	19.94 ± 1.16 c	3.73 ± 0.07 c	0.38 ± 0.03 a	3.30 ± 0.10 b	23.32 ± 0.42 c
T2	2.80 ± 0.14 d	0.33 ± 0.01 b	1.76 ± 0.00 c	17.52 ± 0.88 d	3.59 ± 0.04 d	0.32 ± 0.00 bc	2.07 ± 0.35 c	22.45 ± 0.26 d
T3	1.87 ± 0.21 e	0.30 ± 0.01 b	1.56 ± 0.16 d	11.68 ± 1.34 e	3.45 ± 0.05 e	0.30 ± 0.00 c	1.30 ± 0.20 d	21.57 ± 0.29 e
T4	3.79 ± 0.08 a	0.36 ± 0.00 ab	2.61 ± 0.06 a	23.68 ± 0.51 a	3.92 ± 0.03 a	0.34 ± 0.01 b	3.80 ± 0.10 a	24.50 ± 0.18 a
T5	3.50 ± 0.04 b	0.35 ± 0.01 ab	2.01 ± 0.08 b	21.85 ± 0.23 b	3.83 ± 0.01 b	0.32 ± 0.01 b	3.60 ± 0.00 ab	23.91 ± 0.05 b
LSD ($\alpha=0.05$)	0.267	0.131	0.165	1.668	0.079	0.026	0.348	0.492

Note: The data from Table 7 has been aligned to strictly match the updated treatment key established in Table 6: **T1** (Control), **T2** (6 dS m⁻¹), **T3** (12 dS m⁻¹), **T4** (6 dS m⁻¹+ Proline), and **T5** (12 dS m⁻¹ + Proline).

Potassium is important for stomatal regulation, osmoregulation, and enzymatic activation. Its highly significant decrease confirms the active antagonism of excess sodium in the applied irrigation water against potassium uptake at the root interface. This nutrient lockout cascade finally broke the plant's metabolic capacity, bringing down leaf protein from a healthy 19.94% (T1) to a severely deficient 11.68% (T3), a massive 8.26% loss over the 1.668 LSD threshold, by a wide margin. Reproductive sinks exhibited the same trend of deterioration. Seed protein was reduced to 21.57% with a statistically significant difference when compared to the control (> 0.492 LSD margin). The ionic imbalance mechanism is well documented in the latest agronomic literature. Recent studies showed clearly that high salinity stress significantly decreased the K/Na ratio and reduced the N, P, K, and crude protein contents in faba bean tissues. The main reason for this is the physical replacement of potassium with sodium at critical intracellular sites, which structurally limits protein synthesis and overall metabolic function (Ehtaiwesh & Abuiflayjah, 2024).

3.6.2 Ion Homeostasis and Metabolic Rescue via Proline

The foliar application of proline resulted in a complete overhaul of the stress response, transitioning the biochemical makeup from detrimental to stabilizing, and consequently yielding highly significant nutrient recoveries ($p < 0.05$). Proline treatment was found to selectively increase potassium concentrations in tissues, thereby buffering the plant against the deleterious effects of sodium toxicity. The proline treatment (T5) increased the leaf K to 2.01% under severe salinity, an increase of 0.45% over 1.56% in T3, which is significantly higher than the LSD value of 0.165. Importantly, at moderate salinity (T4), leaf K increased dramatically to 2.61%, which was significantly higher than the freshwater control (0.73% higher than T1). Selective retention of potassium at the molecular level implies that exogenous proline actively modifies membrane electrophysiology, performing a function beyond basic osmotic regulation. The plasma membrane depolarization induced by salinity normally triggers a rapid efflux of K⁺. This may be alleviated by two modes of action of proline: (1) ROS-scavenging activity to prevent lipid peroxidation and K⁺ leakage; (2) upregulation of inward-rectifying K⁺

channels (e.g., AKT1) and high-affinity transporters (e.g., HKT). High K^+ retention (2.61%) indicates that proline is an effective buffer of the membrane potential, maintaining the cytosolic K^+/Na^+ ratio for continuous protein synthesis (El-Ghany & Attia, 2020). Proline protected the fragile nitrogen assimilation pathway by maintaining potassium homeostasis and cellular hydration. Proline treatment increased the leaf protein content to 23.68% in T4 and 21.85% in T5. Both values recover from salinity-induced loss and are statistically significant improvements over the unstressed T1 control (19.94%), comfortably clearing the 1.668 LSD margin. The preservation of leaf physiology is directly related to the quality of the final harvested product. Despite the heavy saline load of 12 dS m^{-1} in the soil, the seeds harvested from the T5 plants had a high viable protein content of 23.91% and nitrogen content of 3.83%. Both metrics were significantly better than the untreated T3 plants and statistically higher than the baseline freshwater control (exceeding their respective LSD thresholds of 0.492 and 0.079). The selective enrichment of macronutrients through proline activity aligns with the latest agricultural trials. Recent studies have shown that the application of exogenous bio-stimulants, especially proline and salicylic acid, significantly enhances N, P, K, and crude protein contents in salt-stressed faba beans by maintaining membrane integrity and preventing the degradation of metabolic enzymes (El-Beltagi et al., 2025). This intervention ameliorates ion toxicity, maintains protein metabolism, and hence ensures both to yield mass and high-value nutritional quality of the faba bean crop.

3.7 Principal Component Analysis (PCA) Modeling of Functional Traits

A principal component analysis (PCA) of the standardized correlation matrix was used to reduce the complex physiological responses of *Vicia faba* L. to different salinity and foliar proline treatments into four principal components. The eigenvalue plot (Figure 4) and variance decomposition (Figure 5) indicate that the model is highly effective at reducing dimensionality, as the first two principal components (PC1 and PC2) account for 98.46% of the total variance. PC1 accounts for 92.28% of the variance and serves as the primary axis, representing overall plant vitality and output. PC2 contributes for extra 6.18% of the variance, providing further insight by reflecting morphological trade-offs between vegetative growth and reproductive development. This parsimonious two-component model provides a superior statistical framework, as PC3 and PC4 account for only 1.54% of the remaining information. PCA compresses almost the entire dataset into a two-dimensional space, effectively delineating the physiological boundaries between high-vigor groups and those under severe osmotic stress. The structural relationships and the ordination of the five treatments (T1–T5) are shown in the structural biplot of PC1 and PC2. PC1 displays positive coefficients for T1, T4, and T5, while exhibiting negative coefficients for T2 and T4 on the horizontal axis (Figure 6).

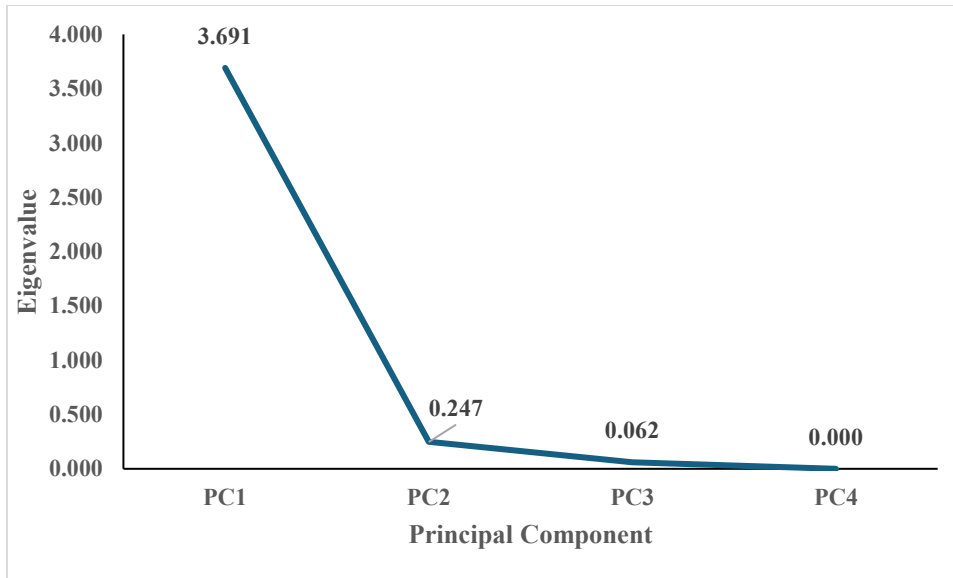


Figure 4. Eigenvalue plot for principal component analysis.

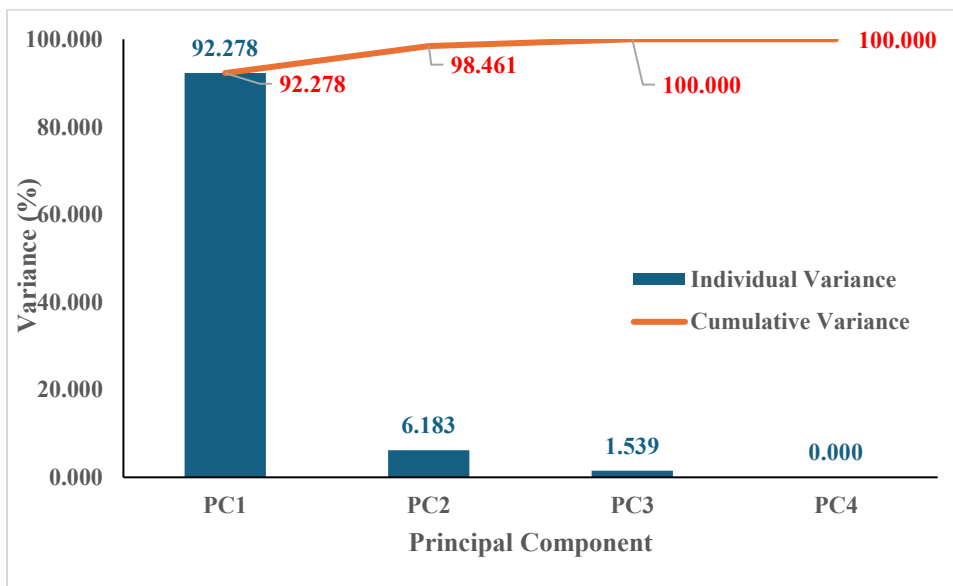


Figure 5. Variance plot for principal component analysis

The heatmaps of the loadings (Figure 7) across PC1 to PC4 successfully mapped the physiological pathways of salt-induced degradation and proline-driven recovery. All four variables have moderate positive and uniform loading (0.47 to 0.51) on PC1, thus validating this component as a general performance factor. This is a combined measure of total plant performance and explains the greatest percentage of variance (80.0%). PC2 is characterized by a strong positive loading for the number of pods (0.87) and a moderate negative loading for plant height (-0.42). Pod weight and yield both had relatively weak negative loadings of -0.19 . Thus, PC2 mainly reflects variation in pod number relative to plant height and mass-based yield traits. PC3 is dominated by a strong positive loading for plant height (0.76), together with negative loadings for pod weight (-0.44) and yield

(−0.45), while the number of pods showed only a weak positive loading (0.17). This component represents a secondary contrast between vegetative growth and yield-related traits; however, its biological importance is limited because it explains only 1.5% of the total variance. PC4 showed contrasting loadings for pod weight (0.71) and yield (−0.71), whereas plant height and pod number had loadings close to zero. Nevertheless, because PC4 explained approximately 0.0% of the variance, it should not be assigned substantive biological interpretation. A value close to 1 indicates a strong association between the variable and the principal component. Values above 0.75 indicate a strong positive correlation, and correlations between 0.5 and 0.74 are considered strong (Samtio et al., 2023).

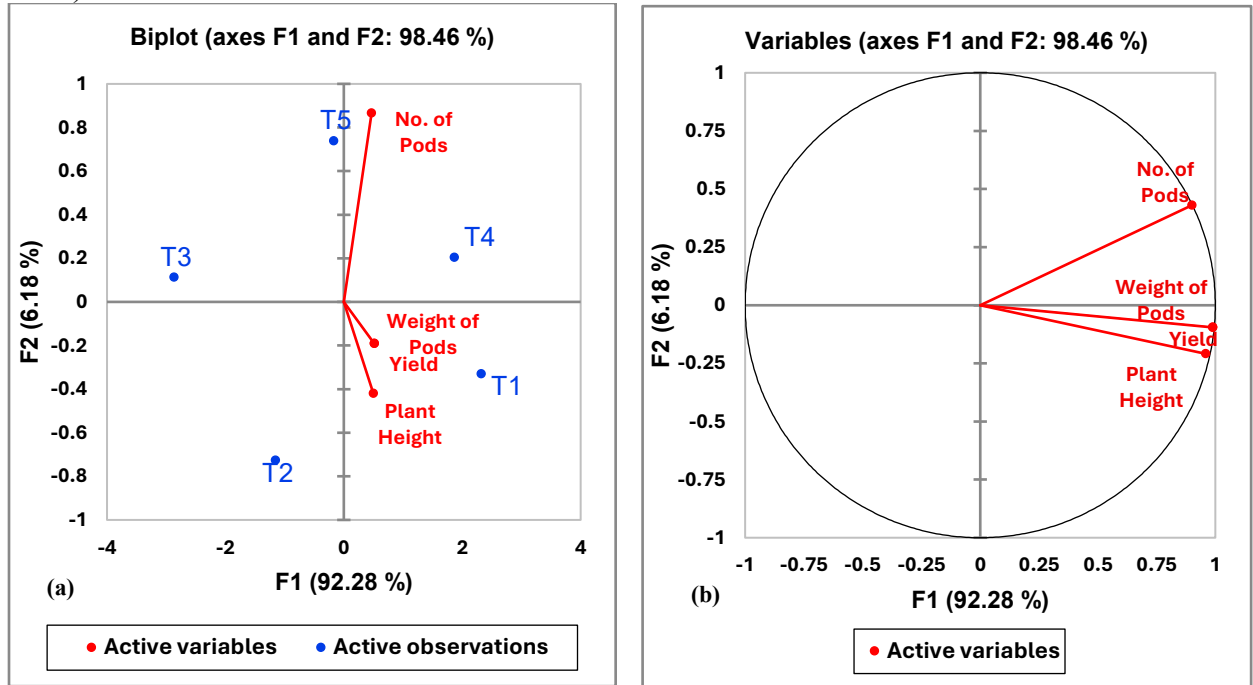


Figure 6. Biplot of PC1 and PC2.

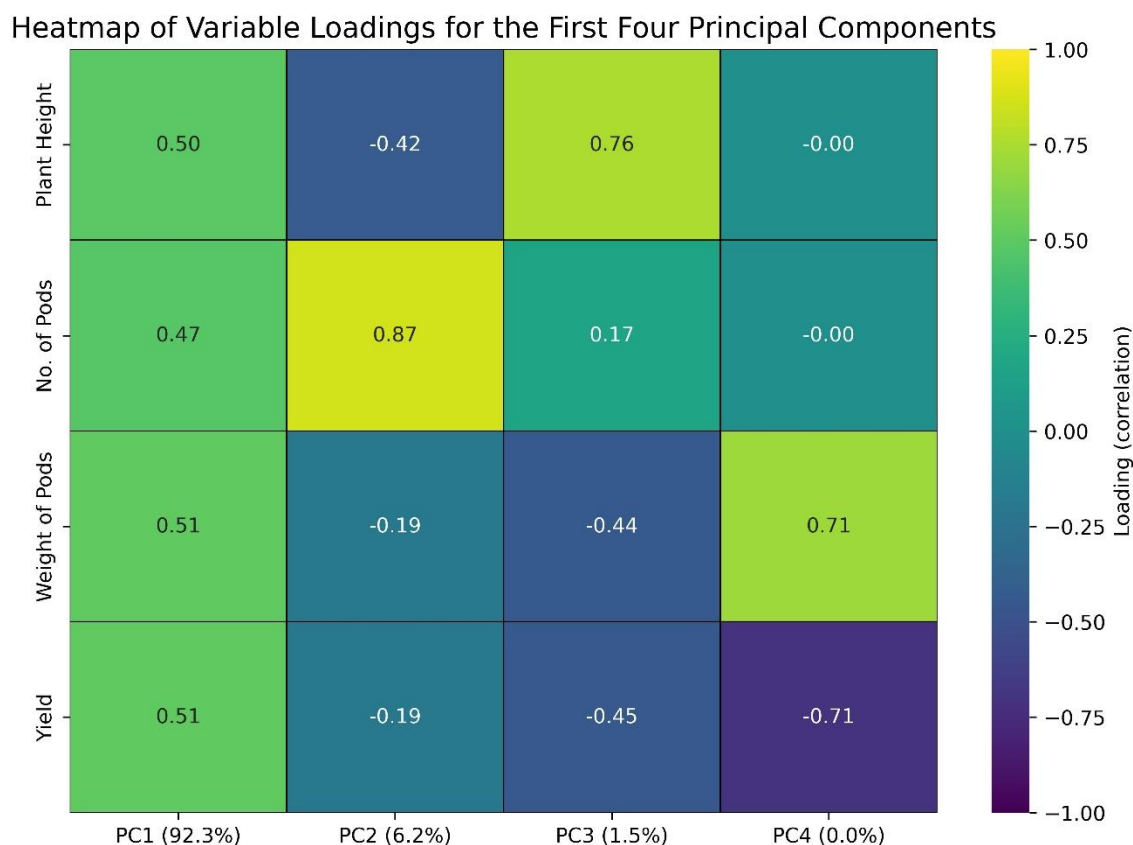


Figure 7. Heatmap of Variable Loadings for First Four Principal Components derived from faba bean growth and yield traits. PC1 and PC2 explained 92.3% and 6.2% of the total variance, respectively, together accounting for 98.5% of the variation among treatments.

3.8 Hierarchical Clustering Validation

The standardized growth and yield parameters (plant height, pod number, pod weight per pot, and total yield) were agglomeratively clustered for the experimental treatments with Ward's linkage and Euclidean distances to systematically evaluate the multivariate phenotypic similarities. The dendrogram's reliability is confirmed by a Cophenetic Correlation Coefficient (CPCC) of 0.724 (Figure 8), indicating good correspondence (0.7 - 0.79) to the original trait dissimilarities (Hinkle, 2003).

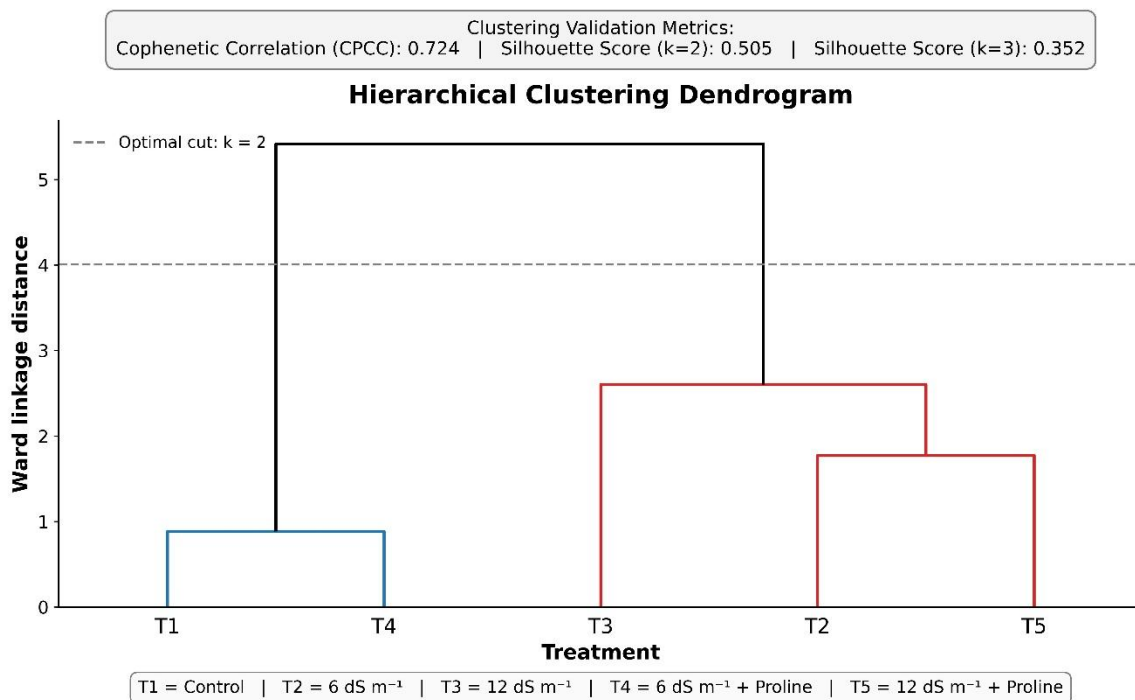


Figure 8. Hierarchical clustering dendrogram (Ward’s linkage, Euclidean distance) classifying five varying salinity and proline treatments based on Faba bean growth and yield metrics (Plant height, No. of pods, Weight of pods/pot, and Yield).

Note: The analysis yielded a Cophenetic Correlation Coefficient of 0.724, indicating adequate preservation of multidimensional distances. Silhouette score validation confirmed a primary two-cluster model ($k=2$, Silhouette Score = 0.505) effectively separating the high-vigor groups (T1, T4) from the stress-impacted groups (T2, T3, T5). T1 = Control ; T2 = 6 dS m⁻¹ ; T3 = 12 dS m⁻¹ ; T4 = 6 dS m⁻¹ + foliar proline spray 500 mg L⁻¹ ; T5 = 12 dS m⁻¹ + foliar proline spray 500 mg L⁻¹.

Additional Silhouette Analysis revealed a bipartite split ($k = 2$) to be the best model to optimize for within-cluster cohesion and between-cluster separation (Silhouette Score = 0.505). The main functional group, Group 1 (High-Vigor Phenotype), included the unstressed freshwater control (T1) and the proline-mitigated moderate-salinity treatment (T4), indicating the potent mitigating effect of exogenous foliar proline on ion toxicity and the quantitative restoration of baseline agronomic vigor.

Moreover, sub-clustering analysis ($k = 3$, Silhouette Score = 0.352) further divided the stress gradient and separated Group 3 (Severe Stress Phenotype), which included the unmitigated severe salinity treatment (T3), from Group 2 (Intermediate Phenotype), which comprised the intermediate performing treatments (T2 and T5). This distinct topological division is consistent with the drastic morphological stunting and yield collapse mathematically represented by an acute load of 12 dS m⁻¹ osmotic stress in the absence of exogenous osmoprotectants, and at the same time, it confirms that proline application buffers phenotypic severity of extreme environmental stress in T5 by shifting it upward into Group 2.

4. Limitations and future directions

Despite providing important insights into the role of exogenous proline in improving faba bean tolerance to salinity, this study has several limitations that should be considered. First, the experiment was conducted under greenhouse pot conditions using a specific clay-loam soil. Although this controlled system enabled the isolation of key physiological and hydrochemical responses, pot cultivation restricts root development and does not fully reproduce the complex microclimatic variation, soil–plant–microbial interactions, and leaching dynamics encountered under open-field conditions.

Second, the experiment evaluated a single concentration of exogenous foliar proline (500 mg L⁻¹) using a fixed weekly application schedule. Although this concentration was selected based on previously reported responses, the absence of a concentration gradient and alternative application timings prevents identification of the minimum effective dose, the optimum application frequency, and the most economically efficient treatment regime. In addition, only one source of faba bean plant material was evaluated; therefore, possible cultivar-dependent differences in proline uptake, metabolism, and salinity response remain to be determined. Future research should compare multiple foliar proline concentrations, application frequencies, developmental stages, and contrasting faba bean cultivars under multi-season and multi-location field conditions in the Nile Delta.

Although the foliar proline material cost was estimated at approximately L.E 21 per treated experimental unit, this pot-scale estimate cannot be directly extrapolated to a cost per feddan. Field-scale economic feasibility should therefore be assessed through multi-season trials and formal partial-budget analysis that incorporates the cost of foliar proline, spray volume, labor, machinery, application frequency, and the market value of the resulting yield and quality gains. Comparisons with alternative salinity-management practices, including soil amendments and other biostimulants, would also help determine the relative economic competitiveness of repeated foliar proline application.

Finally, transcriptomic, proteomic, and ion flux studies are required to elucidate the molecular and biophysical mechanisms by which exogenous foliar proline may influence potassium retention, membrane stability, ion homeostasis, and nitrogen metabolism under salinity stress. Until field-scale agronomic and economic validation is completed, the treatment should be regarded as a promising salinity-mitigation strategy rather than a finalized commercial recommendation.

5. Conclusion

The present study shows that long-term irrigation with severely saline water (12 dS m⁻¹) initiates a cascade of osmotic, ionic, and geochemical hazards that threaten soil structure and crop physiology. Hydrochemical diagnostics indicate that such water is unsuitable for conventional faba bean cultivation due to extreme sodic conditions (SAR: 32.41) and the onset of reverse base exchange, resulting in the depletion of soil-stabilizing cations. This toxicological profile results in severe nutrient lockout without intervention, effectively disabling nitrogen assimilation and disrupting potassium-dependent metabolic pathways.

Weekly foliar application of proline at 500 mg L⁻¹ substantially alleviated the adverse effects of severe salinity by improving plant growth, reproductive performance, potassium accumulation, nitrogen status, and seed protein content. The observed responses suggest that exogenous proline helps maintain ion homeostasis, membrane stability, cellular hydration, and protein metabolism under saline conditions. In particular, the treatment maintained seed protein content at 23.90% and partially restored yield under the highest salinity level. Although the estimated material cost of the proline application was approximately L.E 21 per treated experimental unit, this greenhouse-scale value should be interpreted with caution and cannot be directly extrapolated to a field basis. A reliable assessment of economic feasibility per feddan will require multi-season field trials supported by formal partial-budget analysis that accounts for L-proline cost, spray volume, labor, machinery, application frequency, and the market value of the resulting yield and quality improvements. Ultimately, the work provides a validated, practical intervention for managing marginal water resources. These results offer a replicable model to improve food security and climate resilience in vulnerable, saline-affected agricultural ecosystems worldwide by integrating hydrochemical diagnostics with targeted biochemical stabilization.

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