



Unravelling Salinity-induced Growth and Biochemical Changes in Greengram (*Vigna radiata* L.) with Principal Component Analysis

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ABSTRACT

Background: The impact of global warming on the environment is affecting soil quality and water supplies, which in turn is impacting agricultural production. The rise in salt concentrations in soil and water systems due to increased evaporation rates from warming temperatures and changing precipitation patterns is a major concern. This salinity poses a threat to crop development, especially for sensitive species like the green gram (*Vigna radiata* L.), a protein-rich legume consumed globally. Understanding the effects of salinity on green gram growth and physiological responses is essential due to its importance as a significant food source.

Methods: A pot experiment was conducted at ADACandRI, Trichy, with varying EC values (less than 1, 2, 4, 6, 8, 10 and 12 dS/m) to study the effects of different saline irrigation levels. The experiment utilized the green gram variety VBN 2 in a randomized design with three replications. Growth parameters including plant height, number of leaves and branches, blooming period, root, shoot and leaf lengths were measured. Biochemical characteristics such as protein concentration, reducing sugars, proline and chlorophyll levels were also analyzed. The study evaluated the impact of these characteristics on salinity tolerance using Principal Component Analysis (PCA).

Result: Salinity stress negatively affected growth and metabolic reactions in greengram. The VBN 2 variety exhibited better growth and biochemical reactions at a moderate salinity level of 2 dS/m compared to higher salinity levels, indicating moderate salt tolerance. Plant height, leaf growth and flowering time improved with lower salinity levels but decreased significantly at higher salt levels. Important characteristics such as plant height, chlorophyll content and proline accumulation were key factors in determining the plant's ability to tolerate salt stress, as indicated by PCA analysis.

Key words: Biochemical adaptation, Green gram, Growth, Principal component analysis, Salinity stress.

INTRODUCTION

Global warming is causing significant environmental changes that directly affect water resources and soil quality, impacting agricultural practices worldwide (Bernacchi *et al.*, 2023). Increasing temperatures and changing precipitation patterns are leading to higher evaporation rates, resulting in elevated salt levels in soil and water systems. This salinization issue is particularly severe in arid and semi-arid regions, where high evaporation rates and limited freshwater resources contribute to progressive salinization (Balasubramaniam *et al.*, 2023).

Soil salinization threatens global food security, with projections indicating up to 50% of cultivable land could be lost by mid-21st century (FAO, 2009). Salinity hinders crop growth, reducing productivity and quality (Kaur *et al.*, 2022). The use of saline water for irrigation presents challenges for plant growth, as high salt levels can impede nutrient uptake, reduce water availability and affect photosynthetic efficiency in plants, leading to osmotic and ionic stress (Wu *et al.*, 2023). The potential impact of global warming on agriculture has shifted the focus of agricultural research towards understanding the abiotic and biotic environmental factors affecting crop growth, with a specific emphasis on

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factors influencing crop growth (Patel *et al.*, 2024, Dudhe *et al.*, 2018).

Legumes are the second-largest contributor to global food production, accounting for 27% of main crop consumption and supplying 33% of the world's protein needs (Khatun *et al.*, 2021). Green gram (*Vigna radiata* L.), a protein-rich legume consumed as whole grains or sprouts, plays a crucial role in soil fertility by fixing atmospheric nitrogen and enhancing soil physical properties (Mohan Naik *et al.*, 2020). Environmental challenges, particularly salinity, often hinder the growth and development of green gram. Salinity affects all growth stages, including seed germination, vegetative growth and reproductive stages, significantly impacting productivity (Sehrawat *et al.*, 2019). This connection underscores the importance of researching the effects of growth and biochemical responses of green gram under increasing salinity conditions.

Salinity triggers various growth and biochemical changes in plants. Traits like plant height, leaf count, branch number, flowering time, root and shoot length are critical indicators of stress response. Biochemical compounds, including proline, protein, chlorophyll and sugars, play roles in osmotic adjustment, photosynthesis and energy metabolism under stress (Patel *et al.*, 2024).

Understanding pulse crop responses to salinity is essential for sustaining productivity in affected regions and ensuring food security, especially in semi-arid and coastal areas where saline irrigation is necessary. This study investigates green gram's growth and biochemical responses to salinity through Principal Component Analysis (PCA) to identify key traits associated with salinity tolerance.

MATERIALS AND METHODS

Experimental site and design

The pot experiment was carried out in the Department of Soil Science and Agricultural Chemistry at Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli, Tamil Nadu (Latitude 10.75°N, Longitude 78.60°E) during 2022-2024. The experiment was designed as a completely randomized design (CRD) with three replications and included seven salinity levels (< 1, 2, 4, 6, 8, 10 and 12 dS/m) imposed on the legume crop, green gram (VBN 2).

Preparation of treatment

In this experiment, RO (Reverse Osmosis) water was used as the control due to its very low electrical conductivity (EC), generally around 0.5 dS/m or less, providing a baseline for evaluating the effects of varying salinity levels on growth of the plant. Target salinity levels for irrigation water (ECw) were prepared by diluting seawater (EC ~50 dS/m) with RO water. Seawater, containing key ions like sodium, chloride, calcium, magnesium and sulfate, was chosen to simulate real-world saline conditions in agriculture.

For preparing 10 liters of saline irrigation water at different target electrical conductivity levels, the volumes of seawater and RO water were adjusted as follows: For a target EC of 2 dS/m (T_2)= 0.4 L of seawater + 9.6 L of RO water, 4 dS/m (T_3)= 0.8 L of seawater + 9.2 L of RO water, 6 dS/m (T_4)= 1.2 L of seawater + 8.8 L of RO water, 8 dS/m (T_5)= 1.6 L of seawater + 8.4 L of RO water, 10 dS/m (T_6)= 2.0 L of seawater + 8.0 L of RO water and 12 dS/m (T_7)= 2.4 L of seawater + 7.6 L of RO water.

Solutions were stored in 10 L containers and plants were irrigated manually every three days with 300 mL per pot, ensuring even absorption. Each pot received 9 liter of water during the experiment, enabling uniform moisture and accurate evaluation of salinity effects.

Growth and stress tolerance assessment

During the flowering phase of green gram, several parameters were measured, including plant height, number of branches per plant, number of leaves per plant, flowering time, leaf length, root length and shoot length. Additionally, stress tolerance analyses were conducted, specifically evaluating the plant height stress tolerance index and root length stress tolerance index.

The root length and shoot length of green gram were measured and the following formulas were used to calculate the stress indices, as outlined by Ashraf *et al.* (2004),

• Plant Height Stress Tolerance Index (PHSTI):

$$\text{PHSTI (\%)} = \frac{\text{Plant height of the stressed plant}}{\text{Plant height of the control plant}} \times 100$$

• Root Length Stress Tolerance Index (RLSTI):

$$\text{RLSTI (\%)} = \frac{\text{Root length of the stressed plant}}{\text{Root length of the control plant}} \times 100$$

Biochemical assessment

During the experiment, leaf samples were collected from each replication per treatment at the flowering stage, yielding a total of 42 samples. To maintain the integrity of the biochemical compounds, the samples were promptly stored in a cooler at -4°C prior to conducting the physio-biochemical analysis. The following protocols were used for the analysis.

Proline content analysis

Proline content was determined following the method of Bates *et al.* (1973). A 0.2 g leaf sample was dissolved in 5 mL of 3% sulfosalicylic acid. The homogenate was filtered and glacial acetic acid along with 2 mL of acidic ninhydrin solution was added. This mixture was heated in a water bath at 100°C for 60 minutes and then cooled on ice for five minutes. Toluene was subsequently added and absorbance was measured at 520 nm using a UV spectrophotometer (PerkinElmer Lambda 365). Proline content was reported in $\mu\text{mol/g}$ of fresh sample weight.

Chlorophyll content

Chlorophyll content was assessed using Arnon's method (1949). Leaf samples (0.5 g) were homogenized in 80% ethanol and centrifuged for 3 minutes at 4000 rpm. The absorbance was measured using a UV spectrophotometer (PerkinElmer Lambda 365) at wavelengths of 663 nm and 645 nm. The following formulas were employed to calculate the chlorophyll content. It was expressed in mg/g of fresh sample weight.

$$\text{Chlorophyll a} = [0.0127 \times \text{OD}_{663} - 0.00269 \times \text{OD}_{645}] \times (\text{V/W})$$

$$\text{Chlorophyll b} = [0.0229 \times \text{OD}_{645} - 0.00468 \times \text{OD}_{663}] \times (\text{V/W})$$

$$\text{Total Chlorophyll} = [(20.2 \times \text{OD}_{645}) + (8.02 \times \text{OD}_{663})] \times (\text{V}/(1000 \times \text{W}))$$

Where,

OD= Optical density at the respective wavelengths.

V= Extract volume (mL).

W= Sample weight (g).

Reducing sugar content

Reducing sugars were measured using the DNSA reagent following Miller's method (1959). A 1 mL ethanolic extract of the sample was evaporated and reconstituted with an equal volume of water. To this solution, 1 mL of dinitro-salicylic acid (DNSA) reagent was added and the mixture was incubated in a water bath for 5 minutes. After incubation, 1 mL of 40% Rochelle salt solution was added. Absorbance was measured at 510 nm using a UV spectrophotometer (PerkinElmer Lambda 365) and reducing sugar content was estimated using a glucose standard curve.

Soluble protein content

Soluble protein content was determined following the method of Lowry *et al.* (1951). Absorbance was measured at 660 nm against a reagent blank, using bovine serum albumin as the standard.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) to identify significant mean differences at P d" 0.05. The

least significant differences (LSD) were employed to compare the significance between treatments. The analyses were conducted using the OPSTAT statistical software tool (Sheoran *et al.*, 1998). Furthermore, principal component analysis was performed using the STAR 2.0.1 statistical program (Priyankabhareti *et al.*, 2011).

RESULTS AND DISCUSSION

Impact of Salinity on Growth Parameters and Stress Tolerance

Increasing salinity significantly reduced the growth and flowering characteristics of green gram as irrigation water EC increased. Compared to the control (T_1 , EC <1 dS/m) with a plant height of 48.7 cm, salinity stress caused a progressive decline: 23.6% at EC 4 dS/m (T_3), 37.6% at EC 6 dS/m (T_4), 53.2% at EC 8 dS/m (T_5) and 80% (9.8 cm) at EC 12 dS/m (T_7) (Table 1).

A similar trend was observed in other parameters. The number of leaves declined by 12.6% in T_2 and up to 74% in T_7 . Leaf length decreased by 6.03% at EC 2 dS/m, reaching 64.6% at EC 12 dS/m. Root and shoot lengths decreased by 7.7% and 6.6% at EC 2 dS/m, respectively and by 65.5% and 78.7% at EC 12 dS/m.

Branching and flowering were highly sensitive to salinity. Branch numbers dropped from 5.2 in the control to 4.6 in T_2 (14% decrease) and reached zero at T_7 . Flowering time increased from 33 days in the control to 47 days at EC 10 dS/m, with no flowering at EC 12 dS/m (Table 1).

The plant height stress tolerance index (PHSTI) and root length stress tolerance index (RLSTI) declined with increasing salinity. At EC 2 dS/m, PHSTI and RLSTI were 93% and 92.3%, dropping to 76.4% and 78% at EC 4 dS/m and further to 20.1% and 34.5% at EC 12 dS/m, respectively (Table 1). These reductions highlight the severe impact of salinity on plant height and root length, consistent with findings by Sen *et al.* (2020) and Alsamadany (2022).

Overall, salinity strongly impacted greengram growth and reproduction, likely due to osmotic stress and ionic toxicity, which disrupted water uptake, nutrient absorption and metabolism (Rakavi *et al.*, 2022). Flowering delays

Table 1: Effect of salinity stress conditions on growth attributes and stress tolerances analysis of the green gram.

Treatment details	Plant height (cm)	No. of leaf /Plant	Leaf length (cm)	Root length (cm)	Shoot length (cm)	No. of branches/ plant	Flowering time (days)	Plant height stresstolerance index(%)	Root length stress tolerance index (%)
T_1 Control (EC <1 ds/m)	48.7	16.6	11.6	16.8	31.9	5.2	33	100	100
T_2 EC@ 2 ds/m	45.3	14.5	10.9	15.5	29.8	4.6	34	93	92.3
T_3 EC@ 4 ds/m	37.2	11.3	9.7	13.1	24.1	3.7	37	76.4	78
T_4 EC@ 6 ds/m	30.4	9.8	7.8	11.7	18.7	2.5	41	62.4	69.6
T_5 EC@ 8 ds/m	22.8	8.1	6.4	9.7	13.1	1.8	43	46.8	57.7
T_6 EC@ 10 ds/m	13.8	6.3	5.2	7.6	10.7	1.5	47	28.3	45.2
T_7 EC@ 12 ds/m	9.8	4.33	4.1	5.8	6.8	0	0	20.1	34.5
SE(d)	0.702	0.182	0.150	0.328	0.330	0.110	0.746	1.502	1.368
CD (P=0.05)	1.51	0.39	0.32	0.70	0.71	0.24	1.60	3.22	2.93

suggest salinity also interferes with reproductive phases by delaying flowering and reducing branching (Ahmed *et al.*, 2009).

Effect of salinity on biochemical attributes in green gram

Proline content

The proline content increased significantly with salinity stress. At EC <1 dS/m, proline was 2.5 µmol/g, rising by 112% to 5.3 µmol/g at T₂ and 204% to 7.6 µmol/g at T₃. At EC 12 dS/m (T₇), proline peaked at 19.1 µmol/g, a 664% increase compared to the control (Table 2). This sharp rise highlights the impact of salinity on proline accumulation, a key response to stress (Misra *et al.*, 2005). Proline acts as an osmoprotectant, stabilizing cellular structures, maintaining turgor pressure and balancing osmotic potential, enabling plants to retain water and sustain cellular functions under salinity stress. These findings align with previous research emphasizing proline's role in salinity tolerance (Sen *et al.*, 2020, Pal *et al.*, 2022).

Chlorophyll content

Salinity stress significantly reduced chlorophyll content in green gram. Chlorophyll a decreased by 20.5% at EC 2 dS/m (T₂), 32.6% at EC 4 dS/m (T₃) and 43.2% at EC 6 dS/m (T₄), with a sharp decline to 59.1% at EC 10 dS/m (T₆) and 67.4% at EC 12 dS/m (T₇). Chlorophyll b showed a similar trend, dropping by 19.2% at T₂ and 76.9% at T₇. Total chlorophyll decreased by 20% at T₂, 33.3% at T₃, culminating in a 71% reduction at T₇ (Table 2).

Salinity disrupts chlorophyll synthesis and accelerates degradation due to osmotic and ionic imbalances, damaging chloroplast structures (Panda *et al.*, 2009). The sharp decline in total chlorophyll limits photosynthesis, reducing carbohydrate production necessary for growth and energy reserves for reproduction, causing delays or reductions in flowering (Yasar *et al.*, 2008). This decline directly affects the plant's productivity by reducing light absorption and photosynthetic efficiency, impairing its resilience under stress (Sarkar *et al.*, 2024).

Table 2: Effect of salinity stress conditions on biochemical responses of the green gram.

Treatment details	Proline (µmol/g)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total chlorophyll (mg/g FW)	Reducing sugars (mg/g)	Soluble protein (mg/g)
T ₁ Control (EC <1 ds/m)	2.5	1.32	0.78	2.1	1.4	16.8
T ₂ EC@ 2 ds/m	5.3	1.05	0.63	1.68	1.7	14.2
T ₃ EC@ 4 ds/m	7.6	0.89	0.51	1.4	1.9	11.8
T ₄ EC@ 6 ds/m	9.9	0.75	0.45	1.2	2.5	9.7
T ₅ EC@ 8 ds/m	12.4	0.67	0.33	1	3.3	7.6
T ₆ EC@ 10 ds/m	15.8	0.54	0.29	0.83	3.9	6.8
T ₇ EC@ 12 ds/m	19.1	0.43	0.18	0.61	4.6	5.1
SE(d)	0.205	0.018	0.009	0.022	0.108	0.283
CD (P=0.05)	0.44	0.038	0.019	0.047	0.232	0.607

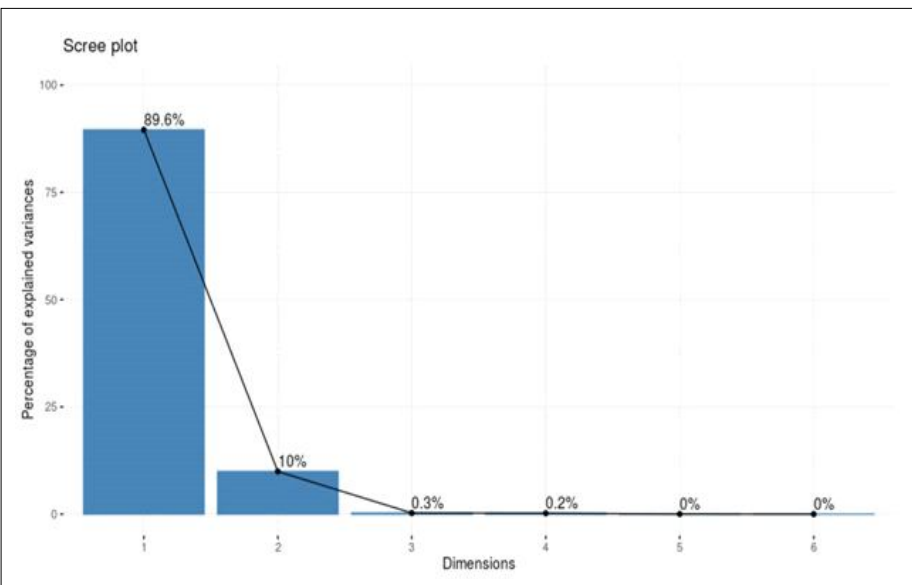


Fig 1: scree plot of variables of growth attributes of green gram under salinity stress.

Reducing sugar content

Salinity stress caused a gradual rise in the reducing sugar content of green gram as the EC of the irrigation water increased. From 1.4 mg/g in the control, levels rose by 21.4% at EC 2 dS/m and reached 4.6 mg/g at EC 12 dS/m, a 228.6% increase (Table 2). This suggests that salinity-induced osmotic and ionic stress triggers sugar accumulation to maintain water balance, protect cellular structures and mitigate oxidative damage, aiding plant survival in high-salinity conditions, though it may still impact overall growth (Arulbalachandran *et al.*, 2009).

Protein content

Soluble protein content decreased with increasing salinity. Starting at 16.8 mg/g in the control, it dropped by 15.5% at EC 2 dS/m and 69.6% at EC 12 dS/m (Table 2). High salinity can disrupt enzyme activities, hinder amino acid uptake, all of which contribute to lower protein synthesis (Sen *et al.*, 2020). Furthermore, the excessive accumulation of salts in plant tissues can lead to oxidative stress, which may cause protein degradation, further reducing the overall protein content. This reduction in protein affects the growth and development of green gram.

Principal component analysis

Principal Component Analysis (PCA) was used to evaluate the genetic diversity within the population. This technique identifies the plant traits that contribute significantly to the variation observed across the population. PCA was applied to reduce the dataset's dimensionality and reveal underlying variables (Mirunalini *et al.*, 2024).

Mean growth Parameters of green gram under salinity stress

Principal component analysis (PCA) of greengram growth parameters under salinity stress revealed that PC1 had an

Table 3: Eigen values of growth attributes of green gram under salinity stress.

Principle component	Eigenvalue	Percentage of variance	percentage of cumulative variance
PC1	8.061	89.568	89.568
PC2	0.896	9.952	99.521
PC3	0.023	0.259	99.779
PC4	0.018	0.2	99.979
PC5	0.002	0.019	99.998
PC6	0	0.002	100

Table 4: Per cent contribution of variables on principal components of growth attributes of green gram under salinity stress.

Variables	PC1	PC2	PC3	PC4	PC5	PC6
Plant height	12.301	0.493	16.585	0.328	0.628	20.077
No. of leaf/plant	12.217	0.361	15.671	46.044	1.754	2.268
No. of branches/plant	12.262	0.149	37.298	7.326	10.862	3.384
Flowering time	1.593	97.296	0.232	0	0.175	0.046
Plant height stress tolerance index	12.301	0.495	16.627	0.345	0.752	19.035
Root length stress tolerance index	12.375	0.056	3.41	6.309	1.174	14.914
Leaf length	12.308	0.38	0.287	22.594	17.459	21.982
Root length	12.374	0.053	3.456	6.585	1.201	17.982
Shoot length	12.27	0.717	6.434	10.469	65.996	0.313

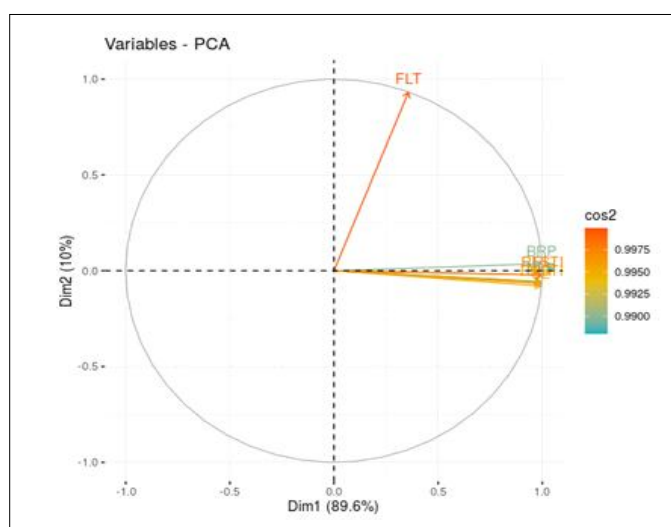


Fig 2: Contribution of variables on principal component of growth attributes of green gram under salinity stress.

eigenvalue of 8.061, explaining 89.56% of the total variation (Table 3). The scree plot showed a sharp decline in eigenvalues after PC1 (89.56%), with PC2 and PC3 contributing 9.952% and 0.259%, respectively (Fig 1).

PC1, the most influential component, was associated with traits like plant height, number of leaves and branches, flowering time, leaf length, root length, shoot length and stress tolerance indices for plant and root length (Fig 2). Flowering time was primarily linked to PC2, while plant height, root length and leaf length were key contributors to PC1, emphasizing their importance in salinity response. Stress tolerance indices significantly impacted both PC1 and PC2, highlighting their role in evaluating performance under saline conditions (Table 4). These findings align

with previous studies on greengram growth traits under stress (Patel *et al.*, 2024).

Table 5: Eigen values of Biochemical responses of green gram under salinity stress.

Principle component	Eigen value	Percentage of variance	percentage of cumulative variance
PC1	5.89	98.171	98.171
PC2	0.096	1.596	99.767
PC3	0.01	0.161	99.927
PC4	0.004	0.06	99.987
PC5	0.001	0.013	100
PC6	0	0	100

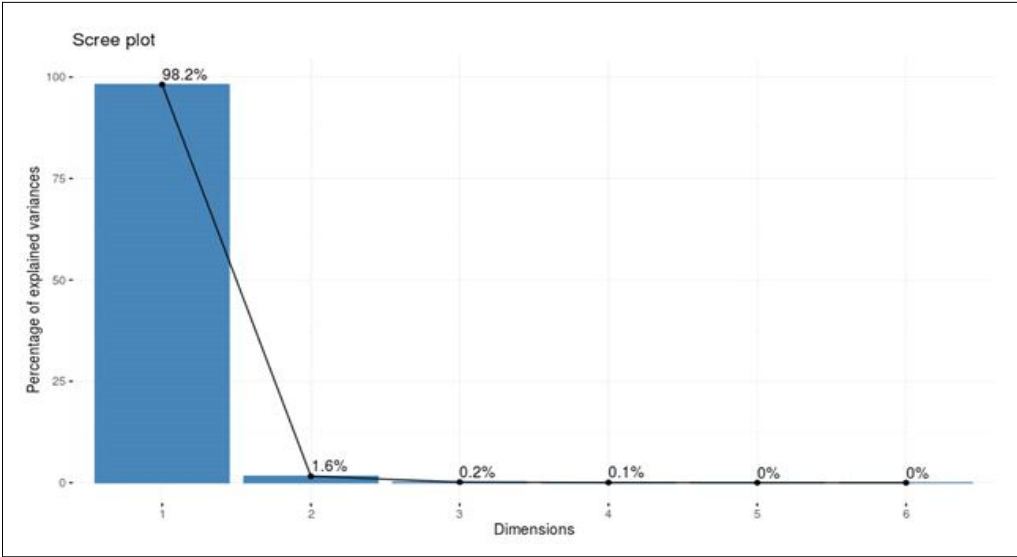


Fig 3: Scree plot of variables of biochemical responses in green gram under salinity stress.

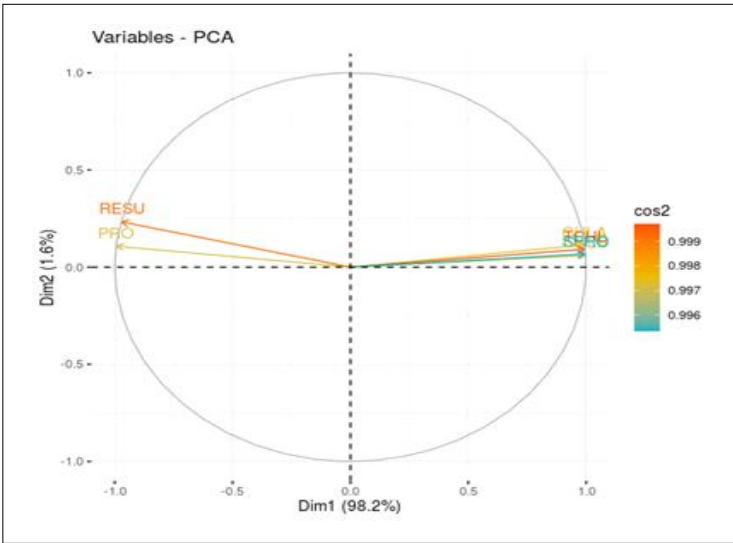


Fig 4: Contribution of variables on principal component of biochemical responses in green gram under salinity stress.

Table 6: Percent contribution of variables on principal components of biochemical responses of green gram under salinity stress.

Variables	PC1	PC2	PC3	PC4	PC5	PC6
Proline	16.732	12.068	25.721	2.3	43.179	0
Chlorophyll a	16.71	13.712	20.115	16.239	9.725	0
Chlorophyll b	16.868	3.701	7.619	59.663	1.421	0
Total chlorophyll	16.827	8.941	2.452	0.505	5.501	0
Reducing sugar	16.044	56.693	4.284	2.845	20.134	0
Soluble protein	16.819	4.886	39.808	18.447	20.04	0

Mean biochemical performance of green gram under salinity stress

PCA analysis showed that PC1, with an eigenvalue of 5.89, explained 98.17% of the total variance, while PC2 contributed only 0.096% (Table 5). The scree plot highlighted PC1 as the dominant component, with subsequent components showing minimal contributions (Fig 3).

PC1 was primarily influenced by proline content, chlorophyll a, chlorophyll b, total chlorophyll, reducing sugars and soluble protein (Fig 4). Proline and chlorophyll-related parameters played vital roles in the salinity stress response, while reducing sugars had the highest contribution to PC2, indicating their importance. Soluble protein was also notable across components, especially PC3 (Table 6). These findings highlight proline and chlorophylls as key biochemical indicators of greengram's resilience to salinity, aligning with similar studies on mungbean (Ogunsiji *et al.*, 2023).

CONCLUSION

The study demonstrated that salinity stress significantly affects the growth and biochemical attributes of greengram, resulting in reduced plant height, leaf and root lengths, branching and flowering. However, the greengram variety VBN 2 showed a better capacity for mild salt tolerance. Among all the salinity treatments, plants subjected to an electrical conductivity (EC) of 2 dS/m exhibited the best growth and biochemical responses, indicating an effective ability to withstand mild salinity stress. VBN 2 displayed notable resilience, maintaining higher values for key parameters such as plant height, number of leaves and flowering time under moderate salinity conditions.

The increase in proline content, alongside a reduction in chlorophyll levels in response to salinity, suggests that plant employs osmoprotective mechanisms to cope with stress. Furthermore, the levels of reducing sugars and proteins exhibited a complex response to salinity stress, with increasing sugar levels potentially serving as an adaptive mechanism. Principal component analysis highlighted the critical traits influenced by salinity, emphasizing the importance of growth and biochemical parameters in assessing the resilience of greengram.

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Disclaimers

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Conflict of interest

On behalf of all authors, we declare that there are no conflicts of interest regarding the publication of this article.

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