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Photosynthetic Trait-Based Evaluation of Lentil Genotypes Under Salt Stress

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Abstract

Lentil (*Lens culinaris*, Medik.), a nutritionally rich pulse crop, is susceptible to soil salinity. At an electrical conductivity (EC) of up to 5 dS m^{-1} (~50 mM NaCl), the crop experiences a significant reduction in yield of approximately 90%. The current emphasis on identifying lentil germplasm with salinity tolerance is essential for ensuring super-food production. Assessment of diversity panels for their ability to withstand salt stress is a leading approach for developing breeding lines and salt-tolerant varieties. One hundred diverse lentil genotypes were evaluated for photosynthetic traits under non-saline (control) and saline (EC = 5 dS m^{-1}) conditions. This study was conducted at ICAR-Central Soil Salinity Research Institute, Karnal, during *rabi* seasons of 2021-22 and 2022-23. Across genotypes, a significant reduction was noticed under salinity in comparison to control for various parameters viz. photosynthetic rate (36.4%), transpiration rate (34.0%), stomatal conductance (34.0%), instantaneous water use efficiency-iWUE (3.7%), chlorophyll content (27.8%), RWC (relative water content) (30.0%), and MSI (membrane stability index (19.0%). The stepwise regression approach revealed that E (Leaf Transpiration Rate), gsw (Stomatal Conductance to Water Vapor), iWUE, inWUE (intrinsic water use efficiency**)**, Chlorophyll, RWC and MSI contributed critically in combating salt stress. A noteworthy positive correlation was noted between Pn, iWUE as well as inWUE under both control and salinity whereas gsw, Pn (Net Photosynthetic Rate), E, and MSI under salinity. Out of eight PCs, initial three principal components (PCs) showed substantial variation. These principal components accounted for 64.6% and 72.2% of the overall variation under normal and saline conditions, respectively. This study provides the fundamental knowledge about how photosynthetic traits respond to salt stress in lentils. The information obtained here will aid in understanding photosynthetic alterations under salt stress.

Key words: Lentil genotypes, Diversity, Salt tolerance, Instantaneous water use efficiency, Photosynthesis, Transpiration

Introduction

Lentil (*Lens culinaris* Medik.) is an important cool-season leguminous crop and a major source of dietary protein, minerals, and micronutrients. India is the world's largest producer of lentil, accounting for nearly one-fourth of global production, with approximately 1.7–1.9 million ha under cultivation (Mandal *et al.,* 2018). The crop is predominantly grown in Madhya Pradesh (≈0.68 million ha), Uttar Pradesh (≈0.58 million ha), Bihar (≈0.17 million ha), West Bengal (≈0.11 million ha), Rajasthan (≈0.09 million ha), and Jharkhand (H"0.05 million ha), which collectively contribute more than 90% of the country's lentil area and production. However, many of these major lentil-growing states are also affected by soil salinity and sodicity. India has about 6.73 million ha of salt-affected soils, including substantial areas in Uttar Pradesh (1.37 million ha), West Bengal (0.44 million ha), Rajasthan (0.38 million ha), Bihar (0.32 million ha), Haryana (0.26 million ha), and Madhya Pradesh (0.13 million ha). Salinity adversely affects germination, water uptake, nutrient acquisition, photosynthetic efficiency, and ultimately grain yield (Singh, 2017). The substantial overlap between major lentil-growing regions and salt-affected areas underscores the necessity of identifying salinity-tolerant genotypes and physiological traits that can be utilized in breeding programs for sustainable lentil production under saline environments (Singh and

Sharma, 2016; Elnaggar *et al*., 2020; Hussain *et al*., 2018).

To mitigate the problem of malnutrition and hidden hunger, pulses being rich in nutrients and protein, play a larger role in human health. Lentil is a *rabi* season nutrition and protein-rich crop. It is one of the most sensitive pulse crops compared to all grain and pulse crops with > 90% yield reduction at ECe above 3 dS m^{-1} (Kokten *et al.,* 2010). Therefore, the identification of resilient lentil germplasm is essential to ensure the production and sustainability of the pulse food industry. Soil reclamation is very costly, hence crop improvement for salinity tolerance is required. Salinity tolerance is a complex polygenic trait that demands coordinated physiological adaptations and multi-genic regulatory networks to mitigate stress (Singh *et al.,* 2021).

Salt stress affects all plant stages indiscriminately, and tolerance across stages is not determined by a single phase of plant development. Principal Component Analysis (PCA), a multivariate technique, analyses data by extracting essential information and representing it using principal components. PCA's effectiveness is dependent on its utilisation of two key mathematical processes: eigen decomposition and singular value decomposition. These techniques enable PCA to extract essential information and effectively decrease the dimension of datasets. This analytical approach is valuable for effectively scrutinising extensive experimental data for genotype assessment (Abdi and Williams, 2010; Allel *et al*., 2016; Chikha *et al*., 2016; Raza *et al.,* 2017). The present study aimed to (i) evaluate the variation in photosynthetic and physiological traits among diverse lentil genotypes under salinity stress, (ii) identify key physiological determinants associated with salinity tolerance, and (iii) assess trait associations through correlation and principal component analyses.

Materials and Methods

Study site

The planting materials consisted of 100 diverse Lentil genotypes gathered from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, Punjab Agricultural University Ludhiana; and Indian Institute of Pulses Research, Kanpur. These genotypes along with check varieties, namely PL-8 (salt sensitive) and PSL-9 & PDL-1(salt tolerant) were grown under non-saline (control) and saline water (ECiw = 5 dS m^{-1}) in the pots twenty seeds per genotype were sown with five plants per genotype were collected for further analysis at ICAR-Central Soil Salinity Research Institute, Karnal (29°43′N, 76°58′E; 245 m above mean sea level) during *rabi* 2021-22 and 2022-23 in completely randomized block design.

The experimental design and photosynthetic traits have been depicted in Fig. 1.

Experimental set up

The diversity set was grown in pots (~ 20 kg) filled with sand culture. To simulate a saline environment, we maintained irrigation with saline water at an electrical conductivity (ECiw) of 5 dS m^{-1}throughout the experiment. Throughout the experiment, we ensured constant salinity levels by draining excess salt from the pots daily (Shekhawat *et al.,* 2025; Singh *et al.,* 2019). To ensure that the sodium absorption ratio (SAR) remained within permissible limits, we used chloride and sulfate salts of sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) for preparing the 5 dS m^{-1} saline irrigation water. Prior to sowing, seeds were surface-sterilised in a 10% sodium hypochlorite solution for 5 minutes with vigorous agitation to eliminate microbial contamination, then thoroughly rinsed with distilled water. Each plastic pot was planted with 20 seeds of each genotype at a depth of 1 centimetre. The pots were designed with drainage at the bottom to facilitate the removal of excess water. In a completely randomised block design, the pots were organised into a factorial experiment. They were irrigated with Hoagland's solution, a nutrient-rich solution, and maintained at maximum field capacity until the seedlings reached the early vegetative stage, typically 14 to 21 days after sowing (DAS). Throughout the experiment, we maintained constant salinity levels by draining excess salt from the pots daily (Singh *et al*., 2019).

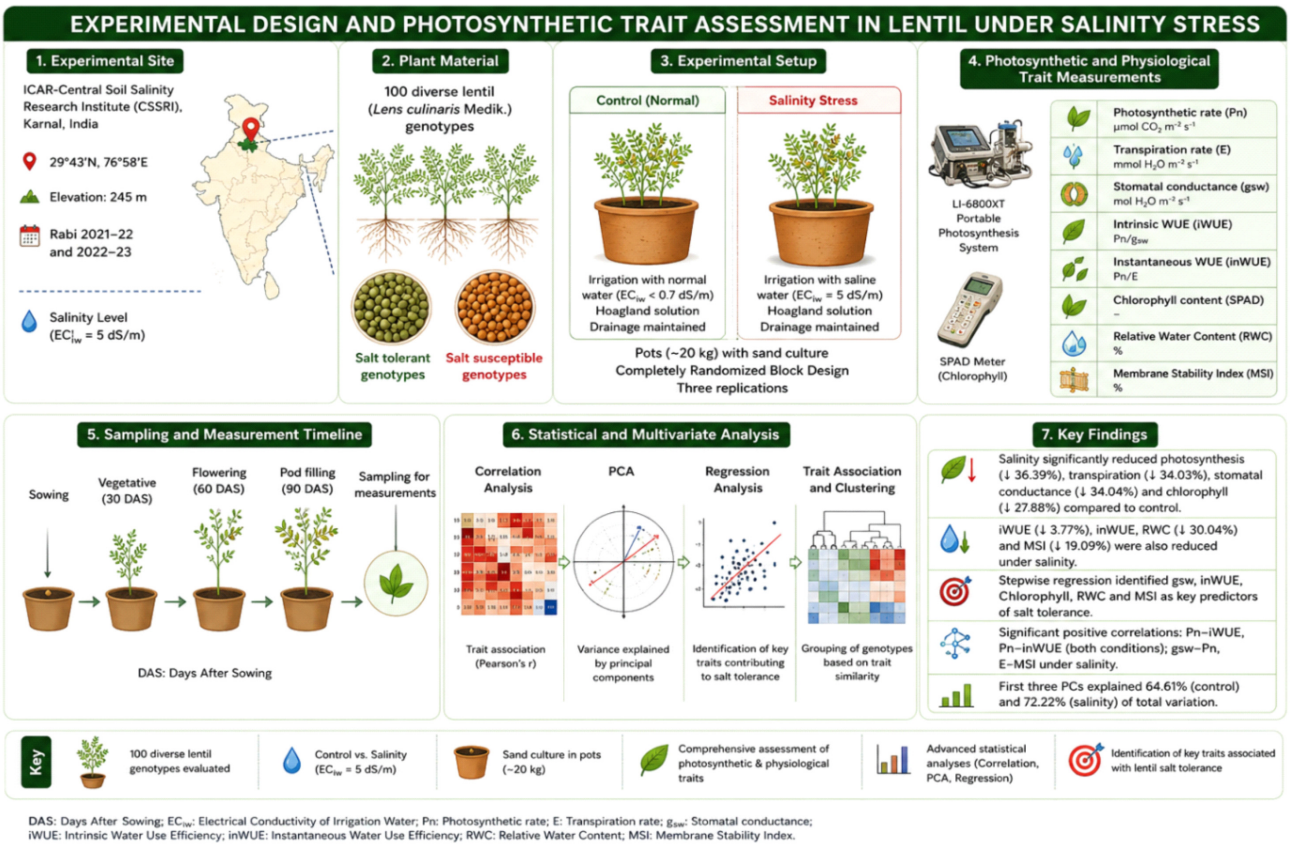

Fig. 1 The experimental design and photosynthetic traits in Lentil

Data collection

Photosynthetic traits

At the seedling stage, three plants from each genotype grown under both control conditions and a salinity regime of ECiw 5 dS m^{-1} were randomly selected. We collected photosynthesis-related data, i.e. photosynthesis rate (Pn), transpiration rate (E), stomatal conductance (gsw), instantaneous water use efficiency (inWUE) and intrinsic water use efficiency (iWUE). To obtain these measurements, a portable photosynthetic system was used, i.e. LI-6800XT infrared gas exchange analyser from Li-COR, USA, following the method described by Singh *et al*. (2019). The data were collected from 1000 hrs to 1200 hrs in sunlight and under specific weather conditions, including photosynthetically active radiation (PAR) of approximately 700 ìmol m-2 s^{-1}, a temperature of around 25±1°C, RH ~ 70%, and an air CO_2 concentration of 355 ìmol mol^{-1}. *In vivo*, chlorophyll content was measured using the SPAD-502 Plus meter (Konica Minolta Ltd., India) at the vegetative stage, with three plants randomly selected from each pot.

Relative water content (RWC) was measured in fully expanded young leaves using a method described by Slavik (1974).

RWC = [Fresh weight (g) – Dry weight (g)]*100/ [Turgid weight (g) – Dry weight (g)]

Membrane stability index (MSI) was assessed following the methodology outlined by Sairam *et al.* (1997). The membrane stability index (MSI) is calculated as:

MSI = [1-(C1/C2)] × 100

Where,

C1 = electrical conductivity of the sample solution heated at 40°C

C2 = electrical conductivity of the sample solution heated at 100°C

Statistical analysis: The statistical analyses, association analysis, and PCA were carried out for all the studied seedling stage traits using the software Minitab 21, SRplot, and the R studio packages (Ahmed *et al.*, 2025).

Results

Photosynthesis involves complex components such as photosynthetic pigments, photosystems, the electron transport chain, and pathways for CO_2 reduction. Salinity stress at any of these processes can significantly impair the plant's overall photosynthetic processes. Salinity stress significantly affects these mechanisms in both tolerant and susceptible plants; however, the percentage reduction was greater in salt-sensitive plants. The box plot of mean values is depicted in Figure 2 and statistical analysis of pooled photosynthetic traits is represented in Table 1.

Photosynthetic rate (µmol $m^{-2}s^{-1}$)

The photosynthetic rate was significantly reduced by 36.39% compared with the control. It ranged from 12.34 to 23.99 µmol $m^{-2}s^{-1}$ with an average value of 20.21 µmol $m^{-2}s^{-1}$ under control conditions and 5.13 to 19.86 µmol $m^{-2}s^{-1}$ with an average value of 12.86 µmol $m^{-2}s^{-1}$ under salinity conditions. The coefficient of variation (CV) among genotypes widened from 14.42% in the control to 25.42% under salinity, highlighting a differential genetic response to salt stress and control conditions among the studied germplasm, as shown in Table 1.

Transpiration rate (mmol H_2O $m^{-2}s^{-1}$)

Transpiration rate ranged from 4.03 to 7.98 mmol H_2O $m^{-2}s^{-1}$ with a mean value of 6.46 mmol H_2O $m^{-2}s^{-1}$ under control conditions and ranged from 1.99 to 6.99 mmol H_2O $m^{-2}s^{-1}$ with a mean value of 4.26 mmol H_2O $m^{-2}s^{-1}$ under salinity. The transpiration rate decreased by up to 34.03% under salinity conditions compared with control conditions.

Stomatal conductance (mol $m^{-2}s^{-1}$)

Stomatal conductance was reduced by up to 33.95% under salt stress compared with the control condition. It ranged from 0.3 to 0.5 mol $m^{-2}s^{-1}$ with an average value of 0.43 mol $m^{-2}s^{-1}$ under control and 0.16 - 0.4 mol $m^{-2}s^{-1}$ with an average value of 0.28 mol $m^{-2}s^{-1}$ under salinity. The coefficients of variation were 11.86% and 29.97% for the control and salinity treatments, respectively.

Instantaneous water use efficiency (µmol/mol)

It ranged from 1.89 to 5.07 µmol/mol with an average of 3.24 µmol/mol under control conditions, and from 1.78 to 4.96 µmol/mol with an average of 3.12 µmol/mol under salinity conditions. A 3.68% reduction was observed

Table 1. Mean physiological traits of 100 lentil genotypes under non-saline (control) and saline irrigation conditions (average of two years data)

Sr. No.	Traits	Environment	Mean	Range	CV (%)	Reduction (%) in mean value under saline condition compared with control
1.	Photosynthetic rate (µmol m^{-2} s^{-1})	Control	20.21 ± 0.29	12.34-23.99	14.42	36.39
		Saline	12.86 ± 0.33	5.13-19.86	25.42	
2.	Transpiration rate (mmol H_2O m^{-2} s^{-1})	Control	6.46 ± 0.12	4.03-7.98	17.79	34.03
		Saline	4.26 ± 0.12	1.99-6.99	28.19	
3.	Stomatal conductance (mol m^{-2} s^{-1})	Control	0.43 ± 0.01	0.3-0.5	11.86	33.95
		Saline	0.28 ± 0.01	0.16-0.4	29.27	
4.	Instantaneous water use efficiency (µmol/mol)	Control	3.24 ± 0.08	1.89-5.07	23.79	3.68
		Saline	3.12 ± 0.07	1.78-4.96	23.87	
5.	Intrinsic water use efficiency (µmol/mmol)	Control	47.70 ± 0.95	25.26-75.4	20	1.8
		Saline	46.84 ± 1.03	28.33-79.04	22.03	
6.	Chlorophyll Content	Control	27.31 ± 0.65	14.91-45.94	23.78	27.75
		Saline	19.73 ± 0.97	4.79-45.28	48.99	
7.	Relative water content (%)	Control	51.13 ± 1.02	37.06-86.76	19.95	30.04
		Saline	35.77 ± 0.81	20.86-54.04	22.64	
8.	Membrane stability index (%)	Control	74.25 ± 0.39	69.38-83.44	5.3	18.99
		Saline	60.15 ± 0.72	47.83-76.3	12.01	

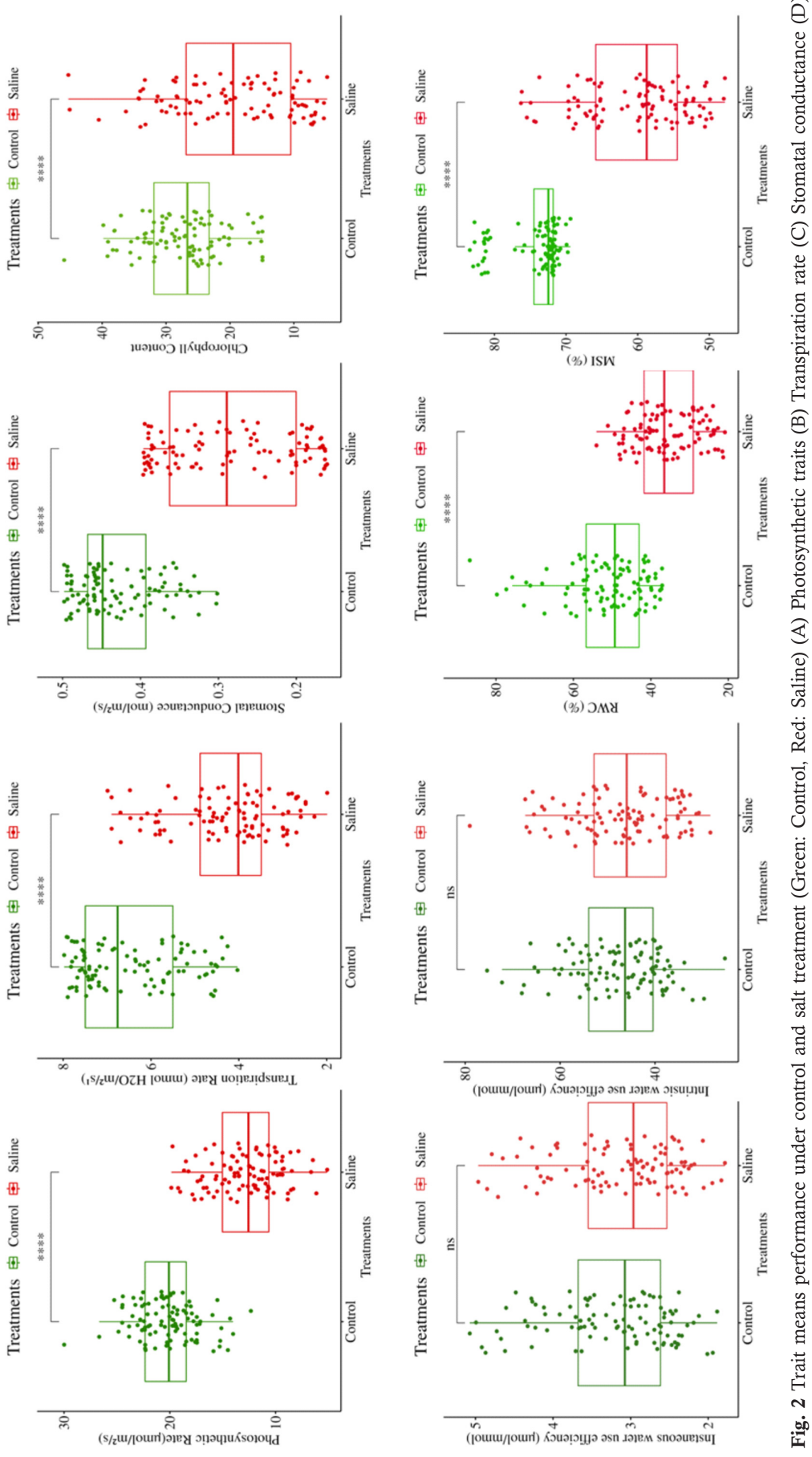

Fig. 2 Trait means performance under control and salt treatment (Green: Control, Red: Saline) (A) Photosynthetic traits (B) Transpiration rate (C) Stomatal conductance (D) Chlorophyll Content (E) Instantaneous WUE (F) Intrinsic WUE (G) Relative Water Content (H) Membrane Stability Index

under salinity in comparison with the control condition.

Intrinsic water use efficiency (µmol/mmol)

It was significantly reduced under saline conditions compared with the control condition. The reduction was up to 1.86% under salinity, with a range from 25.26 to 75.40 µmol $mmol^{-1}$, an average of 47.70 µmol $mmol^{-1}$ under control conditions, and a range of 28.33 to 79.04 µmol $mmol^{-1}$, with an average of 46.84 µmol $mmol^{-1}$. The coefficients of variation were 9.54% and 10.32% for the control and salt stress, respectively.

Chlorophyll content

Chlorophyll content ranged from 14.91 to 45.94 under control conditions, with an average value of 27.31, and it ranged from 4.79 to 45.28 under salinity conditions, with an average value of 19.73. The reduction percentage was 27.75% under salinity compared with the control condition.

Plant water status (%)

The data in Table 1 present the mean RWC under both control and salinity conditions. All genotypes exhibited reduced leaf RWC under this stress. However, this reduction was more evident in susceptible genotypes than in tolerant ones. It ranged from 37.06 to 86.76% under control and from 20.86 to 54.04% under salinity, with average values of 51.13% and 35.77% for control and salinity, respectively. A 30.04% reduction was detected under salinity compared to the control condition.

Membrane stability index (%)

High salinity negatively affects the MSI of plant cells by altering membrane fluidity, creating osmotic stress, causing ionic imbalances, inducing oxidative stress, and potentially leading to cell death (Jamali *et al.*, 2015; Kumar *et al.*, 2015; Senguttuvel *et al.*, 2013). It ranged from 69.38% to 83.44% under control, with a mean of 74.25%, and from 47.83% to 76.30% under salinity, with a mean of 60.15%. A reduction of 18.99% was observed under salt stress compared with the control.

Trait modelling under salinity

To evaluate the impact of constituent variables on photosynthesis (a dependent variable), feasible and stepwise regression analyses were conducted, following the approach outlined by Shannon *et al.* (2000) and Sharma and Sinha (2012). The results of all conceivable regression analyses demonstrated that gsw, E, iWUE and inWUE, significantly impacted photosynthetic rate under salt stress, whereas the remaining attributes exhibited no significant effects (Table 2 and Table 3). Consequently, non-significant traits were excluded during stepwise regression. According to the findings, gsw, inWUE and MSI, collectively explained over 95% of the overall variation in Pn under salt stress conditions. Furthermore, Pn variation was substantially influenced by E, gsw, iWUE, inWUE, Chlorophyll, RWC and MSI, resulting in a cumulative R^2 of 97.19, considered optimal as it adhered to the least Mallow's Cp criteria. The derived equation for estimating the

Table 2. Salinity stress tolerance's regression coefficient, standard error and significance of the prioritized attributes

Dependable variable	Variable	Estimate	SE	t-value	Pr (>|t|)
Net photosynthesis (Pn)	Intercept	-13.34	0.66	-20.07	0.00
	E	1.23	0.23	5.41	0.00
	gsw	23.16	3.52	6.59	0.00
	iWUE	1.65	0.29	5.69	0.00
	inWUE	0.14	0.02	7.18	0.00
	Chlorophyll	0.00	0.01	0.51	0.61
	RWC	0.02	0.01	2.38	0.02
	MSI	0.04	0.01	2.72	0.01

Table 3. Traits modelling for salinity tolerance through multiple linear regressions approach

Variables	Cp	R^2	Adj R^2
MSI	1356.43	55.62	55.16
gsw + inWUE	56.63	95.40	95.30
gsw + inWUE + MSI	43.05	95.87	95.74
E + gsw + iWUE + inWUE	15.05	96.79	96.65
E + gsw + iWUE + inWUE + MSI	9.95	97.01	96.85
E + gsw + iWUE + inWUE + RWC + MSI	6.26	97.18	97.00
E + gsw + iWUE + inWUE + Chlorophyll + RWC + MSI	8.00	97.19	96.97

projected Pn under saline conditions, based on regression coefficients of relevant traits, is as follows:

Predicted photosynthetic rate = -13.34 + (1.23 × E) + (23.16 × gsw) + (1.65 × iWUE) + (0.14 × inWUE) + (0.001 × Chlorophyll) + (0.020 × RWC) + (0.040 × MSI)

Therefore, these identified traits significantly enhance the photosynthetic rate under saline conditions. Targeting these traits in further research, extending to vegetative and harvesting stages, will be crucial for addressing and mitigating salt stress.

Correlation matrix among photosynthetic traits

A substantial and robust positive correlation was identified between photosynthetic rate and iWUE (0.74***), followed by inWUE with the photosynthetic rate (0.55***) and iWUE (0.37***). Similarly, a negative correlation was found between stomatal conductance and iWUE (-0.69***), and between transpiration rate and iWUE (-0.79***) under control conditions. Similarly, under salinity, significant positive correlation was found for photosynthetic rate with membrane stability index (0.75***) and stomatal conductance (0.73***), inWUE with photosynthetic rate (0.34***) and intrinsic water use efficiency (0.23*), transpiration rate with photosynthesis (0.64***), stomatal conductance (0.60***) and membrane stability index (0.59***), stomatal conductance with membrane stability index (0.75***). A significant negative correlation was found between iWUE with stomatal conductance (-0.55***), inWUE with transpiration rate (-0.48***), and iWUE with membrane stability index (-0.21*). The correlation matrix for control and salinity is presented in Figure 3.

Principal Component Analysis

PCA of eight variables measured across 100 genotypes revealed distinct grouping patterns, with genotypes dispersed across all four quadrants of the biplot. This indicates a considerable level of genotypic variability among genotypes. Principal component analysis (PCA) is shown in Figure 3 and indicates the correlation between Pn and inWUE under control conditions and under salinity with Pn, MSI, gsw, and E. Principal component analysis also indicated that the variables iWUE, inWUE and E under control and Pn, gsw, MSI, and Iwue were responsible for most of the variance in the data, whereas RWC and chlorophyll had a smaller contribution to this variance. It was also shown in a table that the contribution of photosynthetic traits, in percentage, was 30.61% for inWUE, 24.34% for Pn, and 24.15% for iWUE under control conditions, and 27.49%, 25.41%, and 24.29% for gsw, MSI, and Pn under salt stress, respectively. PC1, PC2, and PC3 components had eigenvalues exceeding 1, and cumulative variability increased with increasing salt stress. At the control, cumulative variability was 30.42%, 50.83%, and 64.61% for PC1, PC2, and PC3, respectively. Similarly, at salinity, cumulative variability of 38.78%, 59.07%, and 74.22% was noted for PC1, PC2, and PC3, respectively, in PCA. Eigenvalues, variability, and cumulative of lentil traits under normal and salt stress environments, as well as the % contribution of different photosynthetic variables on principal components, are summarized in Tables 4 & 5. Similarly, a PCA diagram for control and salinity is shown in Figure 4.

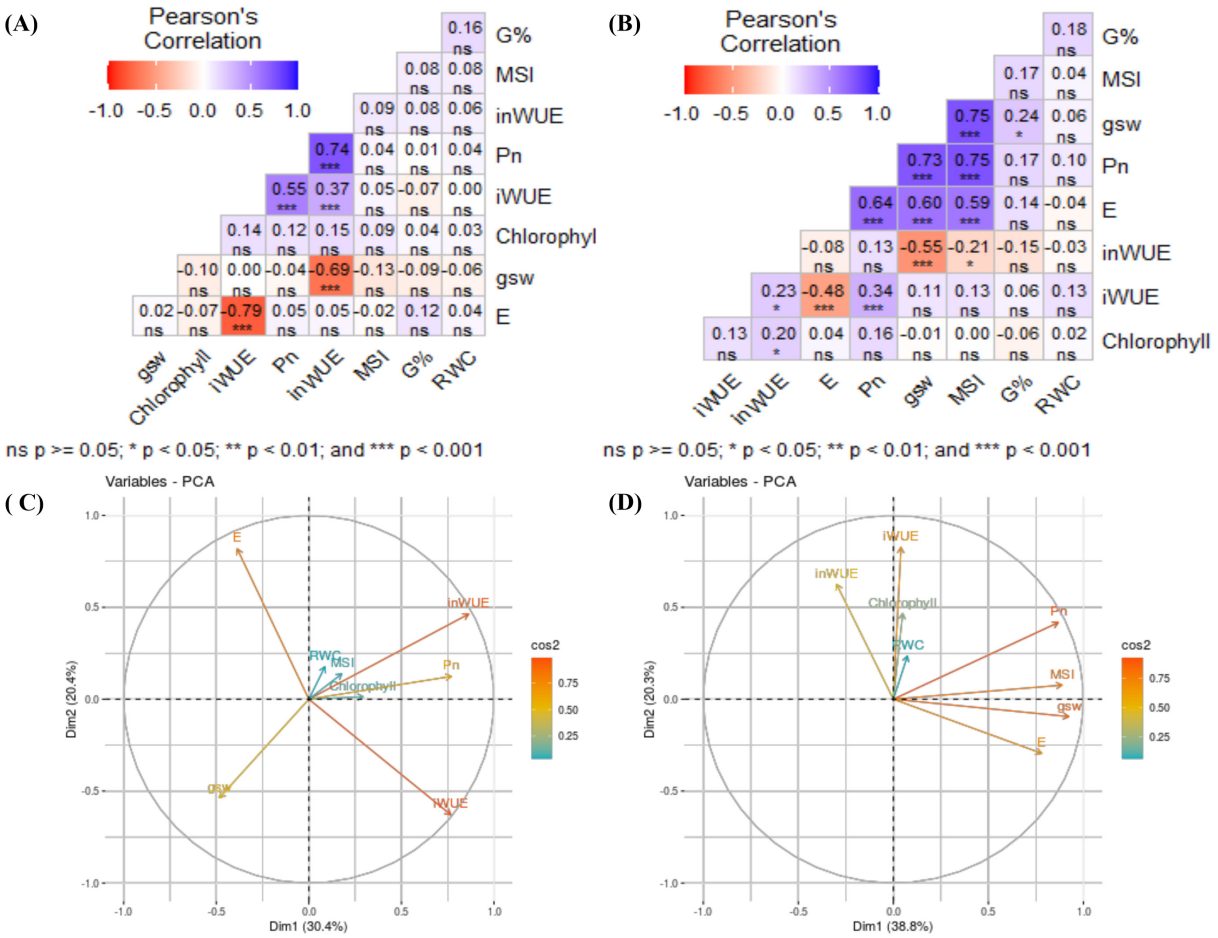

Fig. 3 Diagram depicting the trait association among photosynthetic traits (A). control (B). salinity and Principal Component Analysis (C). Control (D). Salinity. The X and Y axes represent the principal components: Dim 1 (PC1) and Dim 2 (PC2). The color bar indicates the % contribution of each variable to data variance

Table 4. Eigen values, variability, and cumulative variance of Lentil traits under control and salt stress environments

Principal component	Control			Salt stress (5 dS m^{-1})		
	Eigen value	Percentage of variance	Cumulative percentage of variance	Eigen value	Percentage of variance	Cumulative percentage of variance
PC1	2.43	30.42	30.42	3.10	38.78	38.78
PC2	1.63	20.41	50.83	1.62	20.29	59.07
PC3	1.10	13.78	64.61	1.21	15.15	74.22
PC4	1.00	12.45	77.06	0.93	11.67	85.89
PC5	0.94	11.72	88.79	0.84	10.49	96.38
PC6	0.88	10.99	99.77	0.26	3.26	99.64
PC7	0.01	0.14	99.91	0.02	0.19	99.83
PC8	0.01	0.09	100.00	0.01	0.17	100.00

PC1 to PC8 denote the first to eight principal Components obtained from the Principal Component Analysis

Discussion

Salinity negatively impacts the photosynthetic rate by inducing osmotic stress, causing water loss from plant cells, and reducing stomatal opening, limiting carbon dioxide uptake (Mahlooji *et al.* 2018). Excess salt ions can disrupt chloroplast function, impairing light absorption and electron transport chain activity, thereby decreasing photosynthesis (Singh *et al.*, 2023; Zhang *et*

Table 5. Percentage contribution of various photosynthetic variables to principal components

Variables	Control			Salt stress (5 dS/m)		
	PC1	PC2	PC3	PC1	PC2	PC3
PhotosyntheticRate	24.34	0.95	17.25	24.29	10.74	1.27
Transpirationrate	6.16	41.01	6.42	19.64	5.35	19.18
Stomatalconducatance	9.58	17.59	6.91	27.49	0.55	5.42
Instantaneous wateruse efficiency	24.15	24.19	0.15	0.05	42.07	17.36
Intrinsic wateruse efficiency	30.61	13.15	2.07	2.87	24.09	26.92
Chlorophyllcontent	3.55	0.01	11.88	0.08	13.44	14.17
Relative watercontent	0.31	1.91	14.59	0.18	3.40	15.44
Membranestability index	1.31	1.18	40.73	25.41	0.36	0.23

PC1, PC2 and PC3 refer to the first , second and third Principal Components

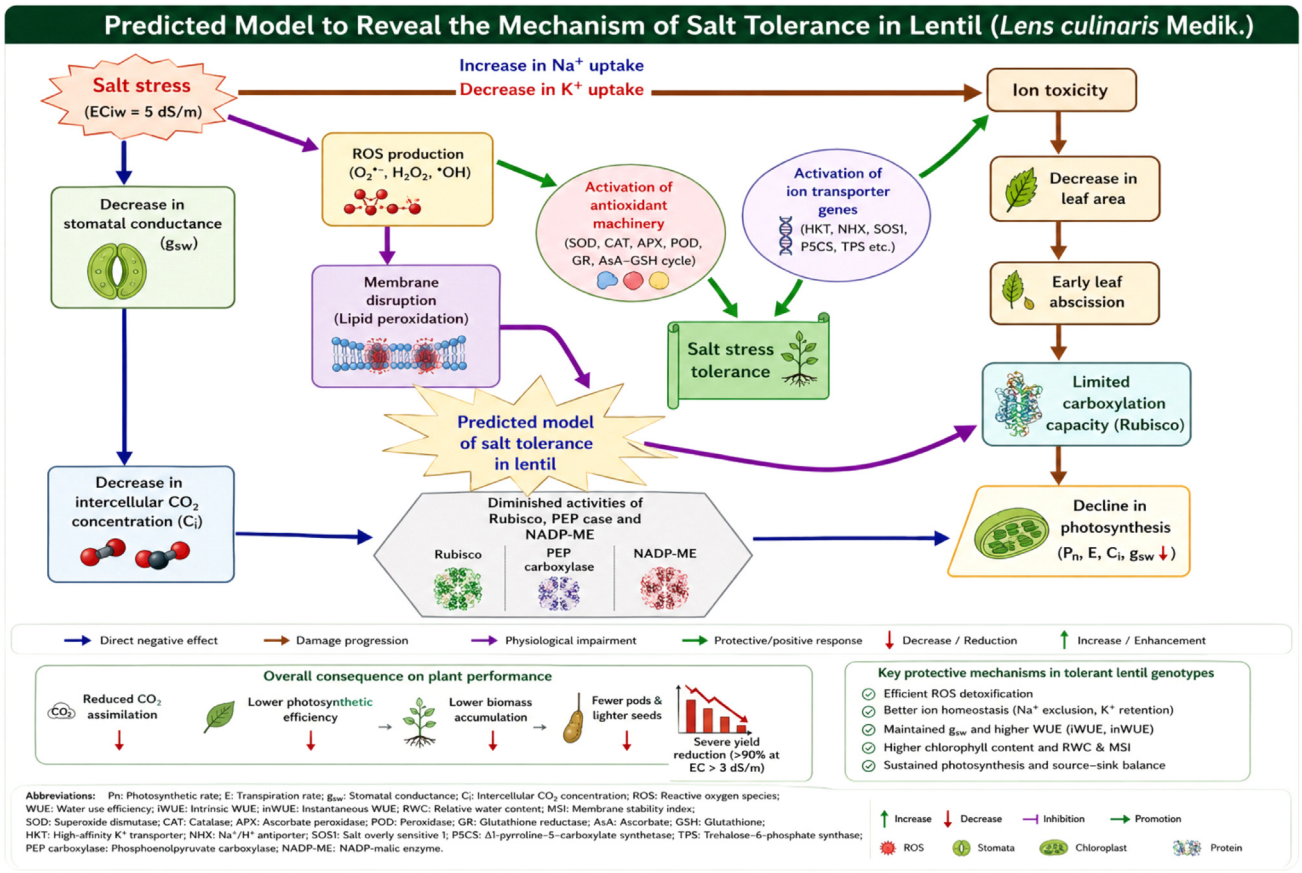

Fig. 4 Proposed mechanism of salt tolerance in lentil under salinity stress. Salt stress increases Naz toxicity, ROS accumulation, and membrane damage, reducing stomatal conductance and photosynthetic efficiency. In tolerant genotypes, antioxidant enzymes and ion transporter genes maintain ion homeostasis, detoxify ROS, and support photosynthetic activity, thereby improving salt tolerance

al., 2018). Nutrient imbalances, such as reduced potassium and magnesium, further hinder photosynthetic enzyme activity (Garcia *et al.,* 2022). Higher salinity in the soil or water creates an osmotic gradient that draws water out of plant cells, resulting in a decrease of turgor pressure and stomatal closure (Lambers *et al.,* 2019; Singh *et al.,* 2020). This limits water vaporization via transpiration, as the plant conserves water to manage salt stress (Hasanuzzaman *et al.,* 2017; Tian *et al.,* 2020). Additionally, salt-induced ion toxicity can damage root cells, reducing their ability to take up water, and further decreasing transpiration. Salt ions accumulate in the guard cells that control stomatal openings, disrupting their function and reducing stomatal conductance

in addition to reduced turgor pressure, stomata closure, and osmotic stress (Lawson *et al*., 2014). These combined effects result in decreased gas exchange, limiting CO_2 uptake and reducing overall stomatal stress in plants (Kashtoh and Baek, 2021; Saito and Uozumi, 2019). Higher salinity can reduce the instantaneous water-use efficiency of plants (Dutt *et al*., 2018; Zhang *et al*., 2016). This reduction occurs because salt stress often leads to less efficient use of available water resources for carbohydrate production and growth maintenance. Chlorophyll content in plant leaves determines photosynthetic capacity, and salinity greatly reduces it (Li *et al*., 2018). Similarly, our findings showed that lentil leaves under extreme salinity stress had a significantly lower greenness index, which in turn led to a discernible drop in seed yield. In the severely salinity-stressed condition (Table 1), the relative water content of the leaf was lowest, which may have been caused by the plant root system being disrupted by restricting water uptake under higher NaCl concentrations. Higher salinity in the soil negatively affects the content of chlorophyll in plants by disrupting nutrient uptake, causing osmotic stress, ion toxicity, oxidative stress, and other physiological changes as shown in Fig. 3 (Arif *et al*., 2020; Hassan *et al*., 2021; Shahzad *et al*., 2019).

Conclusions

Salinity stress significantly reduced photosynthetic and physiological performance in lentil, with marked declines in photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, relative water content, and membrane stability index. Significant genotypic variation was observed among the evaluated lentil germplasm, indicating substantial scope for improving salinity tolerance through breeding. Regression and principal component analyses identified stomatal conductance, transpiration rate, water-use efficiency, chlorophyll content, relative water content, and membrane stability index as key traits associated with salinity tolerance. These traits may serve as reliable physiological markers for identifying and developing salt-tolerant lentil cultivars. Future studies should validate these traits under field conditions and integrate them with genomic approaches to accelerate lentil improvement.

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