

## Trait Prioritization for Salt Tolerance from Physiological Traits on Lentil (*Lens culinaris* Medik.)

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**ABSTRACT:** Lentil (*Lens culinaris*, Medik.), an economical and high-quality protein source for many, is susceptible to salt stress. When exposed to an electrical conductivity (EC) of up to 5 dS/m (~ 50 mM NaCl), it undergoes a notable yield reduction of about 90% compared to other crops. Identifying lentil germplasm with salinity tolerance is crucial for ensuring super-food production and sustaining the lentil industry. Evaluating diversity panels for their capacity to withstand salt stress conditions emerges as a primary approach for developing breeding lines and salt-tolerant varieties. In this investigation, 100 diverse lentil accessions were examined for photosynthetic traits under both control and saline condition (5 dS/m). A significant reduction ( $p < 0.05$ ) was observed under salinity compared to the control. The stepwise regression approach revealed that E, gsw, iWUE, inWUE, Chlorophyll, RWC and MSI contribute critically to reducing salt stress. Nine clusters were formed using manhattan distance method and ward clustering method with highest number of genotypes (22) were found in cluster I followed by 15 (cluster VII), 12 (cluster IV), 10 (cluster III, V, VIII and IX), 7 (cluster VI) and 4 (cluster II). Different genotypes from various clusters can be used as parents in hybridization breeding programme to get transgressive segregants. This study contributes essential knowledge regarding how photosynthetic traits respond to salt stress in diverse lentil genotypes. The information acquired here will enhance our comprehension of photosynthetic alterations under salt stress.

**Keywords:** Lentil, Salt Tolerance, Photosynthesis, Transpiration, Water use efficiency.

### INTRODUCTION

Over 800 million hectares of land currently face salt-related challenges, a figure expected to rise due to climate change (Shahid *et al.*, 2018). Approximately 20% of the world's cultivated land and 33% of irrigated agricultural areas may be adversely affected by high salinity levels, with projections indicating that over half of arable land could become saline by 2050 (Machado and Serralheiro 2017). The impact of salinity on crop production is significant, hindering plant growth, photosynthetic activity, and ultimately leading to reduced yield (Singh and Sharma 2016). This is primarily attributed to salinity's negative effects on water and nutrient uptake by plants (Hussain *et al.*, 2018). Ion toxicity, osmotic stress, and oxidative damage further restrict a plant's ability to absorb water, exacerbating the challenge (Elnaggar *et al.*, 2020). The alarming threat to food and nutritional security in the face of a growing global population underscores the importance of addressing malnutrition and hidden hunger. Pulses, rich in nutrition, play a crucial role in combating these issues. Lentil, a protein and micronutrient-rich *rabi* season crop, are particularly sensitive to salinity, with over 90% yield losses at certain electrical conductivity level (Kokten *et al.*, 2010). Detecting key traits becomes vital to ensure the

sustainability of the pulse food industry, given the high cost of soil reclamation. Salinity has a detrimental impact on the photosynthetic rate by inducing osmotic stress, leading to water loss from plant cells and reducing stomatal opening, which subsequently limits carbon dioxide uptake (Mahlooji *et al.*, 2018). The presence of excess salt ions can disrupt chloroplast function, impair light absorption, and hinder the activity of the electron transport chain, resulting in decreased photosynthesis (Shu *et al.*, 2014; Singh *et al.*, 2023; Tounekti *et al.*, 2012; Zhang *et al.*, 2018). Nutrient imbalances, such as reduced potassium and magnesium, further impede the activity of photosynthetic enzymes (Garcia *et al.*, 2022; Shekhawat *et al.*, 2023). Elevated salt levels in the soil or water establish an osmotic gradient that draws water out of plant cells, leading to reduced turgor pressure and stomatal closure (Lambers *et al.*, 2019). This results in a limitation of water vapor release through transpiration, as the plant conserves water to manage salt stress (Hasanuzzaman *et al.*, 2017; Sharma *et al.*, 2005; Tian *et al.*, 2020). Additionally, salt-induced ion toxicity can inflict damage on root cells, diminishing their capacity for water uptake and further contributing to a decrease in transpiration (Djanaguiraman and Prasad 2012; Munns, 2002). These combined effects result in decreased gas exchange,

limiting carbon dioxide uptake and overall stomatal conductance in salt-stressed plants (Kashtoh and Baek 2021; Pandey *et al.*, 2007; Saito and Uozumi 2019). Elevated salinity levels can decrease the intrinsic water use efficiency of plants (Alkaraki 2000; Dutta *et al.*, 2018; Zhang *et al.*, 2016), as salt stress often leads to less efficient utilization of available water resources for carbohydrate production and growth maintenance. Hybridization followed by selection stands out as a crucial strategy in lentil breeding, with the selection of suitable parents being fundamental to any crop improvement initiative. Accurate insights into the nature and extent of genetic diversity play a pivotal role in guiding plant breeders to choose appropriate parents for targeted hybridization (Samsuddin, 1985). The analysis of genetic divergence serves to quantify the diversity among selected genotypes. Cluster analysis, as advocated by Mellingers (1972), proves to be a suitable method for delineating family relationships that detects the genetic closeness or separation between genotypes. To capitalize on transgressive segregation, it becomes imperative to ascertain the genetic distance between parents, a task facilitated by estimating the Euclidean distance (Joshi *et al.*, 2004). Salt stress, a polygenic trait, affects all stages of plant development, making it essential to identify key traits for salt tolerance. Our present study focuses on the evaluation of germplasm lines for salt tolerance by estimation of different photosynthetic traits and identification of key traits.

## MATERIAL AND METHODS

### A. Study Site

The experimental materials consisted of 100 diverse Lentil genotypes collected from different geographical locations. These genotypes along with checks were grown during consecutive *Rabi* seasons 2021-22 and 2022-23 under control and irrigation water salinity EC<sub>iw</sub> 5 dS/m in the pots with three replications at ICAR-Central Soil Salinity Research Institute, Karnal (29°43'N, 76°58'E; 245 m above the average sea level).

### B. Experimental setup

The diversity set was grown in pots (~20 kg) filled with sand culture. To simulate a saline environment, we maintained irrigation using saline water with an electrical EC<sub>iw</sub> of 5 dS/m throughout the experiment. To ensure that the sodium absorption ratio (SAR) remained within permissible limits, we used chloride and sulfate salts of sodium (Na<sup>+</sup>), calcium (Ca<sup>2+</sup>), and magnesium (Mg<sup>2+</sup>) for preparing the 5 dS/m saline irrigation water. Before planting, the seeds underwent surface sterilization for 5 minutes in a 10% sodium hypochlorite solution and were then rinsed with distilled water. Each plastic pot was planted with twenty seeds from each genotype, at a depth of one centimeter. The pots were designed with drainage at the bottom to facilitate the removal of excess water. In a completely random block design, the pots were organized as part of a factorial experiment. They were irrigated with Hoagland's solution, a nutrient-rich solution, and maintained at maximum field capacity until the seedlings reached an appropriate stage.

Throughout the experiment, we ensured constant salinity levels by daily draining excess salt from the pots (Singh *et al.*, 2019).

### C. Data Collection

**Photosynthetic traits.** At the seedling stage, three plants from each genotype grown under both control conditions and a salinity regime of EC<sub>iw</sub> 5 dS/m were randomly selected. We collected data related to photosynthesis, which included parameters such as Photosynthesis rate (P<sub>n</sub>; μmol m<sup>2</sup> s<sup>-1</sup>), transpiration rate (E; mol m<sup>2</sup> s<sup>-1</sup>), stomatal conductance (g<sub>S</sub>; mol m<sup>2</sup> s<sup>-1</sup>), instantaneous water use efficiency (iWUE; μ mol CO<sub>2</sub>/m mol H<sub>2</sub>O), and intrinsic water use efficiency (inWUE). To obtain these measurements, a portable photosynthetic system was used i.e. known as the LI-6800XT infrared gas exchange analyzer from Li-COR, USA, following the method described by Singh *et al.*, in 2019. The data collection occurred during the hours of 10:00 AM to 12:00 PM, in sunlight, and under specific weather conditions, including photosynthetically active radiation (PAR) of approximately 700 μmol m<sup>-2</sup>s<sup>-1</sup>, a temperature of around 25±1°C, relative humidity at approximately 70%, and an air CO<sub>2</sub> concentration of 355 μmol mol<sup>-1</sup>.

*In vivo*, chlorophyll content was measured by using a portable chlorophyll Meter (SPAD-502 Plus meter - Konica Minolta Ltd., India) at the vegetative stage by randomly selecting three plants in each pot.

**Plant water status and Membrane injury.** The relative water content was calculated through a formula given by Slavik 1974.

Relative water content (RWC) =

$$\frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry Weight}} \times 100$$

The membrane stability index (MSI) was determined according to the method of Premchand *et al.* (1990) and modified by Sairam *et al.* (1997). The membrane stability index (MSI) is calculated as:

$$\text{Membrane stability index (MSI)} = [1 - (C_1/C_2)] \times 100.$$

Where,

C<sub>1</sub> = electrical conductivity of the sample solution heated at 40°C

C<sub>2</sub> = electrical conductivity of the sample solution heated at 100°C

### D. Statistical analysis

The statistical analyses, regression analysis were carried out for all the studied seedling stage traits using software STAR 2.0.1 and MS Excel. Cluster analysis into different clusters was done using software STAR 2.0.1 (IRRI, 2014) following the manhattan method.

## RESULT AND DISCUSSION

### A. Traits modelling under salinity

Photosynthesis, a vital physiological process in green plants, plays a pivotal role in influencing overall plant growth. It involves intricate components such as photosynthetic pigments, photosystems, the electron transport system, and CO<sub>2</sub> reduction pathways. Any stress-induced damage at these levels can profoundly impair the plant's photosynthetic capacity. Salinity stress has a notable impact, leading to significant

reductions in the photosynthetic rate, transpiration, relative water content, membrane stability index, and stomatal conductance in both tolerant and susceptible plants. However, the percentage reduction is more pronounced in salt-sensitive plants. The effects of salt stress on plants in pots are illustrated in Fig. 1.

To evaluate the impact of component variables on Pn (dependent variable), feasible and stepwise regression analyses were conducted, following the approach outlined by Shannon *et al.* (2000); Sharma and Sinha (2012). The results of all conceivable regression analyses demonstrated that gsw, E, iWUE and inWUE, significantly influenced lentil leaves Pn under salt stress, while the remaining traits showed no significant impact (Table 1). Consequently, non-significant traits were excluded during the stepwise regression method. According to the findings, gsw, in WUE and MSI collectively explained over 95% of the overall variation in Pn under salt stress conditions. Furthermore, Pn

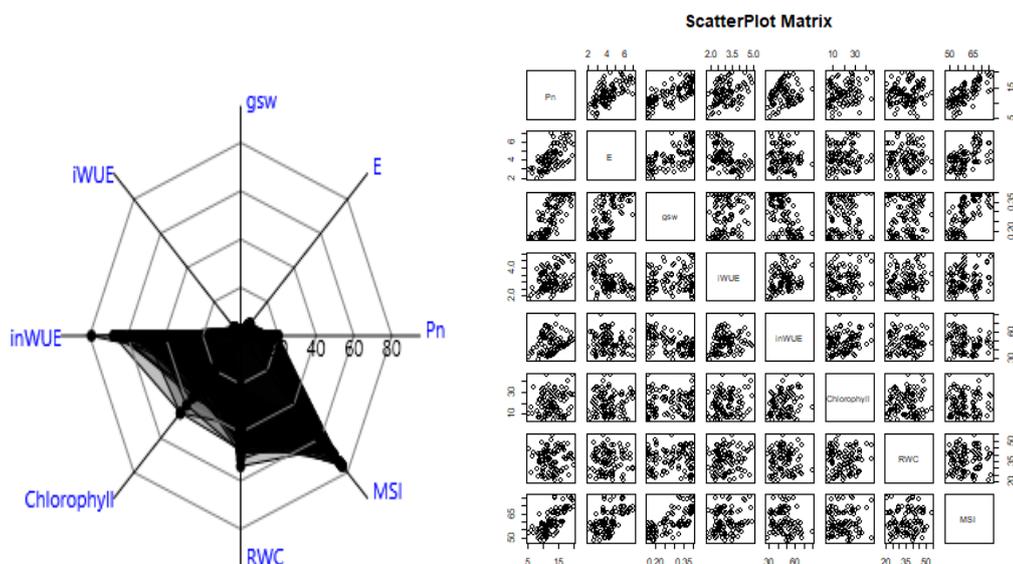
variation was substantially influenced by E, gsw, iWUE, inWUE, Chlorophyll, RWC and MSI resulting in a cumulative R<sup>2</sup> of 97.19, considered optimal as it adhered to the least Mallows' Cp criteria. The derived equation for estimating the projected Pn under saline conditions, based on regression coefficients of relevant traits, is as follows:

$$\text{Predicted photosynthetic rate} = -13.34 + (1.23 \times E) + (23.16 \times \text{gsw}) + (1.65 \times \text{iWUE}) + (0.14 \times \text{inWUE}) + (0.001 \times \text{Chlorophyll}) + (0.020 \times \text{RWC}) + (0.040 \times \text{MSI})$$

Therefore, these identified traits significantly contribute to enhancing 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. Radar graph and scatter plot for physiological traits have been given in Fig. 2.



**Fig. 1.** Pot Experiment depicting the impact of salt stress on plants (A) Tolerant Genotypes (B) Sensitive Genotypes.



**Fig. 2.** Radar graph and Scatter plot matrix of all photosynthetic traits.

**Table 1: Salinity stress tolerance's regression coefficient, standard error and significance of the prioritized attributes.**

Dependable variable	Variable	Estimate	SE	t-value	Pr(> t )
Pn	Intercept	-13.340	0.660	-20.070	0.000
	E	1.230	0.230	5.410	0.000
	gsw	23.160	3.520	6.590	0.000
	iWUE	1.650	0.290	5.690	0.000
	inWUE	0.140	0.020	7.180	0.000
	Chlorophyll	0.001	0.010	0.510	0.612
	RWC	0.020	0.010	2.380	0.019
	MSI	0.040	0.010	2.720	0.008

**Table 2: Traits modelling for salinity tolerance through multiple linear regressions approach.**

Variables	C(p)	R-square	Adj R-sq
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

### B. Cluster Analysis

Nine clusters were created using manhattan distance method and ward clustering method from 100 genotypes (Table 2). Largest number of genotypes (22) were found in cluster I followed by 15 (cluster VII), 12 (cluster IV), 10 (cluster III, V, VIII and IX), 7 (cluster VI) and 4 (cluster II). Cophenetic correlation coefficient was 0.527. It was observed to be more variation among genotypes of all clusters. Different genotypes from various clusters can be used as parents in hybridization breeding programme to get transgressive segregants. The analysis of clusters revealed substantial genetic diversity among the examined lentil genotypes, suggesting a promising potential for enhancement through crossbreeding genotypes from diverse clusters. The arrangement of genotypes from various geographical locations into distinct clusters appeared to be random (Anju *et al.*, 2004). Genotypes with similar origins were found in different clusters, and vice versa, indicating a lack of correlation between geographical region and genetic diversity (Tyagi and Khan 2010). The occurrence of genotypes in clusters across geographical boundaries illustrates that genetic diversity is not only driven by geographical isolation. Several factors contribute to genetic divergence, including changes in breeding material, genetic drift, natural variation, and artificial selection, in addition to ecological and geographical diversification. Therefore, the selection of parents for hybridization should prioritize genetic diversity over geographic diversity to yield more heterotic recombinants and desired transgressive segregants. However, caution is necessary when choosing divergent genotypes, as such crosses may not consistently produce a proportional heterotic response. Initiating a hybridization program involving genotypes from diverse clusters with high means for almost all component traits is recommended. Additionally, these divergent parents should exhibit superior combining ability to ensure results in line with the expected heterotic response (Kumar *et al.*, 2012).

### CONCLUSIONS

The photosynthetic performance in lentils has not been previously assessed. In our current study, we observed that salinity significantly diminishes the activity of the photosynthetic apparatus, directly impacting yield negatively. In staple food crops, photosynthesis remains a plant trait with substantial potential for further improvement. Notably, efforts to enhance lentil crops have commenced with extensive measurements, but there is a need to enhance measurement throughput, covering plants from root to canopy, utilizing high throughput phenotyping facilities. The effectiveness of evaluating the fit of a multiple regression model can be gauged through the application of Mallows' Cp Criterion, where a preference for smaller Cp values indicates lower levels of unexplained error.

### FUTURE SCOPE

The key traits identified in this study can be used in further crop improvement programme as selection criteria for salt tolerance or development of crop ideotype.

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**Conflict of interest.** None.

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