Evaluation of salinity tolerance in chickpea (*Cicer arietinum*): Physiological, biochemical, and yield correlations under variable salinity conditions

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Abstract
Chickpea (*Cicer arietinum*), an ancient leguminous crop, faces significant yield reduction under saline soil conditions. This study aimed to identify chickpea genotypes with improved salinity tolerance by evaluating correlations between physiological and biochemical traits and yield under different salinity levels. The experiment included 10 chickpea genotypes subjected to three salinity treatments (0, 3, and 6 dS/m) in a completely randomized design. Parameters assessed included chlorophyll content (SPAD), relative water content (RWC), membrane injury index (MII), and biochemical markers such as proline and malic acid. Results showed that SPAD values, indicating chlorophyll content, were positively correlated with yield across all salinity levels (0.725 to 0.913). Higher chlorophyll content was associated with better yield, reflecting its role in maintaining photosynthetic efficiency. RWC also demonstrated a strong positive correlation with yield (0.73 to 0.987), suggesting that better water retention supports higher yield under salinity stress. Conversely, MII exhibited a negative correlation with yield (-0.608 to -0.93), indicating that increased membrane damage impairs yield. Total Chlorophyll Content (TCC) and Chlorophyll Stability Index (CSI) showed strong positive correlations with yield parameters (SY/P, TW) under saline conditions, underscoring the importance of chlorophyll maintenance for stress tolerance. Biochemical parameters, particularly proline and malic acid, were positively associated with yield under salinity stress. Proline correlations ranged from 0.775 to 0.799, and malic acid showed correlations up to 0.974 with seed yield, highlighting their roles in osmotic adjustment and stress resilience. This study emphasizes the significance of physiological and biochemical traits in enhancing chickpea salinity tolerance and offers valuable insights for breeding programs aimed at improving crop resilience under adverse conditions.

Keywords: Chickpea, salinity tolerance, physiological traits, chlorophyll content and proline

Introduction
Chickpea (*Cicer arietinum* Linnaeus), a member of the Fabaceae family, is an ancient, self-pollinated, leguminous diploid annual crop (2N=16 chromosomes) that has been cultivated since 7000 BC across various parts of the world (Tekeoglu *et al*., 2000) [10]. The cultivated chickpea species are primarily categorized into two types: desi and kabuli. Desi types feature small, dark brown seeds, whereas kabuli types are characterized by bold, cream-colored seeds. In India, both desi and kabuli chickpea varieties are widely grown, contributing significantly to the country's agricultural economy. Notably, Madhya Pradesh stands out as the single largest producer, accounting for over 40% of the nation's total chickpea production.

However, the productivity of chickpea, like many other crops, is adversely affected by soil salinity. Salt-affected soils, which encompass both saline (high concentrations of chlorides and sulfates of sodium) and alkaline soils (high concentrations of carbonates and bicarbonates of sodium), pose a significant challenge to plant growth and crop yield. Saline soils result from the accumulation of chlorides, sulfates, calcium, magnesium, and potassium in the soil, a process known as salinization. Salinity induces physiological dehydration (water stress) and ion imbalances in plants, leading to several adverse effects such as growth reduction, chlorophyll degradation, decreased photosynthesis, and increased oxidative stress (Toker *et al*., 2007; Zhu, 2001) [11, 15]. In response to salinity stress, plants have evolved various acclimatization and avoidance strategies, including the accumulation of compatible osmolytes, antioxidants, and reactive oxygen species (ROS) scavenging enzymes (Ashraf
and Harris, 2004) [1]. These mechanisms help mitigate the damage caused by oxidative stress and maintain osmotic balance under saline conditions. Despite significant advancements in improving salinity tolerance in various crop species, research on salinity tolerance in chickpea remains limited. Understanding the genotypic responses of chickpea cultivars to salinity stress could provide valuable insights into the physiological and biochemical mechanisms underlying salinity tolerance. This study aims to evaluate the correlation between physiological and biochemical parameters with yield under both normal and saline conditions, thereby identifying potential indicators of salinity tolerance in chickpea. Correlation coefficient analysis will be employed to elucidate the relationships between traits such as leaf chlorophyll content, leaf water potential, and grain yield, offering a deeper understanding of how these traits interact and contribute to salinity tolerance (Narkhede et al., 2017) [9].

Materials and methods
The present investigation was conducted during the rabi season of 2019-2020 to identify the most salinity-tolerant chickpea genotype. The experiment involved 10 chickpea genotypes, including seven released varieties: Anningeri 1, BGD 103, GBM 2, JAKI 9218, JG 11, MNK 1, and NBeG 47, as well as three cultivars from ICRISAT: ICC 96029, ICC 1431, and ICC 5003. The study was carried out in the Department of Crop Physiology, College of Agriculture, Vijayapura, utilizing a completely randomized design (CRD) with three replications.

Experimental site
The pot experiment was conducted at the rainout shelter of the College of Agriculture, Vijayapura, while the laboratory study was carried out in the Department of Crop Physiology. The College of Agriculture, Vijayapura, is located at 16°49’ N latitude and 76°34’ E longitude, with an altitude of 678 meters above mean sea level (MSL).

Salinity treatments
Screening of 10 chickpea varieties for chloride salinity tolerance was conducted using a graded series of NaCl solutions with concentrations of C1 = 0, C2 = 3, and C3 = 6 dSm⁻¹. These salt solutions were prepared according to the method outlined in the USDA Handbook No. 60 (Richards, 1954) [7].

Sowing and salinity treatments
Uniform-sized earthen pots (30x30 cm) were filled with 10 kg of air-dried soil mixed with farmyard manure in a 6:1 ratio. Each pot was fertilized with a dose of 120 kg/ha of nitrogen (N), 60 kg/ha of phosphorus (P), and 60 kg/ha of potassium (K). Prior to sowing, the pots were irrigated with either 2.5 liters of water (control) or salt solutions of varying concentrations. The plants were subjected to three conditions: control (C1) and two salinity treatments (C2 and C3). The salt solutions were prepared using NaCl, with the following concentrations:

- C1: 5 grams of NaCl dissolved in 1 liter of water, resulting in a 3 dSm⁻¹ EC
- C2: 10 grams of NaCl dissolved in 1 liter of water, resulting in a 6 dSm⁻¹ EC
- C3: 15 grams of NaCl dissolved in 1 liter of water, resulting in a 9 dSm⁻¹ EC

The actual salinity values were measured at three stages, and the mean of these values was used to represent the salinity levels for C1, C2, and C3.

Correlation Analysis and Principal Component Analysis Using R Software
Data for this study were collected from chickpea plants subjected to salt stress, covering a range of physiological and agronomic traits, including leaf chlorophyll content, leaf water potential, grain yield, and various biochemical parameters. For correlation analysis, the data were first imported into R software using the read.csv() function. The cor() function was then used to compute the correlation matrix, which was visualized with the corrplot package to display the strength and direction of relationships between traits. This method facilitated the identification of significant correlations and provided insights into the interdependencies of traits under salinity stress conditions (Wei & Simko, 2017) [13].

Results
Correlation Coefficients between Physiological Parameters and Yield
The correlation analysis revealed strong associations between physiological parameters and seed yield per plant (SY/P) across different salinity levels (Table 1). Chlorophyll content, measured by SPAD values, exhibited high positive correlations with SY/P at all salinity levels: 0.725* at 0 dS/m, 0.900*** at 3 dS/m, and 0.913*** at 6 dS/m, indicating that increased chlorophyll content is linked to higher yield. Similarly, Total Chlorophyll Content (TCC) and Chlorophyll Stability Index (CSI) also showed robust positive correlations with yield parameters. TCC correlated strongly with SY/P and test weight (TW) under salinity stress, with coefficients of 0.991*** at 3 dS/m and 0.994*** at 6 dS/m. CSI demonstrated positive correlations with SY/P of 0.985*** at 3 dS/m and 0.995*** at 6 dS/m, highlighting its role in maintaining chlorophyll integrity and yield under stress conditions. Conversely, the Membrane Injury Index (MII) revealed significant negative correlations with yield, underscoring the detrimental impact of membrane damage on plant productivity. MII correlations were -0.608 at 0 dS/m, -0.93*** at 3 dS/m, and -0.90*** at 6 dS/m. Proline and malic acid, two biochemical markers, also exhibited significant positive correlations with yield, particularly under salinity stress. Proline correlations with SY/P were 0.775** at 3 dS/m and 0.799** at 6 dS/m, while malic acid correlations with SY/P were 0.741* at 3 dS/m and 0.974*** at 6 dS/m. These results suggest that maintaining high chlorophyll content, water status, and osmotic adjustment through proline and malic acid are crucial for sustaining yield under saline conditions.
Correlation Coefficients between Total Chlorophyll Content and Chlorophyll Stability Index with Yield Parameters

The correlations between Total Chlorophyll Content (TCC) and Chlorophyll Stability Index (CSI) with yield parameters, including pod weight per plant (PW/P), seed yield per plant (SY/P), and test weight (TW), were analyzed (Table 2). Total Chlorophyll Content (TCC) showed strong positive correlations with yield parameters across all salinity levels. At 0 dS/m, TCC correlated with SY/P at 0.709* and TW at 0.822**. Under 3 dS/m, correlations improved significantly to 0.993*** for SY/P and 0.973*** for TW. At 6 dS/m, TCC correlations remained high, with SY/P at 0.994*** and TW at 0.993***, highlighting the importance of maintaining chlorophyll content for optimal yield performance under salinity stress. Chlorophyll Stability Index (CSI) exhibited significant positive correlations with yield parameters, especially under salinity stress. At 0 dS/m, CSI correlated with SY/P at 0.569 and TW at 0.822**. Under 3 dS/m, correlations were strong at 0.985*** for SY/P and 0.828** for TW. At 6 dS/m, CSI showed high correlations with SY/P at 0.995*** and TW at 0.974***, underscoring the role of chlorophyll stability in supporting yield under stress conditions.

Correlation Coefficients between Biochemical Parameters and Yield Parameters

The study also evaluated the correlations between biochemical parameters, specifically proline (Pr) and malic acid (MA), with yield parameters (Table 3). Proline displayed strong positive correlations with yield parameters under salinity stress. At 3 dS/m, proline correlated with PW/P at 0.771** and SY/P at 0.775**, and at 6 dS/m, correlations were 0.802** for PW/P and 0.799*** for SY/P. These results suggest that increased proline accumulation is associated with improved yield performance under salinity stress. Malic Acid (MA): Malic acid showed significant correlations with seed yield, particularly at higher salinity levels. At 3 dS/m, MA correlated with SY/P at 0.741*, and at 6 dS/m, the correlation increased to 0.974***. This suggests that higher malic acid levels are beneficial for maintaining seed yield under stress.

Discussion

The study's findings underscore the critical role of physiological and biochemical parameters in influencing seed yield under varying salinity levels (Waitt and Levin, 2008). Chlorophyll-related traits, including SPAD values (Fig 1), Total Chlorophyll Content (TCC), and Chlorophyll Stability Index (CSI), all demonstrated strong positive correlations with yield, particularly under salinity stress (Fig 2). Higher chlorophyll content and stability are crucial for sustaining photosynthetic efficiency and, consequently, yield performance. This is consistent with previous studies that highlight the importance of chlorophyll in stress tolerance and yield stability (Smith et al., 2018; Nayyar & Gupta, 2006) [9, 6]. Relative Water Content (RWC) also exhibited positive correlations with yield, emphasizing the significance of plant hydration and turgor maintenance under stress. RWC's role in maintaining cell expansion and overall growth under salinity aligns with findings by Jones et al. (2015) [3]. Conversely, the Membrane Injury Index (MII) showed significant negative correlations with yield, indicating that increased membrane damage negatively impacts yield (Fig 1). This underscores the importance of membrane stability for stress tolerance and yield preservation, as supported by Zhao et al. (2017) [14]. Biochemical parameters (Fig 3) such as proline and malic acid further illustrate the complex interplay between stress tolerance mechanisms and yield. Proline's role as an osmoprotectant and malic acid's involvement in regulating cellular pH and osmotic adjustment highlight their importance in enhancing plant resilience and yield stability under saline conditions (Ashraf & Foolad, 2007; Saeed et al., 2015) [2, 8]. Overall, the results affirm that maintaining physiological and biochemical stability is essential for optimizing yield under stress conditions. These findings support the broader understanding of stress tolerance mechanisms in crops and underscore the need for strategies to enhance chlorophyll content, RWC, and osmoprotectants to sustain productivity in challenging environments (Munns & Tester, 2008) [4].
Table 2: Correlation Coefficients between Total Chlorophyll Content and Chlorophyll Stability Index with Yield Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TCC (0 dS/m)</th>
<th>TCC (6 dS/m)</th>
<th>CSI (0 dS/m)</th>
<th>CSI (6 dS/m)</th>
<th>PW/P(0 dS/m)</th>
<th>PW/P(6 dS/m)</th>
<th>SY/P(0 dS/m)</th>
<th>SY/P(6 dS/m)</th>
<th>TW(0 dS/m)</th>
<th>TW(6 dS/m)</th>
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</thead>
<tbody>
<tr>
<td>PW/P (0 dS/m)</td>
<td>0.347 ± 0.196</td>
<td>0.196 ± 0.374</td>
<td>0.198 ± 0.18</td>
<td>0.305 ± 0.273</td>
<td>1.000</td>
<td>0.828</td>
<td>0.984</td>
<td>0.944</td>
<td>0.982</td>
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</table>

Table 3: Correlation Coefficients between Biochemical Parameters and Yield Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pr (0 dS/m)</th>
<th>Pr (3 dS/m)</th>
<th>MA (0 dS/m)</th>
<th>MA (3 dS/m)</th>
<th>PW/P (0 dS/m)</th>
<th>PW/P (3 dS/m)</th>
<th>SY/P(0 dS/m)</th>
<th>SY/P(3 dS/m)</th>
<th>TW(0 dS/m)</th>
<th>TW(3 dS/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pr (0 dS/m)</td>
<td>1.000</td>
<td>0.855*</td>
<td>0.045</td>
<td>0.011</td>
<td>0.274</td>
<td>0.103</td>
<td>0.038</td>
<td>0.591</td>
<td>0.313</td>
<td>0.137</td>
</tr>
<tr>
<td>Pr (3 dS/m)</td>
<td>0.855*</td>
<td>1.000</td>
<td>0.135</td>
<td>0.001</td>
<td>0.281</td>
<td>0.285</td>
<td>0.398</td>
<td>0.424</td>
<td>0.508</td>
<td>0.423</td>
</tr>
<tr>
<td>MA (0 dS/m)</td>
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<td>0.135</td>
<td>1.000</td>
<td>0.281</td>
<td>0.024</td>
<td>0.001</td>
<td>0.515</td>
<td>0.573</td>
<td>0.423</td>
<td>0.458</td>
</tr>
<tr>
<td>MA (3 dS/m)</td>
<td>0.011</td>
<td>0.281</td>
<td>0.001</td>
<td>1.000</td>
<td>0.312</td>
<td>0.273</td>
<td>0.573</td>
<td>0.038</td>
<td>0.313</td>
<td>0.137</td>
</tr>
<tr>
<td>PW/P (0 dS/m)</td>
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<td>0.024</td>
<td>0.001</td>
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<td>0.591</td>
<td>0.313</td>
<td>0.137</td>
<td>0.103</td>
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</table>

Conclusion

This study elucidates the impact of salinity on chickpea (Cicer arietinum) yield and the associated physiological and biochemical responses. The correlation analysis revealed that higher chlorophyll content (SPAD values and Total Chlorophyll Content), better Relative Water Content (RWC), and lower Membrane Injury Index (MII) are positively correlated with increased seed yield under saline conditions. Additionally, higher Chlorophyll Stability Index (CSI) and increased levels of proline and malic acid were associated with improved yield performance under salinity stress. These findings highlight the critical roles of chlorophyll content, water retention, membrane stability, and osmoregulators in enhancing salinity tolerance in chickpea. The results underscore the importance of these
traits as indicators of salinity tolerance and suggest that breeding strategies focusing on these physiological and biochemical parameters could improve chickpea resilience to saline environments.

Acknowledgement
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Conflict of interest
Authors have declared that no Conflict of interest exist

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