

Proteomic Responses to Salinity Stress: Exploring Protein Fraction Variations in Salt-Sensitive and Salt-Tolerant Genotypes of Chickpea

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Abstract:- The investigation into variations in relative protein fractions under different salinity levels provides insights into the intricate interplay between plant physiology and stress responses. This study aimed to understand the effect of different salinity regimes on proportion of protein fractions namely albumins, globulins, glutelins and prolamins. Two different genotypes (salt tolerant and salt sensitive) of chickpea were investigated. Both genotype exhibited nuanced protein fraction modulation, highlighting dynamic protein allocation in salinity-induced stress. Tolerant genotype showed a more adaptability towards salinity stress as compared to sensitive one. Variations in proportions of different fractions reflected responsive protein distribution shifts under salinity stress. These findings showcase plant protein composition's adaptability to adversity, urging further molecular investigations for potential stress-resilience strategies.

Keywords:- Protein Fractions, Salinity, Salt Tolerant, Salt Sensitive.

I. INTRODUCTION

In a world grappling with the dual challenges of malnutrition and food insecurity, addressing the critical issue of nutrition has taken center stage in global discourse. As populations continue to grow and resources become increasingly strained, ensuring that individuals receive adequate nourishment has become an urgent imperative (Pimentel *et al.*, 1997). Nowhere is this dilemma more pronounced than in countries like India, where a rapidly expanding populace and a diverse socio-economic landscape converge to magnify the complexity of nutritional concerns (FAO, 2017).

Against this backdrop, the role of diet assumes a paramount significance in shaping public health outcomes (Capone *et al.*, 2014). Within the dietary spectrum, legumes have garnered considerable attention due to their potential to offer sustainable solutions to nutritional challenges (Foyer,

2016). Legumes, a class of plant-based foods encompassing beans, lentils, peas, and other similar crops, have emerged as noteworthy candidates for combating malnutrition owing to their rich nutritional profile and environmental sustainability (Temba *et al.*, 2016). Among these legumes, the chickpea, with its unique attributes and multifaceted contributions, stands out as a vital focal point in the search for effective nutritional interventions.

This research paper aims to explore the manifold dimensions of the chickpea's significance in addressing global and Indian nutritional concerns. We will delve into the pivotal role that legumes, particularly chickpeas, play in offering a viable alternative to non-plant diets. By investigating the key attributes of chickpea protein, we will uncover its potential as a nutritional powerhouse.

Furthermore, in the context of the challenges posed by abiotic stresses, the importance of legumes like chickpeas takes on added significance. Abiotic stresses, such as salinity, present formidable obstacles to agricultural productivity, compromising the growth and quality of crops (Sharma and Singh, 2015). Legumes, including chickpeas, have demonstrated remarkable resilience to such environmental stressors, showcasing their capacity to thrive in adverse conditions. However, the impact of salinity on legume growth and nutrient composition is a critical factor to consider, particularly as regions affected by salinity continue to expand due to changing climatic patterns and improper irrigation practices (Essa, 2002).

The subsequent sections of this paper will delve into the nuanced interplay between nutritional inadequacies, the role of legumes as a sustainable dietary source, and the specific attributes that set chickpeas apart. We will also examine the effects of abiotic stresses, with a particular focus on salinity on the proportion of protein fractions of chickpeas. By deciphering the nutritional intricacies of chickpeas, including their protein contribution and distribution, in both optimal and stressful conditions, we aim to shed light on their potential to address the pressing challenges of global malnutrition and

food security. Through this exploration, we hope to contribute to a deeper understanding of the intricate relationship between diet, legumes, protein fractions, and the impact of abiotic stresses, underscoring chickpeas' role as a beacon of nutritional innovation and progress, even in the face of environmental adversity.

II. MATERIAL AND METHODS

The seeds of two cultivars of chickpea, C-235 (salt-sensitive genotype) and CSG- 8962 (salt-tolerant genotype), were procured from Chaudhary Charan Singh Haryana Agricultural University (CCS HAU) Hisar, Haryana and Central Soil Salinity Research Institute (CSSRI) Karnal, Haryana respectively. A pot experiment was conducted in the experimental plot of the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India, in the last week of October to study the effects of salinity. The pots filled with soil for each variety were divided into four sets (i.e., three sets for different salinity levels and one set as control) with five replicates each. Crop thinning was performed to keep five plants per pot. During their vegetative growth, the plants were watered as needed.

➤ *Salinity treatment*

Three different levels of salinity, i.e. 4 dS m⁻¹, 7 dS m⁻¹ and 10 dS m⁻¹, were generated and maintained using Na₂SO₄, CaCl₂ and NaCl in the ratio of 1:2:7 w/v following Richards formulation (1954). A volume 200 ml saline solution was supplied to each pot. For E.C.'s estimation of soils in pots, the soil was mixed with 60 ml of DDW by continuously stirring and left undisturbed for 20-30 minutes. Seeds were harvested at maturity, dried and ground to seed meal. The seed meal was defatted using hexane (10 ml/g seed meal) for protein estimation, fractionation of total seed protein into four fractions, analysis of the content of tryptophan, cysteine and methionine in each fraction and electrophoretic separation of each fraction on SDS gels.

➤ *Fractionation of seed proteins*

The methods described by Croy *et al.* (1984), with little modifications, were employed for seed protein fractionation. Specifically, albumins and globulins were extracted in 50 mM borate buffer at pH 8, followed by dialysis to separate each fraction. Glutelins and prolamins, on the other hand, were isolated using 0.1N NaOH and 70% ethanol, respectively.

➤ *The proportion of four protein fractions*

The four seed protein fractions were quantified using Bradford's method (1976). Subsequently, the final protein concentration in each fraction was determined by referencing a standard curve constructed using bovine serum albumin (BSA).

➤ *Statistical analysis*

The data presented in the tables and figures are represented as the mean value ± standard error (SE) as a measure of variability. A mean of 3 readings was taken in each replicate. Statistical analysis was done using Microsoft Excel version 2010 and Statistical Packages for Social Sciences (SPSS) version 16.0. A Post hoc test (Duncan) using the same software was used to determine the difference among data. One-way ANOVA was employed to assess statistically significant differences among the various estimations.

III. RESULTS

A spectroscopic analytical procedure known as Bradford's method (1976) is used to study the proportion of four protein fractions. The BSA standard curve of (Bovine Serum Albumin) was prepared for unknown protein fraction estimation.

The status of the four protein fractions concerning their relative proportions under varying salinity levels is given in Table 1 and Fig. 1 Thus, the relative proportion of albumins in salt-sensitive plants decreased from 14.4% (control) to 13.0% (at 10 dS m⁻¹), while in the case of globulins, it was noticed from 66.9% to 65.4%. At the same time, the relative proportion of glutelins increased from 13.3% (control) to 16.5% (at 10 dS m⁻¹), and a slight decrease of 5.4% to 5.1% in prolamins was observed. In the case of the salt-tolerant genotype, the relative proportion of albumins decreased from 14.7% to 13.6%, and globulins, glutelins and prolamins increased from 65.7% to 65.8%, 14.5% to 15.4% and 5.1% to 5.2% (control to 10 dS m⁻¹ salinity level) respectively.

Table 1 Effect of salinity on the proportion of four protein fractions in salt-tolerant and salt-sensitive genotypes of chickpea.

Proportion of four fractions (%)					
Genotype	levels of salinity	Seed protein fractions			
		Albumins	Globulins	Glutelins	Prolamins
CSG 8962	Control	14.7 ± 0.12 ^a	65.7 ± 1.29 ^a	14.5 ± 0.10 ^c	5.1 ± 0.05 ^{bc}
	4 dS m ⁻¹	14.2 ± 0.27 ^{abc}	66.0 ± 1.44 ^a	14.9 ± 0.12 ^b	4.9 ± 0.10 ^c
	7 dS m ⁻¹	13.9 ± 0.22 ^{bc}	65.6 ± 1.60 ^a	15.2 ± 0.98 ^b	5.3 ± 0.08 ^{ab}
	10 dS m ⁻¹	13.6 ± 0.30 ^{cd}	65.8 ± 1.37 ^a	15.4 ± 0.39 ^{ab}	5.2 ± 0.13 ^{ab}
C 235	Control	14.4 ± 0.33 ^{ab}	66.9 ± 1.18 ^a	13.3 ± 0.05 ^c	5.4 ± 0.09 ^a
	4 dS m ⁻¹	14.1 ± 0.06 ^{abc}	66.0 ± 0.10 ^a	15.0 ± 0.33 ^b	4.9 ± 0.07 ^c
	7 dS m ⁻¹	13.6 ± 0.28 ^{cd}	65.7 ± 1.57 ^a	15.5 ± 0.22 ^{ab}	5.2 ± 0.09 ^{ab}
	10 dS m ⁻¹	13.0 ± 0.02 ^e	65.4 ± 0.68 ^a	16.5 ± 0.18 ^a	5.1 ± 0.06 ^{bc}

Each value is a mean of three replicates, ± SE and means followed by same letter/s are not significantly different at P ≤ 0.05.

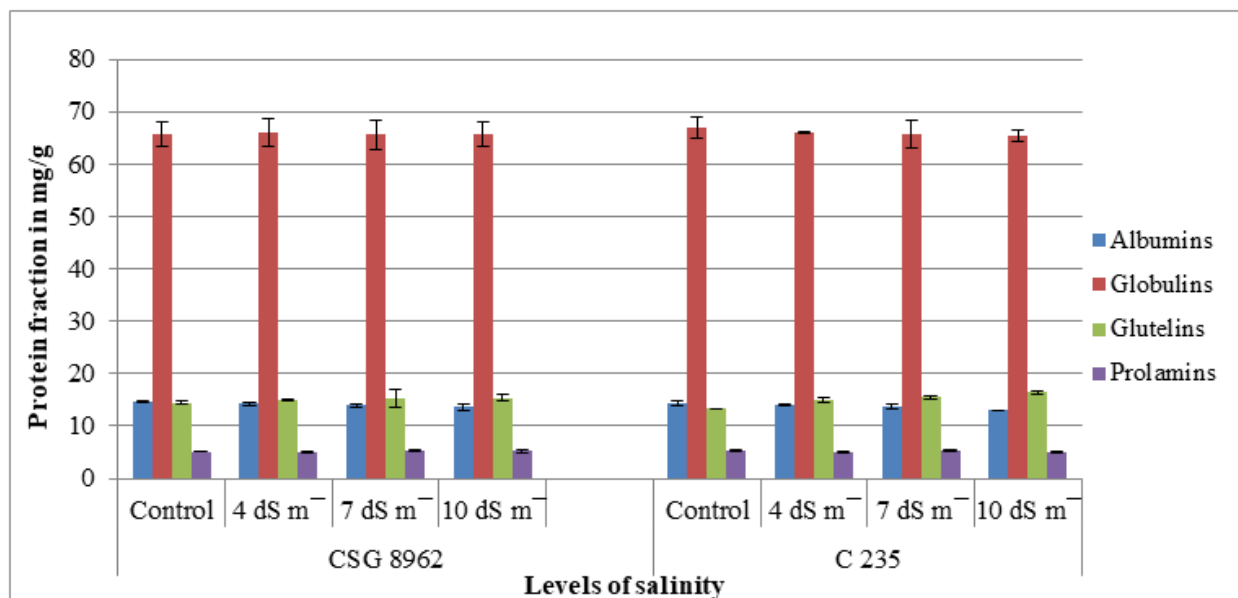


Fig. 1 Effect of salinity on proportion of four seed protein fractions.

IV. DISCUSSION

The investigation into the variations in relative protein fractions under different salinity levels provides valuable insights into the intricate interplay between plant physiology and the stress response. The results presented in Table 1 and Fig. 1 reveal a nuanced modulation of protein fractions in both salt-sensitive and salt-tolerant genotypes, showcasing the dynamic nature of protein allocation in the face of salinity-induced stress (Hussain *et al.*, 2021). In the context of the salt-sensitive plants, the observed decrease in the relative proportion of albumins, from 14.4% (control) to 13.0% (at 10 dS m⁻¹), highlights a responsive shift in protein distribution (Smith *et al.*, 2008). Albumins, with their roles in nutrient transport and defense responses, appear to undergo a

reallocation, potentially reflecting the plant's strategy to redirect resources towards stress mitigation mechanisms (Meena *et al.*, 2017). Similarly, the slight reduction in globulins, from 66.9% to 65.4%, suggests a measured adjustment in protein composition, possibly linked to the plant's adaptive mechanism for optimizing energy utilization during saline stress (Sabagh *et al.*, 2021).

An intriguing contrast emerges in the behavior of glutelins and prolamins within salt-sensitive plants. The significant increase in glutelins, from 13.3% (control) to 16.5% (at 10 dS m⁻¹), suggests a specific response to salinity stress. Glutelins, often associated with seed storage proteins, might be playing an enhanced role in nutrient preservation and mobilization under stressful conditions (Xiong *et al.*, 2021).

On the other hand, the marginal decrease observed in prolamins, from 5.4% to 5.1%, might signify a more stable protein fraction, less prone to fluctuations in response to environmental perturbations.

In the context of the salt-tolerant genotype, the observed changes in protein fractions reflect a distinct response pattern. The marginal decrease in the relative proportion of albumins, coupled with concurrent increases in globulins, glutelins, and prolamins, suggests a finely tuned adjustment in protein composition. This could be indicative of the genotype's ability to efficiently allocate resources towards stress-responsive proteins, thus maintaining its functionality and vitality under saline conditions (Johnson and Puthur, 2021).

The elevation of glutelins in both genotypes under salinity stress is particularly intriguing. This protein fraction, typically associated with storage, may be adopting a multifaceted role in stress adaptation. It could potentially serve as a reservoir for amino acids that contribute to stress-responsive metabolic pathways (Kimbembe *et al.*, 2020). Furthermore, the increased proportion of glutelins might signify a strategic measure to counteract the adverse effects of salinity by enhancing nutrient availability for critical physiological processes (Hassan *et al.*, 2021).

These findings underscore the complexity of protein fraction dynamics in response to salinity stress and highlight the plant's remarkable ability to reconfigure its protein composition to navigate adverse conditions. However, this investigation opens the door to further inquiries. Future studies could delve into the molecular mechanisms underlying these changes and explore the implications of altered protein fractions on broader physiological functions, growth, and yield. Understanding these intricacies could inform the development of targeted strategies to enhance stress resilience in crop plants.

V. CONCLUSION

In conclusion the investigation into the variations in protein fractions under varying salinity levels reveals the remarkable plasticity of plant responses to stress. The observed shifts in albumins, globulins, glutelins, and prolamins emphasize the plant's capacity to dynamically regulate its protein composition to ensure survival and functionality under challenging conditions. These findings contribute to our understanding of the intricate biochemical adaptations that underlie stress tolerance and lay the groundwork for potential applications in crop improvement and sustainable agriculture.

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