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# Optimizing Protein Fractions in Chickpeas: A Salinity Alleviation Approach with Calcium and Potassium Supplementation

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## ABSTRACT

**Background:** Malnutrition is a serious worldwide public health concern that affects both adults and children. It has a substantial negative impact on society and the economy. COVID-19 has exacerbated this problem by reducing household income and access to food. Legumes, especially chickpeas, in particular, are a great source of important nutrients and can help alleviate protein-energy malnutrition. But soil salinity is a big problem for growing chickpeas and, by extension, for food security worldwide. The present study was to estimate the effect of minerals under salinity stress on protein fractions in different genotypes of chickpea. This study explores the potential of calcium (Ca) and potassium (K) supplementation to alleviate salinity stress and improve protein content and quality in chickpeas.

**Methods:** Two chickpea cultivars, salt-sensitive (C-235) and salt-tolerant (CSG-8962), were subjected to varying levels of salinity and mineral treatments (in a ratio of 7:2:1) to examine their effect on protein fractions, which were extracted and estimated.

**Result:** The findings show that, under the conditions of salinity stress, the simultaneous application of calcium and potassium considerably increases the amounts of protein fractions (albumins, globulins, glutelins and prolamins). These results highlight the significance of mineral supplements as a sustainable approach to increasing agricultural yields and fighting malnutrition in salty environments.

**Keywords:** Chickpeas, Legumes, Malnutrition, Protein Fractions.

## INTRODUCTION

Malnutrition, a global health crisis, impacts all ages, hinders economies and is a major cause of child death (WHO, 2020). 45% of child deaths under age five are due to malnutrition (Katharina *et al*., 2017). Legumes, a sustainable and affordable protein source (Kamboj and Nanda, 2018; Bessada *et al*., 2019), can be key in combating malnutrition, especially when incorporated with plant-based diets (Willett *et al*., 2019; Balasubramanian *et al*., 2023).

Legumes are known for their high seed protein content and vital role in human nutrition and agro-ecosystems (Farooq *et al*., 2018). Among legumes, chickpea (*Cicer arietinum* L.) is important for food security and enriching the soil fertility (Bulut *et al*., 2023). It is consumed as a good source of proteins (~16% to 28%), several minerals, phenolics, oligosaccharides, soluble-insoluble fibres, essential nutrients such as antioxidants, biologically active compounds and vitamins (Meena *et al*., 2015). In both area and yield, India dominates the global chickpea market, producing over 65% of the world's supply (Thaware *et al*., 2017). However, its growth, yield and seed protein quality are negatively affected by various abiotic stresses such as heavy metals, heat stress, salinity, drought, *etc*. (Varma and Meena, 2016). Among these stresses, the salinity limits global chickpea production by 8-10% (Ahmed *et al*., 2021). More than 8.7% of soil is salt-affected (833 million hectares) around the globe (FAO, 2021). Therefore, plant scientists are designing several strategies to alleviate the harmful effects of salt stress. However, many of these are adequate but not profitable. As minerals play a crucial role in regulating ion balance and osmotic processes in plants, recently exogenous mineral supply (Ca and K) has been employed to mitigate the salinity stress (Shoukat *et al*., 2023). Therefore, considering the importance of chickpeas in human nutrition and the impact of increasing soil salinity on plants, the current work was undertaken to understand the role of calcium (Ca) and potassium (K) in alleviating the salinity stress and restoring the seed protein quantity/quality in chickpeas.

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## MATERIALS AND METHODS

Chickpea seeds, representing a salt-sensitive genotype (C-235) and a salt-tolerant genotype (CSG-8962), were sourced from Chaudhary Charan Singh Haryana Agricultural University (CCS HAU) in Hisar, Haryana and the Central Soil Salinity Research Institute (CSSRI) in Karnal, Haryana, respectively. In the final week of October, a controlled pot-based experiment was conducted. To examine the effects of salinity stress and its alleviation through minerals, calcium (Ca) and potassium (K) on the field experiment located at the Botany Department, Kurukshetra University, Kurukshetra, Haryana, India. Nine sets of pots filled with soil were maintained for salinity treatment along with mineral treatments (*i.e.*, eight sets for supplying different minerals individually and in combinations and one set as control). The crop was thinned out so that every pot would have five plants in it. Watering was done for crops according to requirements throughout their vegetative growth phase.

Three distinct salinity levels, 4 dS $m^{-}$, 7 dS $m^{-}$ and 10 dS $m^{-}$, were given to plants that were kept using a 7:2:1 w/v ratio of NaCl, $Na_2SO_4$ and $CaCl_2$ in accordance with Richards' formulation (1954). A saline solution of 200 ml was given to each pot. To estimate the E.C. of soil in pots, the soil was blended with 60 ml of DDW, stirring constantly and then allowed to settle for 20 to 30 minutes. For alleviation of salinity, calcium (Ca) and potassium (K) in eight different combinations, *i.e.* $Ca_2$, $K_2$, $Ca_5$, $K_5$, $Ca_2K_2$, $Ca_2K_5$, $K_2Ca_5$ and $K_5Ca_5$ were supplied to plants, where subscripts 2 and 5 represent the two-time and five-times concentrations of respective nutrients. Ca was provided as calcium chloride dihydrate ($CaCl_2.2H_2O$) and K was supplied as potassium sulfate ($K_2SO_4$). The mature seeds were collected, dried and then processed into seed meal. Hexane (10 ml/g seed meal) was used for defatting seed meal in order to estimate its protein content. The total protein in the seed was then fractionated into four fractions.

### Fractionation of seed proteins

Fractionation studies were conducted using an altered version of the proposed method established by Croy *et al.* in 1984. Glutamins and prolamins were extracted in 70% ethanol and 0.1 N NaOH, respectively; albumins and globulins were isolated through extraction using a 50 mM borate buffer (pH 8) and both were then separated by dialysis.

### Estimation of four fractions

Estimation of four fractions was performed using BSA standard curve explained by Bradford formulation (1976).

### Statistical analysis

The variability of the data is expressed as the mean value ± standard error (SE) in the tables and figures. Each replication was measured using a mean of three readings. Statistical Packages for Social Sciences (SPSS) version 16.0 and Microsoft Excel version 2010 were used for statistical evaluation. To find the difference between the data, the same programme was utilised to conduct a post hoc test (Duncan). To determine whether there were statistically significant differences between the different estimations, a one-way ANOVA was used.

## RESULTS AND DISCUSSION

The effects of mineral supply at different levels of salinity on the amount of four protein fractions are shown in Fig 1-4. With the increase in minerals supply, the amount of four fractions increased under different levels of salinity.

Albumins increased from 27.9 to 31.7, 25.8 to 31.0 and 23.9 to 30.2 mg/g seed meal in S.T. genotypes under 4 dS $m^{-}$, 7 dS $m^{-}$ and 10 dS $m^{-}$ salinity levels along with the application of $Ca_2$ to $Ca_5K_5$ respectively (Fig 1). In S.S. genotype, it increased 26.8 to 29.9, 23.8 to 29.7, 21.7 to 27.8 mg/g seed meal at increasing levels of 4 dS $m^{-}$, 7 dS $m^{-}$ and 10 dS $m^{-}$ salinity with change in mineral regime respectively (Fig 1). In case of globulins (Fig 2), they increased under all three increasing salinity levels from

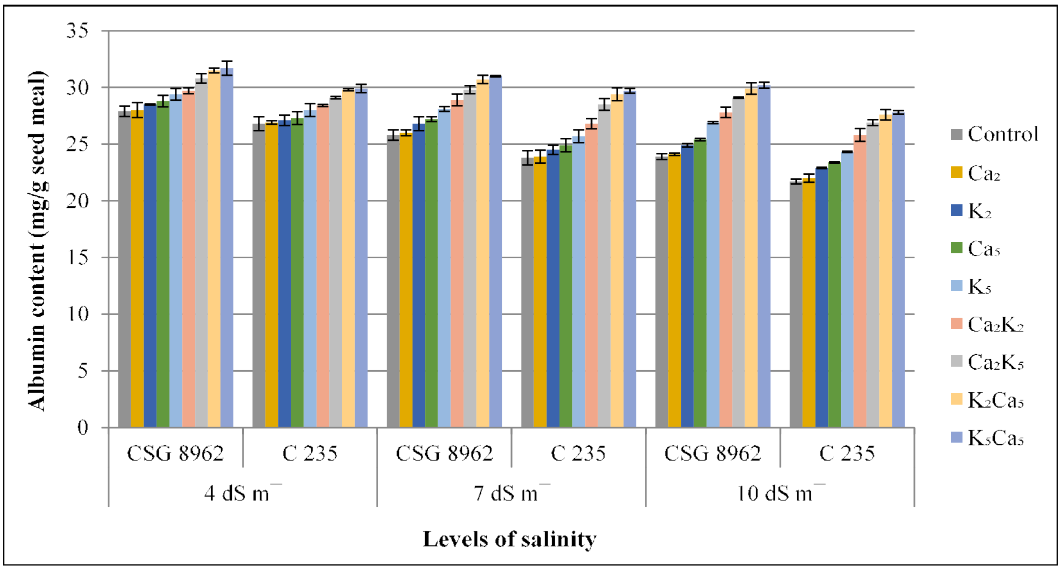

**Fig 1:** Effect of salinity and minerals on the albumin fraction.

119.2 to 124.3, 117.2 to 123.4 and 115.7 to 123.5 mg/g seed meal in S.T. genotype and from 118.4 to 123.3, 115.8 to 121.8 and 113.3 to 121.1 mg/g seed meal in S.S. variety with switching the minerals from $Ca_2$ to $Ca_5K_5$ respectively. Glutelins and prolamins also increased under salinity stress with the use of minerals with the addition of $Ca_5K_5$ over $Ca_2$. Glutelins content improved (Fig 3) at all salinity stress levels from 25.8 to 27.2, 23.5 to 26.1 and 21.7 to 25.7 mg/g seed meal in S.T. while in S.S. genotype it increased from 24.1 to 25.7, 21.8 to 25.5 and 18.7 to 24.5 mg/g seed meal. Prolamins, under all salinity levels, exhibited minor improvements by application of minerals of all combinations, $Ca_2$ to $Ca_5K_5$ (Fig 4). Application of Ca and K individually as well as in combination was found to be gradual in total amount and content of four fractions under increasing levels of salinity stress (Fig 1-4) which aligns with findings of Waraich *et al*. 2012; Wang *et al*. 2013 and Tripathi *et al*. (2014).

Mineral application alleviated the detrimental effect of the salinity in both genotypes, but more so in the case of genotypes that are more sensitive to salinity than those that are tolerant of it, as observed in chickpea and brassica (Mann *et al*., 2019; Naveed *et al*., 2020). It was observed that as salinity level increased along with the application of minerals, the amount of four fractions also improved which aligned with the statement that $K^+$ and $Ca^{2+}$ were added together, the injurious effects of salt stress were significantly reduced (Pathak *et al*., 2020). Also, it was noticed that sensitive genotypes exhibited more improvement than tolerant genotypes (Fig 1-4), as

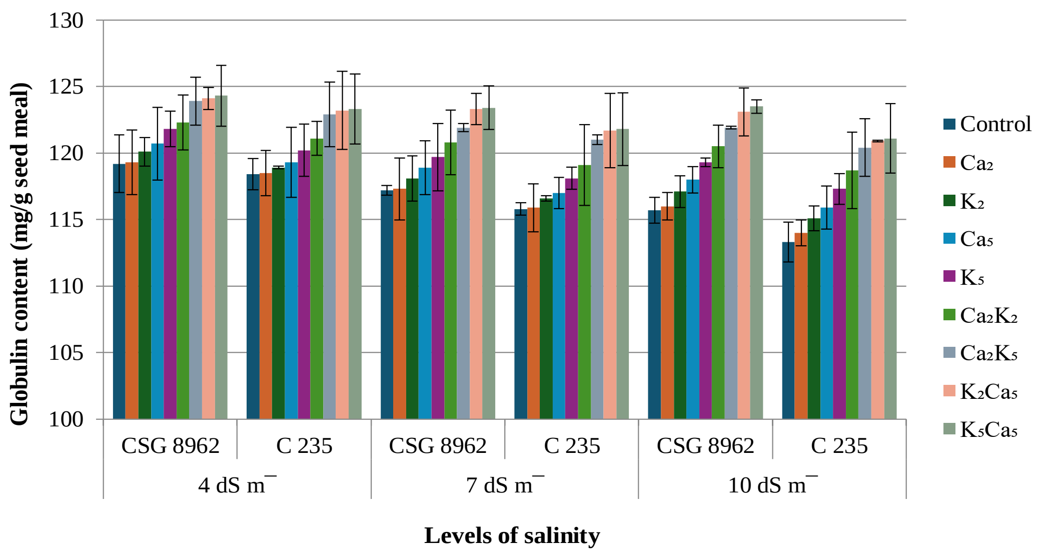

**Fig 2:** Effect of salinity and minerals on the globulin fraction.

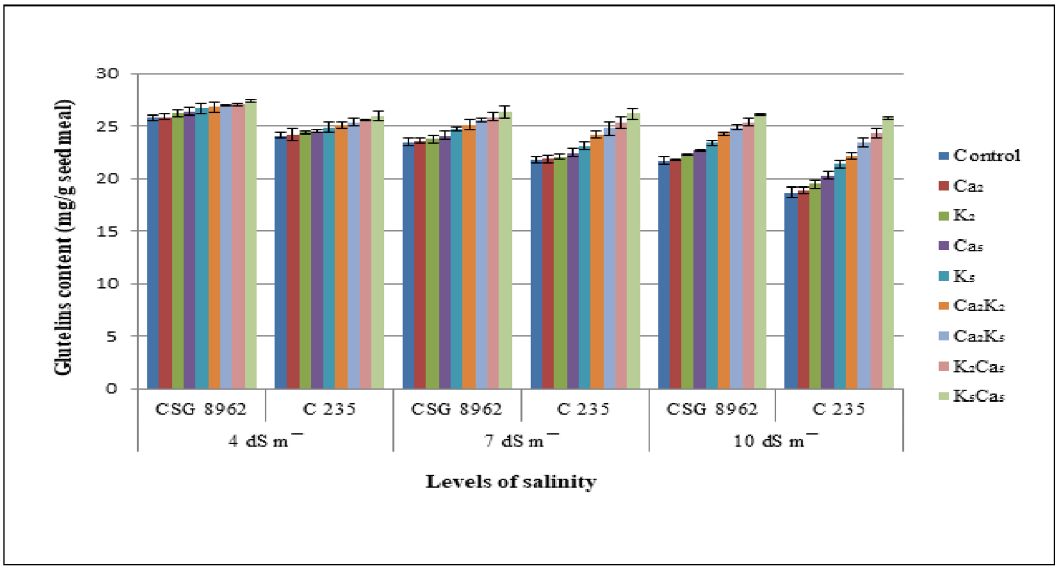

**Fig 3:** Effect of salinity and minerals on the glutelin fraction.

observed by Rahman *et al.* (2005) in wheat, which may be due to improved concentration of $K^+$ over $Na^+$ ions, which improved transpiration efficiency, enriched antioxidant systems by osmotic potential regulations and ultimately enhanced immune responses of plants towards salinity stress (Zhu *et al*., 2022; Rahman *et al*., 2022; Ievinsh *et al*., 2022).

At the maximum stress level (10 dS $m^{-1}$), highest improvement in the amount of all four fractions was noticed under nutrient supply, as observed in rice by Abdel-Haliem *et al.* (2017). Prolamins reflected a minor improvement in the four fractions under increasing levels of salinity with the application of minerals. The combination of minerals ($Ca_5K_5$) proved to be the best way to lessen the impact of salinity in chickpeas, which justified the findings of Shariat-Jafari *et al.* (2009), according to which the addition of $K^+$ and $Ca_2^+$ collectively led to improvements in morphological characteristics and plant development in sorghum, which already proved in maize (Zidan *et al*., 1991).

Calcium was found to minimize the injurious effect of salinity stress on germination in peas, wheat, common sunflower, tomato and wild spinach (Türkmen *et al*., 2004; Liu and Wang, 2010). Calcium binds to organic molecules that have negative groups, like phosphate and carboxyl groups in sugars, proteins and phospholipids. Ca helps to maintain the membrane's integrity in both normal and stressful situations (Maathuis, 2009). Ca also helps plants to absorb nutrients, control hormones and enzymes and keep cell membranes stable to protect them from abiotic stress (Rahman *et al*., 2015). By activating $Ca^{2+}$-dependent stress-responsive genes, ROS play a regulatory role in expressing plant responses to stress, as proved in *Arabidopsis* (Mittler *et al*., 2004). Through the stronger cystolic $Ca^{2+}$ signal, the $Ca^{2+}$ binding protein then adjusts and protects plants' responses to stress conditions (Parvin *et al*., 2019). The Ca-dependent protein kinases (CDPK) control the physiological reactions of plants to abiotic stress, such as stomatal movement, $K^+$ absorption and particular gene expression that respond to stress (Yu *et al*., 2007). Additionally, $Ca^{2+}$ regulates the activity of antioxidant enzymes like SOD, CAT and POD and an accumulation of antioxidants promotes defence against salt stress (Shoresh *et al.* 2011).

Potassium was examined to alleviate abiotic stress in rice, wheat, oats, Indian mustard, cotton, *etc.* (Zain *et al*., 2014; Ahanger *et al*., 2015; Zahoor *et al*., 2017; Singh *et al*., 2019; Rani *et al*., 2021). Under abiotic stress conditions, potassium (K) is an essential macronutrient for physiological development and improved agricultural growth of plants (Wang *et al*., 2017). Additionally, $K^+$ helps to regulate numerous biochemical procedures leading to protein syntheses, metabolism of carbohydrates, enzyme activation and water regulation in plants, as well as photosynthetic processes, transport of the phloem and the exchange of cation-anion balance (Shabala and Cuin, 2008). $K^+$ is important in the osmotic regulations by sustaining the turgor pressure of the cell, regulating enzyme activation, cytoplasmic homeostasis, protein synthesis and membrane potential during salinity stress (Almeida *et al.* 2017). A sufficient supply of minerals is necessary for synthesising proteins, folding and the activation of enzyme systems when they are subjected to salinity stress to reverse damages due to it. Adding K helps raise the potassium-sodium ionic ratio ($K^+/Na^+$), which then makes it easier for higher-affinity $K^+$ transporters to move $Na^+$ and also move $K^+$ along with $Na^+$, which increases $Na^+$ tolerance (Su *et al*., 2015). These processes are necessary for the maintenance of the correct assembly of protein fractions, protein defences and seed quality in legumes (Gharibzahedi *et al*., 2017; Nawaz *et al*., 2020).

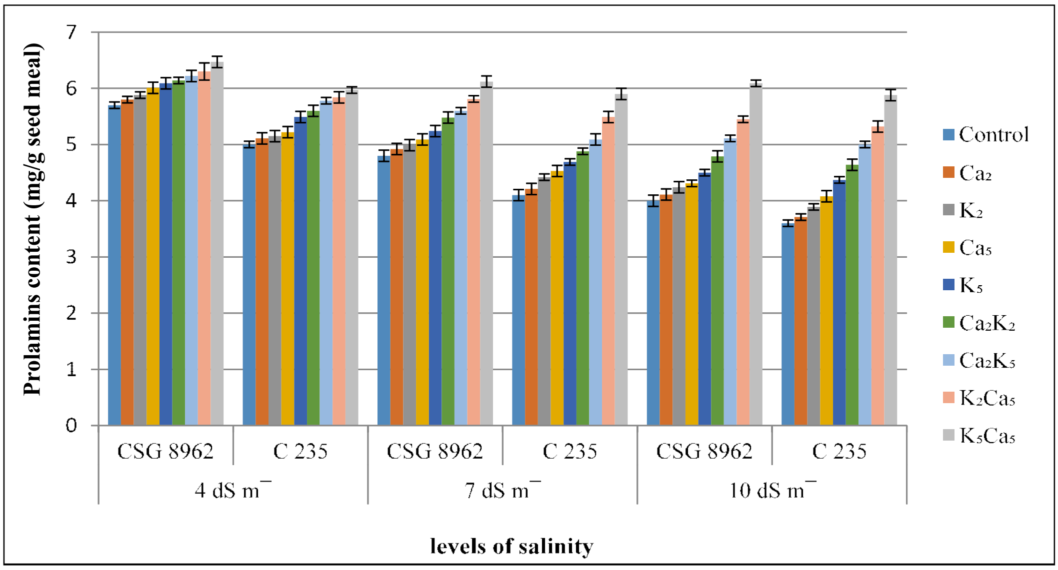

**Fig 4:** Effect of salinity and minerals on the prolamin fraction.

As per the findings of Pfluger and Mengel (1972), the synthesis of the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), involved in photosynthesis, is activated by K. Thus, the increased rate of photosynthesis yields more assimilates, such as amino acids and sucrose, which can be incorporated into storage compounds like seed storage proteins (Egli and Bruening, 2004).

## CONCLUSION

Providing food security is a major concern in agriculture, but different abiotic stresses present a major obstacle to this endeavor. A changing climate exacerbates the negative impact of the stress on plants. Supplying plants with the proper nutrition can assist them in tolerating stress. Nutrients like calcium and potassium, in different combinations, help plants in their osmotic adjustments to stressed abiotic conditions. This strategy provides immediate relief to plants against stress and enhances their seed protein quality. In our study we found that among all tested combinations, $Ca_5K_5$ was most effective in increasing protein content in all four fractions under salinity stress. Improving the seed protein quantity and quality is the primary focus of plant scientists. Supplying plants with a balanced combination of nutrients, including essential amino acids, can additionally enhance their stress tolerance and protein quality. Further studies are required to analyze the effect of additional mineral supply on crop yield and mechanisms of action of these nutrients in plant defense under stress conditions.

### Conflict of interest

The authors declare that they have no conflict of interest.

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