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ORIGINAL ARTICLE **OPEN ACCESS**

Characterization of Seed Protein Fractions in *Cicer arietinum* under Salinity Stress

Sandeep Ghosh, Divya Batra, Amit, Yogesh Kumar*, N.K. Matta
Department of Botany, Kurukshetra University, Kurukshetra, Haryana, 136119, India
*Corresponding author: Email: ykskuk11@gmail.com

ABSTRACT

The world's population faces the challenge of food insecurity with an increasing number of undernourished individuals. Legumes, particularly chickpeas, offer a promising solution as they are rich in protein and essential nutrients. However, climate change-induced salinity stress poses a threat to chickpea productivity and protein quality. This study aimed to understand the impact of rising salinity on chickpea seed protein fractions to enhance human nutrition and food product development. Two chickpea genotypes, one salt-sensitive (C 235) and one salt-tolerant (CSG 8962) were subjected to three different salinity levels (4 dS m^{-1}, 7 dS m^{-1}, and 10 dS m^{-1}). The total seed protein content decreased in both genotypes under salinity stress. The salt-sensitive genotype experienced a more significant decrease in protein content than the salt-tolerant one. The four seed protein fractions (albumins, globulins, glutelins, and prolamins) also showed a decreasing trend with increasing salinity. Amino acid analysis revealed a decline in tryptophan, methionine, and cysteine content in most protein fractions under salinity stress. Electrophoretic analysis showed qualitative and quantitative variations in protein fractions in response to salinity stress. Our study helped to understand the adverse effects of salinity stress on chickpea seed protein fractions and amino acid composition, potentially impacting its nutritional value. Understanding these changes can aid in developing strategies to improve chickpea productivity and nutritional quality in the face of climate change-induced salinity stress.

Keywords: *Amino acids, legumes, protein fractions, salinity.*

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INTRODUCTION

The world's population is growing by ~ 1.1% per year, and if current trends continue, the world's population is projected to reach 9.7 billion by 2050 (1). According to the Food and Agricultural Organisation (FAO), the number of undernourished people in the world reached 821 million in 2017 (2). This rise in food insecurity indicates a significant risk of falling short of achieving the Sustainable Development Goal (SDG) target of hunger eradication by 2030 (2), further, COVID-19 has adversely affected the food-unsecured population (3). Legumes have higher protein content than most plant foods, with about twice the protein content of cereals (4). As they provide dietary proteins and micronutrients, furnish possible benefits in developing countries to meet their growing needs for nutrition (5). Therefore, including food legumes in the diet can improve their health status. Regarding world production, food legumes rank third after cereals and oilseeds, strongly impacting the agroecosystem and human nutrition (6). Nowadays, chickpea is grown in an area of 14.56 million ha in the world with a total yield of 11.5 million tons (7). Legumes are valuable sources of food (protein), feed and forage. They are the source of a wide range of nutrients that include protein with high essential amino acids, complex carbohydrates (~60%), fibres (5–37%), essential minerals and vitamins (8). Further, legumes contain a high concentration of globulins and albumins, 70% and 20%, respectively, with prolamins and glutelins serving as minor protein fractions (9). Climate change and accompanying biotic and abiotic factors impact crop productivity and yield, as evidenced by the diminishing grain productivity of major crops worldwide (10). Drought, heat stress, salinity, and heavy metal (HM) contaminations affect the legume's growth, yield, and quality (11,12). Of these, salt stress is very severe and causes detrimental effects on plant growth, physiological and molecular processes and productivity of economically important crops (13,14,15,16,17). More than 833 million hectares of soil are salt-affected around the globe (8.7% of the planet) (18). It has been estimated that the increase in salinized soils will have devastating effects, resulting in 30% of soil productivity loss within the next 25 years and 50% by 2050 (19). Moreover, severe salt concentration causes many metabolic

disturbances in plants that perturb the cellular redox potential and ultimately lead to cell death (20), generating Reactive Oxygen Species (ROS), ionic toxicity, peroxidation of lipids, enzyme inactivation and degradation of proteins

Chickpea (*Cicer arietinum* L.) is a major leguminous crop that ranks second after the beans with a mean annual production of about 12.8 million tones with approx. 73% of production originates from India (21,22). Chickpea seeds' protein content varies from 16% to 28% (23) and is consumed as a good source of proteins, several minerals like iron, magnesium, calcium, zinc, phosphorus, soluble and insoluble fibre, oligosaccharides, phenolics, and essential nutrients such as vitamins, antioxidants, and biologically active compounds (24). However, salinity stress has been reported to reduce the grain quality and composition (in terms of total yield, oil content, total protein, soluble sugars, loss of P, K and Mg) in soybean, lentil, mung bean, faba bean, punto bean, etc. (6,25,26,27,28,29). Existing research majorly focused on aspects of the total protein content in chickpeas rather than seed protein fractions. Our current research aims to fill this knowledge gap by identifying and characterizing specific protein components, such as albumins, globulins, glutelins, and prolamins, to understand chickpeas' nutritional composition and potential applications better. Therefore, considering the importance of chickpeas in human nutrition and the increasing soil salinity, the present work was proposed to understand how the rising salinity affects seed protein fractions in chickpeas to attain valuable insights into chickpea protein quality, human nutrition, and food product development.

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. 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 Na_2SO_4, $CaCl_2$ and NaCl in the ratio of 1:2:7 w/v following Richards formulation (1954). A volume of 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.

Total seed protein estimation

The semi-micro Kjeldahl method, devised by Peach and Tracey (1956) (30), was used to quantify the total nitrogen in 100mg of seed meal. The calculated nitrogen percentage was multiplied by 6.25, a standard multiplication factor, to determine the total seed protein content.

Fractionation of seed proteins

The methods described by Croy *et al*. (1984) (31), 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.

SDS-polyacrylamide gel electrophoresis

The polypeptide pattern of the four seed protein fractions was studied by electrophoretic analysis on SDS gels, following the discontinuous system developed by Davis (1964) (32) and Ornstein (1964) (33) and the methodology proposed by Laemmli (34). The gels were quantitatively analysed by using GelAnalyzer version 19.1. To simplify our data and graphs, we divided all collected raw values by thousand in our computations.

Tryptophan Estimation

Tryptophan content was estimated using the methodology outlined by Spies and Chambers (35).

Methionine Estimation

Methionine was estimated by following the protocol prescribed by McCarthy and Sullivan (36).

Cysteine Estimation

The analytical approach devised by Goa (1961) was used for the estimation of cysteine content (37).

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.

RESULTS AND DISCUSSION

Total seed protein content and four seed protein fractions

The seed protein content in salt-tolerant (CSG 8962) and salt-sensitive (C 235) chickpea genotypes at varying salinity levels can be seen in Fig. 1. In the salt-sensitive (S.S.) cultivar, the mature protein content showed a considerable decrease from 24.8% in control to 14.8% at the highest level (10 dS m^-) of salinity. In the case of the salt-tolerant (S.T.) genotype, the protein content decreased from 26.4% (control) to 19.1% at 10 dS m^- salinity level. The effects of different salinity levels on the four protein fractions can be seen in Fig. 2. With the increase in the level of salinity from zero in control to 10 dS m^- in the S.S. genotype, albumins decreased from 31.1 to 21.7 mg/g seed meal; the globulins decreased from 123.9 to 113.3 mg/g seed meal. Glutelins and prolamins also followed a decreasing trend with an increase in the level of salinity. In the case of the S.T. genotype, an increase in salinity level also diminished all four protein fractions. Albumins decreased from 31.6 to 23.9 mg/g seed meal, globulins from 124.8 to 115.7 mg/g seed meal, glutelins from 26.2 to 21.8 mg/g seed meal and prolamins from 6.1 to 4.1 mg/g seed meal. The decrease in total seed protein content under salinity stress, as observed in the present study, has also been reported earlier in different crops (6,25,27, 28,38,39,40,41). The decrease in total seed protein was more (40.3%) in the salt-sensitive genotype than salt-tolerant genotype (25.0%) (Fig.1). Plants have developed several mechanisms and strategies to achieve salinity tolerance, including tolerance due to osmo protectants, production of ROS and stress proteins, and finally, by maintaining ion homeostasis. During periods of salt stress, the nitrate (NO_3^-) acquisition from the soil and the N metabolism of grain legumes are affected, resulting in lower grain protein content (25). The decrease in protein content may result from an enhanced rate of protein degradation leading to a decline in protein content (42). Additionally, soil salinity contamination significantly reduces the symbiotic nitrogen fixation (43), which reduces the accumulation of seed storage protein. The salt-tolerant genotype exhibited enhanced salinity tolerance as compared to the salt-sensitive genotype (C 235) due to the activation of genes including late embryogenesis, HI and 219 genes/sequences, as well as non-specific lipid transfer protein, which helped protect the cellular membrane and macromolecules under salinity stress (44).

Amino acids

The four protein fractions analyzed for tryptophan, methionine and cysteine content of each fraction to the total content of each amino acid under increasing levels of salinity are shown in Table 1 - 3. Treatment of C 235 with increasing levels of salinity (from control to 10 dS m^-) resulted in a decrease in total tryptophan content due to albumins (6.47%), glutelins (10.45%) and prolamins (28%). In contrast, the tryptophan content due to globulins increased by 3.26%. In CSG 8962, increased salinity also led to a fall in the total tryptophan content due to albumins (8.43), glutelins (10.39%) and prolamins (24.55%) (Table 1). The content of tryptophan increased by globulins (4.19%). The total methionine due to albumins (18.16%), globulins (13.76%), and prolamins (4.11%) decreased as the salinity increased in salt-sensitive genotype i.e. C 235. After treatment with different salinity levels in the salt-tolerant genotype (CSG 8962), total methionine increased due to glutelins (8.08%). In contrast, methionine content due to albumins (12.48%), prolamins (31.4%) and globulins (12.92%) followed a decreasing trend as shown in Table 2. In both the genotypes, the total cysteine content followed a decreasing trend due to glutelins and prolamins and an increasing pattern due to albumins and globulins with the rise in the level of salinity. The total cysteine content due to glutelins (14.68%) and prolamins (37.4%) followed a decreasing trend with an increase in salt concentration in C 235 genotype, while due to albumins (17.7%) and globulins (9.79%) followed an increasing pattern (Table 3). In both genotypes, a decrease in albumins, glutelins, and prolamins fractions resulted in a reduction in tryptophan content, while a decrease in albumins, globulins, and prolamins led to a decline in methionine content. Additionally, a decrease in glutelins and prolamin fractions contributed to a decrease in the total cysteine content. The downfall in the content of amino acids may be ascribed to a decrease in the nitrogen uptake under salinity stress as reported in earlier studies (45,46). Additionally, decreased rate of protein synthesis, the increased activities of hydrolyzing enzymes, the decreased availability of amino acids, or the denaturation of the enzymes concerned with amino acids and protein synthesis may be responsible for a decrease in protein content (47).

Polypeptide patterns on SDS-gels

The polypeptide patterns of different seed protein fractions of the genotype HC5 and standard protein molecular weight markers are shown in Fig. 3. Both qualitative and quantitative differences were observed in the polypeptide patterns of protein fractions under the influence of increasing levels of salinity on S.T. and S.S. genotypes (Fig. 4 - 5) as founded earlier in different crops such as pea (48), broad bean (49), mung bean (50), lentils (51), etc. Globulins are composed of many polypeptides with molecular weights ranging between *Mr* 13 kDa and 88 kDa in both genotypes. In the S.T. genotype with control treatment, globulin's polypeptides of *Mr* 60 kDa, 53 kDa, 49 kDa, 36 kDa, 30 kDa, 28 kDa, 16 kDa and 13 kDa were intense and dark, followed by those polypeptides which were prominent but relatively of low intensity along with polypeptides with lightly stained bands. In the S.S. genotype, at salinity levels 7 dS m^{-} and 10 dS m^{-}, new bands of globulins appeared with *Mr* 26 kDa, 25 kDa and 11 kDa. Newly formed bands are stress proteins which may be the result of enhanced expression of transcriptional upregulation, believed to play a role in the plant's response and adaptation to the salinity stress (52,53). Albumins were represented by polypeptides of *Mr* 19 kDa to 60 kDa in the S.T. genotype. The polypeptide of *Mr* 19 kDa is intense and darkly stained while the polypeptides of *Mr* 28 kDa are prominent but lightly stained. At the highest level of salinity new band of *Mr* 22, kDa appeared as new in the S.S. genotype. The newly formed polypeptides in the salinity stress may indicate the cell's defensive response to counteract the detrimental effects of salt (54). Furthermore, the identification of novel polypeptides may be associated with NaCl ion toxicity, which can activate signalling cascades impacting gene expression (55). Salinity can lead to genotoxic impairment and structural modifications in DNA, including chromosomal rearrangements, strand breaks, base deletions, pyrimidine dimers, mutations, cross-links, and base alterations (56). The polypeptides in glutelins were lightly stained and ranged between *Mr* 12 kDa to 46 kDa for both genotypes while the polypeptides of prolamins were not visible on the gel. At the maximum level of salinity (10 dS m^{-}), all polypeptides in each fraction become light for both genotypes. This was in accordance with the results of Badran *et al*. (2015) in alfalfa (57). The alterations in intensity patterns may arise due to a series of biochemical and molecular changes in plants under stress, as they endeavour to adapt to the variations in gene expression (58).

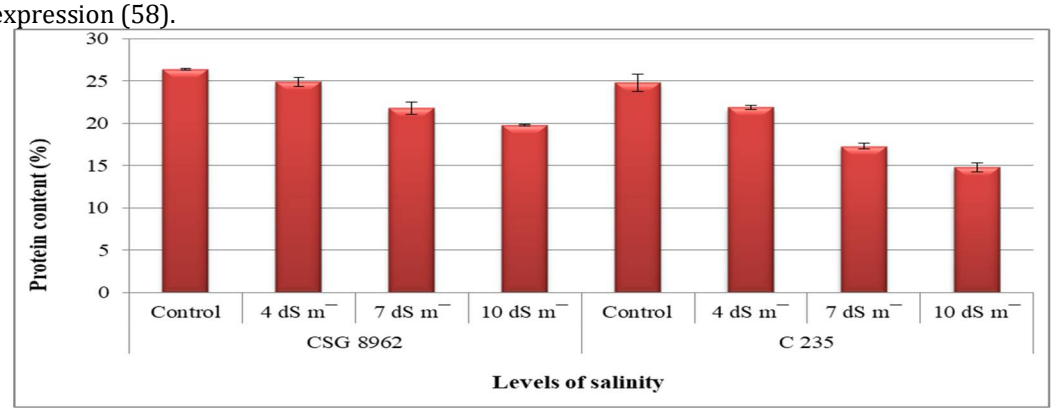

Fig. 1 The seed protein content (%) under different levels of salinity

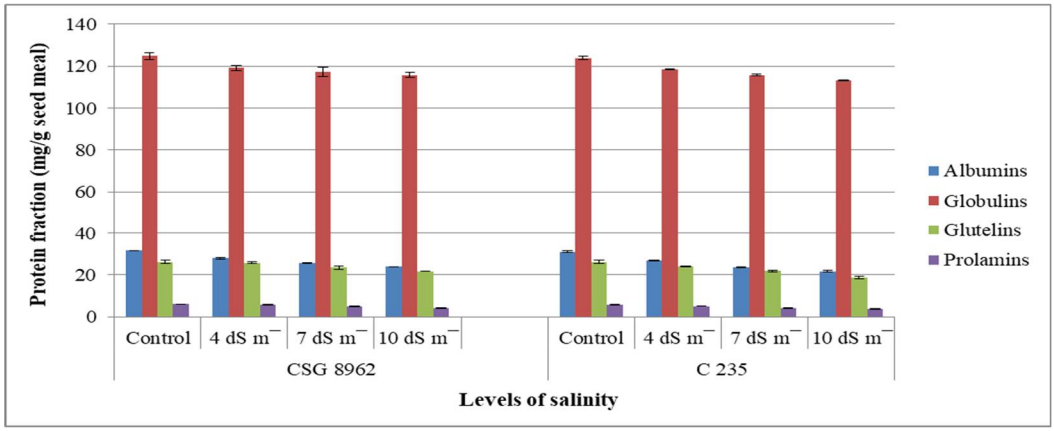

Fig. 2 Effect of salinity on four seed protein fractions

Table 1. Effect of different levels of salinity on total tryptophan content of four protein fractions in salt sensitive and salt tolerant lines.

Genotype	Total tryptophan content (g/100g protein)				
	levels of salinity	Seed protein fractions			
		Albumins	Globulins	Glutelins	Prolamins
CSG 8962	Control	1.41 ± 0.00^{a}	3.172 ± 0.05^{b}	1.395 ± 0.00^{a}	0.395 ± 0.00^{d}
	4 dS m^{-}	1.362 ± 0.02^{b}	3.199 ± 0.07^{b}	1.34 ± 0.00 ^{b}	0.38 ± 0.00^{e}
	7 dS m^{-}	1.319 ± 0.03^{bc}	3.267 ± 0.04^{b}	1.297 ± 0.02^{b}	0.357 ± 0.01^{f}
	10 dS m^{-}	1.291 ± 0.02^{c}	3.305 ± 0.06^{b}	1.25 ± 0.02^{c}	0.298 ± 0.00^{g}
C 235	Control	1.207 ± 0.00^{d}	3.902 ± 0.06^{a}	1.119 ± 0.01^{d}	0.557 ± 0.00^{a}
	4 dS m^{-}	1.173 ± 0.01^{de}	3.957 ± 0.06^{a}	1.08 ± 0.01^{d}	0.493 ± 0.01^{b}
	7 dS m^{-}	1.16 ± 0.00^{de}	4.008 ± 0.09^{a}	1.035 ± 0.01^{e}	0.442 ± 0.00^{c}
	10 dS m^{-}	1.129 ± 0.01^{e}	4.029 ± 0.03^{a}	1.002 ± 0.02^{e}	0.401 ± 0.01^{d}

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

Table 2. Effect of different levels of salinity on total methionine content of four protein fractions in salt sensitive and salt tolerant lines.

Genotype	Total methionine content (g/100g protein)				
	levels of salinity	Seed protein fractions			
		Albumins	Globulins	Glutelins	Prolamins
CSG 8962	Control	1.49 ± 0.03^{a}	3.103 ± 0.03^{a}	1.138 ± 0.03^{g}	0.557 ± 0.00^{b}
	4 dS m^{-}	1.443 ± 0.00^{ab}	3.045 ± 0.08^{a}	1.17 ± 0.01^{fg}	0.45 ± 0.01^{c}
	7 dS m^{-}	1.392 ± 0.03^{b}	2.851 ± 0.06^{b}	1.205 ± 0.01^{ef}	0.401 ± 0.00^{d}
	10 dS m^{-}	1.304 ± 0.02^{c}	2.702 ± 0.06^{b}	1.23 ± 0.00^{e}	0.382 ± 0.00^{e}
C 235	Control	1.415 ± 0.00^{b}	2.855 ± 0.05^{b}	1.289 ± 0.01^{d}	0.607 ± 0.00^{a}
	4 dS m^{-}	1.29 ± 0.02^{c}	2.694 ± 0.00^{b}	1.403 ± 0.02^{c}	0.563 ±0.00^{b}
	7 dS m^{-}	1.207 ± 0.02^{d}	2.531 ± 0.06^{c}	1.536 ± 0.01^{b}	0.41 ± 0.00^{d}
	10 dS m^{-}	1.158 ± 0.02^{d}	2.462 ± 0.05^{c}	1.647 ± 0.00^{a}	0.321 ± 0.01^{f}

Each value is a mean of three replicates, ± SE and means followed by same letter/s are not significantly different at P ≤ 0.0

Table 3. Effect of different levels of salinity on total cysteine content of four protein fractions in salt sensitive and salt tolerant lines.

Genotype	Total cysteine content (g/100g protein)				
	levels of salinity	Seed protein fractions			
		Albumins	Globulins	Glutelins	Prolamins
CSG 8962	Control	0.782 ± 0.01^{d}	1.682 ± 0.02^{cd}	0.817 ± 0.00^{b}	0.305 ± 0.00^{c}
	4 dS m^{-}	0.813 ± 0.00^{c}	1.734 ± 0.03^{bc}	0.776 ± 0.00^{cd}	0.252 ± 0.00^{d}
	7 dS m^{-}	0.869 ± 0.00^{b}	1.807 ± 0.04^{ab}	0.728 ± 0.01^{e}	0.238 ± 0.00^{e}
	10 dS m^{-}	0.908 ± 0.00^{a}	1.878 ± 0.03^{a}	0.659 ± 0.01^{f}	0.201 ± 0.00^{g}
C 235	Control	0.603 ± 0.00^{h}	1.592 ± 0.01^{e}	0.858 ± 0.01^{a}	0.355 ± 0.00^{a}
	4 dS m^{-}	0.659 ± 0.01^{g}	1.645 ± 0.00^{de}	0.802 ± 0.01^{bc}	0.332 ± 0.01^{b}
	7 dS m^{-}	0.687 ± 0.02^{f}	1.697 ± 0.03^{bcd}	0.764 ± 0.01^{d}	0.298 ± 0.00^{c}
	10 dS m^{-}	0.71 ± 0.00^{e}	1.748 ± 0.03^{bc}	0.732 ± 0.01^{e}	0.222 ± 0.00^{f}

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

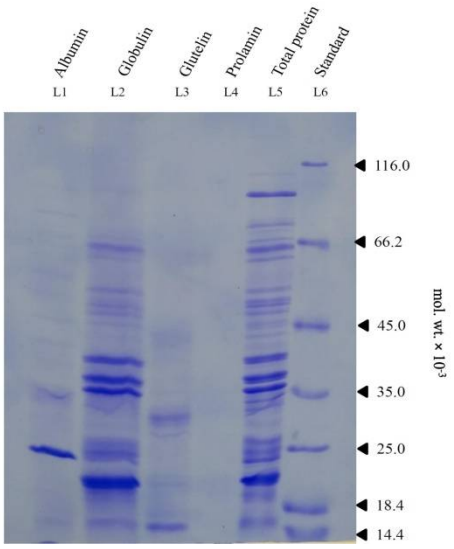

Fig. 3 SDS–PAGE of four seed protein fractions

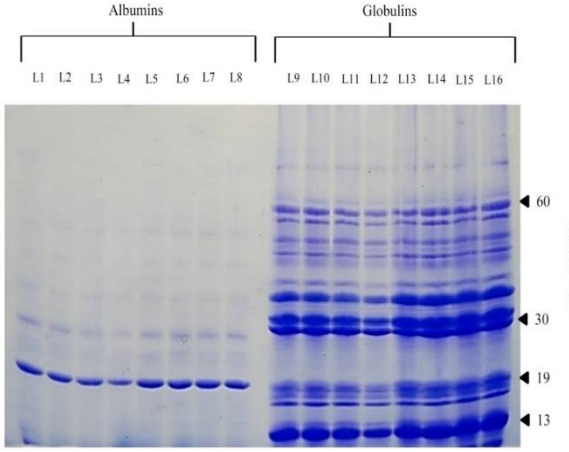

Fig. 4

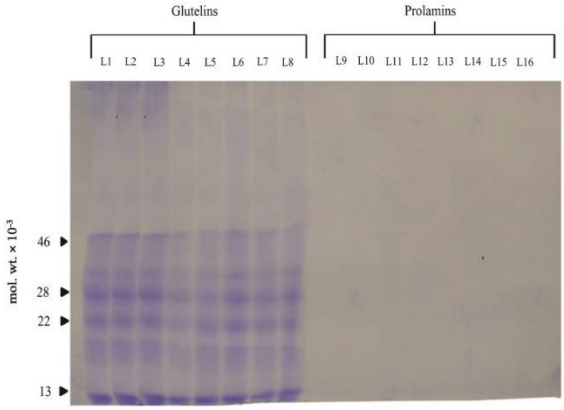

Fig. 5

Fig. 4-5 SDS–PAGE of four fractions from seeds of genotypes **CSG 8962** (L1-L4 and L9-L12) and **C 235** (L5-L8 and L13-L16) grown under different levels of salinity (L1, L5, L9, L13= Control, L2, L6, L10, L14= 4 dS m-, L3, L7, L11, L15= 7 dS m-, L4, L8, L12, L16= 10 dS m-)

CONCLUSION

Salinity stress can lead to a decrease in total seed protein content and alterations in the composition of seed protein fractions, including albumins, globulins, glutelins, and prolamins. This study sheds light on the specific changes in amino acids and polypeptide patterns in chickpeas under salinity stress, which may provide valuable insights into improving chickpeas' nutritional composition and its potential applications in human nutrition and food product development. These findings underscore the importance of understanding the impact of rising salinity on crop protein quality to address the challenges of food security in a changing climate.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that they have no conflict of interest.

AUTHOR'S CONTRIBUTION

Conceptualisation and designing of the research work were carried out by Yogesh Kumar and NK. Matta. Sandeep Ghosh and Divya Batra did field/lab work and data collection; Sandeep Ghosh and Amit performed data analysis and interpreted the results; and finally, the manuscript was prepared by Yogesh Kumar and Sandeep Ghosh.

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