JSML
Journal of Science and Mathematics Letters
ISSN 2462-2052 • eISSN 2600-8718

Research Article

Electrophoretic Polymorphism of Globulin Proteins and its Association with Nutritional and Physicochemical Traits in Chickpea (*Cicer arietinum* L.)

Mahbuba Salmanova[1]*, Gatiba Hasanova[2], Akbar Karimov[3], Saida Hasanova[1], Sevinj Nuriyeva[1]

[1] Department of Cereals and Legumes, Ministry of Science and Education Institute of Genetic Resources, AZ1106, Baku, Azerbaijan
[2] Department of Grain Quality, Azerbaijan Scientific Research Institute of Agriculture, AZ1098, Baku, Azerbaijan
[3] Department of Biochemical Genetics and Genomics, Ministry of Science and Education Institute of Genetic Resources, AZ1106, Baku, Azerbaijan
* Corresponding author: salmanovamahbuba7@gmail.com

ARTICLE HISTORY

Received
28 November 2025
Revised
10 March 2026
Accepted
28 March 2026
Published
11 April 2026

KEYWORDS

Electrophoretic analysis
Genetic diversity
Genotypes
Spectrum
Pattern

ABSTRACT

The aim of the study was to assess the variability and structural features of chickpea globulin proteins across the electrophoretic zones ω, γ, β, and α. The research employed field observations, polyacrylamide gel electrophoresis, densitometric and spectrophotometric analyses, alongside statistical clustering methods. These techniques successfully distinguished four characteristic zones reflecting proteins of varying molecular masses, enabling a comprehensive assessment of intraspecific polymorphism. Our findings revealed that the high-molecular-weight ω-zone exhibits the greatest structural stability, whereas the γ-zone displays the highest variability, indicating the emergence of diverse globulin isoforms. The β-zone, representing the majority of the total protein complex, contained unique marker fractions, while the low-molecular-weight α-zone showed notable intensity variations across specific genotypes. A substantial proportion of the analyzed genotypes demonstrated unique protein fractions, reflecting a high level of genetic heterogeneity while confirming the fundamental structural role of specific, highly recurrent protein spectra. Ultimately, the identification of these distinct protein polymorphism patterns provides a novel, reliable biochemical marker system to accelerate the selection of genetically diverse, structurally resilient, and nutritionally enhanced chickpea cultivars in modern breeding programs.

https://doi.org/10.37134/jsml.vol14.2.10.2026

1. INTRODUCTION

The growing interest in the genetic diversity of chickpea (*Cicer arietinum* L.) is driven by its crucial role as a source of plant protein and its ability to restore soil fertility through nitrogen fixation under changing climate conditions (Bekbayev et al., 2024; Namazbekova et al., 2024). Enhancing chickpea productivity and stress resilience is a global priority (Cordoba et al., 2025); however, limited knowledge of intraspecific variability in local and introduced forms restricts breeding efficiency (Tultabayeva et al., 2023; Valujeva et al., 2022). Seed storage globulins act as reliable biochemical markers that reflect a genotype's adaptive potential, nutritional value, and genetic uniqueness (Grewal et al., 2022; Joshi-Saha et al., 2021; Tiruneh et al., 2025). Previous studies have widely demonstrated that analyzing the polymorphism of globulin fractions, particularly in the highly variable γ- and β-zones, is an effective tool for assessing intraspecific variability, establishing structural characteristics, and selecting high-protein parental lines across various plants and legumes, including soybean (Bayramli, 2024), common bean (Nasrullayeva et al., 2023), and chickpea (Ye et al., 2024; Jesus et al., 2025). Similar biochemical profiling approaches have also proven valuable in highlighting bioactive and metabolite variability across diverse plant systems (Benarba et al., 2025; Molina-Jauregui et al., 2025).

While modern genomic, transcriptomic, and molecular approaches, such as analyzing single nucleotide polymorphisms, as reported by Abaszade et al. (2024), or specific regulatory genes like ROP1 ENHANCER1, as shown by Chakraborty et al. (2023), offer deep insights into the mechanisms controlling genetic heterogeneity and storage protein synthesis, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) remains highly relevant. It uniquely and cost-effectively visualizes the structural and functional polymorphism of translated protein complexes across distinct molecular weight zones, even when complemented by advanced mass spectrometry methods like MALDI-TOF MS, as demonstrated by Di Francesco et al. (2024). Beyond previous chickpea studies that primarily focus on total protein content or utilize advanced omics to map broad genetic diversity, SDS-PAGE provides immediate, phenotypic evidence of structural differentiation at the zonal level. Despite this utility, the specific mechanisms governing the structural stability of the individual ω, γ, β, and α zones across diverse chickpea genotypes remain insufficiently investigated. Similarly, there is a gap in understanding how these specific structural variations correlate with morphological traits, yield, and environmental influences, such as the water deficit effects on globulin expression established by Di Francesco et al. (2025).

Therefore, the aim of this study was to determine the genetic diversity and degree of polymorphism of globulin proteins in different chickpea genotypes through electrophoretic analysis of their profiles specifically mapped to the ω, γ, β, and α zones. The objectives of the study were: (1) to conduct electrophoretic analysis to explicitly characterize and distinguish these four structural zones; (2) to construct idiograms and dendrograms assessing the genetic relatedness of the genotypes; and (3) to identify direct relationships between these compartmentalized protein profiles, yield, and seed quality indicators.

2. METHODOLOGY

Field and laboratory research was conducted in 2024 at the Absheron experimental station of the Institute of Genetic Resources of the National Academy of Sciences of Azerbaijan. The soils of the experimental site are grey-brown and weakly alkaline (pH 7.4-7.6), with a humus content of 1.5-2.0% and low reserves of available phosphorus and potassium. The regional climate is typically arid subtropical, characterised by mild winters and hot summers; the mean annual precipitation amounts to 180-220 mm, most of which falls during the spring period.

The object of the study comprised 76 chickpea genotypes (*Cicer arietinum* L.), including introduced lines from the International Centre for Agricultural Research in the Dry Areas (ICARDA) and local Azerbaijani samples of the same species, which have formed under long-term adaptation to the Absheron climate and are distinguished by the stability of economically valuable traits. The selection of these 76 chickpea genotypes was determined by the need to cover the widest possible range of hereditary variability from ICARDA-introduced lines with confirmed breeding value to local Azerbaijani forms adapted to the arid conditions of Absheron. The genotypes analysed are presented in Table 1.

Table 1. List of studied chickpea genotypes (*Cicer arietinum* L.)

Names and numbers of samples							
1	Flip13-70c	20	Flip13-320c	39	Flip11-21c	58	Flip11-215c
2	Flip13-151c	21	Flip13-330c	40	Flip10-338c	59	Flip11-45c
3	Flip13-153c	22	Flip13-335c	41	Flip11-167c	60	Flip11-72c
4	Flip13-154c	23	Flip13-336c	42	Flip11-76c	61	Flip11-210c
5	Flip13-194c	24	Flip13-338c	43	Flip11-175c	62	Flip10-318c
6	Flip13-227c	25	Flip13-340c	44	Flip11-70c	63	Flip11-16c
7	Flip13-234c	26	Flip13-343c	45	Flip10-332c	64	Flip11-58c
8	Flip13-240c	27	Flip13-356c	46	Flip11-125c	65	Flip11-138c
9	Flip13-247c	28	Flip13-358c	47	Flip11-05c	66	Flip10-345c
10	Flip13-250c	29	Flip13-364c	48	Flip11-208c	67	Flip88-85c
11	Flip13-251c	30	Flip13-369c	49	Flip93-93c	68	Flip11-105c
12	Flip13-253c	31	Flip13-376c	50	Flip11-32c	69	Flip11-01c
13	Flip13-258c	32	İLC-482c	51	Flip11-66c	70	İLC-482c
14	Flip13-261c	33	Flip82-150c	52	Flip11-205c	71	Flip11-216c
15	Flip13-277c	34	Flip88-85c	53	Flip11-140c	72	Flip82-150c
16	Flip13-278c	35	Flip93-93c	54	Flip11-08c	73	Flip11-214c
17	Flip13-282c	36	St Nermin	55	Flip11-198c	74	Sultan
18	Flip13-308c	37	Flip11-12c	56	Flip11-11c	75	Flip11-190c
19	Flip13-314c	38	Flip11-104c	57	Flip11-209c	76	Flip11-15c

2.1. Field Experiment and Morphological Measurements

The field experiment was laid out using a randomized complete block design to provide a clear and robust experimental framework. Sowing took place manually in November 2024, using an inter-row spacing of 45 cm and a spacing of 10 cm between plants. Seedlings emerged 14-18 days after sowing at a mean soil temperature of 10°C. Plants were selected for analysis based on uniformity, the absence of mechanical damage or disease symptoms, and their compliance with the required phenological stage. Physiological and morphological characteristics included plant height, height of the first pod, number of primary and secondary stems, number of pods per plant, pod length and width, and the weight of 100 seeds. Plant height and the height of the first pod were measured with a Stanley 30-287 metal ruler (USA) to an accuracy of 0.1 cm. The number of pods was counted manually on 10 representative plants from each plot. Yield was calculated using the formula (1):

$$Y = \frac{M \times 10000}{S \times 1000}, \quad (1)$$

where Y : yield (t/ha); M : seed mass (kg); S : the plot area (m²).

2.2. Biochemical and Electrophoretic Evaluation

Protein content was determined by the Kjeldahl method (Lynch et al., 1999) using a Kjeltec 8400 automatic analyser (FOSS, Denmark). Oil content was determined by the Crude Fat Determination Soxhlet Method (1998) using a Gerhardt SOXTHERM 416 apparatus (Germany). Tryptophan was measured spectrophotometrically at λ = 540 nm on a Shimadzu UV-1800 (Japan) after reaction with a zinc acetate reagent. For the analysis, 0.5 g of ground seeds from each genotype were used. Proteins were extracted with 0.1 M Tris-HCl buffer (pH 8.0) containing 1% SDS and 2-mercaptoethanol at 60°C for 30 min. After centrifugation (10,000 rpm, 15 min, Eppendorf 5804R, Germany), the supernatant was used for electrophoresis. Fraction separation was carried out using a vertical Bio-Rad Mini-PROTEAN Tetra Cell system (USA) at 120 V for 2 h in 12% polyacrylamide gel. A broad-range pre-stained protein molecular weight marker (10-250 kDa) was loaded onto each gel to accurately calibrate the molecular masses of the separated globulin fractions. To ensure high reproducibility, the electrophoretic analysis for each sample was repeated across three independent runs. Gel image reproducibility and validation were confirmed by verifying the consistent migration of the molecular weight markers and the distinctness of the protein bands across all technical replicates. After staining with 0.1% Coomassie R-250 (Merck, Germany) and destaining in a methanol-acetic acid mixture, the validated gels were fixed and scanned using an Epson Perfection V850 Pro scanner (Japan). The density and position of the bands were assessed using ImageJ v. 1.54f (NIH, USA). Each band was assigned a binary code: “1” : presence and “0” : absence. The number and intensity of zones were determined from the peaks of the densitometric spectrum. The percentage content of individual fractions was calculated according to the formula (2):

$$P_i = \frac{A_i}{\sum A_n} \times 100, \quad (2)$$

where P_i : the proportion of the i-th fraction (%); A_i : the peak area; $\sum A_n$: the sum of the areas of all peaks.

The intensity of zone staining was recorded using a GS-900 Calibrated Densitometer (Bio-Rad, USA), and the gradations of optical density were converted into distribution charts. To visualize the polymorphism of the protein zones (ω, γ, β, and α), ideograms were constructed to represent the frequency of occurrence of each band among the genotypes analysed. Diagram construction and data processing were performed in OriginPro 2022 (OriginLab, USA). The frequencies of occurrence of fractions were calculated as the ratio of the number of genotypes exhibiting a given protein pattern to the total number of samples (%).

2.3. Statistical Analysis

All data processing, diagram construction, and statistical analyses were performed using OriginPro 2022 (OriginLab, USA) and Statistica 12 (StatSoft Inc., USA). Laboratory and field measurements were carried out in three replicates, and data are presented as mean ± standard error (± SE). To evaluate the frequency and distribution of protein patterns across the samples, the index of genetic diversity was determined using Nei's formula:

$$H = 1 - \sum p_i^2, \tag{3}$$

where H : the index of genetic diversity; p_i^2- the frequency of occurrence of the i-th pattern. This specific index was chosen to robustly quantify the degree of polymorphism and genetic heterogeneity within the studied population based directly on allelic/band frequencies.

All 76 lanes on the electrophoretic profile reflected the individual features of the protein composition of each genotype, demonstrating a high level of polymorphism of storage proteins (Bushuk and Zillman, 1978). They also made it possible to distinguish four typical zones (ω, γ, β, and α) corresponding to proteins of different molecular masses. This provided a comprehensive characterization of intraspecific diversity and the structural organization of globulins in chickpea seeds.

To assess the genetic similarity among the 76 genotypes, a binary presence/absence matrix of bands (1/0) was constructed. Genetic distances were calculated using the Jaccard coefficient (4):

$$D_{ij} = 1 - \frac{a}{a+b+c}, \tag{4}$$

where a : the number of matching fractions; b and c : the numbers of mismatches between pairs of samples.

The Jaccard coefficient was utilized because it explicitly ignores joint absences, making it highly appropriate for analyzing binary electrophoretic data. Based on the resulting matrix, a dendrogram was constructed using the unweighted pair-group method with arithmetic mean (UPGMA) in the MEGA X software (Version 10.2.6) and was additionally verified in Statistica 12 (StatSoft Inc., USA). UPGMA was selected over alternative phylogenetic approaches, such as neighbor-joining or maximum likelihood, because the primary objective was to classify genotypes based on overall biochemical similarity and phenotypic clustering rather than reconstructing deep evolutionary lineages.

For visual interpretation of the similarity coefficient, a scale range of 0.5-1.0 was applied. Genotypes with D<0.30 were considered closely related, whereas those with D>0.60 were regarded as genetically distant. Additionally, a normalized matrix of mean trait values was used to construct a dendrogram for yield and quality. Distances between objects were calculated using the Euclidean metric chosen for its suitability in measuring distances between continuous quantitative traits and clustering was performed by the UPGMA method.

To identify functional linear relationships between biochemical traits (protein, oil, and tryptophan content) and morphological traits (plant height, weight of 100 seeds, number of pods), correlations were determined using Pearson's method at $p<0.05$. Pearson's correlation was justified to accurately measure the strength and direction of the linear association between these continuous variables. Significance testing of differences between variants was performed using Analysis of Variance (ANOVA) to compare means across multiple groups. Duncan's multiple range test ($p<0.05$) was subsequently applied for ranking the means, providing a statistically sound framework for identifying specific differences among the grouped genotypes.

3. RESULTS

3.1. Analysis of Protein Fractions of Chickpea Seeds by Electrophoretic Method

The electrophoretic profiling of globulin proteins successfully visualized four distinct molecular weight zones (ω, γ, β, and α) across the 76 chickpea genotypes. The total number of visualized fractions ranged from 18 to 26 per sample, averaging 21 bands. The distribution, structural specialization, and varying levels of intraspecific polymorphism across these zones are summarized in Table 2.

Table 2. Electrophoretic protein profiling zones in chickpea genotypes

Protein Zone	Number of Bands	Variability	Functional Significance
ω-zone	4-6	Minimal; reflects a high level of structural conservation.	Forms a stable hereditary foundation contributing to the seed protein matrix.
γ-zone	5-10	Displays the highest level of polymorphism among the genotypes.	Highly informative marker range providing clear differentiation and diagnostic accuracy for genetic classification (Korotetsky et al., 2010; Bogoyavlenskiy et al., 2012; Kerimkhulle et al., 2021).
β-zone	5-8	High contribution to overall profile variability.	Dominant fractions comprising up to 60% of soluble proteins; serves as a primary reservoir supplying seeds with storage amino acids.
α-zone	Variable	Considerable variability despite possessing weak staining intensity.	Reflects adaptive responses to growing conditions and indicates the energetic stability of seeds.

The primary share of intraspecific polymorphism was driven by the substantial intergenotypic differences found within the γ- and β-zones. While approximately 40% of the evaluated lines shared similar broad spectra, 60% were distinguished by unique fractions across these zones, indicating a high degree of genetic heterogeneity within the collection. In total, the number of visualized fractions in each of the 76 samples ranged from 18 to 26, with an average of 21 bands. The γ- and β-zones demonstrated the greatest differences among genotypes and accounted for the main share of intraspecific polymorphism. Approximately 40% of the lines studied displayed similar spectra, whereas 60% differed by the presence of unique fractions, indicating a high degree of genetic heterogeneity within the collection. Groups of closely related samples (e.g., Flip13-70c, Flip13-153c, and Flip13-154c) were visually distinguishable on the electrophoretic profile, as well as lines with individual profiles, such as Flip13-250c, Flip13-336c, and Flip11-12c (Figure 1).

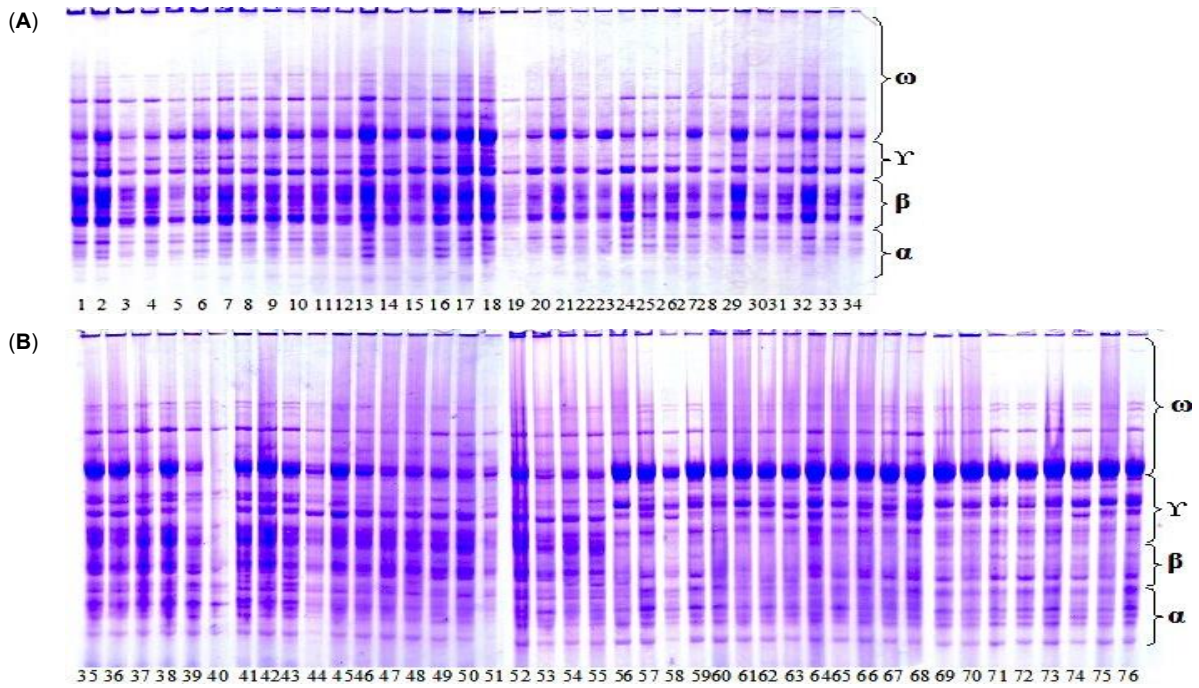

Figure 1. Electrophoretic profiles of globulin proteins in chickpea seeds (A: lanes 1-34; B: lanes 35-76). The analysis reveals the structural conservation of high-molecular-weight globulins in the ω-zone alongside pronounced phenotypic polymorphism in the medium-molecular-weight γ- and β-zones. These central zones exhibit dynamic banding variations and shifting intensity, providing the primary diagnostic markers for intraspecific differentiation and adaptive resilience

Thus, electrophoretic analysis showed that each of the 76 genotypes possessed a unique protein profile, formed by the combination of fractions in the four zones. The greatest diagnostic value belonged to the γ- and β-regions, which ensured reliable differentiation of the samples. The results obtained confirmed that electrophoretic profiles of globulin proteins may be used as a reliable biochemical tool for assessing genetic diversity, identifying cultivars and selecting promising chickpea forms for further breeding.

3.2. Structural Interpretation of Ideograms and Polymorphism of Protein Zones

The samples' electrophoretic profiles revealed 23 spectra and 42 stable patterns, each describing a protein fraction combination in a zone. This level of precision allowed us to determine differences in protein component stability, expression, and inheritance, which helped us assess the lines' intraspecific diversity. Seven spectra and eleven patterns were found in the ω-zone of electrophoretic profiles for high-molecular-weight proteins (Batyrbasheva et al., 2026; Medvedkov et al., 2021; Adekenov et al., 1991). This zone was moderately diverse yet recurred in certain structural combinations. Pattern ω-4 was the most prevalent, reported in 43% of samples, dominating this zone. Pattern ω-1 was the second most common, found in 20% of genotypes, while pattern ω-8 was the rarest, found in only one case (1%). The spectra showed ω-4S in all samples, indicating 100% stability. The spectrum is a major structural element of the ω-zone protein profile due to its universality. In over 60% of genotypes, spectrum ω-3S was found, while ω-1S was only found in 5%. This distribution revealed that conservative protein forms dominated the ω-zone, ensuring the globulin complex's structural integrity. This zone had identical spectra in most samples, indicating protein stability and little variability.

The γ-zone showed the most variation in protein band shape and intensity. The most polymorphic region of the electrophoretic profile had five spectra and sixteen patterns. The most common combination found was pattern γ-3, seen in 43% of genotypes. Pattern γ-8 was observed in 11% of samples, while γ-7 was only found in 1% of cases. The γ-zone's most prevalent spectrum, γ-3S, was discovered in 91% of lines, highlighting its crucial involvement in protein complex assembly. Spectrum γ-4S was found in 71% of samples, while γ-2S was found in one-third. This distribution showed substantial diversity and stable elements in most genotypes. The γ-zone has a complicated internal structure, with 5-10 fractions per sample and varying staining intensity. Most intergenotypic changes were found in the γ-zone, indicating the functional activity and alteration of storage proteins in seeds. Flip13-277c, Flip13-261c, and Flip11-175c showed the most saturated γ-zone ideograms, indicating increased medium-molecular-weight protein production and great adaptability among the examined lines. In the β-zone, where most storage globulins are found, six spectra and seven patterns were seen. This zone had modest polymorphism and a strong frequency asymmetry. The most prevalent pattern was β-2, found in 53.9% of genotypes. Pattern β-1 was found in 24% of samples, whereas β-6 was the least common, occurring in only 1%. The spectra showed 100% prevalence of β-4S, highlighting its crucial function in protein structure in this region. Spectrum β-3S was found in 87% of samples, while β-6S was found in 28%, indicating unusual protein subunit variations. The strong staining intensity and tight fraction arrangement of the β-zone indicate its role in accumulating storage chemicals. Most genotypes had similar profiles, however Flip13-336c, Flip13-250c, and Flip11-12c had additional fractions, generating unique ideograms. These variations suggested breeding selection-relevant biochemical variants of protein complexes.

Five spectra and eight patterns were found in the α-zone of electrophoretic profiles, which comprises low-molecular-weight fractions. The most prevalent were α-3 (36%) and α-1 (29%), whereas α-6 was exceedingly rare (1%). α-2S was found in all genotypes and is essential for the protein composition of this zone. Spectrum α-4S was moderately prevalent (76%), while α-5S was rare (45%). The α-zone showed significant variation in staining intensity, especially in the lower section of the gel where some samples showed intensification of low-molecular-weight fractions. These discrepancies were due to late seed maturity and protein metabolism's sensitivity to agricultural circumstances. The α-zone is important for breeding because its changes can signal seed physiological status and resistance. Ideogram analysis revealed the γ-zone to be the most variable and informative in the protein spectrum, followed by the β-, α-, and ω-zones. The medium-molecular-weight fractions, where genotype-specific patterns emerged, had the most unique combinations. Moreover, the ω-zone remained stable and maintained important conserved features in most samples. Protein gradation reflects functional specialisation: stable high-molecular-weight globulins play a structural role, while γ-zone proteins determine dynamic flexibility and adaptability. Ideograms visualised and systematised sample differences and confirmed that chickpea's globulin profiles have a definite zonal specificity. They found substantial intraspecific polymorphism and that chickpea protein complexes mix stable hereditary elements with flexible variation components that shape genetic diversity (Figure 2).

Thus, protein zone ideograms allowed full characterisation of the intraspecific variety of globulin proteins in the 76 chickpea genotypes and determination of polymorphism in each electrophoretic zone. Most differences and unique fraction combinations were found in the γ-zone, where medium-molecular-weight globulins play a significant role in shaping genotypes' biochemical individuality. Stable storage proteins with moderate variability dominated the β-zone, serving as the primary amino acid reservoir while retaining quantitative changes between lines. The ω-zone had the least variability and formed the protein profile's structural framework. Moderate but significant variability in the α-zone was linked to physiological adaptation and seed maturation (Table 3).

Table 3. Quantitative summary of globulin protein zones in chickpea genotypes

Protein zone	Molecular weight	Fractions (bands)	Spectra	Patterns	Dominant spectra / patterns	Key structural and functional role
ω-zone	High	4-6	7	11	Spectrum ω-4S (100%), Pattern ω-4 (43%)	Highly conserved foundation; limits variability to ensure the structural integrity of the protein matrix.
γ-zone	Medium	5-10	5	16	Spectrum γ-3S (91%), Pattern γ-3 (43%)	Highest level of polymorphism; serves as the primary diagnostic marker reflecting active adaptive variability.
β-zone	Medium	5-8	6	7	Spectrum β-4S (100%), Pattern β-2 (53.9%)	Dominant fraction comprising up to 60% of soluble proteins; primary reservoir for storage amino acids.
α-zone	Low	Variable	5	8	Spectrum α-2S (100%), Pattern α-3 (36%)	Moderate, yet meaningful variability; reflects metabolic differences during the late stages of seed maturation.

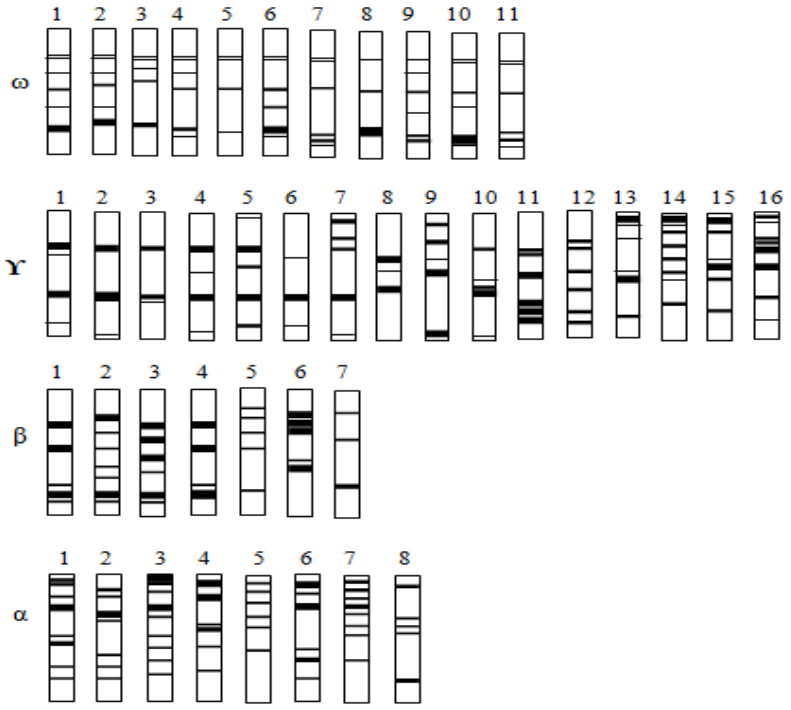

Figure 2. Graphical representation of the variability of globulin fractions in seeds. Ideograms detailing the frequency and distribution of globulin fractions across the ω, γ, β, and α zones. The mapping illustrates the genetic stability of the ω-zone's core framework against the high combinatorial diversity of the γ-zone (which yields 16 distinct patterns), highlighting a clear zonal specialization wherein structural proteins remain stable while metabolic and adaptive proteins fluctuate significantly among genotypes.

3.3. Cluster Analysis of Genetic Similarity based on Protein Patterns

Based on the cluster analysis, all the genotypes examined were grouped into several stable clusters according to the degree of similarity of their protein profiles. The first cluster included six samples; Flip11-214c (No. 73), Flip11-216c (No. 71), Flip82-150c (No. 72), Sultan (No. 74), Flip11-190c (No. 75) and Flip11-15c (No. 76). These genotypes exhibited nearly identical electrophoretic spectra with a high degree of correspondence in the ω- and β-zones, as well as similar γ-fractions of medium intensity. Within this cluster, the similarity of profiles exceeded 85%, indicating close relatedness and a stable type of protein organization. These lines may be regarded as biochemically stable, reflecting a conservative variant of the chickpea protein complex. The second cluster consisted of three genotypes; Flip11-140c (No. 53), Flip11-08c (No. 54) and Flip11-198c (No. 55). They were united by the presence of pronounced β-fractions and well-defined γ-zones that displayed a more complex structure compared with the first cluster. Despite similarities in the main protein ranges, differences in the intensity of

particular bands and slight shifts of the α-fractions were observed, indicating internal variability. The genetic distance within the group was around 0.25-0.28, reflecting close but not identical origins.

The third cluster comprised two samples; Flip11-01c (No. 69) and İLC-482c (No. 70). They were characterized by moderate similarity in protein composition and the presence of rare combinations of γ- and ω-fractions not found in most other lines. These genotypes displayed a specific structure of electrophoretic spectra, including additional bands in the 45-55 kDa range, which made their protein profiles unique. They may be considered transitional forms linking the main clusters and representing interest for hybridization with genetically distant lines.

The fourth cluster consisted of the genotypes Flip11-210c (No. 61) and Flip10-318c (No. 62), which were characterized by a high degree of similarity across all four protein-distribution zones. These samples had simple but clearly defined spectra, reflecting stable inheritance of protein components. The correspondence of spectra within the cluster exceeded 90%, indicating strong genetic proximity. The protein profiles of both lines were marked by pronounced stability of the ω- and β-fractions, making them typical representatives of the conservative direction within the chickpea gene pool. The fifth cluster was represented by two samples; Flip11-138c (No. 65) and Flip88-85c (No. 67). These genotypes had partial matches in the β-zone and pronounced differences in the α-fractions, where additional weak bands were observed. Their spectra were characterized by increased variability of the γ-fractions, reflecting adaptive shifts in protein metabolism. Despite a relatively small number of shared elements, the presence of stable matches in the ω-zone made it possible to group them together with a similarity coefficient of about 0.7.

The remaining samples did not form large clusters and were distributed along separate branches of the dendrogram. Some of them formed small subgroups of two or three genotypes, for example Flip13-277c (No. 15) and Flip13-261c (No. 14), which demonstrated similarity in their γ-fractions, as well as Flip13-336c (No. 23), Flip11-12c (No. 37) and Flip11-125c (No. 46), which were united by shared characteristics in the β-zone. Several genotypes, such as Flip13-250c (No. 10), Flip13-336c (No. 23) and Flip11-05c (No. 47), were isolated and did not enter any of the main clusters, indicating their high genetic distinctiveness and unique protein composition. Overall, the structure of the dendrogram revealed the presence of both closely related groups and solitary genotypes, reflecting a wide range of protein and genetic diversity within the collection studied. The greatest differences were observed between the representatives of the first cluster (Flip11-214c - Flip11-15c) and the isolated lines such as Flip13-250c and Flip11-05c. The spread of similarity coefficient values between them reached 0.45-0.50, indicating substantial divergence in their protein profiles (Figure 3, Table 4).

Table 4. Characteristics of genotype clusters based on electrophoretic protein profiles

Cluster	Number of genotypes	Similarity / distance	Distinctive zonal features and breeding potential
I	6	>85% similarity	Biochemically stable and conservative; marked by highly congruent spectra with strict correspondence in the ω- and β-zones.
II	3	0.25-0.28 genetic distance	Features pronounced β-fractions and complex γ-zones, indicating strong internal variability.
III	2	Moderate similarity	Transitional forms highlighted by unique γ- and ω-fractions in the 45-55 kDa range; ideal for wide hybridization.
IV	2	>90% similarity	Highly conservative lines exhibiting pronounced structural stability across both ω- and β-fractions.
V	2	~0.7 similarity coefficient	Characterized by shared β-zone features but elevated adaptive variability (distinct differences) in the γ- and α-fractions.

Biologically, the clustering patterns reveal distinct adaptive strategies within the chickpea gene pool, where genotypes clustered together share common evolutionary adaptations and functional responses to their environments. Conversely, genetically distant lines serve as critical reservoirs of unique protein alleles that govern specialized physiological functions, such as stress tolerance and metabolic flexibility. For targeted genotype selection, this clustering framework enables breeders to intentionally combine these disparate biological traits. It is highly advisable to carry out hybridization between the most distant functional clusters, specifically pairing the biologically conservative, structurally robust foundational lines of the first cluster (Flip11-214c, Flip82-150c, Sultan) with the highly adaptive, isolated genotypes (Flip13-336c, Flip11-05c, Flip13-250c). This deliberate pairing integrates a stable foundational protein matrix with highly variable, stress-responsive γ-zone globulins. By utilizing this biological divergence, breeders can select parental lines that maximize the recombination of rare functional traits, accelerating the development of climate-resilient cultivars that simultaneously maintain structural integrity and exhibit enriched, nutritionally diverse amino acid profiles. Thus, clustering based on protein profiles clearly demonstrated the internal structure of the chickpea gene pool and confirmed that electrophoretic analysis of globulin proteins is a reliable tool for identification, assessment of relatedness and selection of promising parental pairs for breeding.

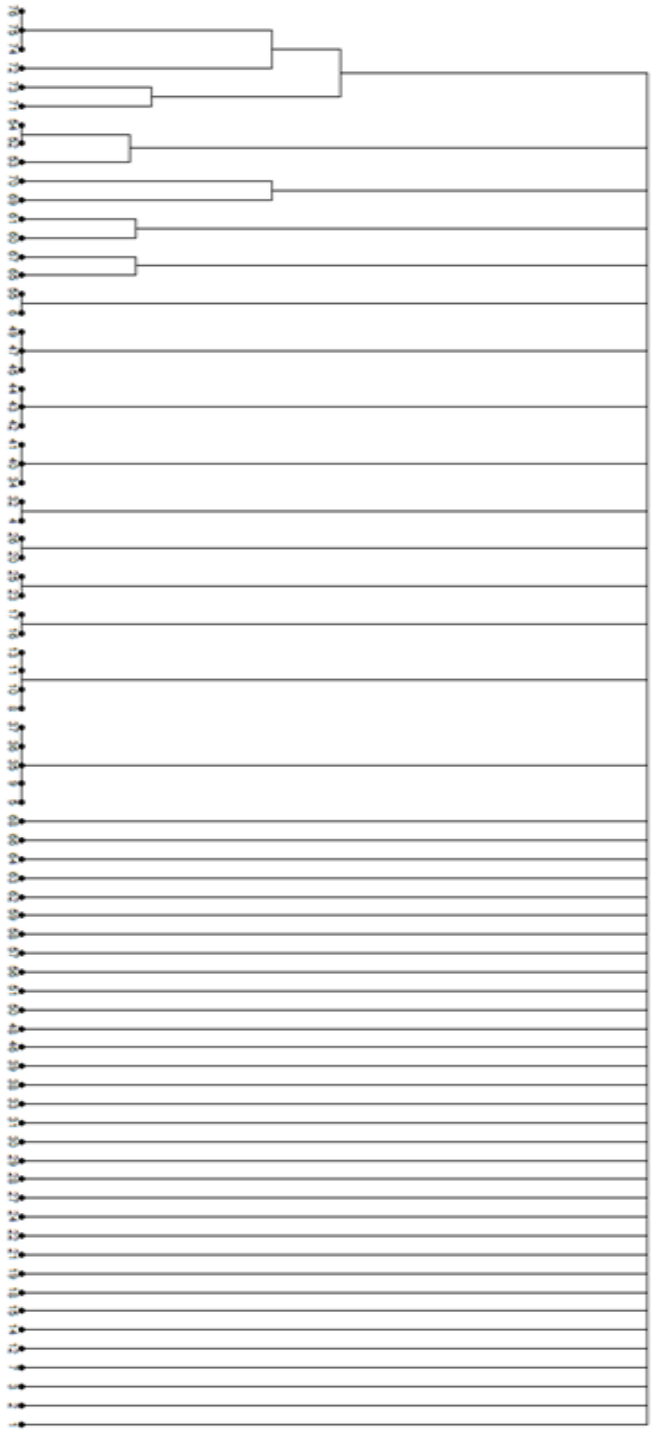

Figure 3. Determination of genetic similarity based on the structure of protein fractions. UPGMA dendrogram constructed from a binary matrix of electrophoretic band presence/absence. The topological clustering demonstrates deep divergence between biochemically conservative clades (e.g., Cluster 1, showing >85% internal similarity) and isolated, highly distinctive lines. This structural disparity provides a targeted basis for selecting genetically distant parental forms to maximize the recombination of rare protein traits.

3.4. Identification of Genotype Clusters according to Morphological and Quality Traits

The first cluster included 28 genotypes that demonstrated similar morphological parameters and moderate differences in yield. It was divided into two subclusters. The first subcluster comprised 13 samples similar in the number of primary and secondary stems, height of the first pod, width and length of the pod, and oil content of the seeds. Among them, Flip13-234c was particularly notable, having shown the highest oil content (11%) while maintaining stable indicators of plant height and the weight of 100 seeds. The remaining genotypes of this subcluster (in particular Flip13-154c, Flip13-250c, Flip13-277c) were characterised by a balanced ratio of biometric parameters and productivity, which made them stable under different growing conditions. The second subcluster included 15 genotypes also similar in plant structure, but with slightly greater variation in the height of the first pod and the number of secondary shoots. This group demonstrated an optimal combination of morphological traits and oil

content, allowing it to be regarded as a morphologically stable type. Overall, the first cluster represented moderately productive forms balanced in terms of biochemical and structural characteristics.

The second cluster, which united 16 genotypes, was also divided into two subclusters with different specializations. The first subcluster consisted of 10 samples characterized by similar values for such traits as pod width and length, number of pods per plant, and overall yield. Among them were ILC-482c, Flip11-45c, Flip11-72c, Flip11-190c and Flip11-15c – lines that demonstrated the largest number of pods and the highest productivity per plant. These samples may be considered a type of multi-podded form with good potential for breeding for yield. The second subcluster included six genotypes grouped according to yield per square metre, oil content and tryptophan levels in the seeds. The most notable representatives here were Flip10-318c, which had the highest yield, and Flip11-11c, distinguished by its tryptophan content. This subcluster comprised samples demonstrating high biochemical potential, making them promising for the development of cultivars with improved nutritional properties.

The third cluster proved to be the largest and most diverse, comprising 32 genotypes that were divided into two subclusters according to productivity characteristics and protein content. The first subcluster, consisting of 9 samples, included lines similar in protein content, number of primary and secondary stems, and pod dimensions. The most notable representatives of this group were Flip11-16c, Flip88-85c, Sultan, Flip11-70c and Flip11-175c, which possessed the highest protein content among all the lines examined. These genotypes combined nutritional value with moderate yield and resilience to external factors, making them typical high-protein forms. The second subcluster, which united 23 genotypes, comprised lines with higher values for plant height, height of the first pod, pod width and length, and the weight of 100 seeds. This group also included the standard cultivar Narmin, used as a control sample. Particularly prominent were the lines Flip11-175c, Flip11-08c, Flip11-198c, Flip11-138c and Flip11-58c, which were distinguished by outstanding plant height and first pod position, traits that have a direct influence on yield. The genotypes Flip11-138c, Flip11-208c and Flip11-209c exceeded the others in pod size, demonstrating the highest values for pod width and length. The heaviest seeds (with a weight of 100 seeds among the highest in the sample set) were found in Flip11-208c, Flip11-209c, Flip11-125c, Flip11-138c, Flip11-105c and Flip11-214c, which allowed them to be classified as highly productive large-seeded forms.

Within the third cluster, genotypes with the highest overall productivity were distinguished. Among them, the smallest Euclidean distance (2.640) was recorded between the pair Flip13-308c and Flip13-330c, indicating the highest degree of genetic and phenotypic similarity between them. These samples displayed not only comparable yield values but also similar seed structure, making them valuable candidates for further selection as parental forms (Figure 4, Table 5).

Table 5. Characteristics of genotype clusters based on morphological and quality traits

Cluster	Number of Genotypes	Productivity & Biometric Traits	Biochemical Traits	Notable Genotypes
I	28	Balanced morphological parameters; moderate yields with structural stability.	Optimal oil accumulation (reaching up to 11%).	Flip13-234c, Flip13-250c, Flip13-277c.
II	16	Characterized by maximum overall yield per square meter and highest number of pods per plant.	Elevated biochemical potential, specifically high tryptophan levels.	Flip10-318c, Flip11-11c, ILC-482c, Flip11-45c.
III	32	Distinguished by maximum plant height, largest pod dimensions, and heaviest 100-seed weights.	Contains lines possessing the absolute highest overall protein content in the sample set.	Flip11-175c, Flip11-208c, Sultan, Flip88-85c.

Overall, the cluster analysis biologically partitions the genotypes into three distinct functional groups, each reflecting a specific physiological strategy and resource allocation trade-off between yield mechanics, plant architecture, and biochemical seed composition. Understanding these biological specializations provides a direct, actionable roadmap for genotype selection in applied breeding programs. Rather than selecting lines based on isolated numerical traits, breeders can utilize these functional clusters to design complementary hybridization strategies. For example, selecting parental lines from the multi-podded, high-tryptophan lineages (Cluster 2) and crossing them with robust, large-seeded, high-protein forms (Cluster 3) offers a targeted pathway to synergistically maximize both agronomic productivity and nutritional biofortification in future commercial chickpea cultivars.

Thus, Flip11-72c, ILC-482c and Flip11-15c, characterised by a high number of pods per plant, were assigned to the second cluster in the productivity analysis but were positioned separately in the protein composition analysis. Similarly, Flip11-205c and Flip11-08c, which exhibited increased protein content, fell into the second cluster of the protein analysis, whereas Flip11-138c and Flip88-85c belonged to the fifth. The genotypes Flip11-72c and Flip11-210c, assigned to the fourth cluster of the protein dendrogram, were characterised by high tryptophan content.

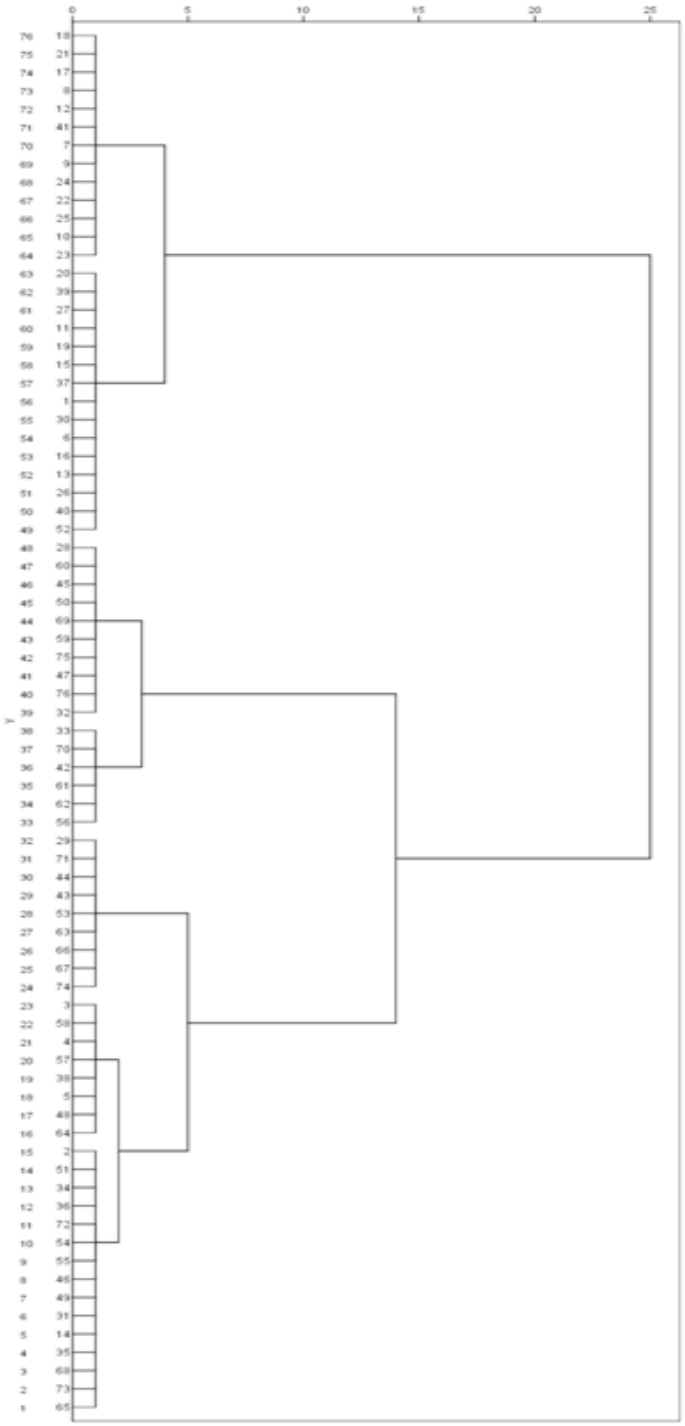

Figure 4. Multifactorial clustering of samples according to productivity and seed composition. UPGMA dendrogram segregating genotypes based on a normalized matrix of agro-morphological productivity and biochemical seed composition. The distinct branching reflects specialized trait accumulations, effectively separating multi-podded, high-tryptophan lineages (Cluster 2) from robust, large-seeded forms that maximize total protein concentration (Cluster 3).

3.5. Integrative Analysis of Globulin Polymorphism and Adaptive Breeding Potential

The electrophoretic analysis successfully confirmed the presence of pronounced intraspecific polymorphism within chickpea globulin proteins while simultaneously maintaining the overall zonal structure of the entire protein complex (Mukhametov et al., 2022; Adenekov et al., 1992; Hajiyeva et al., 2025). The upper part of the generated electrophoretic profiles, corresponding precisely to the ω-zone, was notably characterised by minimal variability and remarkably high reproducibility of specific spectra. This clearly indicates the profound genetic stability of these high-molecular-weight fractions across all samples (Amourah et al., 2024; Salah et al., 2023). The fundamental conservatism of this specific region heavily reflected its critical structural role in properly forming the dense seed protein matrix and reliably preserving its vital morphophysiological integrity. Minor biochemical variations occasionally observed in a very few isolated samples were most probably directly associated with minor isoform modification and subsequent post-translational structural changes. Comparison of all evaluated zones consistently

revealed a highly regular and predictable structural organisation of the entire protein spectrum. The remarkably stable fractions found in the ω- and β-zones clearly provide a deeply conservative foundation. Conversely, the highly dynamic γ- and α-zones actively form the true evolutionary core of genetic variability. Such strict zonal organisation directly determines the inherent adaptive potential and essential genetic differentiation of diverse chickpea lineages. This absolutely confirms the immense practical significance of utilizing detailed electrophoretic profiles in targeted breeding research. In the area of applied phenotypic analytics, modern investigators have continuously evaluated genetic diversity using various advanced scientific tools. For example, Mesfer Alshamrani et al. (2022) successfully combined several biochemical, physiological, and complex molecular markers in their extensive analysis of sesame. They successfully identified numerous general patterns of widespread genetic variability. However, their approach did not include specific electrophoretic protein characterisation.

The present comprehensive study demonstrated that detailed electrophoretic ideograms across the ω, γ, β, and α zones provide significantly more accurate genotype differentiation. This successfully enables the critical linking of unique structural protein features with highly inheritable agronomic traits. This ultimately renders our empirical results substantially more informative compared to traditional visually based phenotypic approaches. Within similar biochemical-breeding research directions, Ohanenye et al. (2022) meticulously examined the unique properties of various legume proteins heavily situated within the broad context of modern food technology and functional nutritional value. In their insightful study, it was clearly shown that intense physical processing methods rapidly alter the complex tertiary structure of essential globulins. Such intensive treatments ultimately increase rapid digestibility but simultaneously reduce the critical native stability of these crucial proteins. In sharp contrast to these earlier technological studies, our present dedicated work strictly focused on evaluating completely natural protein variations without introducing any external technological intervention.

This methodical approach provided a deeper understanding of the inherent genetic stability characterizing these diverse chickpea protein complexes. In the closely related analytical context of complex genetic-structural studies, Seo et al. (2025) successfully employed rather different experimental strategies for thoroughly investigating analogous plant proteins. They meticulously examined wild oat by powerfully combining both sequencing and electrophoresis to appropriately assess broad genetic diversity. However, within their specific methodology, distinct protein fractions were surprisingly considered merely as a secondary confirmatory marker. The present study expanded upon this by detailing intricate protein differences at the critical zonal level. Our robust experimental framework successfully identified highly specific electrophoretic migration patterns alongside completely stable, highly reproducible ideograms.

Consequently, this refined approach provided empirical evidence highlighting structural variability alongside distinct functional differentiation. This ultimately makes the resulting analytical methodology more precise and directly applicable within practical crop breeding programs. Furthermore, in the fundamental context of highly specialized structural-functional research, Sinha et al. (2023) deliberately focused heavily on investigating the innate functional nature alongside the specific nutritional value characterizing various seed proteins. Sinha et al. clearly demonstrated that internal seed protein reserves are formed partly from unique amyloid and distinct amyloid-like micro-structures. These unique microscopic elements effectively ensure the biologically necessary, extremely gradual release of vital amino acids throughout the prolonged germination stage.

This fascinating theoretical approach correctly emphasized the crucial physiological role played by incredibly stable protein aggregates regarding the essential energetic support required by developing seedlings. However, their otherwise excellent study significantly did not comprehensively cover any intraspecific differences occurring specifically within the underlying structural framework of typical globulins. Jha et al. (2024), by contrast, focused their efforts on formulating advanced strategies to enhance the overall chickpea nutritional profile.

4. CONCLUSION

The analysis of 76 chickpea genotypes revealed pronounced polymorphism and considerable intraspecific diversity in globulin proteins. Electrophoretic profiling effectively differentiated four structural protein zones, identifying the γ-zone as the most highly variable and the ω-zone as the most structurally stable. By integrating these biochemical profiles with agro-biological data, the study successfully established strong correlations between specific protein fractions, yield, and seed quality traits. Practically, the results indicate that approximately 60% of the evaluated lines possess unique protein and biochemical characteristics highly suitable for targeted breeding. These findings provide a reliable framework for advancing specific breeding goals: developing biochemically rich, high-tryptophan cultivars by utilizing isolated genotypes; breeding high-yield, multi-podded architectures from structurally

stable lines; and creating biofortified, high-protein forms by leveraging medium-molecular-weight globulin alleles from heterogeneous samples. Moving forward, because the current findings rely on a single growing season, subsequent research must evaluate the impact of environmental factors on protein profile variability over multiple years. Future studies will also expand the genotype sample and incorporate molecular DNA markers to clarify deeper genetic relationships and firmly validate these electrophoretic models.

ACKNOWLEDGEMENT
In addition to presenting the results of their research, scientists also discussed the most relevant issues in science with colleagues from other countries. In conducting this work, authors received significant support from the Director-General of the Genetic Resources Institute of the Ministry of Science and Education of the Republic of Azerbaijan.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTION
Mahbuba Salmanova: Conceptualization, Methodology, Investigation, Supervision. Gatiba Hasanova: Data Collection, Investigation, Writing Original Draft. Akbar Karimov: Data Collection, Software, Visualization. Saida Hasanova: Software, Validation, Formal Analysis. Sevinj Nuriyeva: Writing – Reviewing and Editing, Methodology, Supervision.

DATA AVAILABILITY
The data that support the findings of this study are available on request from the corresponding author.

DECLARATION OF GENERATIVE AI
Not applicable.

ETHICS
Not applicable.

REFERENCES

Abaszade Z, Gasimov K, Shahmuradov I. (2024). Study of single nucleotide polymorphism in the encoding exons of the AGT gene of an arterial hypertension patient from Azerbaijan. *Journal of Life Sciences and Biomedicine*, 6(79), 11–21. doi:10.59849/2710-4915.2024.1.11

Adekenov SM, Gafurov NM, Turmukhambetov AZh. (1991). Chemical modification of estafiatin. *Chemistry of Natural Compounds,* 27(5), 571–575. doi:10.1007/BF00630357

Adenekov SM, Kupriyanov AN, Turmukhambetov AZh, Gafurov NM, Dzhazin KA. (1992). Austricin from Artemisia juncea. *Chemistry of Natural Compounds,* 28(6), 633–634. doi:10.1007/BF00630453

Amourah A, Frasin BA, Salah J, Al-Hawary T. (2024). Fibonacci Numbers Related to Some Subclasses of Bi-Univalent Functions. *International Journal of Mathematics and Mathematical Sciences,* 2024, 8169496. doi:10.1155/2024/8169496

Batyrbasheva Zh, Asanaliev A, Imanaliev A, Sultanbaeva V. (2026). Amino acid composition of chickpea (Cicer arietinum L.) varieties cultivated in the Kyrgyz Republic. *Bulletin of the Kyrgyz National Agrarian University,* 24(1), 18–27. doi:10.63621/bknau./1.2026.18

Bayramli O. (2024). Delineating genetic diversity in soybean (*Glycine max* L.) genotypes: Insights from a-page analysis of globulin reserve proteins. *Advances in Biology & Earth Sciences*, 9(2), 259–266. doi:10.62476/abes9259

Bekbayev K, Mirzoyan S, Toleugazykyzy A, Tlevlessova D, Vassilian A, Poladyan A, Trchounian K. (2024). Growth and hydrogen production by Escherichia coli during utilization of sole and mixture of sugar beet, alcohol, and beer production waste. *Biomass Conversion and Biorefinery,* 14(1), 909–919. doi:10.1007/s13399-022-02692-x

Benarba B, Belhouala K, Korkmaz C, Taş-Küçükaydin M, Küçükaydın S, Duru ME. (2025). Phytochemical composition and biological activities of ephedra alata Decne.: Antioxidant, anti-inflammatory and anticancer potentials. *Journal of Science and Mathematics Letters*, 13(2), 33–47. doi:10.37134/jsml.vol13.2.3.2025

Bogoyavlenskiy A, Berezin V, Prilipov A, Usachev E, Korotetskiy I, Zaitceva I, Kydyrmanov A, Sayatov M. (2012). Characterization of pigeon paramyxoviruses (newcastle disease virus) isolated in Kazakhstan in 2005. *Virologica Sinica,* 27(2), 93–99. doi:10.1007/s12250-012-3234-0

Bushuk W, Zillman RR. (1978). Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. *Canadian Journal of Plant Science*, 58(2), 505–515. doi:10.4141/cjps78-076

Chakraborty A, Junaid A, Parida SK, Bhatia S. (2023). Integrated genomic approaches delineate a novel role of ROP1 ENHANCER1 in controlling seed protein content of chickpea. *Journal of Experimental Botany*, 74(3), 817–834. doi:10.1093/jxb/erac452

Cordoba HA, Sadohara R, Gali KK, Zhou J, Hart J, Alexandre AS, Cichym J, Wilker J, Dohle S, Mukankusi C, Warkentin T, Rajcan I, Hoyos-Villegas V. (2025). Breeding for plant-based proteins in pulse and legume crops: Perspectives, challenges and opportunities. *Crop Science*, 65(4), e70137. doi:10.1002/csc2.70137

Crude Fat Determination – Soxhlet Method. (1998). Meat Industry Services. [accessed 23.02.2026].

Di Francesco A, De Santis MA, Lanzoni A, Pittalà MG, Saletti R, Flagella Z, Cunsolo V. (2024). Mass spectrometry characterization of the SDS-PAGE protein profile of legumins and vicilins from chickpea seed. *Foods*, 13(6), 887. doi:10.3390/foods13060887

Di Francesco A, Lanzoni A, De Santis MA, Pittalà MG, Saletti R, Flagella Z, Cunsolo V. (2025). In-depth shotgun proteomic characterization of chickpea (*Cicer arietinum* L.): A case study on the genotype Pascià grown using two water regimes. *ACS Agricultural Science & Technology*, 5(7), 1274–1284. doi:10.1021/acsagscitech.4c00705

Grewal SK, Sharma KP, Bharadwaj RD, Hegde V, Sidhu SK, Singh S, Jain P, Rasool S, Arya D, Agrawal P, Mondal B. (2022). Characterization of chickpea cultivars and trait specific germplasm for grain protein content and amino acids composition and identification of potential donors for genetic improvement of its nutritional quality. *Plant Genetic Resources*, 20(6), 383–393. doi:10.1017/S147926212300028X

Hajiyeva A, Aliyeva R, Hajiyeva A, Myskovets I, Samadov B. (2025). Efficiency of using Bioorganic Preparations to Protect Pine Stands from Pests and Diseases: Lessons from the Application of Basidiomycetes. *Evergreen,* 12(3), 1722–1735. doi:10.5109/7388860

Jesus JV, Pinho ÉV, Silva CM, Rios VR, Santos HO, Nascimento WM. (2025). Validation of morphological and molecular descriptors in the identification of chickpea cultivars for cultivar registration. *Journal of Seed Science*, 47, e202547024. doi:10.1590/2317-1545v47297871

Jha UC, Nayyar H, Thudi M, Beena R, Vara Prasad PV, Siddique KH. (2024). Unlocking the nutritional potential of chickpea: strategies for biofortification and enhanced multinutrient quality. *Frontiers in Plant Science*, 15, 1391496. doi:10.3389/fpls.2024.1391496

Joshi-Saha A, Misra G, Reddy KS. (2021). Quality improvement in chickpea. In: Gupta DS, *Gupta S, Kumar J (Eds.),* Breeding for Enhanced Nutrition and Bio-Active Compounds in Food Legumes, *Cham: Spring*er. doi:10.1007/978-3-030-59215-8_2

Kerimkhulle S, Kerimkulov Z, Bakhtiyarov D, Turtayeva N, Kim J. (2021). In-field crop-weed classification using remote sensing and neural network. In: *2021 IEEE International Conference on Smart Information Systems and Technologies,* 1–6. Nur-Sultan: IEEE. doi:10.1109/SIST50301.2021.9465970

Korotetsky IS, Bogoyavlensky AP, Prilipov AG, Usachev EV, Usacheva OV, Turmagambetova AS, Zaitseva IA, Kydyrmanov A, Shakhvorostova LI, Kh Sayatov M, Borisov VV, Pchelkina IP, Gerilovich AP, Berezin VE. (2010). Molecular genetic characteristics of the Newcastle disease virus velogenic strains isolated in Russia, Ukraine, Kazakhstan, and Kirghizia. *Voprosy Virusologii,* 55(4), 29–32.

Lynch JM, Barbano DM. (1999). Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *Journal of AOAC International*, 82(6), 1389–1398. doi:10.1093/jaoac/82.6.1389

Medvedkov Y, Nazymbekova A, Tlevlessova D, Shaprov M, Kairbayeva A. (2021). Development of the juice extraction equipment: physico-mathematical model of the processes. *Eastern-European Journal of Enterprise Technologies*, 1(11 (109)), 14–24. doi:10.15587/1729-4061.2021.224986

Mesfer Alshamrani S, Safhi FA, Alshaya DS, Ibrahim AA, Mansour H, Abd El Moneim D. (2022). Genetic diversity using biochemical, physiological, karyological and molecular markers of *Sesamum indicum* L. *Frontiers in Genetics*, 13, 1035977. doi:10.3389/fgene.2022.1035977

Molina-Jauregui PP, Mérida-Reyes MS, Ribeiro da Silva AJ, Perez Sabino JF. (2025). Unveiling the phytochemical richness of Guatemalan Spondias: Comparative analysis of phenolics, flavonoids and antioxidant potential. *Journal of Science and Mathematics Letters*, 13(2), 174–183. doi:10.37134/jsml.vol13.2.13.2025

Mukhametov A, Mamayeva L, Yerbulekova M, Aitkhozhayeva G. (2022). Fatty Acid Profile of Functional Emulsion-Based Food Products Containing Conventional and Unconventional Ingredients. *Italian Journal of Food Science,* 34(2), 89–97. doi:10.15586/ijfs.v34i2.2181

Namazbekova S, Sodonbekov I, Samsaliev A, Chakaeva A. (2024). Chickpea diseases in the Chui valley of Kyrgyzstan. *Bulletin of the Kyrgyz National Agrarian University,* 22(4), 49–54.

Nasrullayeva M, Kalbiyeva Y, Isgandarova R, Huseynova T, Mammadova Sh. (2023). Study of genetic diversity of globulin proteins in common bean (*Phaseolus vulgaris* L.) genotypes. *Znanstvena Misel Journal*, 78, 4–9. doi:10.5281/zenodo.7980516

Ohanenye IC, Ekezie FG, Sarteshnizi RA, Boachie RT, Emenike CU, Sun X, Nwachukwu I, Udenigwe CC. (2022). Legume seed protein digestibility as influenced by traditional and emerging physical processing technologies. *Foods*, 11(15), 2299. doi:10.3390/foods11152299

Salah J, Rehman HU, Buwaiqi IA. (2023). Inclusion results of a generalized mittag-leffler-type poisson distribution in the k-uniformly janowski starlike and the k-janowski convex functions. *Mathematics and Statistics,* 11(1), 22–27. doi:10.13189/ms.2023.110103

Seo H, Won H, Lee S, Jeong JS, Jo J, Han S, Ha B-K, Lee KJ. (2025). Exploration of the genetic diversity of *Avena Fatua* L. (wild oat) through genotyping-by-sequencing and SDS-PAGE. *BMC Plant Biology*, 25(1), 1358. doi:10.1186/s12870-025-07315-x

Sinha N, Zahra T, Gahane AY, Rout B, Bhattacharya A, Basu S, Chakrabarti A, Thakur AK. (2023). Protein reservoirs of seeds are amyloid composites employed differentially for germination and seedling emergence. *The Plant Journal*, 116(2), 329–346. doi:10.1111/tpj.16485

Tiruneh A, Ptaszek P, Żmudziński D, Tarko T. (2025). Peas (*Pisum sativum* subsp. *arvense* Asch) and Beans (*Vicia faba* var. *minor*) as source of quality plant proteins. *Molecules*, 30(9), 2009. doi:10.3390/molecules30092009

Tultabayeva T, Tokysheva G, Zhakupova G, Konysbaeva D, Mukhtarkhanova R, Matibayeva A, Mukhametov A, Zamaratskaia G, Makangali K. (2023). Enhancing nutrition and palatability: The development of cooked sausages with protein hydrolysate from secondary raw materials for the elderly. *Applied Sciences*, 13(18), 10462. doi:10.3390/app131810462

Valujeva K, Pilecka-Ulcugaceva J, Skiste O, Liepa S, Lagzdins A, Grinfelde I. (2022). Soil tillage and agricultural crops affect greenhouse gas emissions from Cambic Calcisol in a temperate climate. *Acta Agriculturae Scandinavica Section B: Soil and Plant Science,* 72(1), 835–846. doi:10.1080/09064710.2022.2097123

Ye J, Shi N, Rozi P, Kong L, Zhou J, Yang H. (2024). A comparative study of the structural and functional properties of chickpea albumin and globulin protein fractions. *Food and Bioprocess Technology*, 17(10), 3253–3266. doi:10.1007/s11947-024-03323-1