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## QUANTITATIVE ANALYSIS OF FREE AMINO ACIDS IN *PHASEOLUS VULGARIS* L. SEEDS

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**Summary.** This study analyzed the free amino acid composition of six *Phaseolus vulgaris* L. cultivars using high-performance liquid chromatography. The results revealed considerable variation among cultivars, with physiologically important amino acids such as aspartic and glutamic acids being predominant across all samples. Notably, the cultivar Belaya fasol exhibited an exceptionally high glutamine content (8.47 mg/g), while alanine, a key component of energy metabolism, showed consistently elevated levels (3.95–7.96 mg/g) in all cultivars. Essential amino acids, including leucine, isoleucine, threonine, and valine, displayed cultivar-specific patterns, reflecting differences in protein quality and nutritional potential. The findings highlight the significant biochemical diversity within common bean germplasm and underscore the nutritional value of these cultivars. Furthermore, the identified amino acid profiles provide a valuable basis for breeding programs aimed at biofortification, improved protein quality and the development of high-value common bean varieties for both food security and health-oriented applications.

**Keywords:** *Phaseolus vulgaris* L., free amino acids, Glutamine, Aspartic acid, protein quality, biofortification

## КОЛИЧЕСТВЕННЫЙ АНАЛИЗ СВОБОДНЫХ АМИНОКИСЛОТ В СЕМЕНАХ *PHASEOLUS VULGARIS* L.

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**Аннотация.** В этом исследовании был проанализирован состав свободных аминокислот шести сортов *Phaseolus vulgaris* L. с использованием высокоэффективной жидкостной хроматографии. Результаты показали значительные различия между сортами, причем во всех образцах преобладали физиологически важные аминокислоты, такие как аспарагиновая и глутаминовая кислоты. Примечательно, что сорт Белая фасоль отличался исключительно высоким содержанием глутамина (8,47 мг/г), в то время как уровень аланина, ключевого компонента энергетического обмена, был стабильно повышен (3,95–7,96 мг/г) у всех сортов. Незаменимые аминокислоты, включая лейцин, изолейцин, треонин и валин, представлены в зависимости от сорта, что отражает различия в качестве белка и питательном потенциале. Полученные данные свидетельствуют о



значительном биохимическом разнообразии зародышевой плазмы фасоли обыкновенной и подчеркивают питательную ценность этих сортов. Кроме того, выявленные аминокислотные профили обеспечивают ценную основу для селекционных программ, направленных на биофортификацию, улучшение качества белка и выведение ценных сортов фасоли обыкновенной как для обеспечения продовольственной безопасности, так и для целей здравоохранения.

**Ключевые слова:** *Phaseolus vulgaris* L., свободные аминокислоты, глутамин, аспарагиновая кислота, качество белка, биофортификация

### Introduction

Currently, at the global level, extensive research is being conducted to improve the economically valuable traits of common bean (*Phaseolus vulgaris* L.) through the integration of genetic and physiological approaches. In modern breeding programs, a primary focus is on enhancing the genetic diversity of beans, developing new high-performing cultivars and thoroughly investigating the key genetic, physiological and biochemical traits expressed in these improved lines.

In this context, the inheritance mechanisms of valuable agronomic traits, their variability, the expression of quantitatively controlled traits regulated by polygenes, and their transmission across generations are being rigorously analyzed. Additionally, determining the correlational relationships among various traits, assessing genetic distances between populations through cluster analysis, and evaluating the genetic potential of recombinant forms for use in breeding programs constitute critical aspects of current research efforts.

Consequently, these studies aim to facilitate the development of novel,

high-yielding, stress-resilient, and nutritionally and economically significant cultivars, thereby providing promising prospects for future breeding strategies.

At present, the common bean (*Phaseolus vulgaris* L.) is cultivated on a wide scale worldwide. According to FAO data of the United Nations, common bean occupies approximately 24-28 million hectares, accounting for 17% of leguminous crops. The extensive distribution of *Phaseolus vulgaris* L. varieties across large areas is primarily attributed to their valuable biochemical composition and the relatively low agronomic input required for their cultivation to meet market demand [1].

This crop is recognized as an excellent source of nutrition, owing to its high protein content, abundant protein fibers, complex carbohydrates, vitamins (including folic acid), and minerals such as Cu, Ca, Fe, Mg, Mn, and Zn, which are essential for daily human dietary requirements [2]. Wild relatives of the common bean are believed to have originated in South and Central America, subsequently leading to the domestication of two main



genotypes in the southern and northern regions of each continent. Each of these domesticated genotypes encompasses several smaller subpopulations. Both genotypes established secondary centers of genetic diversity in Africa and Asia, contributing to the parallel global spread of the species [3].

Morphologically, the presence of colored streaks (e.g., purple or pink) on the external surface of the flower petal serves as a significant marker in *Phaseolus vulgaris* L. In fact, this trait has been utilized to differentiate Andean and Mesoamerican genotypic groups. For instance, colored streaks are characteristic of Mesoamerican genotypes, whereas South American genotypes (e.g., from Chile, Nueva Granada, and Peru) typically lack such markings [4].

Ranalli and Mari [5] analyzed the inheritance of several traits in *Phaseolus vulgaris* L., including seed coat color, plant height, and 100-seed weight. Seed coat color was shown to depend on the interaction of at least two loci (P and R), with the R allele demonstrating epistatic effects over certain  $\tilde{N}$  genotypes. Seed coat color is controlled monogenically, plant height segregates in a 3:1 ratio, while 100-seed weight exhibits polygenic inheritance.

The genetics of petal streaks in common bean and their relationship with seed coat color have not been extensively investigated. Previous studies focused primarily on the

association between petal color and seed coat or the inheritance of anthocyanin pigmentation in petals. For example, in common bean, light pink petal color from PI 527735, combined with gray-white seeds produced by the gri gene, revealed interactions of recessive alleles at the P locus with other genes [6, 7]. Recent studies have highlighted that seed coat color is predominantly associated with the dominant P gene [8]. Furthermore, in Central American cultivars, black "Longyundou 4" and white "Longyundou 5" phenotypes demonstrated involvement of two independent genes with dominant epistatic effects [9]. Bassett and Miklas identified two additional dominant genes (Prp i-2 and t bp) from the G07262 line responsible for blue vein patterns in white flowers [10].

Diego Viteri and Angela Linares [11] suggested that the presence or absence of purple streaks on petals can serve as a practical morphological marker to select seeds with light red or white color in breeding programs. Research also emphasized that the development of genotypes tolerant to abiotic stresses is closely associated with extensive root system architecture. Studies involving six parental lines and 30 F<sub>1</sub> hybrids investigated the occurrence of heterosis in root spread, revealing that its absence could be explained by relatedness among genetic groups and certain epistatic interactions [12]. P. Balasubramanian et al. reported



interspecific hybridization between wild and stress-sensitive common bean accessions, producing hybrids adaptable to cold and short-season environments, with in vitro methods facilitating the successful recovery of some crosses [13].

QTLs associated with disease resistance, grain and fruit quality, and yield traits from cereals (wheat, rice, barley, oats, maize, common bean, chickpea, mung bean), fruit trees (apple, pear, banana), and vegetables (potato, tomato, pumpkin) have been introgressed into desired genotypes, enabling the development of improved cultivars and breeding lines [14].

Bilira et al. [15] genetically characterized common bean populations using 102 genotypes analyzed with luminescent SSR markers, identifying 192 alleles with an average of 14.8 per locus. No synonymous genotypes were detected, though maximum heterozygosity was observed at three loci. SSR markers proved highly effective for molecular characterization, germplasm conservation, and marker-assisted selection in local common bean resources.

Common bean is a rich source of proteins and micronutrients, especially iron and zinc, and represents a cost-effective food resource. Biofortification is critical for developing varieties with enhanced Fe/Zn concentrations to improve nutrition in developing countries. A strong negative phenotypic

correlation between Fe/Zn content and yield presents a challenge for breeders. Several populations, including biofortified parental lines, have been analyzed for QTL mapping and GWAS to identify genomic regions associated with yield and micronutrient accumulation [16].

Studies on Lima beans involved 241 controlled crosses generating 42 hybrid combinations, with SSR markers facilitating hybrid selection [17]. Heat and drought stress pose major constraints for developing protein- and mineral-rich common bean cultivars. Conversely, tepary bean (*P. acutifolius* L. Gray) is naturally adapted to hot and arid conditions, although its genetic and molecular potential has been underutilized. Recent molecular-genetic studies on tepary bean, *P. vulgaris*, and *P. parvifolius* aim to exploit these species for hybridization to confer drought tolerance while retaining desirable agronomic traits [18, 19].

Sookyeong Lee et al. [20] analyzed seed color in 200 common bean accessions, classifying them in an HTML-based color table for prediction of seed composition and efficient utilization of protected genetic resources. Seeds were categorized into seven color groups: yellow-green, yellow, brown, red, white, gray, and indigo. Concentration-dependent distributions were observed, with red showing the highest correlation, while gray and yellow-green were



independent of concentration. Dominant pigments influencing seed color include chlorophylls (yellow-green), carotenoids (yellow), and anthocyanins (brown, red, white, gray, indigo). This classification method can be extended to other agricultural crops for germplasm management and breeding purposes.

It is well established that protein serves as an essential and irreplaceable biological macronutrient. Protein deficiency in the organism leads to impaired metabolic processes, reduced immune function, stunted growth, disrupted endocrine activity, and a variety of other adverse effects. Conversely, excessive protein intake can result in altered sensitivity of the nervous system and dysfunction of internal organs [21, 22].

Common bean (*Phaseolus vulgaris* L.) provides not only substantial energy—approximately 340 kcal per 100 g - but also satisfies up to 35% of the daily protein requirement. In addition, it contains high concentrations of vitamins

and micronutrients. For instance, common bean seeds possess 4-10 times more iron (Fe) and 2-3 times more zinc (Zn) compared to cereal grains such as maize, wheat, and rice [23, 24].

Due to its high protein content, common bean is widely considered a valuable dietary component. Its bioactive compounds and strong antioxidant capacity are also associated with the prevention of chronic diseases, including diabetes, cardiovascular disorders, and inflammatory bowel conditions. In this study, the physicochemical properties and bioactive compounds of 155 *Phaseolus vulgaris* L. accessions were characterized. Seed composition was analyzed for protein, fat, carbohydrates, antioxidants, total phenolics, flavonoids, anthocyanins, and phytic acid. Additionally, biostatistical analyses such as cluster and correlation analyses were performed, resulting in the identification of ten accessions with particularly high protein content [25, 26; 27].

### Materials and methods

The research was conducted using both local and foreign cultivars of *Phaseolus vulgaris* L. as the experimental material. The formation of free amino acids with phenylthiocarbonyl (PTC) derivatives was analyzed using high-performance liquid chromatography

(HPLC), following the method described by Steven, Cohen, and Daviel [28]. The experiments were carried out at the Laboratory of Protein and Peptide Chemistry, Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan.

### Results and discussion

In the study conducted by Vronska L.V. and Demyd A. Ye. [29],

the amino acid composition of five white-seeded *Phaseolus vulgaris* L.



accessions was analyzed using chromatography. The analysis identified aspartic acid, glutamine, glycine, valine, tyrosine, leucine, and other amino acids. Among free amino acids, proline (12.47 mg/g) was found at the highest concentration, while among bound amino acids, glutamine exhibited a maximum content of 3.41 mg/g. Essential amino acids were also quantified, with glycine (1.02 mg/g), serine (1.04 mg/g), valine (0.80 mg/g), phenylalanine (0.67 mg/g), threonine (0.66 mg/g), leucine (0.63 mg/g), and isoleucine (0.58 mg/g) detected. The total amino acid content in the extracts was recorded at 3.2%.

Ángel Ramón et al. (2020) analyzed protein content and amino acid composition in 46 common bean populations. The study revealed that certain populations exhibited high levels of isoleucine, threonine, methionine, arginine, serine, alanine, tyrosine, and cysteine. In other populations, aromatic amino acids were predominant, whereas some groups showed elevated concentrations of sulfur-containing amino acids [30].

This study presents a comprehensive analysis of free amino acid accumulation patterns in six cultivars of *Phaseolus vulgaris* L. - Beybi Lima, Ravot, Vir, Kalipso, Solnishko, and Belaya fasol using high-performance liquid chromatography (HPLC). The resulting amino acid profiles provide precise insights into

protein quality, metabolic activity during seed maturation, physiological resilience, and the overall nutritional and functional value of the cultivars. The data indicate that each cultivar possesses a distinct “metabolic signature,” a finding consistent with earlier studies while also expanding current understanding through several novel observations.

One of the most notable results is the consistently high accumulation of glutamic acid across all cultivars. Particularly elevated concentrations were observed in Vir (2.56 mg/g) and Belaya fasol (2.03 mg/g), exceeding the average values (1.2-1.4 mg/g) previously reported by Vronska L.V. and Demyd A.Ye. Such elevated levels suggest enhanced nitrogen assimilation and active protein biosynthesis pathways during seed development. Of special interest is the extraordinarily high glutamine content in Belaya fasol (8.47 mg/g) - a concentration significantly above the commonly reported 0.8-1.5 mg/g range. This finding implies an exceptional capacity for organic nitrogen storage and conversion into reserve proteins, reflecting an advanced nitrogen regulatory system in this cultivar. From a biomedical perspective, glutamine plays crucial roles in maintaining intestinal epithelial integrity, supporting immune cell energetics, and mitigating inflammatory responses. Therefore, Belaya fasol emerges as a promising cultivar for



functional foods and nutraceutical applications (Fig. 1, 2).

Asparagine, another major nitrogen-transporting amino acid, accumulated at particularly high levels in Ravot (1.53 mg/g) and Kalipso (1.78 mg/g). These results align with the findings of Viteri and Linares (2017), who demonstrated that asparagine accumulation increases under abiotic stress as part of an adaptive nitrogen mobilization strategy. The elevated asparagine levels observed here suggest that Ravot and Kalipso may possess enhanced tolerance to environmental stresses such as drought, salinity, or temperature fluctuations, providing a valuable foundation for stress-resilient breeding programs (Fig. 3, 6).

Alanine levels were markedly high, especially in Kalipso (5.97 mg/g) and most notably in Belaya fasol (7.96 mg/g), far exceeding typical literature values (2-2.5 mg/g). Alanine functions as a central intermediate in pyruvate metabolism and glucose-alanine cycling. Such elevated concentrations indicate heightened metabolic activity and energy turnover during seed filling. Increased alanine accumulation also suggests involvement in osmoprotection and rapid metabolic adjustments, further emphasizing Belaya fasol's exceptional biochemical activity.

Sulfur-containing amino acids - cysteine and methionine - showed significant variation among cultivars. The remarkably high cysteine content in

Ravot (2.08 mg/g) indicates a highly active glutathione antioxidant system, which is central to cellular redox homeostasis. These amino acids play key roles in detoxifying reactive oxygen species, stabilizing cellular membranes, and protecting proteins from oxidative damage. Meanwhile, the elevated methionine concentration in Belaya fasol (1.00 mg/g) enhances the biological value of its protein fraction, given that methionine is often a limiting amino acid in legumes. This trait significantly improves the nutritional quality of Belaya fasol and its relevance in dietary applications.

Aromatic amino acids - phenylalanine and tyrosine - serve as essential precursors to phenolic compounds, flavonoids, and anthocyanins. The high tyrosine content in Vir (2.02 mg/g) suggests active phenylpropanoid biosynthesis, contributing to antioxidant capacity and potential health-promoting properties. Belaya fasol likewise demonstrated elevated phenylalanine (0.45 mg/g), indicating a strong capacity for secondary metabolite production and reinforcing its suitability for functional food purposes (Fig. 1, 5).

Histidine levels were unexpectedly high in all cultivars (4.38-6.38 mg/g), surpassing the typical values reported for common beans (2.5-3 mg/g). This has significant nutritional implications, as histidine is essential for hemoglobin synthesis, growth



processes, inflammation modulation, and immune support. Elevated histidine concentrations position these cultivars - especially Belaya fasol - as valuable resources for food products targeting children, adolescents, and individuals requiring enhanced nutritional support.

The branched-chain amino acids (BCAA) - leucine, isoleucine, and valine - were most concentrated in Belaya fasol, highlighting its potential relevance for sports nutrition. BCAAs are essential for muscle protein synthesis, energy production during physical activity, and neurological balance through neurotransmitter regulation. Their abundance reinforces Belaya fasol's suitability for specialized high-protein and performance-oriented food formulations.

Proline accumulation was highest in Kalipso (1.24 mg/g), consistent with its known role as an osmoprotectant. Proline stabilizes proteins and membranes under dehydration, regulates cellular osmotic balance, and protects cells against abiotic stresses. This finding suggests that Kalipso is particularly well adapted to drought conditions and may serve as a promising genetic resource for breeding stress-tolerant cultivars.

Overall, the comparative analysis of amino acid profiles confirms the diverse nutritional, physiological, and biochemical potential of the studied cultivars. Belaya fasol stands out due to its exceptionally high levels of

glutamine, alanine, methionine, and BCAAs, indicating superior nutritional quality and strong applicability in food, medical nutrition, and biotechnology sectors. Ravot presents high cysteine and asparagine levels, supporting its potential for stress tolerance and antioxidant enrichment. Kalipso exhibits elevated proline, indicating strong ecological adaptability, while Vir displays a metabolically active aromatic amino acid profile linked to enhanced phenolic biosynthesis.

The analysis of free amino acids in the six bean cultivars examined revealed substantial variation both in the total content and in the distribution of individual amino acids. According to the results, the total amount of free amino acids was highest in Belaya fasol (37.05 mg/g) and lowest in Vir (19.44 mg/g). Such differences are attributable to the genetic characteristics of the cultivars, the maturity level of the seeds, the activity of nitrogen metabolism pathways, and differences in enzymatic transformations of amino acids. In general, the variation in total free amino acids directly influences the nutritional value of the seeds, the availability of substrates for protein synthesis, and the overall metabolic activity of the cultivars.

Regarding qualitative composition, alanine, glutamic acid, asparagine, and particularly histidine were identified as the dominant free amino acids across all cultivars. Alanine



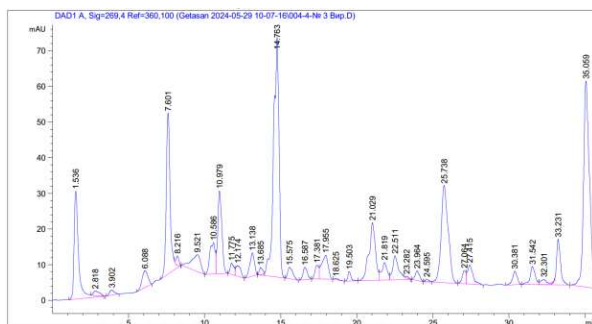
was consistently abundant (3.95–7.96 mg/g), reflecting its central role as a key metabolite in transamination reactions. In seeds, alanine functions not only as an energy-related molecule but also as a component of stress-protection mechanisms, as it easily converts to pyruvate and supports metabolic adaptation. Its remarkably high concentration in *B. fasol* suggests intensive nitrogen assimilation or enhanced turnover of proteinaceous compounds.

Histidine levels were also high in all cultivars (4.38–6.38 mg/g). As reported in the literature, this aligns with the tendency of bean seeds to accumulate aromatic amino acids with antioxidant properties. Histidine serves as a precursor for histamine and contributes to cellular protection, pH buffering, and metal ion chelation. Its abundance in the free form indicates a high degree of biochemical maturity in the seeds.

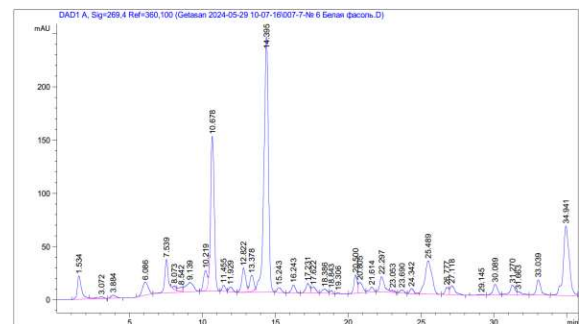
Asparagine (0.69–1.78 mg/g) and glutamine (0.15–8.47 mg/g) are the

principal forms of nitrogen transport and storage in legumes. One of the most striking differences in the dataset is the exceptionally high glutamine content in *B. fasol* -8.47 mg/g - which is 5–10 times higher than in the other cultivars. This suggests elevated activity of the glutamine synthetase/glutamate synthase pathway, potentially associated with enhanced nitrogen fixation, stress tolerance, or increased protein turnover. Asparagine also plays a major role as a storage metabolite during seed development and showed consistently high levels across the cultivars, fully consistent with known physiological patterns in legumes.

Cysteine and tyrosine occurred at moderate concentrations and are involved in redox regulation and the biosynthesis of phenolic compounds. The highest cysteine concentration was found in Ravot (2.08 mg/g), which may indicate active synthesis of sulfur-containing metabolites, including glutathione, a major antioxidant component in plants.



1



2

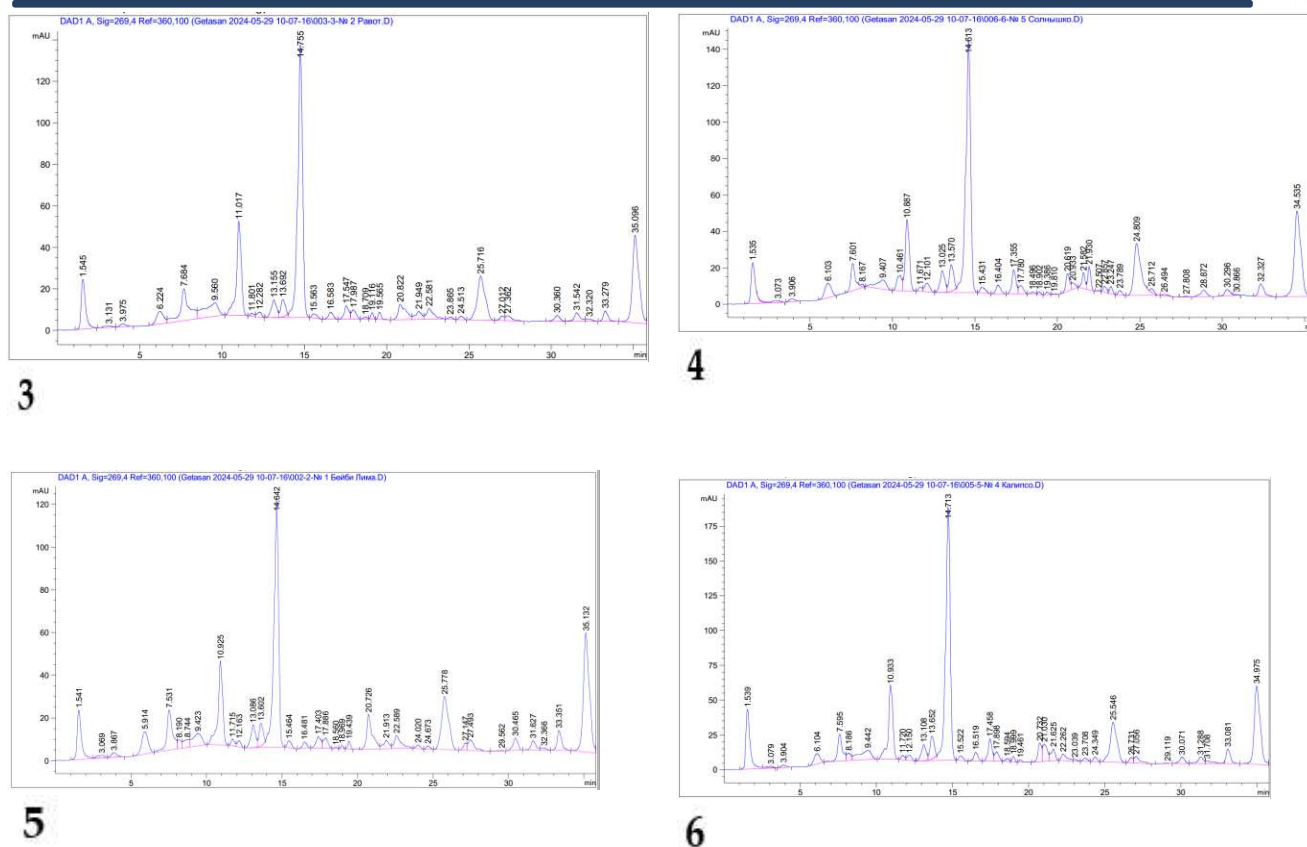


Fig 1. Analysis of free amino acids in common bean varieties: 1. Vir 2. Belaya fasol 3. Ravot 4. Solnyshko 5. B. lima 6. Kalipso

Among the essential amino acids, threonine, valine, leucine, isoleucine, lysine, methionine, tryptophan, and phenylalanine were present in low to medium amounts, consistent with patterns observed in other legumes. Methionine, typically the most limiting amino acid in legumes, ranged from 0.46 to 1.00 mg/g - well within the 0.2–1.2 mg/g range reported in the literature. Although present in small amounts, methionine strongly contributes to the biological value of bean proteins. Lysine (0.20–0.58 mg/g), which is often deficient in cereals, appeared in sufficient quantities in the studied cultivars; thus, combining beans with cereal products can substantially improve protein quality in the human diet. Moderate concentrations of

tryptophan and phenylalanine were also detected, both of which are precursors for key metabolites such as serotonin, nicotinamide, and phenolic compounds.

A comparative evaluation among cultivars revealed that Belaya fasol possesses the most metabolically active profile, with high nitrogen storage and markedly elevated amounts of free amino acids. Conversely, Vir exhibited the lowest total free amino acid content, possibly reflecting a higher conversion of amino acids into structural proteins or reduced metabolic activity. These differences are valuable for breeding programs targeting improved nutritional quality and enhanced protein profiles in bean cultivars.

Comparison of the findings with published data indicates that the



observed patterns fully align with international studies on *Phaseolus vulgaris*. Numerous reports highlight alanine, glutamate, asparagine, and histidine as the most abundant free amino acids in common beans [31]. The present results confirm that the amino acid profiles differ significantly among

cultivars and constitute a major determinant of their nutritional value. The presence of essential amino acids underscores the potential of these bean cultivars to serve as valuable sources of high-quality protein for human nutrition.

### Conclusion

In conclusion, the results provide comprehensive evidence that these *Phaseolus vulgaris* cultivars possess valuable amino acid compositions with implications for nutrition, health, stress physiology, and crop improvement. Their distinct metabolic characteristics make them suitable genetic materials for breeding programs, biofortification

initiatives, functional food development, and further biochemical research. These findings not only enrich the current understanding of amino acid diversity in common bean germplasm but also establish a solid scientific basis for future applied and theoretical investigations.

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