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Evaluation of Productivity and Seed Amino Acid Composition of Sainfoin (*Onobrychis viciifolia* Scop.) Cultivated in the Almaty Region of Kazakhstan

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ABSTRACT

Background: In Kazakhstan, the rapid expansion of animal husbandry has made the development of a stable, high-quality forage base a key priority and since its introduction into field production, sainfoin has gained widespread recognition as a promising crop due to its favorable biological traits. The aim of this study was to examine the sainfoin (*Onobrychis vicifolia* Scop.), variety Almaty 2, developed due to domestic selection efforts, along with 24 additional variety samples from the collection material. These patterns were evaluated for key economically valuable traits, including productivity, seed protein content and amino acid composition.

Methods: A field assessment of the local breeding material was conducted using phenological observations and measurements. The samples were ranked according to their total productivity of green and dry mass and subject to cluster and correlation analyses.

Result: Five specimens out of the 24 sainfoin variety samples demonstrated a high level of productivity based on dry mass yield. The highest crude protein content was recorded in sample No. 4 (10.7%), while the Almaty 2 variety showed a slightly lower value of 9.9%. Comparative amino acid analysis of sainfoin seeds revealed elevated levels of essential amino acids in sample No. 4, particularly methionine (512.8 mg/100 g), phenylalanine (890.1 mg/100 g), threonine (418.7 mg/100 g) and histidine (4453.3 mg/100 g), compared to Almaty 2 and previously reported data for wheat seeds. Some amino acids in wheat protein are considered limiting, supporting the classification of the studied Kazakhstan sainfoin varieties as sources of high-quality protein suitable for food supplements and combined food and forage use.

Key words: Amino acid composition, *Onobrychis vicifolia* scop., Productivity, Sainfoin seeds, Sainfoin.

INTRODUCTION

In the context of agricultural intensification, the search for and introduction of highly productive forage crops with high nutritional value and resistance to adverse environmental factors is of particular significance. One of such promising crop is sainfoin (*Onobrychis viciifolia* Scop.), a perennial leguminous plant that is highly resistant to drought and disease. Sainfoin is known for its ability to improve soil structure by enriching it with nitrogenous compounds owing to its extensive root system, which penetrates deeply into the soil. This powerful root system contributes to the plant's overall viability and enhances the soil's protection against erosion of various origins (Sakhraoui *et al*., 2024; Ertuş, 2021; Sariyildiz and Savaci, 2020).

Sainfoin plants are cultivated as a valuable and nutritious feed, highly regarded as one of the best honey plants and a source of medical preparations. In animal husbandry, sainfoin is used to produce feed and grass meal, possessing superior nutritional properties, compared to other forage crops. Perennial sainfoin has an advantage over annual forage crops during spring-summer droughts due to its drought resistance. This resistance stems from its ability to absorb moisture from deeper soil layers, facilitated by a well-developed root system (Batkhishig, 2023; Sakhraoui *et al*., 2024).

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In Kazakhstan, with the intensive development of animal husbandry, creating a robust and high-quality forage base is a critical challenge. Sainfoin was introduced into field forage production in the 1950s and, due to its biological characteristics, has become widely adopted as a promising crop. A previously compiled register identifies of popular varieties and species used in forage production, selected for their suitable technological qualities summarized those efforts (Bulatova *et al.*, 2015). However, modern data on this crop and its varietal characteristics for zoning in Kazakhstan's diverse climatic zones are lacking. One approach to developing popular and adapted varieties is the introduction and expansion of variety samples and lines with enhanced nitrogen-fixing capacity and productivity based on selective breeding.

Increased sown areas of sainfoin and other legumes can be achieved through the breeding of nitrogen-fixing, high-yielding, rareripe varieties that are suitable for mechanized cultivation and harvesting and resistant to temperature fluctuations.

The use of forage grain legumes in crop rotations can play a key role in improving the physico-mechanical properties of soil and enhancing its fertility, simultaneously reducing the need for nitrogen fertilizers. Introducing sainfoin into cultivation helps set up a positive humus balance in crop rotations, positioning this crop as one of the best precursors for cereals. This is because sainfoin can accumulate up to 200 kg/ha of nitrogen in the arable layer, thereby increasing not only the yield of cereals but also the quality of their grain (Xiang *et al.*, 2024; Tufenkci *et al.*, 2006).

Sainfoin seeds are a valuable source of plant-based protein, which plays a critical role in providing animals with a complete diet. Several studies have emphasized that the protein fraction of sainfoin seeds is highly digestible and biologically valuable, making it an important component of animal rations, particularly in organic and pasture-based livestock systems (Craine *et al.*, 2024; Andaç *et al.*, 2025; Craine *et al.*, 2023).

The amino acid profile of sainfoin seeds can vary significantly depending on the genotype, climatic and soil conditions, cultivation techniques and harvest stage. In this regard, studying the amino acid composition of seeds from different sainfoin varieties and lines is particularly relevant to identify forms with the highest protein value.

Currently, another strategic issue is functional nutrition, suggesting that specific products not only meet the body's basic needs for proteins, fats and carbohydrates but also provide additional health benefits (Wiyono *et al.*, 2024). For instance, sports nutrition includes products and supplements with high protein and amino acid content, as well as various beverages and shakes containing dietary fiber, probiotics, antioxidants and vitamins (Kostrakiewicz-Gierałt, 2024). In this context, the use of well-known agricultural crops, particularly legumes, which are rich in essential biologically active compounds, is highly relevant for the production and enrichment of functional foods. Therefore, studying crops such as sainfoin deserves special attention (Andaç *et al.*, 2025). Moreover, sainfoin is not only used as livestock feed but also serves as a functional additive in the confectionery industry, derived from its seeds and leaves. Thanks to its advanced root system, sainfoin absorbs elevated levels of nutrients from the soil. Sainfoin contain several macro- and microelements, such as P, Ca, Mg, K, Đ, Fe, Mn, Fe, Cu, Ni and Zn in concentrations compared to other legumes (Kaplan *et al.*, 2019, Kidambi *et al.*, 1989; Baker *et al.*, 1952; Kidambi *et al.*, 1990). Other authors also declare that the carotene content, which is presented by neoxanthin, violaxanthin, lutein, zeaxanthin, 9z-β-carotene and 13z-β-carotene, is equal to 246 mg/ kg^{-1} DM in fresh mass (Rufino-Moya, 2022). Recent studies have shown that this crop possesses additional beneficial properties, due to its unique tannin composition, which provides antihelmintic effects, enhances protein absorption and prevents tympany in cattle (Carbonero *et al.*, 2011).

However, it is known that by-products of nutritional components and plant raw materials may contain hazardous or potentially hazardous compounds. Therefore, upon developing recommendations for new unconventional raw materials in food production, detailed studies of their chemical and biochemical composition are necessary, which will be in the focus of our future research.

The relevance of this article results from the need for a comprehensive evaluation of the productivity, amino acid content and protein value of seeds from certain sainfoin varieties to identify the most promising specimens for their use in breeding programs and as a source of high-quality forage, the stock of food ingredients and biologically valuable raw materials.

The aim of this study is to conduct a comparative assessment of the productivity, amino acid content and protein value of seeds from the Almatinsky 2 variety of Kazakhstani selection and several sainfoin varieties (*Onobrychis viciifolia* Scop.), with an emphasis on identifying genotypes with an optimal balance of agronomic and biochemical characteristics.

MATERIALS AND METHODS

Plant material collection and experimental site

The experimental work was carried out at the Laboratory of Plant Genetics and Breeding, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University (Almaty, Kazakhstan). Seed material of the Almatinsky 2 variety and 24 accessions of common sainfoin (*Onobrychis viciifolia* Scop.), along with experimental plots, were provided by the Forage Crops Laboratory of the Research Institute of Agriculture and Plant Growing, Almalybak, Almaty Region, Kazakhstan. The Almatinsky 2 variety was developed through repeated mass selection of populations under open pollination, using superior sainfoin specimens and was included in the State Register in 1980. This variety is characterized by lodging resistance and a vegetative period of 86-103 days (from sowing to full seed maturity), 52-70 days (from sowing to the first cut) and 30-42 days (from the first cut to the second cut).

Structural analysis

Structural analysis of sainfoin was conducted during the period from 2023 to 2025 based on several characteristic parameters, following the methodological guidelines for breeding perennial grasses developed by the V.R. Williams All-Union Research Institute of Forage (Dospekhov, 2014). For each biometric parameter, the mean value, standard error of the mean and correlation coefficient were calculated.

Statistical analysis

Data were analyzed using correlation, one-way analysis of variance (ANOVA) and cluster analyses, performed with the software packages GraphPad Prism 9, Past 4, SPSS and Microsoft Excel 2021 (Sokal and Rohlf, 2013).

Protein content analysis

Protein and amino acid content were analyzed in 2024. Protein content in the samples was determined using the Kjeldahl method, following standard analytical procedures outlined by the Association of Official Analytical Chemists (AOAC, 2000). Dry, homogenized seed material (1.0 g) was treated with concentrated sulfuric acid (H_2SO_2) in the presence of a catalyst (potassium sulfate and copper sulfate) to mineralize organic nitrogen into ammonium sulfate. The samples were then neutralized with sodium hydroxide (NaOH), subjected to the sublimation (steam distillation) to release ammonia, which was trapped in a boric acid solution and titrated with a standard solution of hydrochloric acid (HCl) to quantify nitrogen content. Crude protein content was calculated by multiplying the total nitrogen content by a conversion factor of 5.95:

$$\text{Protein (\%)} = \text{Nitrogen (\%)} \times 5.95$$

To ensure accuracy and reproducibility, all measurements were performed in triplicate (Horwitz, 2000).

Amino acid content analysis

Total amino acid (AA) content in seed samples was quantified using high-performance liquid chromatography (HPLC) following acid hydrolysis. Amino acid derivatization was performed using pre-column phenylisothiocyanate (PITC) chromatography. Separation of amino acid derivatives by HPLC required two mobile phases. Mobile phase A consisted of 99% HPLC-grade acetonitrile and 1% acetic acid, whereas the mobile phase B comprised 99.9% HPLC-grade water, 0.1% acetic acid and 0.1 M sodium acetate. All the buffers were filtered through 0.2 µm membrane filters and degased. Chromatographic separation of amino acid fractions was conducted using a Shimadzu Prominence LC-20 system (Shimadzu, Japan), equipped with a UV detector (SPD-20A) and a fluorescence detector (RF-10AXL). The HPLC system included a binary pump (LC-20AD), an autosampler (SIL-20AC), a degasser (DGU-20A5) and a column oven (CTO-20A), controlled by LCSolution software.

HPLC separation

Samples and standards were separated using a Thermo Hypersil GOLD C18 HPLC column (150 mm × 4 mm, 5 µm). UV detection of the fraction peaks was performed at the wavelength of 254 nm. The mobile phase flow rate was 0.8 mL/min. The total HPLC run time for separating amino acid derivatives in each sample or the blank was 43 minutes.

Pre-column derivatization

Pre-column derivatization of amino acids was carried out by using phenylisothiocyanate (PITC). Sample hydrolysis (1,000 µL) was performed in 1 M HCl at 110°C for 13 hours. The hydrolyzed sample was dried under vacuum, then redissolved in 150 µL of 0.1 M NaOH, 50 µL of deionized water and 350 µL of PITC reagent (propanol/PITC/triethylamine [8:1:1, v/v/v]). The reaction mixture was incubated at room temperature for 30 minutes. Subsequently, PITC was removed under a nitrogen atmosphere and the resulting amino acid derivative mixture was dissolved in 1.5 mL of water, filtered through a 0.45 µm syringe filter and injected into the HPLC system (injector diameter: 10 µm).

Amino acid standards

Amino acid standards (L-aspartic acid [Asp], L-glutamic acid [Glu], L-serine [Ser], L-asparagine [Asn], L-histidine [His], L-arginine [Arg], L-threonine [Thr], L-alanine [Ala], L-proline [Pro], L-cysteine [Cys], L-tyrosine [Tyr], L-valine [Val], L-methionine [Met], L-cystine [Cys-Cys], L-isoleucine [Ile], L-leucine [Leu], L-phenylalanine [Phe] and L-lysine [Lys]) were obtained from Titan Biotech Ltd. All standards were commercially available, of pharmaceutical grade and had a purity of ≥ 99%.

Preparation of standard solutions

The above-mentioned standards were accurately weighed and dissolved in 0.1 M HCl to prepare stock standard solutions for each amino acid (1 mg/mL). Each 1 mL standard solution was evaporated under vacuum and derivatized as described above. Calibration solutions were prepared by serial dilution to achieve five concentrations for each amino acid: 1, 10, 25, 50 and 100 µg/mL (Avino *et al.*, 2006; Prasad, 2017).

RESULTS AND DISCUSSION

To investigate the symbiotic capacity of sainfoin varieties and hybrids in planned studies, it was essential to identify accessions that surpass the reference variety Almatinsky 2 in terms of green (fresh) mass, dry mass, forage quality and protein content. According to Mohajer *et al.* (2012), high-yielding genotypes typically combined favorable morphological traits, supporting the integration of multivariate analysis for more efficient genotype evaluation in forage improvement programs.

Growth characteristics and productivity of selected sainfoin accessions from the Kazakhstani collection were evaluated under the conditions of Almaty Region. Field observations were conducted from 2023 to 2025 on experimental plots and production fields at the Kazakh Research Institute of Agriculture and Plant Growing, Almalybak, Almaty Region, Kazakhstan. Twenty-four numbered sainfoin accessions were selected for the study: 41872, 87, 40816, 1663, 40937, 1544, 40998, 40822, 41241, 40935, 40994, 42300, 38623, 40790, 1, 2, 3, 4, 5, 6, 7, 8, 10, along with the reference variety Almatinsky 2. The evaluation focused on key agronomic parameters, including plant height, foliage (number of leaves per plant), productive tillering (number of productive shoots per plant), green mass yield and dry mass yield. Quantitative analysis of 50 plants per accession yielded the following results for each parameter (Fig 1).

Comparative analysis of plant height and foliage among the studied sainfoin accessions revealed minor variations in these parameters (Fig 1a, b). The average plant height was approximately 67 cm. Sainfoin, a herbaceous perennial, can reach stem heights of up to 1 meter.

Foliage significantly influences the forage quality of grasses and is indicative of overall productivity. Compared to alfalfa, sainfoin exhibits greater foliage and fleshy stems (Sokal *et al.,* 2013). Relative to the reference variety Almatinsky 2, all numbered accessions showed increased foliage, which consistently corresponded to their plant height values (Fig 1a, b). Fig 1 illustrates the dynamics of foliage (%), which aligns with plant height (cm). A significant positive correlation was observed between these two traits, with a correlation coefficient of r = 0.60±0.02 (Fig 2).

Plant height and productivity

The reference variety Almatinsky 2 exhibited a reduced foliage index (39.0±0.5%) with minimal relation to plant height (69.0±0.8 cm). In contrast, the accession No. 3 displayed high, consistent values for both plant height (79.0 ±0.7 cm) and foliage (51.6±0.9%) (Fig 1a, b). However, this correlation between foliage and plant height did not correspond to increased yields of green or dry mass (Fig 1d).

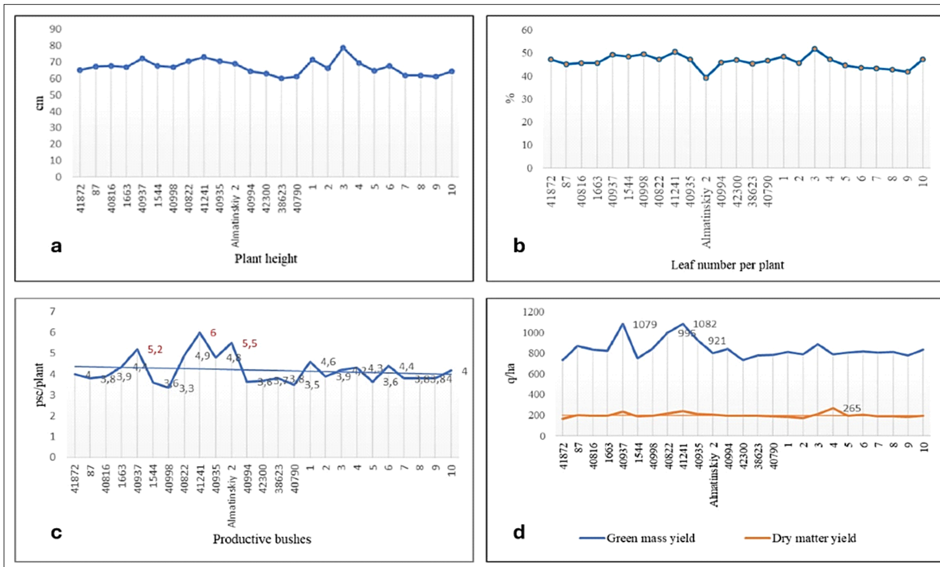
Productive tillering, defined as the ability of plants to form additional shoots, is a critical breeding trait influencing crop productivity. Analysis of the tillering determined three accessions - 40937, 41241 and 40935, that exhibited significantly greater green and dry mass yields compared to the reference variety Almatinsky 2 (Fig 1c). A substantial positive correlation was observed between tillering and green mass yield in sainfoin, with a correlation coefficient of r = 0.70±0.02 (P<0.01) (Fig 2). However, no significant correlations were detected between other trait pairs, such as foliage and dry mass yield or foliage and tillering (Fig 2).

Dry mass yield is a key indicator of the breeding value of forage crops. To evaluate this parameter, one-way analysis of variance (ANOVA) was conducted to identify the most promising genotypes for the yield potential. Sainfoin dry mass is a valuable animal feed-stuff, enriched with vitamins and minerals, including vitamin C and calcium, which are particularly beneficial for young cattle (Craine *et al.,* 2023).

Dry mass yield data (Fig 1d) indicated that most tested accessions exhibited stable mean values comparable to the trend line of the reference variety Almatinsky 2. However, accessions 40937, 40822, 4, 41241 and 40935 showed considerable deviations in dry mass yield relative to the reference variety, as determined by ANOVA (Fig 3).

To classify the studied plant collection's variety samples into groups similar in a set of characteristics, the cluster analysis method was applied. For this, the Ward method with the Euclidean distance as a tool for measuring the extent of similarity was used. The clustering data are presented as a dendrogram (Fig 4).

The following characteristics were considered as important economically valuable characteristics of sainfoin:



a) by plant height, b) leaf number per plant, c) productive bushes, d) yield of green mass and dry matter yield.

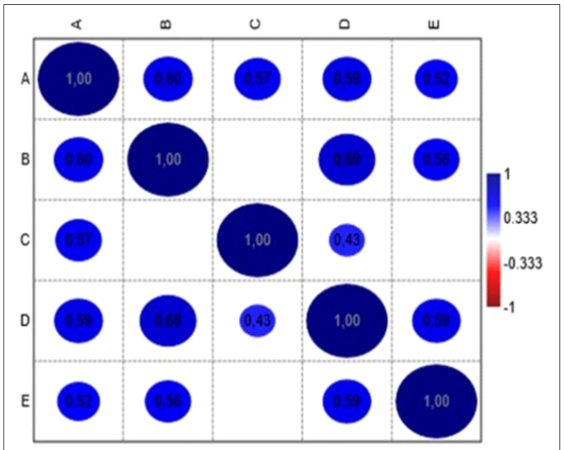
Fig 1: Distribution of chosen sainfoin samples (cv. Almatinskiy 2, 41872, 87, 40816, 1663, 40937, 1544, 40998, 40822, 41241, 40935, 40994, 42300, 38623, 40790, 1-10).

plant height, tillering, foliage, green and dry productive mass. Based on the cluster analysis, 6 clusters were obtained. In this case, the variety samples forming a single cluster had a similar set of characteristics. The first and second clusters included 4 sainfoin variety samples (40937, 41241, 40935, 40822), which were characterized by a tall stem, high tillering, green and dry mass (Table 1).

Cluster grouping of accessions

The third cluster comprised seven sainfoin accessions (38623, 40790, 9, 2, 41872, 1544, 42300), characterized by low productivity in terms of green and dry mass yields.

Accession No. 4 was ascribed to a distinct fourth cluster, exhibiting average values for all traits except dry mass yield, which was notably high (Table 1). This accession matched the reference variety Almatinsky 2 across all evaluated parameters, except its superior dry mass yield, indicating its high forage quality.



A - Plant height; B - Productive bushes; C - Leaf number per plant; D - Yield of green mass; E - Dry matter yield.

Fig 2: Correlation matrix by different studied characters.

The fifth cluster included the reference variety Almatinsky 2 and six sainfoin accessions, all displaying similar average values for all traits, comparable to the control.

The sixth cluster consisted of six accessions (40998, 40994, 10, 40816, 87, 3). These accessions differed from the reference variety in dry mass yield, although the differences were generally insignificant or did not exceed the control (Fig 3). For other evaluated parameters, the values were either comparable to or slightly higher than the control, with no substantial impact on green or dry mass yields.

Selection of promising genotypes

Cluster analysis enabled the grouping of 25 sainfoin accessions based on multiple traits, identifying clusters of closely related genotypes. Based on the results of analysis of variance (ANOVA) and cluster analysis, five accessions, such as 40937, 40822, 41241, 40935 and 4 were selected from the 25 evaluated for their superior performance compared to the reference variety Almatinsky 2 (Table 1). These accessions were identified as the most promising for further breeding and genetic studies.

Protein content and amino acid profile

Given the growing importance of functional nutrition, this study also assessed the crude protein content in seeds of the reference variety Almatinsky 2 and accession No. 4, selected as the top-performing accession. Additionally, the amino acid composition of seed proteins from both the reference variety and accession No. 4 was determined (Table 2, Fig 5). The accession No. 4 surpassed the reference variety in crude protein content, with the values of 10.7% and 9.9%, respectively.

Comparative analysis of the amino acid profiles of the two evaluated accessions (Almatinsky 2 and No. 4) revealed significant quantitative differences in the content of both essential and non-essential amino acids (Table 2).

Accession No. 4 exhibited higher concentrations of most amino acids compared to the reference variety

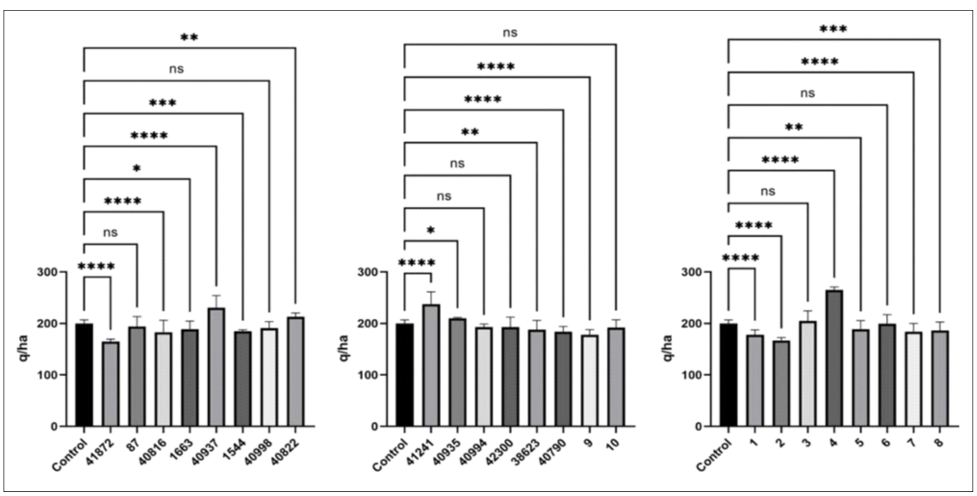


Fig 3: Comparative analysis of dry matter yield of sainfoin, cv. Almatinskiy 2 (reference variety) and 24 specimens of the Kazakh collection.

Almatinsky 2, suggesting greater biological, forage and nutritional value. Particular attention was focused on essential amino acids, which are critical for complete protein nutrition. Noteworthy, accession No. 4 contained significantly higher levels of histidine (4453.33 µg/100 g vs. 3472.40 µg/100 g in Almatinsky 2). Histidine is the essential substance for tissue growth and development, hemoglobin synthesis and immune system function (Wu, 2010).

Accession No. 4 also exposed substantially higher levels of other essential amino acids, including methionine (512.82 µg/100 g vs. 283.64 µg/100 g in the reference variety), which is involved in methylation, detoxification and cysteine biosynthesis (Brosnan and Brosnan, 2006) and lysine (260.67 µg/100 g vs. 144.23 µg/100 g), which is crucial for growth, antibody formation and calcium absorption (Martínez, 2017; Matthews, 2020). Some studies point out the synergistic role of lysine and vitamin C in synthesizing L-carnitine, which enhances muscle oxygen utilization, improves endurance and supports bone growth and collagen production in connective and bone tissues (Furusawa *et al*., 2008).

Furthermore, accession No. 4 has indicated the elevated levels of branched-chain amino acids (BCAAs),

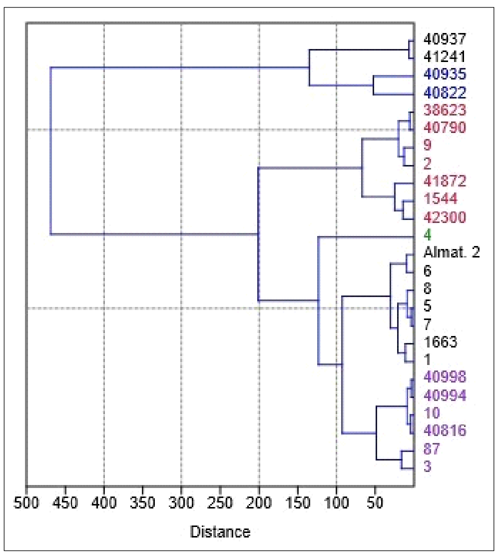


Fig 4: Dendrogram of clustered sainfoin collection samples by economically valuable characters using Ward's method.

specifically leucine and isoleucine. These amino acids, constituting up to 35% of muscle tissue, play a central role in skeletal muscle metabolism, recovery from physical exertion and activation of protein biosynthesis (Newsholme and Blomstrand, 2006). The increased content of these amino acids enhances the potential value of accession No. 4 for sports nutrition and therapeutic diets.

Arginine content in accession No. 4 (5324.16 µg/100 g) also significantly exceeded that of the reference variety (3943.21 µg/100 g). Arginine is vital for regulating vascular tone, wound healing and nitric oxide synthesis, particularly under physiological stress (Wu *et al*., 2009).

Additionally, accession No. 4 exhibited higher levels of cystine, alanine, aspartic acid and other amino acids compared to Almatinsky 2, indicating an enriched protein composition or greater bioavailability.

Accession No. 4 had lower tyrosine content (230.71 µg/100 g) compared to Almatinsky 2 (409.36 µg/100 g), but significantly higher phenylalanine content (890.08 µg/100 g vs. 511.73 µg/100 g). The phenylalanine-to-tyrosine ratio (tyrosinylation coefficient) in accession No. 4 was 3.86, more than three times higher than that of the reference variety (1.25). This elevated ratio may reflect greater comprehensive stability (mechanical, thermal and chemical) of membrane proteins in accession No. 4 compared to the control (Scriver *et al*., 2001). It may also indicate increased activity of enzymes involved in the tyrosine biosynthesis (Zhumabaeva *et al*., 2017).

Comparison with wheat

Given the potential use of sainfoin seed powder in flour-based confectionery products (Tarasenko, 2015), a comparative analysis was conducted between the experimentally determined key amino acid contents in seeds of accession No. 4 and wheat seeds, based on literature data (Zenkova, 2019). Fig 5 presents the amino acid profiles of accession No. 4 (blue) and wheat (orange), expressed in mg/100 g of product (Fig 5).

Accession No. 4 showed higher methionine content comparing to the wheat, suggesting greater biological value of the former in this respect. Methionine is a limiting amino acid in cereals and its elevated presence can improve the overall amino acid profile of a product. Methionine plays a critical role in metabolism, protein

Table 1: Comparative study of five selected sainfoin varieties from Kazakhstani collection based on the main economically valuable characteristics (2023-2025).

Samples	Plant height, cm	Bushiness, pcs/plant	Leaf number per plant, %	Yield of green mass, q/ha	Dry matter yield, q/ha
Almatinsky	269.0±0.8	5.5±0.1	39.0±0.5	800.2±4.6	200.2±1.2
40937	72.4±1.1	5.2±0.1	49.0±0.5	1078.7±18.7	230.4±4.3
40822	70.5±0.4	4.9±0.2	47.1±0.4	995.0±1.9	213.0±1.3
41241	73.0±0.9	6.0±0.2	50.3±1.0	1082.1±19.7	237.7±4.3
40935	70.7±0.3	4.8±0.2	47.0±0.2	921.0±0.3	210.0±0.3
No.	469.6±1.2	4.4±0.1	47.0±0.8	785.0±3.2	265.0±1.1

biosynthesis and the reaction of transmethylation as the methyl group donor (*via* S-adenosylmethionine). It is also involved in the synthesis of biomolecules, such as cysteine, creatine and glutathione, thereby supporting the cellular antioxidant defense. Methionine deficiency can impair growth and protein metabolism, particularly in humans and animals consuming grains with low amounts of this amino acid (Brosnan and Brosnan, 2006).

By contrast, glutamic acid and proline contents were significantly higher in wheat, attributed to the elevated levels of glutelins and prolamins, which determine the technological properties of wheat flour. Oppositely, sainfoin accession No. 4 disposed lower levels of these amino acids, suggesting differences in protein composition and functional properties, when compared to the wheat grain. Cystine was present in substantial amounts in accession No. 4 (~1800 mg/100 g), whereas wheat contained significantly smaller amounts of that amino acid. Cystine and proline are amino acids playing substantial biological roles in plant stress adaptation.

Analysis revealed that sainfoin accession No. 4 excelled wheat in the content of several biologically valuable amino acids, namely arginine, histidine and aspartic acid. Arginine and histidine play critical parts in protein metabolism and immunomodulation, specifying the high nutritional and functional value of this sainfoin accession. Higher arginine content, which supports nitric oxide synthesis and nitrogen metabolism regulation and the increased histidine levels, essential for hemoglobin synthesis and tissue growth, are particularly remarkable.

Accession No. 4 also has demonstrated a significant accumulation of aspartic acid (2985.1 mg/100 g) compared to wheat grain (598.3 mg/100 g). Aspartic acid is imperative for energy metabolism, brain function and the synthesis of other amino acids. Aspartate contributes to ATP generation, acts as a neurotransmitter, supports the functioning of the central nervous system and participates in DNA replication and transcription. Moreover, D-aspartate can temporarily stimulate testosterone synthesis (Roshanzamir and Safavi, 2017).

Accession No. 4 has surpassed wheat by the content of phenylalanine and threonine, amino acids that are limited in wheat protein but decisive for its enhanced biological value. Phenylalanine (890.08 mg/100 g, as previously discussed) and threonine (418.73 mg/100 g)

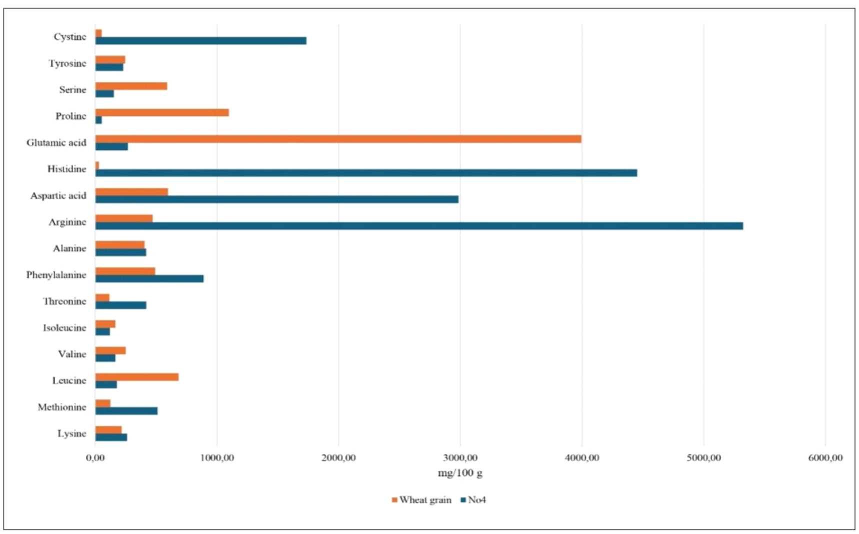


Fig 5: Comparative analysis of amino acid profiles from seeds of sample No. 4 of sainfoin and wheat seeds (based on average values from literature data).

Table 2: Amino acid composition of sainfoin seeds of the cv. Almatinsky 2 (No. 2) and sample No. 4.

AA	Aspartic acid	Glutamic acid	Serine	Asparagine	Histidine*	Arginine
No. 2	2092.08	337.51	250.88	2118.13	3472.40	3943.21
No. 4	2985.12	268.43	152.63	1818.90	4453.33	5324.16
AA	Threonine*	Alanine	Proline	Cysteine	Tyrosine	Valine*
No. 2	325.19	313.05	55.35	72.00	409.36	219.24
No. 4	418.73	417.45	54.01	125.39	230.71	165.26
AA	Methionine*	Cystine	Isoleucine*	Leucine*	Phenylalanine*	Lysine*
No. 2	283.64	1297.42	98.01	88.44	511.73	144.23
No. 4	512.82	1735.76	118.64	178.73	890.08	260.67

AA - Amino acid, *Essential amino acids samples amino acid content, mg/100 g.

levels in sainfoin were obviously higher than those in wheat flour (491.8 mg/100 g and 116.7 mg/100 g, respectively). Threonine is an essential amino acid that neutralizes toxins, supports fat metabolism in the liver and serves as the key component of collagen (Lee and Kim, 2019; Wen *et al*., 2023).

Comparative analysis depicts the balanced amino acid profile of sainfoin accession No. 4 relative to wheat grain, positioning this sanfoin specimen as a promising candidate for developing functional food products or combined protein mixtures in dry or liquid forms. Increased antioxidant activity under stress conditions may reflect an adaptive metabolic response, highlighting the species as a promising source of biologically active compounds (Beyaz and Yıldız, 2020). This, in turn, supports its potential application in the development of functional feed additives.

CONCLUSION

This study demonstrated that sainfoin (*Onobrychis viciifolia* Scop.) grown under the agroecological conditions of the Almaty Region shows stable productivity and high protein quality. Significant differences between the Almatinsky 2 variety and accession No. 4 indicate substantial genetic variability and strong breeding potential.

Accession No. 4 exhibited an improved amino acid profile, with higher levels of essential amino acids (methionine, phenylalanine, threonine and histidine) compared to the control and wheat, highlighting its value as a high-quality protein source. The observed amino acid balance further supports its potential use in functional foods and specialized nutrition.

Overall, the studied sainfoin accessions represent promising genetic resources for forage production and breeding programs aimed at enhancing protein composition. They may also serve as alternative protein sources to complement cereal-based diets, particularly in regions with similar environmental conditions.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare no conflict of interests.

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