Cultivating Crops as Alternative Protein Sources: Exploring Three Diverse Cereal Grain Varieties

Termesztett növények termesztése alternatív fehérjeforrásként: Három különböző gabonafajta vizsgálata

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Abstract: Protein-based plants, a key trend in food system transformation, hold immense potential to foster a sustainable and nutritious food system cycle. This research, which compares three ancient cereal grain varieties, aims to address pressing issues of environmental degradation, food security, and public health. The findings from these alternative protein sources promise nutritional benefits and offer diversity cultivation advantages, inspiring a promising future for our food systems and public health.

The study stands out for its meticulous examination of cereal grain varieties, including proso millet (Gyöngyszem, Biserka, and Rumenka), winter wheat (SE15), and buckwheat (Hajnalka). These distinct varieties, registered with the Institute of Research and Study Farm, University of Debrecen, Nyíregyháza, were subjected to diverse cropping systems under the control of N fertilizer, particularly (N 27 % CAN), with a rate of (80 kg N/ha - 300 kg/ha CAN), applied for each cereal grain for the 2021 and 2022 cultivation seasons. The statistical results, which showed a significant variance attributed to a variety group ($P \leq 0.05$), confirm confidence in the reliability of the findings.

Accordingly, the results revealed that the crude protein contents fluctuated among the tested varieties. They were 16.7 g/kg to 19.0 g/kg for proso millet varieties, 19.3 g/kg for winter wheat, and 17.4 g/kg for buckwheat. However, the extra crude protein in the buckwheat crop was linked to the adverse effect of the amount of N fertilizer used. This was evident as the control sample measured 18.4 g/kg and then dropped to 16.5 g/kg, indicating a negative correlation between N fertilizer and protein content. The findings also indicated that Hungarian proso millet varieties have abundant protein contents, which was a reason to examine the colour profile and sort the three respective varieties based on the varied colour profiles, which can contribute to predicting the physical properties that can affect of developed final product.

Overall, protein-based plants are emerging strategies that could contribute to the green revolution, SDGs targeting, quarantine of natural food sources, and protection against uncommunicable diseases, moreover, supporting that the finding outcomes could benefit the breeders, nutritionists, and industrial sector in a wide manner.

Keywords: *cereal grain, N fertilizer, crude protein, diverse varieties, proso millet*

Összefoglalás: A fehérjealapú növények, amelyek az élelmiszerrendszer átalakításának egyik fő irányvonala, óriási potenciállal rendelkeznek a fenntartható és tápláló élelmiszer-rendszer körforgásának előmozdításában. Ez a kutatás, amely három ősi gabonafajtát hasonlít össze, a környezetromlás, az élelmezésbiztonság és a közegészségügy égető kérdéseit kívánja kezelni. Az alternatív fehérjeforrásokból származó eredmények táplálkozási előnyöket ígérnek és sokszínű termesztési előnyöket kínálnak, ígéretes jövőt inspirálva élelmiszerrendszereink és a közegészségügy számára.

A tanulmány kiemelkedik a gabonafajták, köztük a prozofű (Gyöngyszem, Biserka és Rumenka), az őszi búza (SE15) és a hajdina (Hajnalka) aprólékos vizsgálatával. Ezeket a Debreceni Egyetem Nyíregyházi Kutató- és Tanulmánygazdaság Intézetében bejegyzett különböző fajtákat a 2021-es és 2022-es termesztési idényben az egyes gabonafélékre kijuttatott N-trágya, különösen (N 27 % CAN), (80 kg N/ha - 300 kg/ha CAN) mennyiségben történő N-trágyázás ellenőrzése mellett különböző vetésrendszerekben vetettük ki. A statisztikai eredmények, amelyek a fajtacsoportnak tulajdonított szignifikáns eltérést mutattak ($P \leq 0,05$), megerősítik az eredmények megbízhatóságába vetett bizalmat.

Ennek megfelelően az eredmények azt mutatták, hogy a nyersfehérje-tartalom a vizsgált fajták között ingadozott. A prozofű fajták esetében 16,7 g/kg és 19,0 g/kg, az őszi búza esetében 19,3 g/kg, a hajdina esetében pedig 17,4 g/kg volt. A hajdina termésében a többlet nyersfehérje azonban a N-trágya használatának kedvezőtlen hatásával függött össze. Ez nyilvánvaló volt, mivel a kontrollminta 18,4 g/kg-ot mért, majd 16,5 g/kg-ra csökkent, ami negatív korrelációt jelez a N-trágya és a fehérjetartalom között. A megállapítások azt is jelezték, hogy a magyar prozofű fajták bőséges fehérjetartalommal rendelkeznek, ami indokolta a színprofil vizsgálatát és a három megfelelő fajta különböző színprofilok alapján történő válogatását, ami hozzájárulhat a fizikai tulajdonságok előrejelzéséhez, amelyek hatással lehetnek a kifejlesztett végtermékre.

Összességében a fehérjealapú növények olyan feltörekvő stratégiák, amelyek hozzájárulhatnak a zöld forradalomhoz, a fenntartható fejlődési célok eléréséhez, a természetes élelmiszerforrások karanténjához és a nem fertőző betegségek elleni védelemhez, továbbá támogatva, hogy a megállapítások eredményei széles körben hasznára válhatnak a nemesítőknek, a táplálkozástudományi szakembereknek és az ipari ágazatnak.

***Kulcsszavak**: gabonafélék, N-trágya, nyersfehérje, különböző fajták, proso köles*

1. Introduction

The insertion of alternative plant protein sources has emerged as a crucial measure in pursuing sustainable and nutritious food systems. Considering environmental, food security, and public health factors (Sobczak et al., 2023). Therefore, the emergence of alternative protein-based plant sources has become a critical pathway for agriculture nutrient management (Langyan et al., 2022). In recent years, the demand for plant-based proteins has increased due to an increasing population that has adopted a variety of dietary preferences and a heightened awareness about the side effects of animal product diets (Hefferon et al., 2023). However, the principal objective is to create superior protein alternatives that can adequately fulfil the needs and preferences for healthful and ecologically sustainable food options, alleviate the environment associated with conventional meat production, and enhance food security (Henchion et al., 2017). the Grain cereals development is one of the top strategies in agricultural sectors. And can enhance their protein content and potential to alleviate the burden on global protein supplies (Taskinen et al., 2022). Sorghum, millet, and quinoa are emerging as sustainable protein sources, altering protein production and consumption. They employ their protein-rich kernels instead of the traditional carb-rich diets (Khalfalla et al., 2024; Medina Martinez et al., 2020). Organisations have highlighted intentions to maximise protein output and nutritional quality in cereal grains via modern agricultural methods, genetic alterations, and value-added processes, boosting collaboration between politicians, researchers, and farmers. (Khalfalla et al., 2024). Substitute crops have the capacity to offer money and economic prospects in developing nations. (Tabbita et al., 2017). Numerous grains, including winter wheat, proso millet, and buckwheat, can considerably boost plant protein sources. Their nutrient-dense and high protein content makes them ideal diet supplements (Banta et al., 2021). Individuals with gluten sensitivity may consume meals that do not contain wheat (Leser, 2013). Plants rich in protein contribute to about 60% of the global protein supply, offering a diverse array of amino acids and minerals essential for a balanced diet (Kowalczewski et al., 2023).

The research examined the crude protein content of winter wheat, buckwheat, and proso millet grown under a varied cropping scheme. The results help establish the investigated cereal grain as a viable substitute for protein-based plants as a potential unique source to insert into the human diet.

2. Material and Methods

2.1. Reporting the environmental conditions

We assessed environmental factors, including temperature and precipitation, to provide a more complete evaluation of the temporal and precipitation patterns over the years during the study's conduct. The collection also included mean values from the prior three decades and pinpointed the exact times of growth for each cereal grain. The winter season began on October 15, 2020, and proved beneficial for developing and expanding the winter wheat crop. The 197.3 mm of rain in October offered optimum conditions for planting 200 kg/ha of winter wheat. The region saw above-average rainfall during the buckwheat planting season on May 3, 2021, and proso millet planting on May 20, 2021. Specifically, there was an excess of 20.2 mm in April and 36.6 mm in May, which exceeded the normal rainfall for both months over several years.

In contrast, precipitation in June was modest, totalling just 14.9 mm, 61.1 mm below average. As a result, rainfall for the rest of the growing season was below normal. The autumn of 2021 had extremely little rainfall, with just 44.5 mm recorded before the winter wheat harvest on July 16, 2022. Only 1.7 mm of rain fell during winter wheat sowing on October 18, 2021. During the 2022 planting season, proso millet and buckwheat received 237.7 mm of precipitation. Only 81.5 mm of precipitation fell throughout the growth of these two crops. Proso millet growth was marked by severe drought, with rainfall dropping 180 mm below normal from May to September. Proso millet's growing season in 2022 had an overall total of 182.8 mm of precipitation. However, rainfall distribution was quite unequal, with 82% of the rainfall falling in September, which corresponded with the harvesting season for buckwheat (September 27, 2022) and proso millet (September 25, 2022).

2.2. Agronomic practice

The soil samples were taken 30 centimetres below the surface the sandy soil with a pH of 7.5 set this region apart. Soil characteristics of the test site are shown in Table 2. Winter wheat (Triticum aestivum L.), proso millet (Panicum miliaceum L.), and buckwheat (Fagopyrum esculentum L.) are the cereal crops that were examined. Soil construction properties are shown in Tables 2 and 3. The selected cereal crops' agricultural practices are detailed according to the nitrogen fertiliser application rate (80 kg N/ha, 300 kg/ha CAN).

Table 1. Characteristics of soil chemical construction for 2020, 2021, and 2022 production years

Property	Range of content
pH-KCl	7.3
KA	26
Salt content %	<0.02
CaCO3 %	<0.1
Humus %	0.6
NO3-NO2-N mg/kg	4.01
P2O5 mg/kg	126.0
K2O mg/kg	108.0
Zn mg/kg	0.33
Cu mg/kg	1.82
Mn mg/kg	37.6

Source: Nyíregyháza Study Institute.

**AL-K2O is potassium-oxide, KA represents soil cohesion number, AL-P2O5 is phosphorus-pentoxide (ammonium lactate solution)*

2.3. Investigation of crude protein levels

As the initial procedure of all preparations, the buckwheat, proso millet, and winter wheat samples were successfully differentiated. Before processing in a Retsch SK-3 hammer mill manufactured by Retsch GmbH Haan in Germany, the samples were dried and passed through a 1mm filter. The ground samples were stored in the Chemistry Central Laboratory of the University of Debrecen at a temperature of 25 °C in preparation for further analysis. Using the Kjeldahl technique, flour samples derived from cereal grains underwent nitrogen content testing. To create a digestion tube, 14 millilitres of sulphuric acid (H_2SO_4) were combined with a 1-gramme sample containing two S/3,5 Kjeltabs catalyst tablets. The homogenised samples were heated for two hours in a tube using a block heater at temperatures ranging from 420 to 430 degrees Celsius. This process was carried out to induce oxidation and decomposition of the materials. After digestion, the samples were let to cool down and then measured using converter 6.25, following the guidelines outlined in ISO 20483:2013.

2.4. Analysis of the colour profile

Utilising a Konica Minolta Camera (Chroma metre, CR-410, Minolta Co. Ltd., Japan, 2002), the hues of the husked grains were quantified as L* (whiteness), a* (redness), b* (yellowness), and Y (brightness) values.

2.5. Statistical tests

The statistical analysis was conducted using SPSS software (version 28.1). The data were assessed for normality using the Anova One Way test to determine the statistical variance between the dependent variables (varieties) based on the independent variable (total protein). The results were interpreted based on a significance level of $P \leq 0.05$.

3. Results and discussion

According to Table 2. the crude protein showed a significance variance ($P < 0.05$) based on the variety, while no significance variance ($P > 0.05$) based on the Year, Treatment, Year*Treatment, and Variety*Treatment. The result was aligned with the report (Khalfalla et al., 2024), the study showed that the variation of the crude protein among diverse varieties, did not show statistical variance ($P > 0.05$).

Table 2. Statistical variance of the factor effecting crude protein level among the tested varieties

Factor	DF	M.S
Year	1	7.140250^{NS}
Variety	4	10.532***
Treatment	1	1.332^{NS}
Year*Treatment	1	1.9802^{NS}
Variety*Treatment	4	21.596500^{NS}

**Values calculated from two replication readings based on the dry matter,*

****p < 0.001, NS: not statistically significant.*

The frequency of the nitrogen mineral accumulation in the diverse cereal grains, was presented in Figure1, as clarification of total protein distribution before the applied rate of N fertilizer (control) and after the applied rate of N fertilizer (Treatment) attributed to the respective variety.

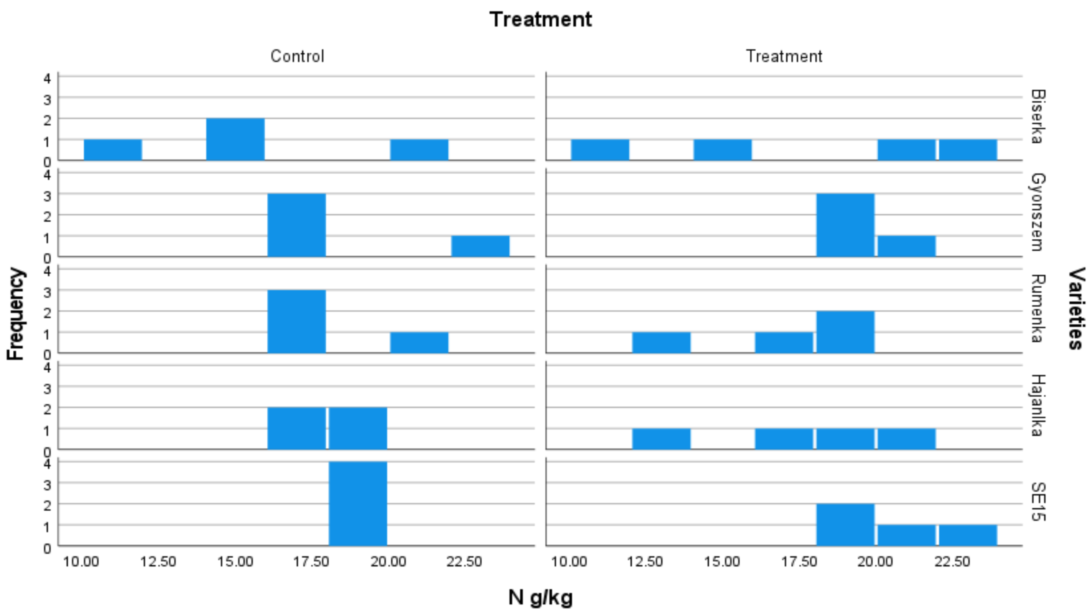

Figure 1. *Frequency of the crude protein distribution among the investigated varieties based on the treatment, P>0.05*

The crude protein findings indicated that the applied N fertilizer fluctuated among the examined cereal grains (Figure 2), positively impacting the protein contents in SE15 (W) variety Gyöngyszem (M) variety, and Biserka (M) variety, according to control and treated grain varieties, ranging from 18.4 g/kg to 20.2 g/kg, 18.6 g/kg to 19.5 g/kg, and 15.9 g/kg to 17.6 g/kg.

On the other hand, the applied rate of N fertilizer (Treatment) as shown in Tables1, with no significance value ($P < 0.05$) showed a reverse impact on the Hajanlka (B) variety and Biserka (M) variety when compared to the control to treated cereal grains, which ranged from 18.4 g/kg to 16.5 g/kg and 17.7 g/kg to 16.8 g/kg, respectively. The finding indicated that is the the applied rate of N fertilizer was over dose to the buckwheat (Hajanlka variety), as was aligned to (Selzer & Schubert, 2021), the study reported that is catch crop has capability to nutrient retention such as N mineral.

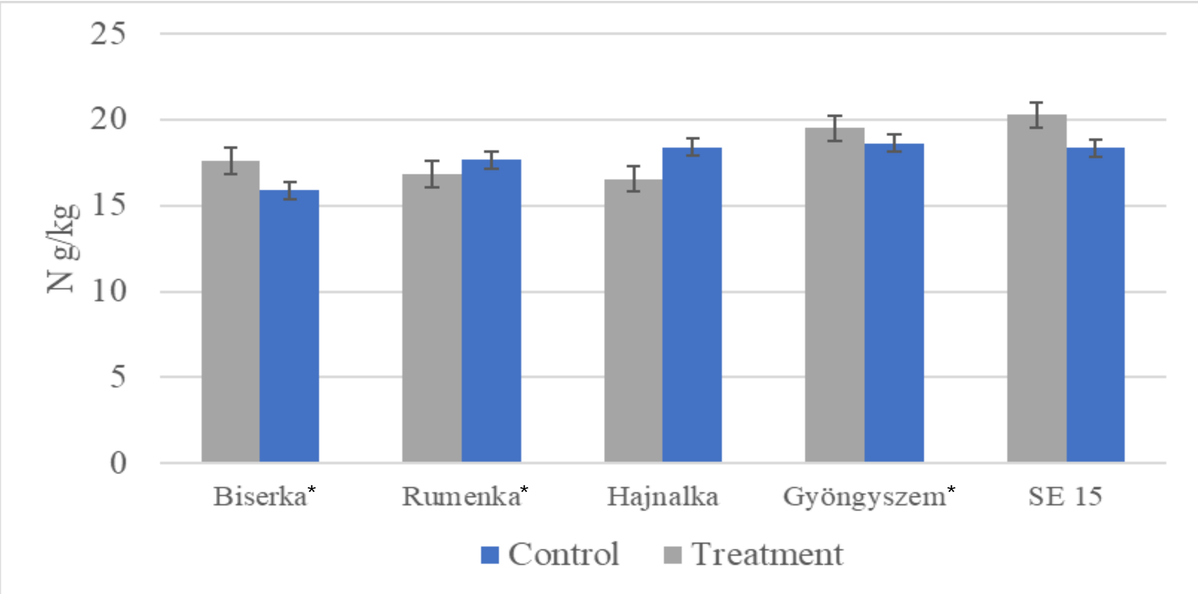

Figure 2. *Average of crude protein among diverse cereal grains for 2020-2022 cultivation seasons*

**Values mean±SD calculated from three replication readings, on the dry basis, $P < 0.05$.*

According to Figure the hierarchical cluster showed five clusters of the correlation between crude protein and varieties, which indicated the strong correlation between the proso millet varieties in the first cluster, while the second cluster involved winter wheat and buckwheat. The revealed relationship between the varieties as supporting of the measurement's precision and accuracy, which can help the breeder enhance the agronomic practice of crop divarication. And help the nutritionist the benefit from the similarity of the physical and chemical properties of the tested cereal grains. Which can be supporting to previous research conducted by (Medina Martinez et al., 2020).

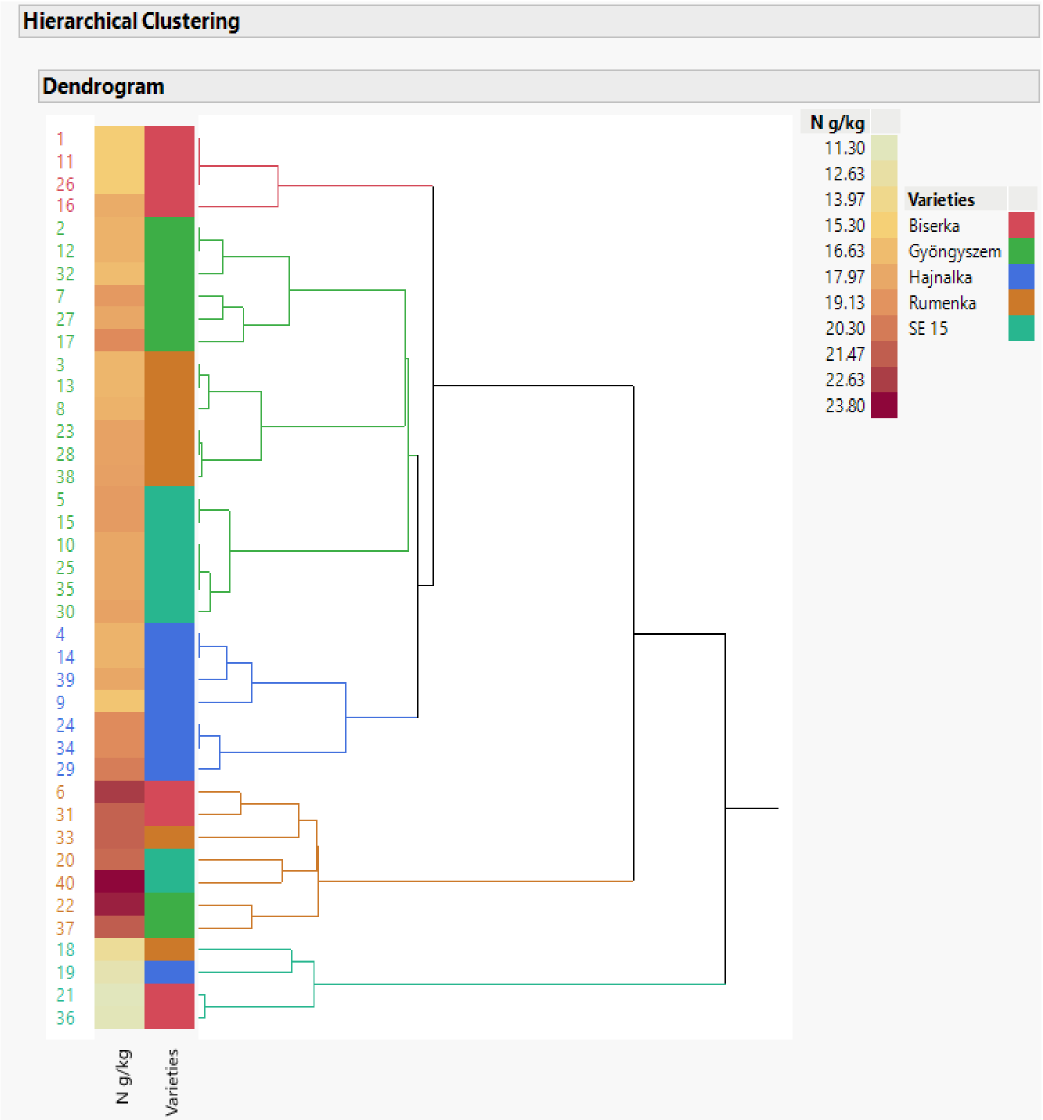

Figure 3. *Relationships between the crude protein contents and the tested cereal grain varieties*

Based on the distinct color of the proso millet varieties, Gyöngyszem (white), Rumenka (red), and Biserka (yellow), Figure 4 presents the average of the colour parameter spectrum profile, as findings indicated the same spectrum of b* (Yellowness). As evidence of the accuracy of the test, the Gyöngyszem variety (white) showed highest average of the Y (brightness) and a lowest average of a* (redness) in contrast, to Rumenka variety (red), which showed highest average of (darkness) at spectrum (<65). The findings were linked to (Nayik et al., 2023), the author reported that the The colour measurements provide insights on the nutritional content, processing compatibility, and industrial applications of sorghum. These measures are regulated by genetic regulation and the antioxidant characteristics of cereal grains.

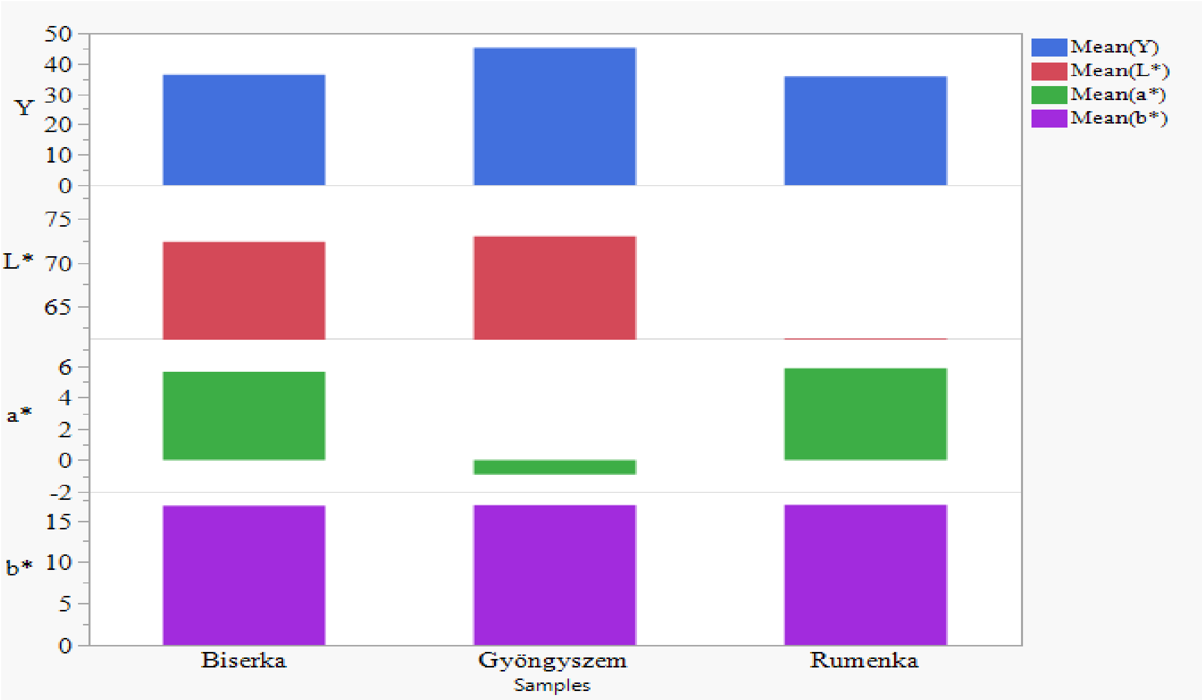

Figure 4. *Color properties of the proso millet varieties*

**Average of the color spectrum calculated from three replication readings, $P < 0.05$*

4. Conclusion

The research was undertaken in response to the increasing need for plant-based proteins fueled by environmental and health issues arising from extensive protein animal sources. Cereal grains, commonly eaten and protein-enriched, could supply potentially rich protein resources. The current research analyzed the crude protein levels of cultivating diverse cereal grains under varied applied N fertilizer dosages based on the respective crops. The European Union's directives prioritize promoting these alternatives in the food business sector, and we utilized diverse cereal grains, such as wheat, buckwheat, and proso millet, for the protein plant source by comparing the protein level in each cereal grain, which can provide good insight and estimation about the total protein content in diverse Hungarian cereal grain varieties. Furthermore, the study outcomes can facilitate and reflect the general evaluation of applied agricultural practices for cereal grain for human consumption; accordingly, we can enhance the yield quantity and quality of production.

Consequently, we evaluated the colour profile features of several varieties of proso millet. Plant breeding procedures may be used to improve cereal grains' protein content and quality. This method enhances the development of a food system that is both environmentally sustainable and able to withstand and recover from challenges. However, the author recommended researching the protein characteristics of the tested grain varieties.

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