

POTENTIAL OF OIL-TYPE SUNFLOWER GENOTYPES AS PROTEIN SOURCES: VARIABILITY ANALYSIS AND BREEDING IMPLICATIONS

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Abstract

Sunflower (*Helianthus annuus* L.) is one of the world's major oilseed crops, and the growing demand for plant-based protein sources has renewed interest in its seed proteins for both food and feed applications. This study evaluated the variability of protein content in 100 oil-type sunflower genotypes to identify promising candidates for breeding programs focused on enhanced nutritional quality. Protein content exhibited considerable variation, ranging from 15.08 to 27.37%, mean 20.25%; CV = 11.27%), indicating substantial genetic diversity. K-means cluster analysis grouped the genotypes into four distinct clusters, representing low-, moderate-, elevated- and high-protein groups. Cluster 4 included 19 genotypes with the highest mean protein content (23.75%), with genotype 100 (Oliva) showing the maximum value (27.37%), highlighting its potential as a superior donor in breeding for high-protein oil-type cultivars. These findings demonstrate strong genetic potential for improving protein content in sunflower and provide valuable insights for breeding programs aimed at developing nutritionally enhanced, high-protein hybrids with improved applicability in food and feed production.

Key words: breeding, sunflower, protein

Introduction

Sunflower (*Helianthus annuus* L.) is an oil-seed crop of the Composite (*Asteraceae*) family and the third most widely grown oilseed crop globally, following soybean and rapeseed. For the 2022/2023 season, world sunflower production was estimated at 58.57 million tonnes, with the majority produced in Russia, Ukraine, and countries of the European Union (FAO, 2025). Sunflower seeds consist of a kernel and a hull, and typically contain 35-55% oil and 10-27% protein (Cvejić et al., 2021; González-Pérez and Vereijken, 2007). Sunflower oil is rich in unsaturated fatty acids (oleic and lin-

oleic acids), and tocopherols (α -tocopherol), which contribute to its oxidative stability and nutritional value. Due to its favorable chemical composition, pleasant flavor, and light color, approximately 90% of sunflower oil production is directed toward food applications (Cvejić et al., 2021; Li et al., 2024). While sunflower is predominantly cultivated for its oil, there is growing recognition of the potential uses of sunflower meal as a valuable source of protein. Sunflower meal contains 40-50% protein, depending on the extraction method (Dauguet et al., 2016; González-Pérez and Vereijken, 2007),

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and is traditionally used as animal feed, often replacing soybean meal in poultry, swine, and ruminant diets (Dauguet et al., 2016; Murru and Calvo, 2020). Sunflower proteins contain high levels of sulfur amino acids, cysteine and methionine, but are low in the essential amino acid lysine. In contrast to soybean and rapeseed, sunflower does not contain major antinutritional or toxic compounds, making it suitable for human consumption and food applications (Nenova and Drumeva, 2012; Murru and Calvo, 2020; Kaur and Ghoshal, 2022; Li et al., 2024). Sunflower protein has been successfully incorporated into various food products, including bakery products, pasta, cookies, cereal, granola bars, baby formula, and plant-based milk, demonstrating its strong potential for broader utilization in the food industry (Kaur and Ghoshal, 2022; Li et al., 2024; Murru and Calvo, 2020).

Sunflower breeding traditionally prioritized obtaining hybrids with high yield, high oil content, and a favourable fatty acid composition (e.g., high-oleic genotypes). In recent years, advances in breeding and processing technologies have increasingly emphasized approaches to enhance protein content, thereby expanding the potential applications of sunflower beyond animal feed (Jocić et al., 2015; Cvejić et al., 2021; Miklič, 2022). Studies indicate that the oil and protein contents of sunflower seeds are often negatively correlated, which complicates the simultaneous improvement of both traits (Kandil et al., 1990; González-Pérez, 2015). Although protein content varies among genotypes, it is also strongly influenced by

environmental conditions and agronomic practices (Mrdja et al., 2012; Dauguet et al., 2016). Drought and water deficit during the flowering and seed filling phase significantly affect both protein content and amino acid composition (González-Pérez, 2015), while agricultural practices such as nitrogen and phosphorus fertilization have been used to increase protein and oil levels, and alter amino acid profiles (Steer et al., 1984; Ali et al., 2012).

Given the increasing demand for sustainable plant-based resources, breeding programs are placing renewed emphasis on genotypes that combine high oil content with improved protein quantity and quality, ultimately enhancing the competitiveness and economic value of sunflower as both an oil and protein crop for the food and feed applications (Rani et al., 2017; Jocković et al., 2019; Miklič, 2022).

Our study aimed to determine the grain protein content of different sunflower genotypes to evaluate their potential for developing improved hybrids with enhanced nutritional and economic value. By identifying genotypes that exhibit both high oil yield and elevated protein levels, support to breeding programs is provided through improved understanding of protein content variability and identification of traits responsive to selection. Such improved hybrids are expected to contribute to more efficient agricultural use, and to provide high value raw materials for the food and feed industries, thereby strengthening the role of sunflower as both a major oilseed crop and a promising protein source.

Materials and methods

A collection of 100 sunflower genotypes was used to determine protein and moisture contents. These genotypes represent elite genetic material developed at the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS). They are all oil-type genotypes but differ in morphological, phenological, and physiological characteristics and have different intended uses. The largest group consisted of conventional oil-type (OT) genotypes (1–6, 8–22, 23–28, 31–33, 47–52, 65, 75, 77, 89–95), followed by high-oleic (HO) genotypes (7, 54–58, 61–64, 66–70, 80, 86, 87, 96–100), genotypes tolerant to imidazolinone (IMI) herbicides (41–46, 53, 59, 60, 71, 79, 82–85, 88) and genotypes tolerant to sulfonylurea (SU) herbicides (29, 30, 34–40, 72–74, 76, 78, 81).

Seed samples for protein analysis were collected from plants grown in an experimental trial at IFVCNS in Rimski Šančevi, near Novi Sad, Serbia (latitude 45°20' N and longitude

19°51' E). The experimental trial was established as a randomized block design with three replications, using ten plants per replication as the sampling unit. After harvest, seed samples were collected for moisture and protein content analysis.

The moisture content of sunflower seed samples was determined using oven-drying method according to the international standard ISO 665:2000. Approximately 5 g of seeds was weighed in previously dried and weighed containers. The samples were dried in a laboratory oven at 103±2 °C for 6 h. The protein content of the seed samples was assessed using the Dumas combustion method (AOAC 992.23), which provides a fast and reliable determination of total nitrogen. Statistical analyses were performed using the R statistical software (R Core Team, 2024) within the RStudio environment.

Results and discussion

Protein and moisture content of the 100 oil sunflower genotypes are presented in Table 1 and 2. Protein content ranged from 15.08 to 27.37%, with a mean of 20.25% and a coefficient of variation of 11.27%, confirming a substantial level of genetic diversity (Table 1). These findings are consistent with previously reported ranges by Dauguet (2016) and Cvejić (2021), suggesting a comparable genetic influence on protein content. The protein content of the conventional oil-type hybrids recorded in our study (15.08–24.41%) is consistent with previously reported ranges for oil-type sunflower (10–27%) published by Dijanović (2003) and González-Pérez (2015). The protein content of imidazolinone-tolerant genotypes in our study ranged from 15.46% to 24.08%, whereas sulfonylurea-tolerant genotypes exhibited protein content between 17.68% and 23.62%. These values are higher than those reported by Gabriel-Anton (2019), who found protein content of 18.00 to 19.10% for imidazo-

line-tolerant genotypes and 17.20 to 17.70% for sulfonylurea-tolerant genotypes. In high-oleic sunflower hybrids, protein content ranged from 17.61% to 27.37%, which is similar to the protein content of 15.63–24.37% found in high-oleic hybrids by Triboui-Blondel (2019). Moisture content ranged from 5.00 to 9.50%, with a mean of 6.37% and a coefficient of variation of 9.70, indicating notable variability among genotypes for this trait as well (Table 2).

K-means cluster analysis (Figure 1 and Table 2) ($k=4$) was applied to classify sunflower genotypes based on seed protein content, resulting in four clearly defined clusters that captured the overall variability present within the germplasm and provided a structured framework for breeding decisions. Cluster 1 contained 21 genotypes (10 oil type genotypes, 5 imidazolinone-tolerant genotypes, 5 high-oleic type genotypes, and 2 sulfonylurea-tolerant genotypes) with the lowest mean protein content (mean=17.40%), ranging from 15.08% to

18.37%. Genotypes in cluster 2 (19 oil type genotypes, 7 high-oleic type genotypes, 6 sulfonylurea-tolerant genotypes, and 4 imidazoline-tolerant genotypes) exhibited moderately higher protein content (mean=19.39%), ranging from 18.50% to 20.16%, while cluster 3 comprised 24 genotypes (13 oil type genotypes, 5 high oleic type genotypes, 4 imidazoline-tolerant genotypes, and 2 sulfonylurea-tolerant genotypes) characterized by intermediate to high protein content (mean=21.27%), ranging from 20.41% to 22.46%. Cluster 4 contained 19 genotypes (7 high-oleic type genotypes, 5 sulfonylurea-tolerant genotypes, 4 oil type genotype and 3 imidazoline-tolerant genotypes) with the highest mean protein content (mean=23.75 %), ranging from 22.72% to 27.37%. This cluster represents the most valuable source of high-protein germplasm for quality-oriented breeding programs. A notable subset of genotypes within this cluster 4 (55, 56, 58, 64, 98, 99, 100) also exhibited high oleic acid content, clearly demonstrating dual-use potential, providing both high-quality

oil and elevated seed protein. Genotype 100 showed the highest protein content (27.37%), highlighting its exceptional value as a dual-purpose genotype that combines premium oleic oil with elevated seed protein. This genotype belongs to the high-oleic group and is already commercially released as the hybrid Oliva, confirming its practical relevance for breeding and production. These results are consistent with the findings of Cvejić et al. (2021), where the hybrid NS Oliva was identified as a promising dual-purpose type due to its high oleic acid concentration and comparatively higher protein content than other evaluated hybrids. Cluster 4 included all sunflower hybrid types (OT, SU, IMI, and HO), indicating a diverse genetic base with strong potential for breeding programs to develop multifunctional hybrids with high oil and protein content. Such hybrids offer significant agricultural value by improving crop productivity and enhancing nutritional quality, helping meet the growing demand for alternative protein sources and for sustainable agriculture.

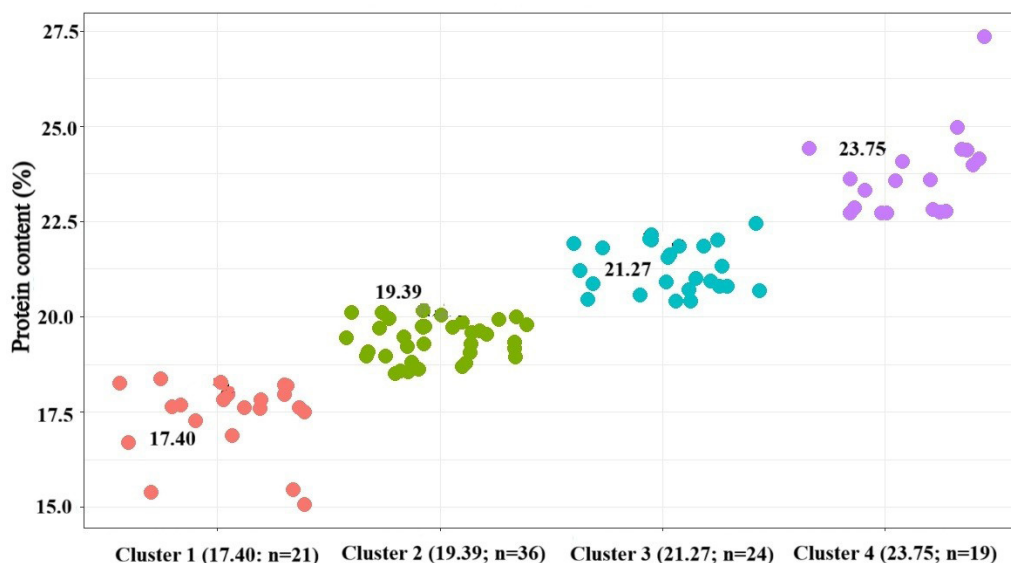


Figure 1. K-means cluster analysis of protein content in sunflower genotypes

Grafikon 1. Klaster analiza srednjih vrednosti sadržaja proteina suncokretovih genotipova

Sunflower hybrids generally contain lower seed protein levels compared to other major oil crops. Soybean remains the benchmark, with protein concentrations typically ranging from 40% to 45%, which is significantly higher than sunflower (Medić et al., 2014; Guo et al., 2022). Among other oilseed species, conventional sunflower types also show lower protein content than camelina (23.8–30.8%) and mustard (24.1–33.6%) (Hossain et al., 2019). When compared to rapeseed (canola), whose protein content varies between 17–26%, most sunflower genotypes fall within a similar range.

Despite this general trend of lower protein concentration in sunflower, substantial genetic variability exists within the crop. Certain genotypes exceed the typical range, demonstrating the potential for breeding sunflower as a dual-purpose source of both oil and protein.

For example, genotype 100 reached a protein content of 27.37%, surpassing rapeseed and approaching levels reported for camelina and mustard. Such cases indicate that sunflower, although traditionally regarded as an oil crop with modest protein content, harbors underutilized diversity that can be exploited to enhance seed protein levels without compromising agronomic or oil quality traits (Hossain et al., 2019; Chmielewska et al., 2021). This variability highlights an important opportunity: while most commercial hybrids are optimized for oil yield, targeted breeding for increased protein could broaden sunflower's value, enabling the development of dual-purpose cultivars suited for both food and feed applications.

Table 1. Distribution of sunflower genotypes into clusters based on protein content

Tabela 1. Raspodjela genotipova suncokreta u klastere na osnovu sadržaja proteina

Cluster 1		Cluster 2		Cluster 3		Cluster 4	
genotype	protein	genotype	protein	genotype	protein	genotype	protein
1 (OT)	15.08	82 (IMI)	18.50	9 (OT)	20.41	36 (SU)	22.72
91 (OT)	15.38	87 (HO)	18.56	97 (HO)	20.41	38 (SU)	22.73
88 (IMI)	15.46	61 (HO)	18.57	25 (OT)	20.46	72 (SU)	22.73
90 (OT)	16.70	20 (OT)	18.63	8 (OT)	20.58	53 (IMI)	22.75
5 (OT)	16.89	26 (OT)	18.70	66 (HO)	20.69	64 (HO)	22.77
79 (IMI)	17.28	70 (HO)	18.79	3 (OT)	20.72	45 (IMI)	22.82
27 (OT)	17.51	11 (OT)	18.80	33 (OT)	20.80	58 (HO)	22.86
75 (OT)	17.59	92 (OT)	18.94	44 (IMI)	20.81	56 (HO)	23.32
86 (HO)	17.61	84 (IMI)	18.96	37 (SU)	20.88	94 (OT)	23.58
4 (OT)	17.63	24 (OT)	18.97	31 (OT)	20.92	39 (SU)	23.61
85 (IMI)	17.63	14 (OT)	19.05	47 (OT)	20.95	73 (SU)	23.62
74 (SU)	17.68	6 (OT)	19.08	83 (IMI)	21.00	21 (OT)	23.99
63 (HO)	17.82	28 (OT)	19.17	12 (OT)	21.22	42 (IMI)	24.08
7 (HO)	17.83	17 (OT)	19.22	59 (IMI)	21.34	18 (OT)	24.15
71 (IMI)	17.96	78 (SU)	19.28	62 (HO)	21.55	98 (HO)	24.38
77 (OT)	17.97	69 (HO)	19.29	81 (SU)	21.62	50 (OT)	24.41
93 (OT)	18.18	19 (OT)	19.33	57 (HO)	21.82	55 (HO)	24.43
67 (HO)	18.22	34 (SU)	19.46	54 (HO)	21.86	99 (HO)	24.97
15 (OT)	18.25	10 (OT)	19.48	60 (IMI)	21.86	100 (HO)	27.37

Extension of Table 1
Nastavak Tabele 1

Cluster 1		Cluster 2		Cluster 3		Cluster 4	
genotype	protein	genotype	protein	genotype	protein	genotype	protein
46 (IMI)	18.27	32 (OT)	19.54	22 (OT)	21.93		
35 (SU)	18.37	29 (SU)	19.58	52 (OT)	22.01		
		65 (OT)	19.63	48 (OT)	22.02		
		43 (IMI)	19.71	16 (OT)	22.15		
		89 (OT)	19.72	51 (OT)	22.46		
		80 (HO)	19.73				
		76 (SU)	19.74				
		96 (HO)	19.74				
		40 (SU)	19.80				
		68 (HO)	19.86				
		23 (OT)	19.94				
		30 (SU)	19.95				
		95 (OT)	20.00				
		13 (OT)	20.05				
		49 (OT)	20.11				
		2 (OT)	20.12				
		41 (IMI)	20.16				
number of geno- types	21	number of genotypes	36	number of geno- types	24	number of geno- types	19
min	15.08	min	18.50	min	20.41	min	22.72
max	18.37	max	20.16	max	22.46	max	27.37
mean	17.40	mean	19.36	mean	21.27	mean	23.75

*IMI -imidazolin herbicide-tolerant genotypes; SU-sulfonylurea herbicide-tolerant genotypes; HO-high oleic type genotypes, OT-oil type genotypes

Based on moisture content, the sunflower genotypes were grouped into three distinct k-means clusters (Figure 2; Table 2). Cluster 1 comprised 57 genotypes (34 oil type genotypes, 11 high-oleic genotypes, seven sulfonylurea-tolerant genotypes, and five imidazoline-tolerant genotypes), with lowest mean moisture content of 5.97%, ranging from 5.00% to 6.30%. Cluster 2 included 35 genotypes (10 oil type genotypes, nine high-oleic genotypes, nine imidazoline-tolerant genotypes, and seven sulfonylurea-tolerant genotypes) with inter-

mediate moisture levels, showing a mean of 6.72% and a range of 6.35% to 7.14%. Finally, Cluster 3 contained eight genotypes (two oil type genotypes, three high-oleic genotypes, two imidazoline-tolerant genotypes and one sulfonylurea-tolerant genotypes) with the highest moisture content, with a mean of 7.77% and a range of 7.40% to 9.50%. These results clearly differentiate the genotypes based on their moisture characteristics, which may have implications for seed storage, oil extraction efficiency, and their suitability for breeding programs.

Table 2. Distribution of sunflower genotypes into clusters based on moisture content
 Tabela 2. Raspodela genotipova suncokreta u klastere na osnovu sadržaja vlage

Cluster 1				Cluster 2		Cluster 3	
Genotype	MC	Genotype	MC	Genotype	MC	Genotype	MC
27 (OT)	5.00	9 (OT)	6.13	78 (SU)	6.35	36 (SU)	7.40
5 (OT)	5.53	29 (SU)	6.13	96 (HO)	6.41	50 (OT)	7.40
92 (OT)	5.54	66 (HO)	6.13	85 (IMI)	6.43	44 (IMI)	7.45
23 (OT)	5.56	52 (OT)	6.14	84 (IMI)	6.44	59 (IMI)	7.51
72 (SU)	5.59	49 (OT)	6.16	73 (SU)	6.45	48 (OT)	7.56
81 (SU)	5.69	69 (HO)	6.16	13 (OT)	6.48	57 (HO)	7.64
22 (OT)	5.70	71 (IMI)	6.16	32 (OT)	6.50	56 (HO)	7.68
65 (OT)	5.70	63 (HO)	6.18	70 (HO)	6.50	55 (HO)	9.50
1 (OT)	5.71	63 (HO)	6.18	95 (OT)	6.50		
93 (OT)	5.71	97 (HO)	6.18	21 (OT)	6.53		
28 (OT)	5.74	87 (HO)	6.20	39 (SU)	6.54		
35 (SU)	5.74	20 (OT)	6.21	76 (SU)	6.56		
4 (OT)	5.75	67 (HO)	6.21	42 (IMI)	6.59		
11 (OT)	5.77	94 (OT)	6.22	53 (IMI)	6.60		
80 (HO)	5.78	8 (OT)	6.23	51 (OT)	6.62		
31 (OT)	5.81	16 (OT)	6.23	40 (SU)	6.65		
12 (OT)	5.85	43 (IMI)	6.24	98 (HO)	6.69		
26 (OT)	5.88	68 (HO)	6.24	34 (SU)	6.71		
30 (SU)	5.88	82 (IMI)	6.26	60 (IMI)	6.72		
6 (OT)	5.89	74 (SU)	6.27	88 (IMI)	6.73		
47 (OT)	5.9	83 (IMI)	6.27	61 (HO)	6.74		
89 (OT)	5.91	3 (OT)	6.28	91 (OT)	6.74		
15 (OT)	5.92	86 (HO)	6.30	41 (IMI)	6.80		
33 (OT)	5.93			90 (OT)	6.81		
7 (HO)	5.94			46 (IMI)	6.83		
75 (OT)	5.94			37 (SU)	6.85		
79 (IMI)	5.94			14 (OT)	6.90		
17 (OT)	5.99			58 (HO)	6.97		
62 (HO)	6.01			100 (HO)	7.00		
77 (OT)	6.02			99 (HO)	7.01		
19 (OT)	6.04			10 (OT)	7.02		
25 (OT)	6.07			45 (IMI)	7.03		
18 (OT)	6.09			2 (OT)	7.08		
24 (OT)	6.09			54 (HO)	7.13		
38 (SU)	6.11			64 (HO)	7.14		
number of genotypes	57			number of genotypes	35	number of genotypes	8

Extension of Table 2
Nastavak Tabele 2

Cluster 1				Cluster 2		Cluster 3	
Genotype	MC	Genotype	MC	Genotype	MC	Genotype	MC
min	5.00			min	6.35	min	7.40
max	6.30			max	7.14	max	9.50
mean	5.97			mean	6.72	mean	7.77

*IMI – imidazolin herbicide-tolerant genotypes; SU – sufonylurea herbicide-tolerant genotypes; HO – high oleic type genotypes; OT – oil type genotypes; MC – moisture content

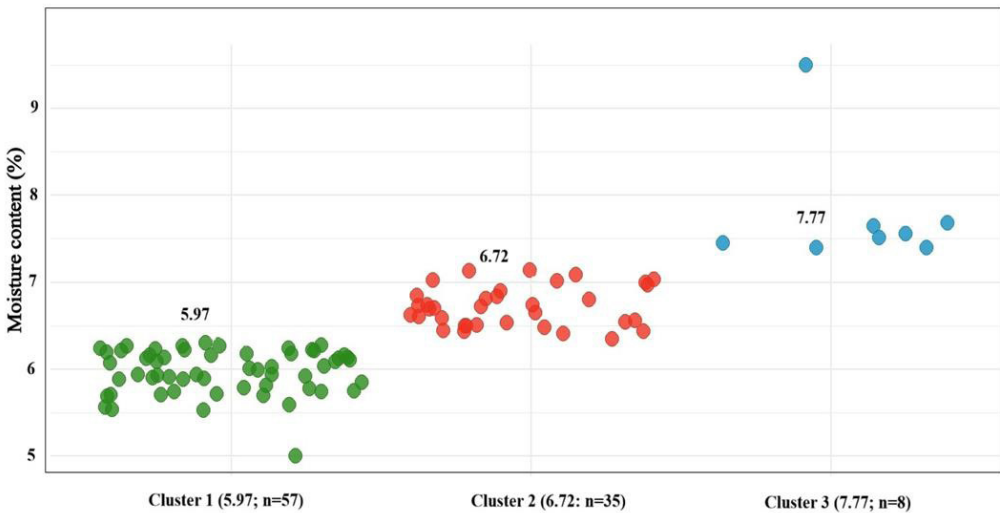


Figure 2. K-means cluster analysis of moisture content in sunflower genotypes
Grafikon 2. Klaster analiza srednjih vrednosti sadržaja vlage suncokretovih genotipova

Sunflower seeds are typically stored at a moisture content of 6 to 7%. Higher moisture content increases water and enzyme activity, promotes microbial growth, leading to degradation of seed nutritional quality, reduces germination rates, and lower oil extraction efficiency (Huang et al., 2021). Genotypes from clusters 1 and 2 require less intensive post-harvest drying and more favorable storage conditions compared with those from cluster 3. However, lower moisture content may negatively affect germination under water-lim-

ited conditions. In contrast, higher moisture content in genotypes from cluster 3 may indicate increased water-absorption capacity and more robust root development, contributing to improved drought adaptability. Nevertheless, these genotypes require careful drying prior to storage to prevent microbial spoilage (Nithya et al., 2017). Overall, the observed differences emphasize the importance of moisture content not only for post-harvest management but also for selecting genotypes adapted to specific environmental conditions and production systems.

Conclusion

The global demand for protein and alternative protein sources continues to rise, and sunflower seeds represent a promising source of plant-based protein for both the feed and food industries.

The present study demonstrated substantial genetic variability in protein and moisture content among one hundred oil-type sunflower genotypes, confirming the wide diversity available within existing genotypes for improving seed nutritional quality. Protein content ranged from 15.08% to 27.37% and was differentiated into four k-means clusters. The protein content of 19 genotypes in cluster 4 showed the highest mean (23.75%), with genotype 100 (Oliva) recording the maximum value of 27.37%, ex-

ceeding the commonly reported maximum for confectionery-type sunflower, further emphasizing its value as a high-protein source. These results highlight significant potential for developing high-protein oil-type sunflower hybrids. Moisture content also showed notable variability, ranging from 5.00% to 9.50% and forming three statistically distinct k-means clusters.

These findings provide a strong foundation for targeted breeding strategies and further genetic enhancement to develop sunflower hybrids with high protein and oil content, contributing to agricultural sustainability and global protein security.

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Author contributions

Conceptualization, N.G. and S.C.; Methodology, N.G. and M.A.; Data analysis, M.A. and M.J.; Resources, S.J., M.J. and N. Ć.; Supervision, N.G. and S. J.; Visualization, M.A.

and N.Ć.; Funding acquisition, S.C.; Writing — original draft, N.G., S.C and M.A.; Writing — review & editing, N.G. and S.C.

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POTENCIJAL KORIŠĆENJA GENOTIPOVA ULJANOG SUNCOKRETA KAO IZVORA PROTEINA: ANALIZA VARIJABILNOSTI I MOGUĆNOSTI OPLEMENJIVANJA

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Sažetak

Suncokret (*Helianthus annuus* L.) predstavlja jednu od najvažnijih uljanih biljnih vrsta u svetu, a sve veća potražnja za biljnim izvorima proteina ponovo je usmerila interesovanje prema proteinima suncokretovog semena, kako za potrebe ishrane ljudi, tako i za stočnu hranu. Istraživanje se bavi ispitivanjem varijabilnosti sadržaja proteina u 100 genotipova uljanog suncokreta kako bi se identifikovali obećavajući genotipovi za programe oplemenjivanja usmerene na poboljšanje nutritivnog kvaliteta. Sadržaj proteina je pokazao značajnu varijabilnost, u rasponu od 15,08 do 27,37%, (prosek 20,25%; CV= 11,27%), što ukazuje na prisustvo izražene genetičke varijabilnosti. K-means klaster analiza grupisala je genotipove u četiri jasno definisana klastera, koji predstavljaju grupe sa niskim, umerenim, povišenim i visokim sadržajem proteina. Klaster 4 je obuhvatio 19 genotipova sa najvišim prosečnim sadržajem proteina (23,75%), pri čemu je genotip 100 (Oliva) pokazao maksimalnu vrednost (27,37%), što ističe njegov potencijal kao superiornog donora u oplemenjivanju uljanog tipa suncokreta sa visokim sadržajem proteina. Dobijeni rezultati pokazuju važan genetski potencijal za poboljšanje sadržaja proteina u suncokretu i pružaju vredne uvide za programe oplemenjivanja usmerene na razvoj nutritivno poboljšanih, visokoproteinskih hibrida sa primenom u proizvodnji hrane i stočne hrane.

Ključne reči: oplemenjivanje, suncokret, proteini

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