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Biophysical and Morphometric Characteristics of Sunflower Achenes: Implications for Industrial Processing and Byproduct Utilization

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Abstract: The main focus of industrial sunflower processing is oil production, in which the pericarp is most often treated as a byproduct or biological waste. However, sunflower pericarps have shown significant potential for alternative applications. Bridging the gaps in knowledge of the properties of achenes and their byproducts would improve the efficiency of industrial processes and open new possibilities for utilizing the pericarp as a biological resource. In this work, we analyzed biophysical and morphometric properties of the achenes of eight sunflower genotypes. Their byproducts indicate a complex interrelationship among the analyzed traits. The basic achene color of the tested genotypes was gray, with dark to light shades. Larger achenes had larger seeds and a higher weight, while more spherical achenes had a higher proportion of pericarp. Additionally, achenes with a smaller cavity between the seed and the pericarp had a higher germination percentage. Genotypes with a thinner and softer pericarp had higher oil content, while greater thickness contributed to its reduction. Pericarp hardness was proportional to the number of sclerenchyma layers, not to the percentage of sclerenchyma. These findings suggest that pericarp structure can be a key determinant for both oil yield and byproduct valorization, enabling the selection of genotypes for specific industrial applications.

Keywords: achene; pericarp; color; size; weight; hardness; sclerenchyma; germination; oil content

Academic Editor: Alessandro Suardi

Received: 25 March 2025
Revised: 20 April 2025
Accepted: 23 April 2025
Published: 25 April 2025

Citation: Ovuka, J.; Jocković, J.; Jocković, M.; Jocić, S.; Gvozdenac, S.; Krstić, M.; Jovičić, D. Biophysical and Morphometric Characteristics of Sunflower Achenes: Implications for Industrial Processing and Byproduct Utilization. *Agronomy* **2025**, *15*, 1035. https://doi.org/10.3390/agronomy15051035

1. Introduction

Sunflower (*Helianthus annuus* L.) produces dry fruits classified as achenes, in which the seed, rich in oil, is enclosed in a protective outer layer known as a pericarp or a hull. Their chemical composition and nutritive value are influenced by numerous factors, most notably genotype, followed by soil type, cultivation practices, processing methods, and, increasingly, climatic conditions [1]. Since the seed contains most of the oil, it represents the main focus of industrial processing using pressing or solvent extraction methods [2,3]. However, the pericarp, which accounts for about 30% of the total achene weight, is often treated as a byproduct or biological waste in the sunflower oil industry [4,5]. Despite this, sunflower pericarps have shown significant potential for alternative applications, such as the production of biosorbents for removing cationic dyes and heavy metals [6–10], as a renewable energy source in the form of biomass pellet fuel due to their high heat output [11,12], or as composite materials, preserving natural wood reserves [13]. After oil extraction, the hull

can serve as an alternative carbon source for producing valuable biopolymers and natural antioxidants [14,15]. These compounds have potential applications in creating materials, dietary fibers, food additives, and preservatives [16].

The structural and mechanical properties of the pericarp are crucial for protecting the seed during development, dispersal, storage, and germination [17,18]. Composed primarily of sclerenchyma tissues, the pericarp provides mechanical protection through thickened, lignified secondary cell walls in the sclerenchyma, which contain a high content of lignin (20–25% dry weight; Seiler [19]). The hardness of the pericarp, often determined by lignification and polysaccharide-based structures, ensures protection against physical, chemical, and biological damage, enabling the seed to withstand various environmental challenges [16,20,21]. Additionally, the presence of the phytomelanin layer within the sunflower pericarp significantly contributes to the mechanical support of the achene [22,23]. These structural properties play a key role in industrial processes such as dehulling, where "hullability" measures the ease of hull/pericarp separation from the seed. This parameter is influenced by the biophysical and biochemical characteristics of the pericarp [17,24].

The quality and stability of vegetable oils, such as sunflower oil, are influenced by minor components like waxes. Wax is primarily located in the hull of sunflower seeds and can constitute up to 3% of its composition [25,26]. During the oil extraction process, a certain amount of wax transfers into the oil, and its concentration depends on the degree of hull removal and the extraction method [27,28]. Waxes tend to crystallize, creating cloudiness, a physical condition that impacts the stability of the oil and reduces its marketability [29]. Given that waxes present in the pericarp transfer into the oil during extraction, they affect its stability and product quality [27]. Understanding how the physical properties of the pericarp, including seed hullability, influence the presence of waxes can help optimize industrial processes [30]. Knowledge of the physical properties of achenes and pericarps, as well as their correlation with seed hullability potential, can significantly contribute toward optimizing industrial processes. This connection enables reductions in energy costs and improvements in extraction efficiency and oil quality, while minimizing the negative impact of waxes on the stability and commercial value of the product [31].

Knowledge of the physical and mechanical properties of sunflower achene, seeds, and pericarp is essential for optimizing industrial processes, particularly compression during dehulling and oil extraction [32]. Seed pericarp can transfer pigments to the extracted oil, lead to higher energy consumption, and produce products with lower nutritional quality [33]. Removing the pericarp could reduce the wax content and improve the color of the oil but may also reduce the fiber content and increase the protein in the meal [34]. Therefore, in order to improve the quality of the extracted oil, it is important to fully remove the pericarp from the seeds before the extraction process. Previous research results indicate that achene characteristics such as size and bulk density [35–37], pericarp and oil content [38,39], and moisture content [40–43] directly affect dehulling ability. Compression involves complex phenomena, including the elastic behavior of seeds, which directly affects energy efficiency and the equipment's performance in cracking the pericarp and grinding seeds. Studies [44,45] have demonstrated that the elastic modulus of sunflower achenes and their seeds increases with the achene size, while moisture content and loading rates significantly influence mechanical traits. Such insights enable accurate modeling of seed behavior under compression, facilitating the design of more efficient equipment and reducing production losses [46].

The analysis of sunflower achenes'/seeds' morphometric properties, as well as the strength of pericarps, allows us to understand how these characteristics affect industrial processing, energy consumption, and oil quality, and give an insight into the potential alternative uses of pericarp. This approach allows the improvement of industrial process

efficiency and opens up new possibilities for utilizing the pericarp as a biological resource. The aim of this study was to analyze the biophysical and morphometric properties of achenes and their byproducts in eight sunflower genotypes, in order to better understand their role in industrial processing and the potential of the pericarp as a biological resource. The research included an assessment of the variability in achene size, weight, and shape, as well as the relationship between these traits and the proportion of pericarp and seed. Particular attention was given to pericarp thickness and hardness, the number of sclerenchyma layers, and their impact on seed germination and oil content. On the basis of the results obtained, the goal was to identify genotypes with specific physical characteristics that offer potential for different industrial applications.

2. Materials and Methods

2.1. Experimental Material and Sample Preparation

Material used for this research included the seeds of eight (8) sunflower genotypes (L1–L8) from the breeding program of the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia. The samples were obtained in the vegetative season of 2023 from the experimental site at Rimski šančevi (45°19′59″ N; 19°50′51″ E). The Rimski šančevi site is located at an altitude of 84 m and is characterized by a moderate continental climate [47]. It is characterized by a chernozem type of soil located at the southern border of the Southern Panonian Basin, with a 26.55% clay, 34.31% silt, 39.14% sand, 0.98% organic carbon, and 0.15% nitrogen at a depth of 53 cm, and a pH value of 7.7 [48–50]. The average annual temperature is 11.9 °C and the annual precipitation is 647.3 mm, while in the cropping season of 2023, the average temperature from March until September was 18.3 °C, with precipitation of 410.9 mm [51].

The experiment was sown in a completely randomized block design, with three replicates. The basic plot included four rows, 5.0 m long with 70 cm × 25 cm plant spacing and 10.5 m^2 in size. During the growing season, standard cultivation measures were applied for conditions without irrigation. Harvesting was carried out manually at the stage of physiological maturity (R9) by cutting heads from the two middle rows, excluding the edge plants. The achenes were separated from the heads and cleaned of impurities mechanically and then left for 3 months in storage to pass dormancy. For each genotype, a sample of 0.5 kg per replicate was made and used for analysis.

2.2. Morphometric Analysis of Achenes, Pericarps, and Seeds

Three principal dimensions, namely the length, width, and thickness of the achene and seed, were analyzed in a sample of 20 randomly selected and manually dehulled achenes per replicate (three replicates in total) to determine variations in achene and seed size. Length (L) was measured at the longest, width (W) at the widest, and thickness (T) at the thickest part of the achene/seed using a digital Vernier caliper with an accuracy of ±0.01 mm.

To determine the shape of the achene and seed, the sphericity coefficient (ϕ) was calculated according to Mohsenin [52]. First, the equivalent diameter (D_e) was calculated using Equation (1)

$$D_e = (L \times W \times T)^{1/3} \quad (1)$$

where L, W, and T are the length, width, and thickness of seed/kernel, respectively, and then the sphericity coefficient was calculated according to Equation (2):

$$\phi = D_e \times L^{-1} \quad (2)$$

The 100-achene and -seed weight was calculated on the basis of dry weight by measuring and calculating the mean value of 2 × 100 randomly selected and manually dehulled achenes per replicate, with a precision of 0.01 g. The pericarp content was calculated as a percentage (%) of the total weight (g) by manually removing the pericarp from 5 g of achenes per replicate, and the seed content was calculated based on it. For pericarp morphometric analysis, 15 achenes per genotype were taken. Cross-sections of the pericarp in the central part of the achene were manually prepared as semi-permanent preparations, placed on a microscope slide, immersed in glycerin, and covered with a cover slip. To demonstrate lignin in the sclerenchyma tissue of the cross-sections, a solution of phloroglucinol was used, which gives a positive result in a reddish-orange color. The pericarp analysis was performed using a KERN light microscope with a KERN camera and a program for image analysis. The analyzed parameters included (i) the thickness of the pericarp, (ii) the thickness of the epidermis with the cuticle, (iii) the thickness of the hypodermis, and (iv) the thickness of the sclerenchyma, as well as (v) the number of sclerenchyma layers. Based on the values obtained, the percentage contributions of individual tissues were calculated and presented. In all analyzed genotypes, the presence of the parenchyma was noted, appearing as a very thin layer of collapsed cells. Since the parenchyma consists of cells with extremely thin cell walls, these cells undergo significant compression during seed development due to the expanding seed. This process results in a reduction in the structural integrity and thickness of the parenchyma layer, making its quantification challenging. Additionally, the lack of a clearly defined and consistent parenchyma structure under these conditions prevented its precise measurement, and it was therefore not included in the total pericarp thickness.

2.3. Biophysical Analysis of Achenes, Pericarp, and Seeds

2.3.1. Pericarp Hardness and Color

The pericarp hardness was assessed by a needle penetration test, which is based on the application of force (N) required to puncture the achene pericarp. The test was performed at the Laboratory for Biosystem Engineering, Faculty of Agriculture, University of Novi Sad, using a TMS-Pro texture analyzer (Food Technology Corporation, Sterling, VA, USA). Forty-five achenes per genotype (3 × 15), previously visually inspected for visible cracks in the pericarp, were tested in a horizontal orientation by placing a needle in the center of the achene under the following conditions: pre-test speed, 60 mm/min; test speed, 30 mm/min; test distance, 2 mm with a 50 N load capacity cell. The pericarp color observations were performed using a stereoscopic microscope (STEMI 2000, ZEISS AG, Oberkochen, Germany).

2.3.2. Moisture Content

Achene moisture content was calculated in a sample per replicate according to ISO Standards (ISO 665:2020) [53]. Values are presented in percentages.

2.3.3. Oil Content

Seed oil content was determined by extraction of the whole ground achene with n-hexane (≥99% purity, J.T. Baker, Phillipsburg, NJ, USA) in Soxhlet apparatus (Soxtherm 2000, Gerhardt, Reichshof, Germany) according to a reference method (ISO 659:2009) [54]. The ground sample (2 g) was placed in an extraction thimble, which was then covered with cotton wool and subjected to extraction with n-hexane. The extraction was performed under the following conditions: a temperature of 150 °C, an extraction time of 120 min, and five automatic cycles. Values are presented in percentages.

2.3.4. Germination

The germination test was preceded by the preparation of achenes. First, achenes were surface-sterilized with a 3% sodium hypochlorite (NaClO) solution for 10 min, then rinsed three times for 10 min in distilled water and dried for 36 h at 18 ± 1 °C. Germination was tested under controlled laboratory conditions in a growth chamber (SWGC-450, Witeg Labortechnik GmbH, Wertheim, Germany) at 25 ± 1 °C, with a 12 h light/12 h dark photoperiod and an light intensity of ~1250 lux. Four replicates per one hundred seeds were placed in a filter paper moistened with distilled water and wrapped into rolls. Total germination was determined after 10 days, evaluating typical seedlings according to the ISTA rules [55] for sunflower.

2.3.5. Statistical Analysis

The statistical analysis was conducted using PAST 4.11 software, employing both the univariate and multivariate methods. Basic statistical indicators were calculated, including mean values, standard errors, correlation coefficients, and coefficients of variation. Duncan's test was applied to assess differences in morphometric parameters among the genotypes, with a significance level of $p \leq 0.05$. Additionally, discriminant component analysis (DCA) was used to determine whether the samples could be grouped into distinct categories. For the discriminant analysis, only selected traits were used, which included the following: achene length (LA), achene width (WA), achene thickness (TA), achene sphericity (ϕs), pericarp percentage (%P), pericarp thickness (TP), epidermis with cuticle percentage (%EC), hypodermis percentage (%HY), sclerenchyma percentage (%SC), number of sclerenchyma layers (NoS), pericarp hardness (HP), and moisture percentage (% HUM). Since the analysis involved a combination of traits measured in different units and on different scales, we initially tested the data for normality using the Shapiro–Wilk test. The results indicated that the majority of traits did not follow a normal distribution ($p < 0.05$). To meet the assumptions of parametric statistical methods, a logarithmic transformation of the data was subsequently performed using PAST software. Pearson's correlation was applied to examine the relationships among the analyzed traits.

3. Results

The analyzed sunflower genotypes exhibited significant differences in the morphometric and biophysical traits of the achene and seed, providing insight into achenes' potential for selecting genotypes with better agronomic traits (Table 1). The basic color of the pericarp was gray, ranging from light gray to dark gray, with some showing intermediate shades. The darkest seed coat color was observed in genotype L1, while genotype L3 had the lightest gray shade (Figure 1). The weight of 100 achenes ranged from 3.21 g in genotype L8 to 8.5 g in L5, with an average value of approximately 4.89 g. Achenes of most genotypes weighed between 4.2 g and 5.6 g. The seed percentage, indicating achene filling, was the lowest in L1 (56.3%) and the highest in L4 (77.2%), with an average of approximately 70% across genotypes. The percentage of the pericarp also played a key role in determining seed filling, with the highest percentage observed in L1 (43.7%), correlating with the lowest seed percentage (56.3%), while the lowest pericarp percentage was recorded in L4 (22.8%), accompanied by the highest seed percentage (77.2%). Seed germination showed high values across genotypes, averaging around 92%. The highest germination rates were recorded in L3 (97%), L5 (96%), and L6 (96%), while genotypes L1 and L8 exhibited lower values (82% and 87%, respectively). The oil content in the seeds was the highest in L3 (49.8%) and the lowest in L1 (24%), further highlighting differences in seed quality among the genotypes. Seed moisture content ranged from 4.2% in L4 to 5.1% in L3 and L6. These results indicate a complex inter-relationship among the analyzed traits (Table 1).

Table 1. Biophysical characteristics of sunflower achenes/seeds (mean value ± standard error, coefficient of variation (CV), %).

Genotype	Achene Weight (g)	Moisture Content (%)	Seed				% of Pericarp
			Weight (g)	%	% of Germination	% of Oil	
L1	4.88 ± 0.12 ^{c} (4.26)	4.3 ± 0.1 ^{cd} (4.9)	2.75 ± 0.08 ^{d} (5.45)	56.3 ± 0.98 ^{e} (1.76)	82 ± 1.0 ^{ab} (2.5)	24 ± 0.7 ^{d} (5.5)	43.7 ± 0.57 ^{a} (2.27)
L2	4.91 ± 0.08 ^{c} (2.93)	4.9 ± 0.05 ^{ab} (1.5)	3.68 ± 0.14 ^{c} (6.95)	74.9 ± 1.85 ^{abc} (4.29)	88 ± 1.7 ^{ab} (3.9)	39.2 ± 1.7 ^{ab} (8.4)	25.2 ± 1.85 ^{cde} (12.8)
L3	5.56 ± 0.13 ^{b} (4.24)	5.1 ± 0.1 ^{a} (2.8)	4.13 ± 0.07 ^{b} (3.0)	74.3 ± 1.0 ^{abc} (2.47)	97 ± 0.4 ^{a} (0.8)	49.8 ± 1.7 ^{c} (8.7)	25.7 ± 1.06 ^{cde} (7.14)
L4	4.6 ± 0.03 ^{cd} (1.08)	4.2 ± 0.2 ^{d} (5.1)	3.55 ± 0.10 ^{c} (5.0)	77.2 ± 1.8 ^{a} (4.0)	94 ± 0.5 ^{a} (1.0)	40.7 ± 1.8 b^{c} (8.9)	22.8 ± 1.8 ^{e} (13.7)
L5	8.5 ± 0.15 ^{a} (3.0)	5.0 ± 0.05 ^{a} (1.4)	6.53 ± 0.11 ^{a} (3.09)	76.9 ± 0.5 a^{b} (1.17)	96 ± 1.25 ^{a} (2.6)	36.7 ± 1.1 ^{c} (5.9)	23.1 ± 0.52 ^{de} (3.9)
L6	4.28 ± 0.06 ^{de} (2.7)	5.1 ± 0.1 ^{a} (2.8)	2.91 ± 0.04 ^{d} (2.61)	68.1 ± 0.72 ^{d} (1.83)	96 ± 48.7 ^{a} (3.9)	48.2 ± 0.9 ^{a} (3.9)	31.9 ± 0.72 ^{b} (3.91)
L7	4.18 ± 0.14 ^{e} (6.0)	4.9 ± 0.05 ^{ab} (1.4)	2.98 ± 0.04 ^{d} (2.56)	71.5 ± 6.52 ^{cd} (3.57)	95 ± 0.9 ^{a} (1.9)	40.7 ± 0.9 ^{bc} (4.6)	28.6 ± 1.5 ^{bc} (8.9)
L8	3.21 ± 0.04 ^{f} (2.37)	4.6 ± 0.05 ^{bc} (1.5)	2.35 ± 0.05 ^{e} (3.68)	73.0 ± 0.68 ^{bc} (1.61)	87 ± 0.7 ^{b} (1.9)	44.6 ± 1.4 ^{ab} (6.2)	26.9 ± 0.68 ^{cd} (4.39)

Means indicated with different letters in the same column are significantly different ($p < 0.05$).

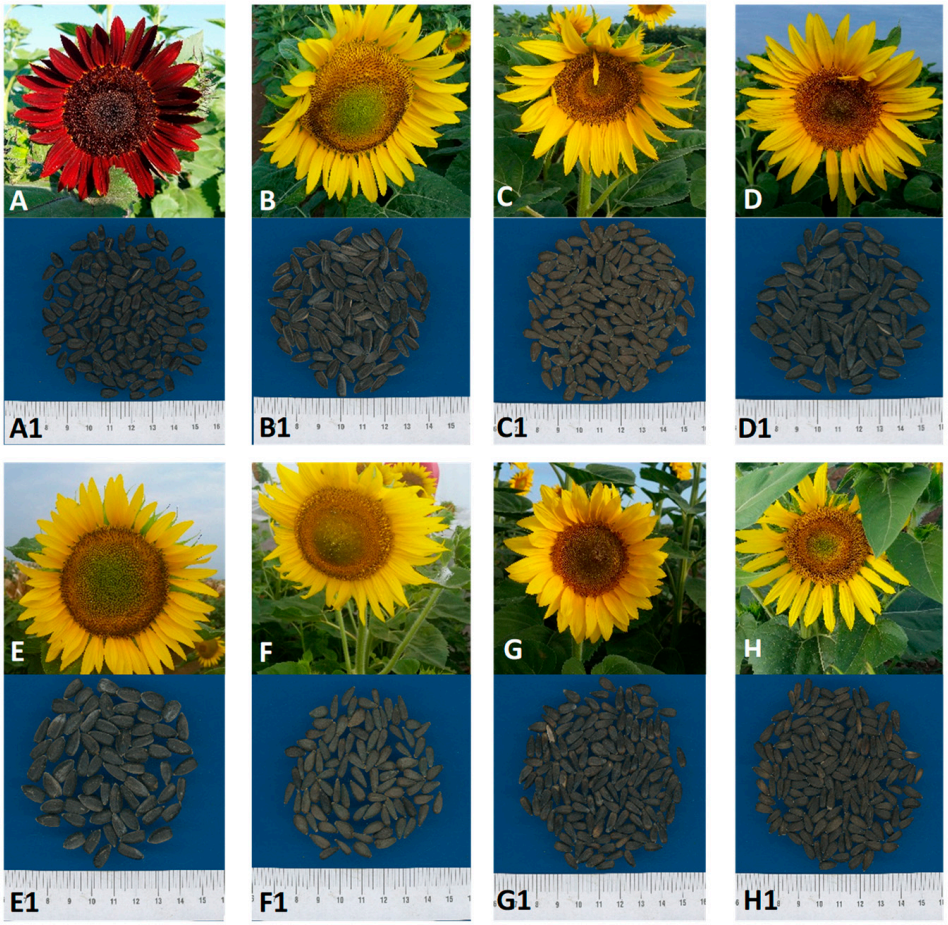

Figure 1. Presentation of the examined sunflower genotypes: (**A**,**A1**)—L1; (**B**,**B1**)—L2; (**C**,**C1**)—L3; (**D**,**D1**)—L4; (**E**,**E1**)—L5; (**F**,**F1**)—L6; (**G**,**G1**)—L7; (**H**,**H1**)—L8.

The analyzed sunflower genotypes exhibited significant differences in achene and seed dimensions (Table 2). Achene length ranged from 8.4 mm in genotype L1 to 11.3 mm in L5, with an average value of approximately 9.6 mm across all genotypes. Achene width varied from 3.97 mm (L7) to 6.2 mm (L5), with a mean of around 5.1 mm, while achene thickness was highest in L5 (4.3 mm) and lowest in L8 (2.7 mm), averaging 3.6 mm. These parameters indicate that genotype L5 consistently exhibited the largest achenes across all measured dimensions. Achene sphericity, which reflects the ratio of length, width, and thickness, ranged from 0.53 in L8 (the lowest value, indicating a more elongated shape) to 0.61 in L5 and 0.62 in L1 (the highest value, indicating a more compact shape).

Table 2. Achenes' and seeds' morphometric characteristics in the examined sunflower genotypes (mean value ± standard error, coefficient of variation (CV), %).

Genotype	Achene Length (mm)	Achene Width (mm)	Achene Thickness (mm)	Achene Sphericity (ϕs)	Seed Length (mm)	Seed Width (mm)	Seed Thickness (mm)
L1	8.4 ± 0.08 ^{g} (8.3)	4.8 ± 0.07 ^{c} (11.4)	3.33 ± 0.04 ^{c} (9.9)	0.62 ± 0.004 ^{a} (5.9)	6.94 ± 0.06 g (7.5)	3.12 ± 0.04 ^{c} (10.6)	2.3 ± 0.03 ^{bc} (11.8)
L2	9.7 ± 0.06 ^{c} (4.8)	4.65 ± 0.05 ^{c} (9.55)	3.22 ± 0.03 ^{c} (8.2)	0.56 ± 0.003 ^{b} (4.2)	8.31 ± 0.07 ^{c} (6.6)	3.5 ± 0.04 ^{b} (9.8)	2.4 ± 0.03 ^{b} (11.6)
L3	10.81 ± 0.07 ^{b} (5.3)	4.98 ± 0.05 ^{b} (9.3)	3.47 ± 0.04 ^{b} (9.5)	0.55 ± 0.004 ^{b} (6.14)	9.0 ± 0.09 ^{b} (8.2)	3.42 ± 0.04 ^{b} (9.9)	2.4 ± 0.04 ^{b} (14.1)
L4	9.75 ± 0.07 ^{c} (5.9)	4.65 ± 0.06 ^{c} (12.2)	3.31 ± 0.03 ^{c} (7.86)	0.57 ± 0.003 ^{ab} (4.4)	7.86 ± 0.07 ^{d} (6.98)	3.36 ± 0.05 ^{b} (11.7)	2.37 ± 0.03 ^{b} (9.96)
L5	11.3 ± 0.08 ^{a} (5.9)	6.2 ± 0.05 ^{a} (6.9)	4.3 ± 0.04 ^{a} (7.48)	0.61 ± 0.003 ^{a} (4.2)	9.35 ± 0.09 ^{a} (8.0)	4.13 ± 0.05 ^{a} (10.42)	2.85 ± 0.05 ^{a} (14.3)
L6	9.3 ± 0.09 ^{d} (7.5)	4.2 ± 0.06 ^{d} (11.4)	3.0 ± 0.05 ^{d} (13.7)	0.56 ± 0.004 ^{b} (6.1)	7.34 ± 0.09 ^{ef} (10.43)	3.21 ± 0.05 ^{c} (12.7)	2.23 ± 0.04 ^{c} (15.0)
L7	9.0 ± 0.06 ^{e} (5.2)	3.97 ± 0.06 ^{e} (11.7)	2.85 ± 0.05 ^{e} (14.5)	0.54 ± 0.004 b^{c} (6.8)	7.4 ± 0.07 ^{c} (7.53)	3.2 ± 0.04 ^{c} (10.2)	2.26 ± 0.03 b^{c} (12.9)
L8	8.8 ± 0.08 ^{f} (7.72)	4.0 ± 0.06 d^{e} (11.5)	2.7 ± 0.06 ^{f} (17.8)	0.53 ± 0.006 ^{c} (9.0)	7.2 ± 0.09 ^{d} (10.6)	2.97 ± 0.05 ^{d} (15.0)	1.8 ± 0.03 ^{d} (15.2)

Means indicated with different letters in the same column are significantly different ($p < 0.05$).

Similarly, seed length varied between 6.94 mm in L1 and 9.35 mm in L5, with an average of approximately 8.1 mm. Seed width ranged from 2.97 mm (L8) to 4.13 mm (L5), with a mean value of 3.6 mm, whereas seed thickness was most pronounced in L5 (2.85 mm) and smallest in L8 (1.8 mm), with an average of 2.3 mm. These findings suggest that genotypes with larger achenes generally had larger seeds, reinforcing the relationship between achene and seed size. This trend is particularly evident in L5, which displayed the most robust achenes and seeds, whereas L8 exhibited the smallest dimensions across multiple traits (Table 2).

The analyzed sunflower genotypes showed statistically significant differences in the morphometric and physical properties of the pericarp (Table 3). In all genotypes, the pericarp was composed of the epidermis with a cuticle, hypodermis, sclerenchyma, and parenchyma, with varying degrees of development of these tissues among the genotypes. The pericarp thickness ranged from the thickest in L1 (298.3 µm) to the thinnest in L6 (144.1 µm), with L1 having a significantly thicker pericarp than L6 by more than 150 µm (Figure 2). Other genotypes, such as L3 (203.7 µm), L4 (191.1 µm), and L8 (193.6 µm), had intermediate thickness values. The percentage of the epidermis with the cuticle was the highest in L6 (7.3%) and L2 (6.5%), while it was the lowest in L1 (4.3%). The percentage of the hypodermis ranged from the highest in L1 (18.1%) to the lowest in L5 (4.8%) and L8 (4.9%). The sclerenchyma layer was dominant in the structure of the pericarp, with the highest percentage in L8 (90.4%) and L5 (90.3%), while L1 had the lowest percentage (78.2%).

The relationship between the percentage of sclerenchyma and the number of sclerenchyma layers was not always positive, as genotypes with higher percentages of sclerenchyma, such as L8 and L5, had fewer sclerenchyma layers (8 and 6.2 layers, respectively), while L1, despite having the lowest percentage of sclerenchyma, had the highest number of layers (9 layers). This indicates that the number of sclerenchyma layers is not necessarily directly related to the proportional share of sclerenchyma. A higher percentage of sclerenchyma is expected to lead to greater hardness. However, L1 had a lower percentage of sclerenchyma, yet had more layers and higher hardness. Pericarp hardness ranged from the highest in L1 (2.0 N) to the lowest in L6 and L3 (0.29 N, 0.33 N), which corresponds to the number of sclerenchyma layers in these genotypes (Table 3).

Table 3. Pericarps' morphometric and physical characteristics in the examined sunflower genotypes (mean value ± standard error, coefficient of variation CV (%)).

Genotype	Pericarp Thickness (µm)	% of Epidermis with Cuticle	% of Hypodermis	% of Sclerenchyma	No. of Sclerenchyma Layers	Pericarp Hardness (N)
L1	298.3 ± 5.2 ^{a} (6.8)	4.3 ± 0.26 ^{c} (23.6)	18.1 ± 0.7 ^{a} (14.4)	78.2 ± 0.7 ^{c} (3.2)	9 ± 0.09 ^{a} (4.2)	2.0 ± 0.20 ^{a} (22.2)
L2	166.4 ± 7.7 ^{b} (17.9)	6.5 ± 0.4 b^{c} (25.9)	6.2 ± 0.5 ^{bc} (34)	88.3 ± 0.7 ^{ab} (3.2)	7.7 ± 0.4 ^{b} (17.9)	0.89 ± 0.06 ^{cd} (15.2)
L3	203.7 ± 6.9 ^{b} (12.2)	6.3 ± 0.4 ^{ab} (21.9)	5.8 ± 0.5 ^{c} (29.1)	88.6 ± 0.7 ^{b} (3.0)	5.0 ± 0.2 ^{e} (15.8)	0.31 ± 0.04 ^{e} (26.7)
L4	191.1 ± 6.9 ^{b} (13.5)	5.6 ± 0.4 ^{bc} (31.5)	5.4 ± 0.4 ^{bc} (26.8)	89.9 ± 0.8 ^{b} (2.5)	7.6 ± 0.2 ^{b} (9.7)	1.11 ± 0.06 ^{c} (13.5)
L5	185.5± 9.1 ^{b} (19.1)	4.9 ± 0.4 ^{bc} (34.8)	4.8 ± 0.4 ^{c} (30.9)	90.3 ± 0.6 ^{ab} (2.5)	6.2 ± 0.25 ^{cd} (15.4)	0.66 ± 0.05 ^{d} (16.7)
L6	144.1 ± 2.3 ^{c} (6.2)	7.3 ± 0.5 ^{a} (25.8)	7.5 ± 0.6 ^{b} (32.1)	86.5 ± 0.9 b^{c} (4.2)	5.57 ± 0.2 ^{de} (12.0)	0.29 ± 0.01 ^{e} (10.9)
L7	153.7 ± 11.8 ^{c} (29.9)	6.3 ± 0.4 ^{ab} (24.2)	5.0 ± 0.4 ^{c} (48.1)	89.9 ± 0.7 ^{ab} (3.0)	6.4 ± 0.3 ^{c} (20.3)	0.7 ± 0.05 ^{d} (16.3)
L8	193.6 ± 8.0 ^{b} (16.2)	5.6 ± 0.3 ^{bc} (19.8)	4.9 ± 0.34 ^{c} (26.3)	90.4 ± 0.6 ^{a} (2.5)	8 ± 0.2 ^{b} (9.4)	1.67 ± 0.08 ^{b} (10.3)

Means indicated with different letters in the same column are significantly different ($p < 0.05$); No.—number.

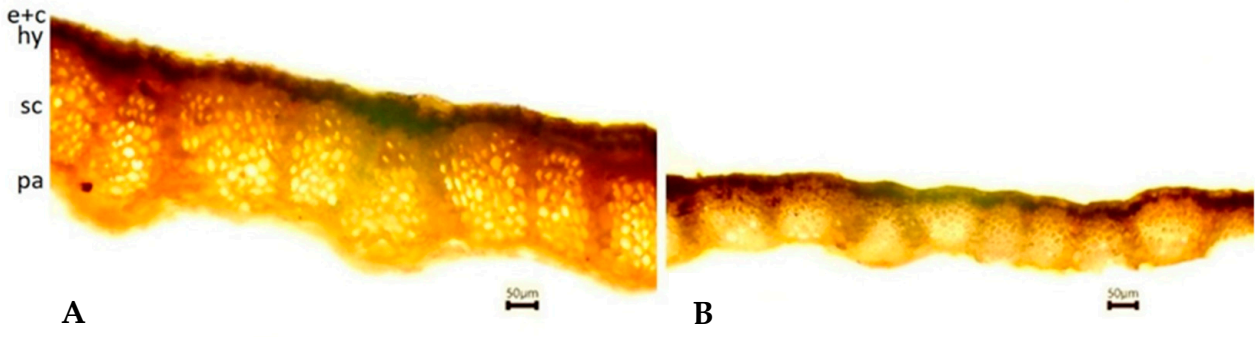

Figure 2. Cross-sections through the middle part of the achene pericarp: (**A**) L1; (**B**) L6. e + c, epidermis with cuticle; hy, hypodermis; sc, sclerenchyma; pa, parenchyma.

The results of the discriminant component analysis (DCA) performed on selected morphometric and biophysical traits of sunflower achenes and seeds are presented in Figure 3 and Table 4. The first discriminant axis (DC 1, 63.1%) primarily separates the samples on the basis of achene width (WA), which has the highest contribution along this axis, followed by achene length (LA). On this axis, genotypes L5 and L3 are positioned in the positive zone, characterized by longer and wider achenes, whereas genotypes L1, L6, L7,

and L8 are found in the negative zone, with smaller achene dimensions (Table 4, Figure 3). The second discriminant axis (DC 2, 20.0%) is mainly influenced by pericarp thickness (TP) and the percentage of sclerenchyma (%SC), which differentiate genotypes along this axis. Likewise, the pericarp thickness (TP) was the main separation feature in the third discriminant axis (DC 3). The percentage of epidermis with cuticle (%EC) and hypodermis (%HY) also contribute to the separation, indicating that the structural components of the pericarp play a key role in distinguishing genotypes along this axis. On the second axis, genotype L1 stands out in the positive zone, exhibiting the thickest pericarp, the lowest percentage of epidermis with cuticle and sclerenchyma, and the highest proportion of hypodermis (Figure 3, Table 4).

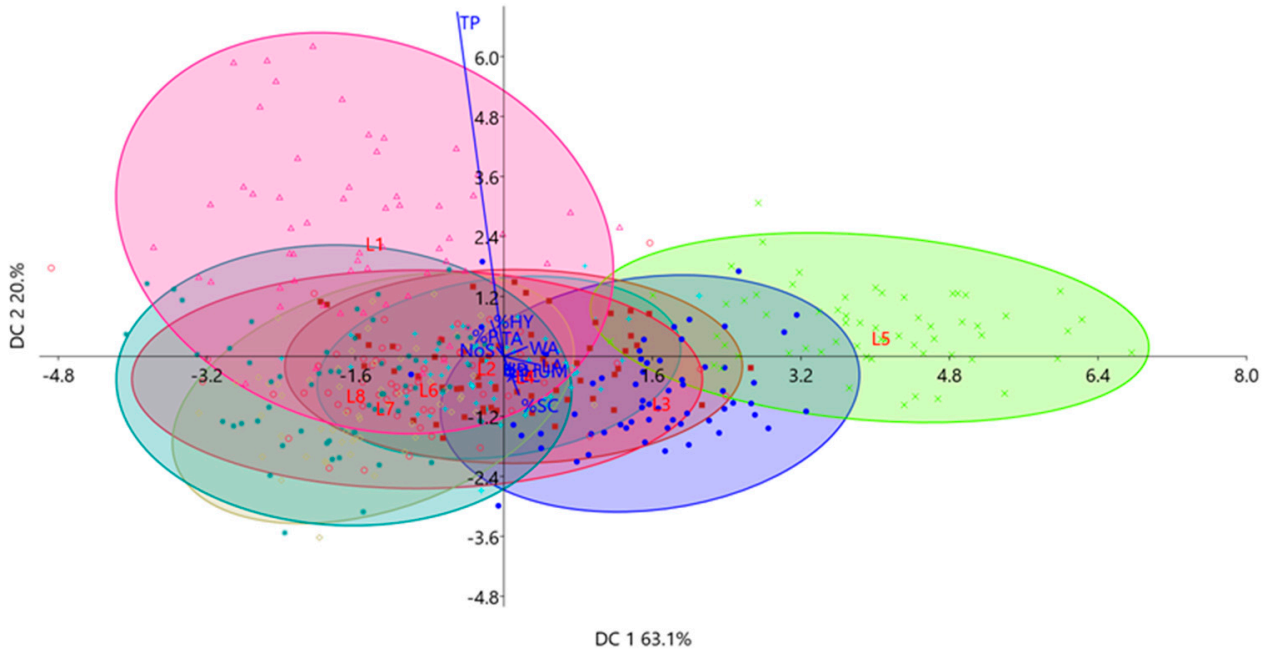

Figure 3. Scatter plot obtained using DCA and the position of centroids in the space of two discriminant axes, based on the characteristic morphometric and biophysical traits of sunflower achenes and seeds of the studied sunflower genotypes.

Table 4. Loading levels of the first three canonical axes in the discriminant analysis based on selected morphometric and biophysical traits of achenes and seeds in the studied sunflower genotypes.

Characteristics	DC 1	DC 2	DC 3
Achene length (LA)	**0.790**	−0.218	0.156
Achene width (WA)	**0.829**	0.247	−0.039
Achene thickness (TA)	0.237	0.169	−0.002
Achene sphericity (ϕs)	0.005	0.025	−0.001
Pericarp percentage (%P)	−0.094	0.226	0.095
Pericarp thickness (TP)	−0.655	**8.994**	**3.625**
Epidermis with cuticle percentage (%EC)	0.005	**−0.128**	0.048
Hypodermis percentage (%HY)	−0.179	**0.930**	0.363
Sclerenchyma percentage (%SC)	0.213	**−1.003**	−0.468
Number of sclerenchyma layers (NoS)	−0.081	0.187	−0.137
Pericarp hardness (HP)	−0.012	0.031	−0.019
Moisture percentage (% HUM)	0.002	−0.005	0.0015

The correlation analysis reveals significant relationships among various morphometric and biophysical traits of sunflower achenes and seeds (Figure 4). Achene dimensions (length, width, and thickness) are positively correlated with seed dimensions, indicating

that larger achenes generally contain larger and heavier seeds (r > 0.65). Achene weight (WGA) and seed weight (WGS) show a strong correlation (r ≈ 0.97), suggesting that heavier achenes also contain heavier seeds. The sphericity of achenes (ϕs) is significantly correlated with achene and seed width and thickness (0.74, 0.25), while it is negatively associated with the seed percentage (%S, r ≈ −0.30), implying that more spherical achenes have a higher pericarp proportion. The seed and pericarp percentages are inversely related (r ≈ −1.00), while pericarp thickness (TP) is positively correlated with the percentage of the hypodermis (%HY) as well as with pericarp hardness (HP, r > 0.5), confirming that a greater number of sclerenchyma layers contribute to a harder seed coat. Pericarp hardness shows a negative correlation with germination percentage (%Ger, r ≈ −0.48), suggesting that a tougher pericarp may affect seed germination. Additionally, oil content (%O) is negatively correlated with pericarp thickness and the number of sclerenchyma layers (r > −0.5), indicating that genotypes with a thicker and tougher pericarp tend to have lower oil content. Seed percentage (%S) is positively and statistically significantly associated with germination percentage (%Ger, r > 0.8), suggesting that better filled achenes also have better germination potential. These findings highlight key relationships among the physical properties of achenes and seeds, which can significantly influence genotype selection for improved agronomic traits, seed quality, and germination success.

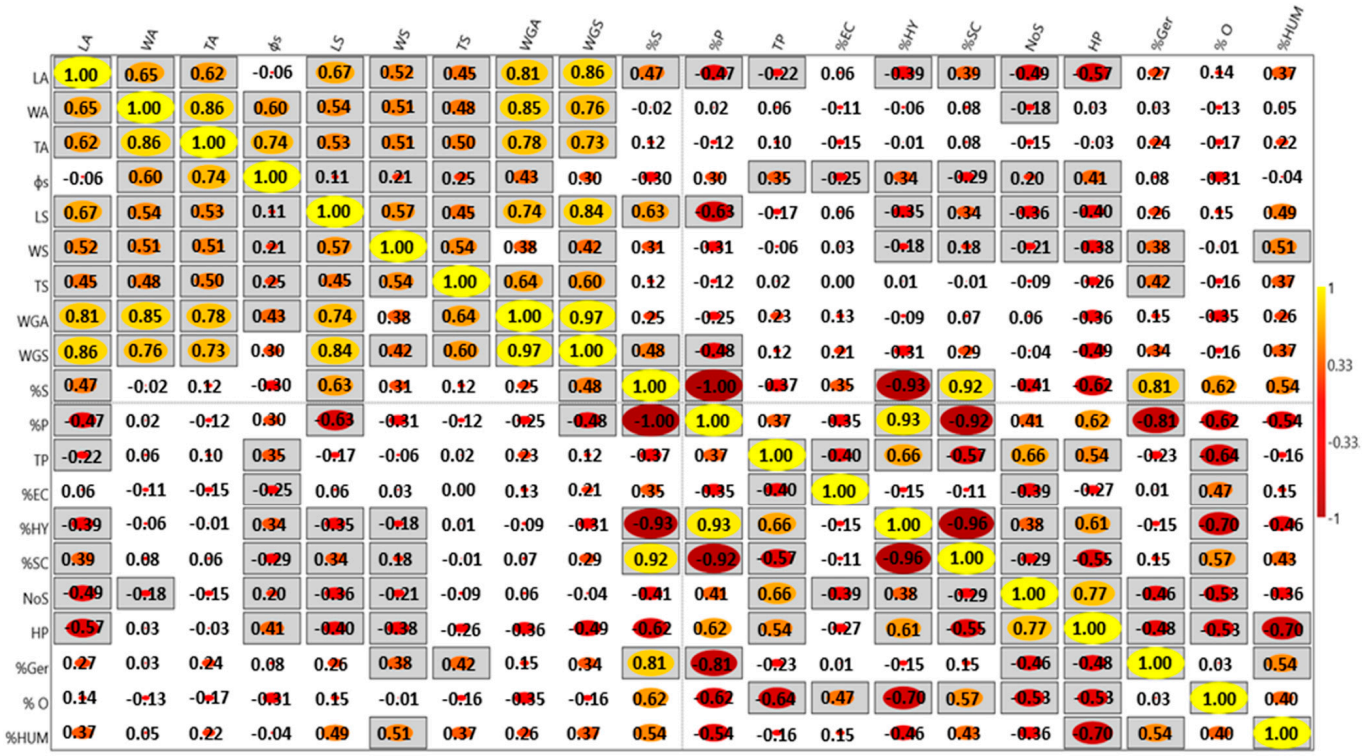

Figure 4. Correlation analysis (Pearson's correlation) $p \leq 0.05$. Values in grey square are statistically significant. LA—achene length; WA—achene width; TA—achene thickness; ϕs—achene sphericity; LS—seed length; WS—seed width; TS—seed thickness; WGA—achene weight; WGS—seed weight; %S—seed percentage; %P—pericarp percentage; TP—pericarp thickness; %EC—epidermis with cuticle percentage; %HY—hypodermis percentage; %SC—sclerenchyma percentage; NoS—number of sclerenchyma layers; HP—pericarp hardness; %Ger—germination percentage; %O—oil percentage; %HUM—moisture percentage.

4. Discussion

Understanding the biophysical and morphometric properties of achenes, including pericarp thickness and strength, pericarp percentage, oil content, and germination, is crucial for optimizing industrial processes. On the basis of these characteristics, it is possible to

identify genotypes that are optimal for alternative uses of the pericarp, as well as those that are more suitable for oil extraction and processing, thus increasing the sustainability of industrial production [32].

The color of the pericarp is an important trait in the sunflower breeding process and plays a significant role in the selection of a desirable sunflower genotype [56,57]. The basic gray color of the pericarp of the investigated genotypes had different intensities, ranging from the darkest in genotype L1 to the lightest in genotypes L3 and L6. Dedio [58] and Nadkarni et al. [59] state that genotypes with a darker pericarp are used for oil production, due to their higher oil content compared with sunflowers with a lighter pericarp color, which is in contrast to the results of our study, where genotypes L3 and L6 had both the lightest pericarp color and the highest oil content, and genotype L1 had the opposite. It is precisely because of the dark color of the pericarp that genotype L1 could be an excellent substitute for natural wood in the production of polymer composites, since pericarp powder can give them a wood-like appearance, as stated by Stănescu and Bolcu [13]. In addition, through further research, it could be analyzed as a potential natural source of food colorant in accordance with the research of Vaccari et al. [60] and Özdemir and Keleş [61], or as a potential source of phytomelanin for industrial use. According to Škorić [62], the color is determined by the amounts and combinations of pigments found in the individual layers of the pericarp. The white color comes from the complete absence of pigments in the pericarp layers, which, in combination with the phytomelanin layer, gives the pericarp a gray color. In their research, Özdemir and Keleş [61] conclude that in addition to influencing the color of the pericarp, phytomelanin also has antioxidant properties, so the pericarp of genotypes rich in this rare pigment can be a source of pure phytomelanin and have a significant role in the cosmetic, pharmaceutical, and food industries. Similarly, in line with the research by Tostain et al. [63], which indicates a reduction in the environmental footprint during the dehulling process, our results support the circular bioeconomy approach, where the pericarp is recognized as a valuable byproduct. This approach not only enables better use of the entire plant material but also contributes to the sustainability of industrial production and reduces the need for its incineration. In this regard, our work shows that genotypes like L1, which have the highest pericarp content and the thickest pericarp, can be particularly useful for industrial applications. Given the high pericarp content, L1 may have significant potential for biomass production or other industrial materials, thus reducing the environmental footprint and enhancing the sustainability of industrial production. In accordance with the work of Cui et al. [11], which analyzes the potential of sunflower pericarp as a raw material for biomass pelletization, and Demir et al. [64], which addresses the emissions generated during the incineration of sunflower hulls, our research on the utilization of sunflower pericarp as a biological resource can contribute to reducing the environmental footprint. The same approach is stated by Stănescu and Bolcu [13], as well as Irez [65], who see an opportunity for a more sustainable industry in the development of natural fiber composite materials, as a replacement either for materials from limited natural resources or for conventional materials. While Demir et al. [64] and Lunguleasa et al. [12] highlight emissions during the burning of the hulls and Zakrzewska et al. [15] offer a solution to the problem of waste management through thermal conversion of biomass, our work demonstrates that better utilization of this byproduct, such as its potential for biomass or biochemical substances, can reduce the need for incineration and thus lessen its negative environmental impacts.

By applying multivariate discriminant analysis, there was a clear separation of genotypes characterized by larger dimensions of the achene compared with genotypes with smaller dimensions. According to the first discriminant axis (the axis that explains the largest share of variability), characteristics such as achene width and achene length had the

greatest influence on the differences between genotypes. Traits such as pericarp thickness and percentage of sclerenchyma also influenced the differences among the tested genotypes, according to the second discriminant axis.

Analyzing the results of their own studies on achene structure and lipid accumulation, Mantese et al. [66] found that by the maturity stage, the thickness and number of sclerenchyma cell layers decreased proportionally to the increase in oil content in the genotypes studied. In line with these findings, the results of our study confirm that genotypes with fewer sclerenchyma layers and softer pericarp (such as L3 and L6) have a higher oil content and potentially better processing efficiency, and also support the previous findings of Dauguet et al. [67] regarding the negative correlation between hullability and oil content. Hernandez and Belles [24] found that increased lignin content in the sclerenchyma significantly contributes to pericarp strength, increasing its mechanical resistance but simultaneously reducing its ability to be efficiently hulled. Sharma et al. [40] and Malik and Saini [30] point out that the presence and strength of cellulose fibers in the pericarp directly affect the magnitude of the force required for initial cracking. Similarly, our results show that a higher number of sclerenchyma layers directly contributes to greater pericarp strength, with genotypes having the thickest pericarp (L1, 298.3 µm) exhibiting the highest resistance to mechanical breakage (2.0 N). On the other hand, genotypes with thinner pericarps and fewer sclerenchyma layers had lower strength values but higher oil content. Since different sunflower genotypes exhibit variations in the physical and mechanical properties of achenes, their proper selection can improve processing efficiency and final product quality. Genotypes with fewer sclerenchyma layers of pericarps and higher oil content ensure better oil extraction efficiency, while genotypes with tougher pericarps and higher mechanical tissue content may have potential for use in biomass production or other industrial applications of the pericarp. This approach enables better utilization of the entire plant material, reduces waste, and increases the economic sustainability of sunflower processing. These findings are supported by Tostain et al. [63], who highlight that during the dehulling process, the pericarp can be used as biomass, thus reducing the ecological footprint of sunflower production. In addition to industrial processing, the proper management of byproducts, such as the pericarp, can contribute to sustainable agricultural production and waste reduction in the oil extraction process. In the context of circular bioeconomy, the pericarp can have additional value as a raw material for biomass, biochemical substances, or food additives. Therefore, understanding the physical properties of seeds and the pericarp not only contributes to optimizing industrial processing but also opens up opportunities for further valorization of sunflower byproducts. Dimić [68] and de Figueiredo et al. [69] also point out that physical seed characteristics, including pericarp thickness and resistance to breakage, play a crucial role in the efficiency of the dehulling process, which can impact further sunflower processing. Additionally, de Figueiredo et al. [69] state that hybrid sunflower seeds with smaller dimensions and a higher pericarp content have better dehulling efficiency, which is confirmed by our results for genotypes with smaller achene dimensions, such as L6 and L8. On the other hand, the size of the achene and the structure of the pericarp play an important role in imbibition and the germination rate. In our study, pericarp hardness showed a negative correlation with germination, which was lowest in L1 and L8 (82% and 87, respectively), suggesting that the harder pericarp may hinder the water uptake process. Germination showed a positive and statistically significant correlation with the percentage of seeds, suggesting that better filled achenes have a higher germination potential. Namely, during the study, the highest germination was observed in the L5 and L3 genotypes, which had the largest achene and seed dimensions, as well as weight. In addition, these two genotypes also had the softest pericarp, which was not affected by the thickness of the pericarp itself but by the smallest number of sclerenchyma

layers. These results are consistent with the conclusion of Ahmed et al. [70] that, in general, large seeds have better field performance than small seeds, and also with the conclusion of Hernandez and Orioli [71] that the pericarp is considered an important pathway for the passage of water to the seed. A detailed investigation of the physical properties of sunflower seeds is crucial, as these properties must be well understood when designing various equipment for industrial seed processing. These characteristics should also be well known when designing machines and systems used in different processes, such as classification and quality assessment of agricultural products [72,73]. According to this approach, understanding the physical characteristics of seeds and the pericarp is not only crucial for efficient industrial processing but also significant for the design of equipment and systems used for classification and quality assessment of agricultural products.

5. Conclusions

This study shows that genotypes L3 and L6 are more suitable for oil extraction, and genotype L1 is promising for the industrial use of pericarp. Further studies and coverage of multiple environments, along with chemical profiling of pericarp compounds, would confirm the proposed uses. Identifying these genotypic profiles on the basis of the biophysical and morphometric properties of achenes can support a more rational use of resources and expanded utilization of byproducts in the sunflower production chain.

Author Contributions: Conceptualization, J.O. and J.J.; methodology, J.O., J.J., S.G. and D.J.; software, J.J.; validation, J.O., J.J., S.G. and D.J.; formal analysis, J.O., J.J. and M.J.; investigation, J.O., J.J., M.J., S.G., M.K. and D.J.; resources, M.J., S.J. and M.K.; data curation, J.O. and J.J.; writing—original draft preparation, J.O. and J.J.; writing—review and editing, J.O., J.J., M.J., S.J., S.G., M.K. and D.J.; visualization, J.O. and J.J.; supervision, M.J. and S.J.; project administration, J.O. and J.J.; funding acquisition, J.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Acknowledgments: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (grant number 451-03-136/2025-03/200032) and by the Science Fund of the Republic of Serbia (#6691, New biorational methods for stored seed pest control and protection: To serve and prevent—SafeSeed). This work was performed as part of the activities of the Centre of Excellence for Innovations in Breeding of Climate Resilient Crops—Climate Crops, Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, Novi Sad, Serbia. The authors would like to thank Milivoj Radojčin for providing equipment for conducting experiments at the Laboratory for Biosystem Engineering, Faculty of Agriculture, University of Novi Sad, Serbia.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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