Article

Assessing the Potential of Old and Modern Serbian Wheat Genotypes: Yield Components and Nutritional Profiles in a Comprehensive Study

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Abstract: Creating wheat genotypes characterized by high grain yield, high protein content, and favorable amino acid composition is the main goal of breeders, especially in developing countries where wheat is a staple food. An experiment with 20 wheat genotypes, released through breeding activities in the Serbian region at different periods and adapted to its pedoclimatic conditions, was conducted with the aim of determining the genetic potential of the analyzed genotypes for grain yield and quality. Due to the divergence of the examined wheat germplasm, the factor of genotype had the largest share in the variation of all yield parameters (>66%). The genotypes Zadruga and Agrounija exhibited superior abilities for overall grain yield. Also, genotype Zadruga stood out in a distinct cluster group due to high values of both thousand grain weight and grain yield per plant. A continuous improvement in protein content was found, with newer genotypes having 17.13% higher protein content compared with older genotypes. Genotype Sloga stood out with the highest protein content (13.93%). On the other hand, the old genotype Balkan was distinguished by the highest content of nonessential amino acids (61.5 g 100 g^{-1} protein), which makes it a good genetic resource. Genotypes Agrounija (32.62 g 100 g^{-1} protein) and Tanjugovka (32.47 g 100 g^{-1} protein) had the highest content of essential amino acids. The highest AAS value was established for tryptophan (1.81) and the lowest for lysine (0.61). Genotypes Tanjugovka and Zadruga had the highest AAS, i.e., protein completeness. The genotypes Zadruga, Tanjugovka, Agrounija, and Sloga have demonstrated high-yield capacity and possess a favorable amino acid profile, making them promising candidates for enhancing the nutritional quality of wheat and potentially benefiting human health.

Keywords: AAS; grain yield components; essential amino acids; nonessential amino acids; protein completeness

1. Introduction

Wheat is a staple food for 40% of the global population, serving as a daily protein source for approximately 2.5 billion people, particularly in less-developed countries [1]. Its widespread consumption, nutritional contribution, versatility, and economic importance make wheat a vital crop for ensuring food security and meeting the dietary needs of a significant portion of the world's population [2]. The global population is expected to reach around 9.7 billion by 2050, and as a result, the demand for food, including staple

crops like wheat, is projected to increase by 60% [3]. The projected 60% increase in demand highlights the need to enhance both the yield and quality of wheat grains. To meet this demand, researchers, agricultural scientists, and farmers are actively working on various strategies and technologies to improve wheat production. Examining agronomic and quality traits in a wide range of wheat germplasm is a valuable approach when selecting genotypes as desirable genetic resources for breeding purposes [4–8]. This approach allows researchers and breeders to identify and understand the diverse characteristics present within the wheat gene pool, which can be utilized to develop improved varieties with enhanced yield and quality characteristics [9,10]. Grain yield in wheat is predominantly sink-limited because the capacity of developing grains to store and utilize assimilates (such as sugars) is the main factor that determines final grain yield [11–13]. The number and size of developing grains on the wheat spike can affect the overall sink strength, and wheat genotypes with a greater number of grains or larger grains generally have a higher sink strength, allowing them to attract and utilize more assimilates [14,15]. Therefore, studying yield components related to grain number and size could help to better understand the genetic basis for increasing wheat production [4,8,13,16–19].

In addition to increasing grain yields, one of the main objectives of plant breeders is to improve their nutritional quality [20–23], especially due to the rising demand for plant proteins, the rising popularity of organic and vegan diets, and health considerations [20,24]. This involves identifying and selecting the specific nutrients that should be increased in the crop to address nutritional deficiencies or health concerns [24]. In addition, the creation of crops with improved nutritional quality requires the identification of genetic resources with high values of quality traits from available germplasm [25]. As one of the major staple food sources around the world, wheat provides approximately 20% of the calories and proteins in the daily human diet [26]. The nutritional quality of protein is determined by the amount and composition of amino acids, both nonessential and essential ones. These amino acids are required in the human body to regulate the growth, maintenance, repair, and replacement of tissues, as well as to synthesize enzymes, hormones, and the immune system [27–29]. Proteins are composed of twenty amino acids: nonessential (aspartic acid, glutamic acid, serine, glycine, alanine, arginine, tyrosine, cysteine, and proline), which can be synthesized by the human body, and essential (lysine, isoleucine, leucine, phenylalanine, threonine, tryptophan, valine, histidine, methionine, and cysteine), which cannot be synthesized by the human body [27,29,30]. The amino acid profile of wheat seems to be very unbalanced, with wheat proteins being particularly weak in some essential amino acids, especially lysine, followed by threonine and methionine [29,31–34]. A balance between the essential amino acids in the protein complex is crucial from the aspect of nutritional value [35], particularly since if one essential amino acid is deficient, the others will be metabolized and expelled, which may restrict human growth [29].

Many researchers have studied protein and starch content as quality indicators in wheat [36–39]. However, a small amount of research was focused on the study of the amino acid profile in wider germplasm of wheat genotypes from different years of approval. Better knowledge and understanding of the protein quality and content of essential and nonessential amino acids in different wheat genotypes can be of great importance not only for plant breeders but also for producers and the food industry [33].

The objectives of this study were as follows: (i) examination of the influence of genotype, year, and genotype × year interaction on grain yield components; (ii) assessment of the improvement of protein content over time in the analyzed wheat genotypes; (iii) identification and quantification of essential and nonessential amino acid concentrations in wheat genotypes; and (iv) identification of wheat genotypes bred in the Serbian region and adapted to those pedoclimatic conditions that can serve as a suitable genetic resource in breeding for high grain yield and nutritional quality.

2. Materials and Methods

2.1. Experimental Design and Plant Material

This study comprises twenty genetically divergent winter wheat (*Triticum aestivum* sp.) genotypes released by the Institute of Field and Vegetable Crops in Novi Sad, the Centre for Small Grains in Kragujevac, and PFZ Inđija (Table 1). The germplasm included in this scientific study was meticulously selected based on a comprehensive analysis of prior research conducted by Zečević et al. [5,21,22,40], Knežević et al. [23,41], and Hristov et al. [42]. These earlier studies extensively evaluated the yield and quality characteristics of various wheat genotypes. By building upon the insights gained from these investigations, we aimed to identify genetic resources characterized by their remarkable combination of high yield, elevated protein content, and favorable amino acid composition. Moreover, the selection of germplasm used in this study was based on a careful consideration of the diversity inherent within the evaluated genotypes. Additionally, in our selection criteria, we incorporated a temporal dimension by including genotypes from different years of approval, with the aim of examining whether the yield and nutritional quality of wheat improved over the decades and establishing whether older genotypes can be good selection material for improving nutritional quality. The experiment was established in Kraljevo (43°43′25″ N, 20°41′13″ E) during the two vegetation seasons (2015/2016 and 2016/2017), according to a randomized complete block design (RCBD) with three replications. The seeds of different varieties were sown at a distance of 0.10 m in six rows of 1.0 m length at a distance of 0.2 m.

Table 1. Wheat germplasm used in this research.

No.	Genotype	Pedigree	Year of Approval
1.	Evropa 90	Talent/NSR–2	1971
2.	Gružanka	Leonardo/Argento	1972
3.	Zastava	Bezostaja/Abbodanza	1973
4.	KG–56	Bezostaja 1/Halle Stamm//Bezostaja 1	1975
5.	Orašanka	Bezostaja 1/Halle Stamm	1976
6.	Balkan	Bačka/Bezostaja 1/Mironovskaya 808/NS 433/Skor.35	1979
7.	Jugoslavija	NS 646/Bezostaja 1//Aurora	1980
8.	Oplenka	Kavkaz/KG–56	1982
9.	Lasta	Dunav/NS611/NS736/Stepnjač K30/NS 736/Lcs32//Bezostaya 1/Aurora/Mironovskaya 808/Jubil50	1987
10.	Agrounija	Kavkaz/Zlatna Dolina/KG–56	1988
11.	Rodna	NS 646/Bezostaya 1//Aurora/Partizanka	1988
12.	Tanjugovka	Jugoslavija/Partizanka	1988
13.	Zadruga	Jugoslavija/Balkan	1989
14.	Proteinka	NS 2726/3/Mačvanka 1	1990
15.	NSR–5	[(NSR-1/Tisa)/Partizanka)]/Mačvanka 1	1991
16.	Gruža	KM 20/KM192-75/KG 56	1991
17.	Milica	Zelengora/Mačvanka 2//Partizanka	1992
18.	Sloga	NS 2986/ZG 628/77	1993
19.	Dejana	NS 7016/NS7001	1994
20.	Tera	NS 2979/5-1/NS 3000/Rana niska	1995

2.2. Analysis of Grain Yield Components

Harvesting was performed at the stage of physiological maturity when the grain moisture was below 14%. After the harvest, agronomic traits such as the number of grains per spike, grain weight per spike, thousand grain weight, and grain yield per plant were examined.

2.3. Analysis of Protein Content

The total protein content in the wheat samples was determined using Kjeldahl's method, specifically following the procedure outlined in AOAC Official Method 979.09 [43]. This method is used for nitrogen (N) content determination, which is then used to calculate

the protein content by multiplying it by a coefficient of 5.7. The resulting value represents the protein content as a percentage (p%).

2.4. Analysis of Amino Acid Content

For the extraction of amino acids in all wheat genotypes, representative samples of wheat seeds were milled, and 0.5 g of the resulting flour was utilized for amino acid extraction. The amino acid analyses were performed using ion exchange chromatography, following the method established by Spackman et al. [44]. An automatic amino acid analyzer, the Biochrom 30+ (Biochrom, Cambridge, UK), was employed for this purpose. This method involved the use of strong cation exchange chromatography for amino acid separation, followed by the ninhydrin color reaction and photometric detection at 570 nm (with additional measurements at 440 nm for proline).

The representative samples of wheat seeds were subjected to acid hydrolysis using 6 M hydrochloric acid (HCl) sourced from Merck, Darmstadt, Germany, at a controlled temperature of 110 °C for a duration of 24 h. This hydrolysis process effectively cleaves the proteins, yielding their constituent amino acids. To preserve the integrity of amino acids and prevent thermal degradation, the hydrolyzed wheat samples were promptly cooled to room temperature. Subsequently, the hydrolyzed wheat samples were dissolved in 25 mL of loading buffer, with a pH maintained at 2.2, obtained from Biochrom, Cambridge, UK. The prepared samples were filtered through a 0.22 μm pore-size polytetrafluoroethylene (PTFE) filter (Plano, TX, USA). The resulting filtrate was transferred into vials provided by Agilent Technologies, USA, and stored in a refrigerator until analysis. The analysis was conducted in triplicate, and the concentration of amino acids was expressed in grams per 100 grams of protein (g 100 g^{-1} protein).

The evaluation of essential amino acids in the test material was performed through the amino acid score (AAS) methodology. This scoring system enables the determination of the nutritional quality of proteins present in the test material by comparing the content of each essential amino acid in wheat samples with the recommended intake values established for adults by the Food and Agriculture Organization [45]. The AAS is calculated according to the relevant formula:

AAS = essential amino acid content (%)/recommended essential amino acids (%)

2.5. Meteorological Conditions

The data on meteorological conditions (Figure 1) were obtained from the website of the Republic Hydrometeorological Institute of Serbia [46].
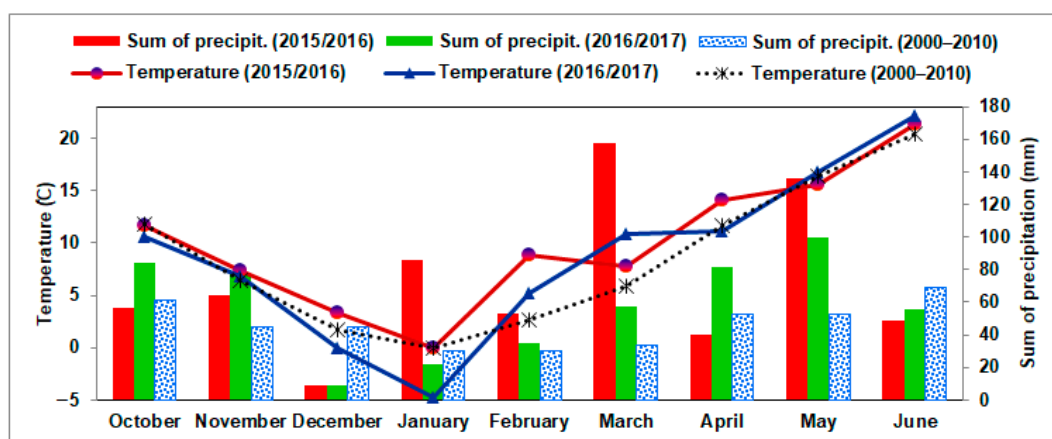

Figure 1. Monthly mean temperatures and sum of precipitation at the locality of Kraljevo during the experiment.

The sum of precipitation in both growing seasons was higher than the multi-year average for the analyzed locality, with a significantly higher sum of precipitation recorded

in the 2015/2016 season compared with 2016/2017 (651 and 522.6 mm, respectively). October and November were characterized by an appropriate sum of precipitation and mean temperatures in both vegetation seasons. The surface layer of the soil was of favorable heat and moisture, which enabled appropriate germination, emergence, and tillering of plants. Temperatures were slightly higher than the multi-year average characterized by December (3.3 °C) in the 2015/2016 growing season. In January of the given season, a significant amount of precipitation (86.2 mm) in the form of snow was recorded, which protected the plants from freezing. On the other hand, December and January in 2016/2017 were marked by a lack of precipitation and significantly lower temperatures (0 °C in December and −4.7 °C in January) compared with the multi-year average. However, these conditions did not harm the wheat, which was in the phenophase of tillering.

February was characterized by warm weather in both vegetation seasons (8.8 °C in 2015/2016 and 5.2 °C in 2016/2017) and a higher sum of precipitation (52.7 mm in 2015/2016 and 35 mm in 2016/2017) compared with the multi-year average, which influenced the earlier start of vegetation. Also, the warm weather with a favorable amount of precipitation continued in March and April, which allowed the intensive growth of plants in both vegetation seasons.

The increased amount of precipitation in May (135.9 mm in 2015/2016 and 100 mm in 2016/2017), combined with the temperatures within the multi-year average, enabled appropriate flowering and fertilization. In both vegetation seasons, June was marked by a significantly lower amount of precipitation compared with the multi-year average. However, the water reserves in the soil from the previous months favored the grain filling. Higher average temperatures in June, especially in the last decade, accelerated the ripening of wheat and the harvest in both analyzed seasons (Figure 1).

2.6. Statistical Analysis

An analysis of the phenotypic variability of grain yield components, protein content, and amino acid composition was performed using the analysis of variance (ANOVA). Multiple comparisons of genotypes in relation to analyzed traits were performed using the LSD test at two levels of statistical significance (1% and 5%). This analysis was performed using the program IBM SPSS Statistics, Trial Version 22.0 [47].

A correlation matrix analysis using the principal component method (PCA) was performed to express the interrelationships among grain yield components and amino acids. The cluster heatmap analysis was created using the function heatmap in the R programming language, which enabled hierarchical clustering of rows (genotypes) and columns (yield components), respectively. The rows and columns of the heatmap were rearranged based on their similarities, and dendrograms were added to the heatmap to visualize the clustering. The hierarchical clustering algorithm was based on the Euclidean distance between the rows or columns of the scaled data matrix. The algorithm recursively merges the most similar rows or columns based on the distance metric until all objects are assigned to a cluster. The resulting dendrogram represents the hierarchical structure of the clusters. The PCA and cluster heatmap analysis were performed using the R Project for Statistical Computing, Version 4.2.0, 2022-04-22 ucrt [48].

3. Results and Discussion

3.1. Grain Yield Components

Grain yield is a complex quantitative trait influenced by multiple factors and their interactions. Also, it is the result of various yield components that interact in a multiplicative manner, leading to the final grain yield [4,17,18,49,50]. In this study, the influence of genotype, year, and their interaction on the variation of the number of grains per spike, grain weight per spike, thousand grain weight, and grain yield per plant was analyzed (Figure 2). Genotype has the highest impact on the variation of yield components, ranging from 66.7% for grain weight per spike to 80.7% for the number of grains per spike. This is in agreement with the results published by Knežević et al. [41], examining the influence of

genotype and year on the variation of the number of grains per spike in different wheat genotypes, nine of which were used in this study. The mentioned authors state that the share of the genotype factor in the variation of the number of grains per ear was 63.92%, while the share of the year factor was the smallest (2.72%). A similar observation was made by Zečević et al. [51] for the thousand grain weight, where they found that the factor of genotype had the greatest single influence on this trait, followed by sowing density, while the factor of the year had the lowest influence. The predominant share of the genotype factor in the total variability of the studied grain yield components within a given population is a key observation because it emphasizes the genotypic variability of the population. Genetic variability in a population is crucial because it allows the population to adapt to changes in the environment. It plays a significant role in crop breeding programs as well. Without genetic variability, a population would struggle to cope with environmental challenges and could hinder the development of improved crop varieties [10].
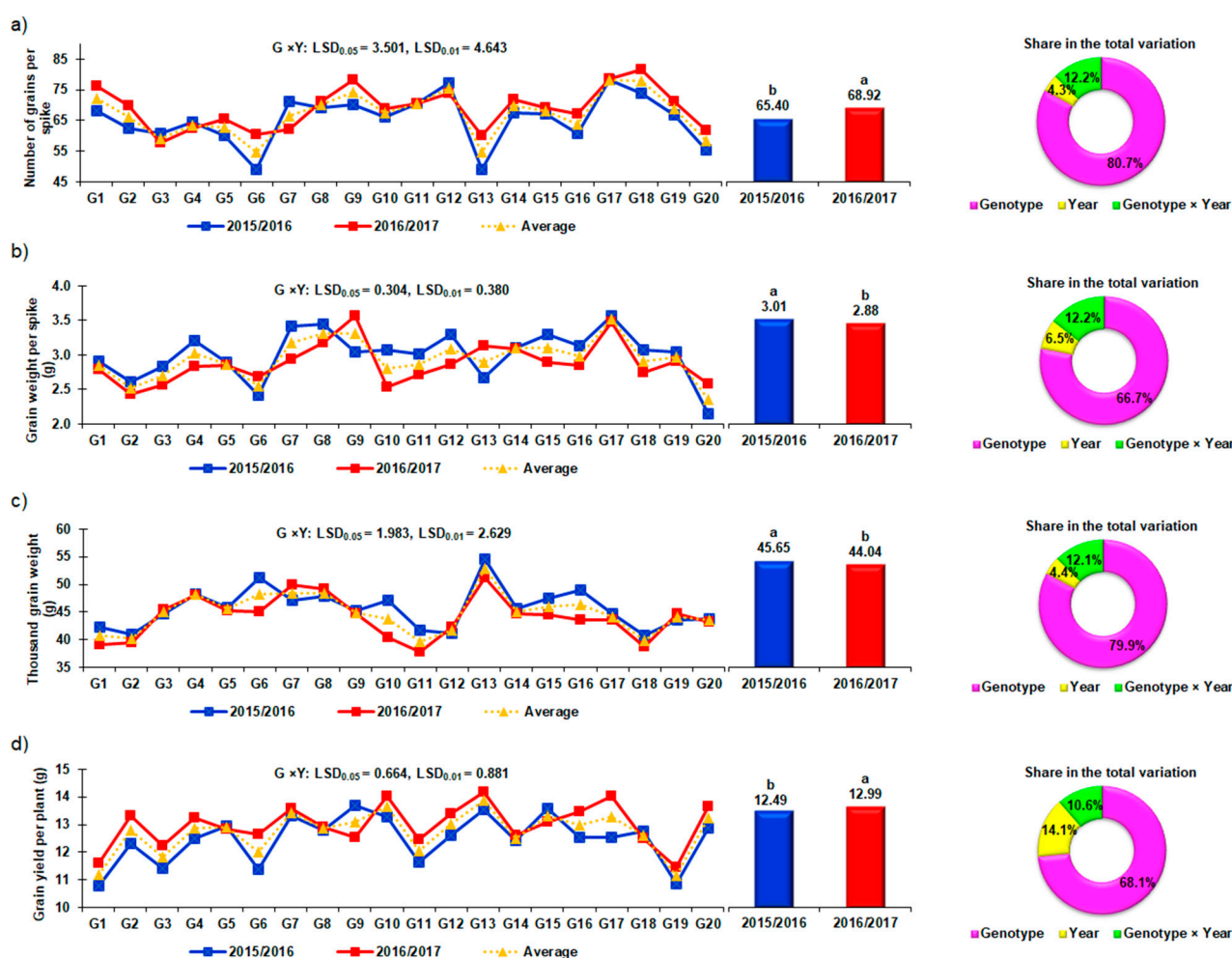

Figure 2. The mean value of agronomic traits such as: (**a**) the number of grains per spike, (**b**) grain weight per spike (g), (**c**) thousand grain weight (g), and (**d**) grain yield per plant (g) in twenty wheat genotypes grown in two vegetation season; and share of genotype, year, and genotype × year interaction in the total variation of analyzed traits. Note: G1—Evropa 90, G2—Gružanka; G3—Zastava; G4—KG-56; G5—Orašanka; G6—Balkan; G7—Jugoslavija; G8—Oplenka; G9—Lasta; G10—Agrounija; G11—Rodna; G12—Tanjugovka; G13—Zadruga; G14—Proteinka; G15—NSR-5; G16—Gruža; G17—Milica; G18—Sloga; G19—Dejana; G20—Tera; Different letters (a,b) indicate significant differences (*p* < 0.05) in grain yield components between years.

The factor of the year had the smallest effect, contributing less than 7% to the variation in the number of grains per spike, grain weight per spike, and the thousand grain weight.

The share of the year factor in the variation of grain yield per plant is slightly higher (14.1%), but it is still significantly lower than the share of the genotype factor. Mitura et al. [52] found that the factor of the year had a smaller impact on the variation of wheat grain yield compared with the factors of genotype and tillage system. Their study highlighted that the weather conditions during the years of the research did not significantly influence the wheat yield. The reason genotype had a stronger influence on the variation of the analyzed traits compared with the factor of the year is that the genotypes in the studied germplasm showed significant differences in their characteristics. In other words, the genotypes vary widely, leading to a more significant impact on yield components than the year-to-year variations. In addition, the reason for the pronounced influence of the genotype factor is that both growing seasons were favorable for wheat production, in which the analyzed genotypes could express their genetic potential. This finding is in contrast to prior research by Zečević et al. [17], where the year factor exhibited the highest contribution (48.71%) to the variability in the number of grains per spike, while the genotype factor had a relatively diminished impact (15.48%). The dominant share of the environmental factor in the variation of grain yield per plant was established by Mohammadi et al. [53] and Popović et al. [54]. However, the reason for this is pronounced differences in climatic parameters between the examined seasons and/or treatments. Popović et al. [54] point out that the large sum of squares for the environments indicated that the environments were diverse, with large differences among environmental means.

The share of genotype × year interaction in the total phenotypic variation of the analyzed traits ranged from 10.6% for grain yield per plant to 12.1%, i.e., 12.2% for the other analyzed traits (Figure 2). The significance of the influence of the interaction of factors points to the conclusion that there has been a change in the ranking of genotypes over the examined seasons. Zecevic et al. [17], Knežević et al. [41], and Banjac et al. [55] established a slightly higher proportion of the interaction of genotype and year/treatment in the variation of the number of grains per spike: 28.88, 29.89, and 24.75%, respectively. This could be due to varying environmental conditions, which have led to different levels of trait expression among genotypes in different years/treatments. In our study, the significance of the genotype × year interaction is primarily attributed to differences in the mean values of individual genotypes rather than widespread variation across the genotypes. In other words, although the interaction is significant, the majority of genotypes displayed relatively minor variation in yield components across different seasons. The most substantial difference in mean values between seasons was found in the genotype Balkan for the number of grains per spike, the genotype Agrounija for grain weight per spike, the genotypes Balkan and Gruža for thousand grains weight, and the genotype Milica for grain yield per plant. These particular genotypes exhibited the most pronounced fluctuations in trait expression across the different years, contributing significantly to the observed genotype × year interaction.

On average for both seasons, the highest grain weight per spike (>3.3 g) was observed in Milica (G17), Lasta (G9), and Oplenka (G8), while the lowest values (<2.6 g) were found in Tera (G20), Gružanka (G2), and Balkan (G6) genotypes. Similarly, the highest number of grains per spike (>75.0) was found in genotypes Milica (G17), Tanjugovka (G12), and Sloga (G18), whereas genotypes Zadruga (G13) and Balkan (G6) had the lowest values (<55.0). For the thousand grain weight, Zadruga (G13) and Balkan (G6) had the highest values (>48.0 g), while Evropa 90 (G1), Gružanka (G2), Rodna (G11), and Sloga (G18) had the lowest values (<41.0 g). In terms of grain yield per plant, genotypes Zadruga (G13) and Agrounija (G10) showed the highest values (>13.5 g), whereas genotypes Evropa 90 (G1) and Dejana (G19) had the lowest values (<11.2 g) (Figure 2). Comparing the approval periods for wheat genotypes (1971–1979, 1980–1989, and 1990–1995), it is evident that there was a consistent increase in the number of grains per spike over time. The newer genotypes, approved in the 1990s, exhibited the highest average values for this trait. This is in alignment with findings by Achilli et al. [8], who observed a consistent increase in the number of grains per spike when comparing wheat genotypes across three distinct periods

of approval. However, when it comes to other yield components, such as grain weight per spike, thousand grain weight, and grain yield per plant, the medium-old genotypes (1980–1989) showed the highest average value. The old genotypes, approved in the 1970s, had the lowest average values for all analyzed yield components, except for the thousand grain weight, where the newer genotypes had the lowest values (Figure 2). Similarly, Royo et al. [56] observed a 2–5% decrease in the thousand grain weight in modern wheat genotypes. This reduction could be attributed to several factors, including whether this trait was prioritized in breeding programs and its potential negative correlation with the number of grains per spike.

In order to analyze the interrelationship among the wheat genotypes and analyzed grain yield components, correlation matrix analysis by the principal component method (PCA) and cluster heatmap analysis were performed (Figure 3a,b).
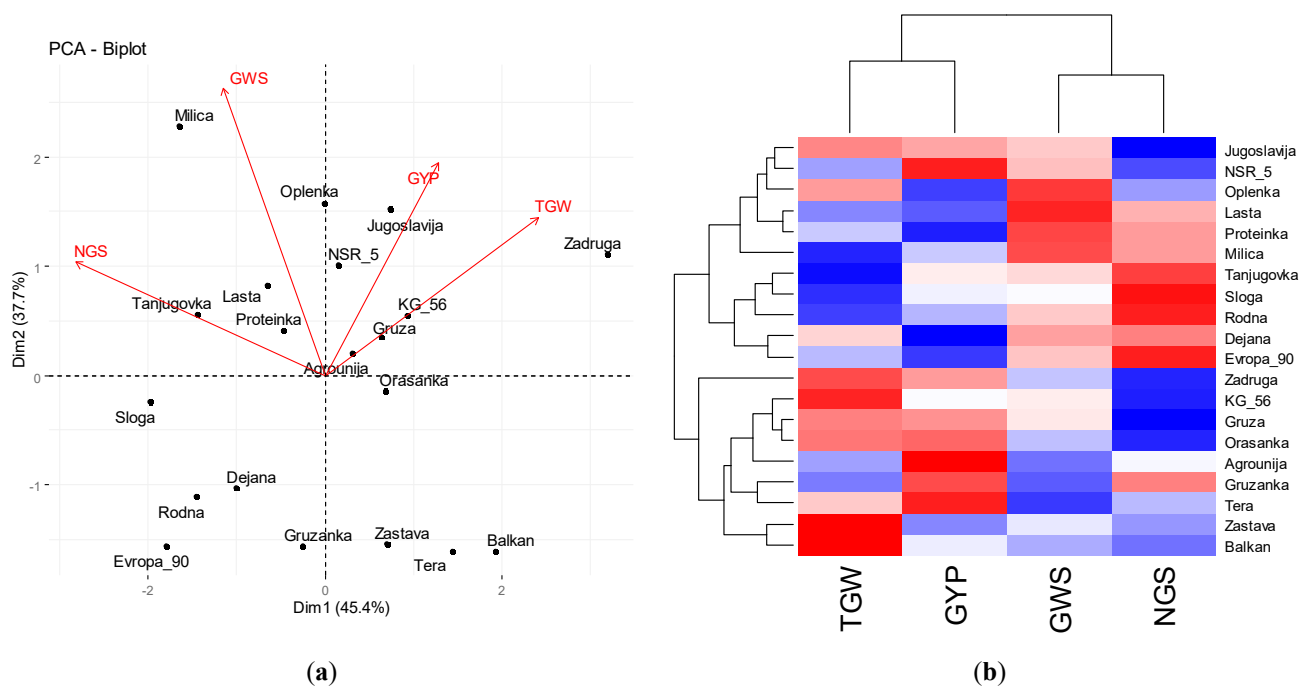

Figure 3. Principal component analysis (PCA) (**a**) and cluster heatmap analysis (**b**) for agronomic traits (NGS—number of grains per spike, GWS—grain weight per spike, TGW—thousand grain weight, and GYP—grain yield per plant) of twenty bread wheat genotypes.

The first principal component (PCA$_1$) explained 45.4% and PCA$_2$ explained 37.7% of the total variance of the analyzed agronomic traits (Figure 3a). This is in agreement with the results obtained by Ali et al. [57]. Positive values of both PCA axes positioned the thousand grain weight and grain yield per plant in the second quadrant of the biplot, with a sharp angle (<90 °C) between the vectors of these two traits (Figure 3a). Also, these two traits are located in the same cluster. The results are consistent with those reported by Janmohammedi et al. [6], Ali et al. [57], and Verma et al. [58]. All three studies identified a positive correlation between thousand grain weight and grain yield, suggesting that an increase in thousand grain weight could lead to an increase in wheat grain yield. Grain weight per spike and number of grains per spike had negative values on the PCA$_1$ and positive values on the PCA$_2$ axes and were positioned in the first quadrant of the biplot. Sourour et al. [59] also found an equal grouping of these two traits, which had negative PCA$_1$ axis values. The number of grains per spike and the thousand grain weight are positioned at an obtuse angle (>90°), showing a negative correlation between these traits. Similar findings were reported by Philipp et al. [4] and Zečević et al. [60]. Also, the cluster heatmap analysis classifies these two traits into different clusters (Figure 3b). Previous research by Philipp et al. [4] indicated that the individual grain size, particularly grain width

and grain area, had a more significant impact on the thousand grain weight compared with the number of grains per spike.

The red color on the cluster heatmap graph represents high values of the trait, the blue color indicates low values, and the white color represents average values (Figure 3b). The first cluster group includes the genotypes Jugoslavija, NSR-5, Oplenka, Lasta, Proteinka, and Milica, which are characterized by high values of grain yield per plant (Jugoslavija and NSR-5) and high values of grain weight per spike (Milica, Oplenka, Lasta, and Proteinka). The genotypes Tanjugovka, Sloga, Rodna, Dejana, and Evropa 90 belong to the same cluster group, showing high values of the number of grains per spike. On the other hand, the genotype Zadruga stands out in a distinctive cluster group due to its high values of both thousand grain weight and grain yield per plant. This unique trait combination of the genotype Zadruga offers a valuable resource for plant breeding. Genotypes KG-56, Gruža, Orašanka, Agrounija, Gružanka, Tera, Zastava, and Balkan are grouped together in another cluster. Among these, Gružanka, Tera, Zastava, and Balkan exhibit high values for one trait (either grain yield per plant or thousand grain weight), but the values of other traits are generally very low, positioning them below the abscissa of the biplot (Figure 3a,b). The conducted analyses confirm the existence of a significant diversity of constitutive genotypes in terms of the assessed traits. This diversity provides a good basis for plant breeding.

3.2. Protein Content

The protein content exhibited considerable variability among the examined wheat genotypes, with a pronounced statistically significant distinction observed between them ($p < 0.05$) (Figure 4). Similarly, the research conducted by Laze et al. [61] underlined the significant influence of the wheat genotype on the protein content variation. The authors highlighted that this variance in protein content among genotypes could be attributed to differences in their genetic variability. Therefore, exploring genetic resources to identify wheat genotypes with high grain protein content is the most efficient way to improve the nutritional value of wheat grains [62].
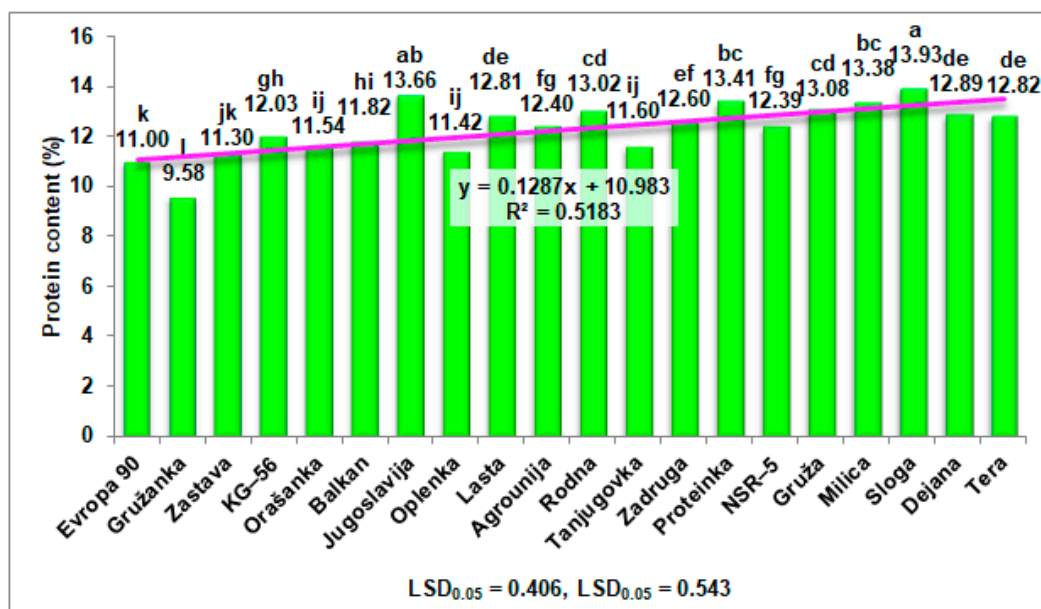

Figure 4. Protein content (%) in whole grain of twenty bread wheat genotypes. Note: Bars marked with different letters differ significantly ($p < 0.05$).

This study reveals that the protein content in wheat has steadily increased over the past decades ($R^2 = 5.813$), Figure 4.

Newer genotypes (Proteinka, NSR-5, Gruža, Milica, Sloga, Dejana, and Tera) showed a significantly higher average protein content (13.12%) compared with older wheat genotypes

created in the 1970s (Evropa 90, Gružanka, Zastava, KG-56, Orašanka, and Balkan), with an average protein content of 11.21%. Accordingly, the newer genotype Sloga exhibited the highest protein content (13.93%), making it a valuable genetic resource for breeding programs. Similarly, the older genotype Jugoslavija attained a high protein content (13.66%) and can be a useful genetic resource for improving protein content. On the other hand, the older genotype Gružanka had the lowest protein content (9.58%) (Figure 4). The results are consistent with the findings of Anjum et al. [63], who established a noticeable trend of significant increases in protein content in wheat varieties during the last decades.

The average protein content obtained in this research is higher than the protein content found in earlier research by Hristov et al. [42] studying the quality components of 20 wheat genotypes, among which there are 5 genotypes included in our research. Nonetheless, the study by the aforementioned researchers delineated that the newer genotypes, such as Milica (11.8%), Gruža (11.5%), and Tera (11.1%), exhibited the highest protein content among analyzed wheat genotypes, whereas the older genotypes Evropa 90 and NSR-5 exhibited a significantly lower value of protein content (11.0%). This is consistent with our results. Furthermore, the genotypes with high protein content did not react to extreme environmental conditions and can be recommended for cultivation in southeast Europe [42].

3.3. Nonessential Amino Acid Content

The total content of nonessential amino acids (aspartic acid, serine, glutamic acid, proline, glycine, alanine, cystine, tyrosine, and arginine) was 57.86 g 100g^{-1} and made up 65.67% of the total amino acids (Figure 5a). Similar results were obtained by Siddiqi et al. [33] and Tomičić et al. [64]. The aforementioned amino acids are linked to gluten proteins (gliadin and glutenin) and play an important role in the end-product use of wheat flour [33].
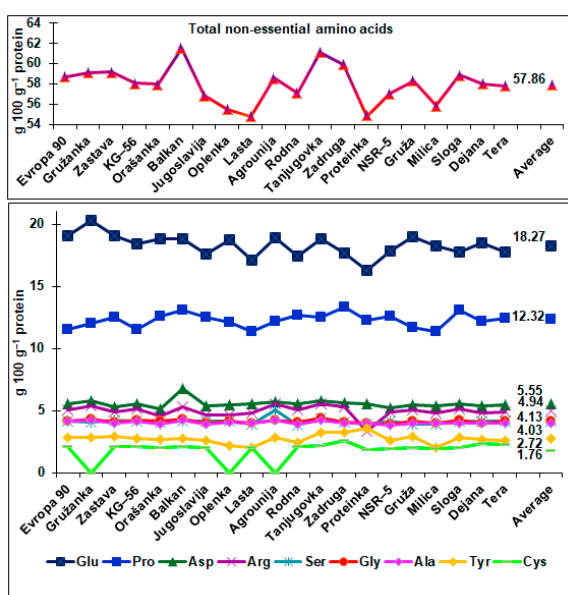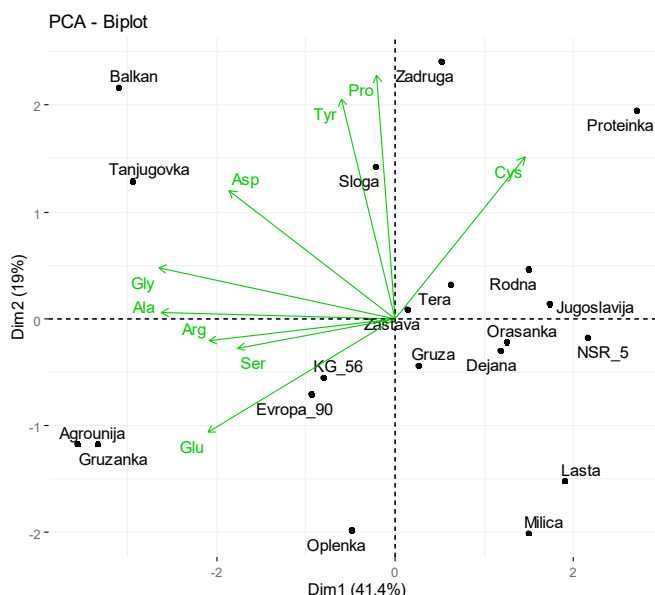
(**a**)   (**b**)

Figure 5. Mean values (g 100 g^{-1} protein) of nonessential amino acid content (**a**) and principal component analysis (**b**) for nonessential amino acids (Glu—glutamic acid, Pro—proline, Asp—aspartic acid, Arg—arginine, Ser—serine, Gly—glycine, Ala—alanine, Tyr—tyrosine, and Cys—cysteine) in the whole grain of analyzed bread wheat genotypes.

Glutamic acid is the most abundant nonessential amino acid in the analyzed wheat genotypes, which is in accordance with the results obtained by Siddiqi et al. [33], Knežević et al. [31,65], Laze et al. [61], and Alijošius et al. [66]. Glutamic acid has a main role in the central metabolism of many organisms, including nitrogen assimilation, amino acid biosynthesis, and cofactor production [67,68]. Furthermore, glutamic acid, in conjunction with pro-

line, serves as the fundamental amino acid within all cereal protein fractions, notably in storage proteins [65]. The average glutamic acid content was 18.27 g 100 g^{-1} protein, which is lower compared with the content established by Tsochatzis et al. [32] and Siddiqi et al. [33] in wheat genotypes (33.3 and 41.2 g 100 g^{-1} protein, respectively). In contrast, Anjum et al. [69] reported significantly lower glutamic acid levels ranging from 6.29 to 12.03 g 100 g^{-1} protein in Pakistani wheat genotypes. After glutamic acid, the next predominant nonessential amino acid is proline, with an average content of 12.32 g 100 g^{-1} protein across the assessed wheat genotypes. This observation is consistent with the recent findings of Sułek et al. [70], where they determined that glutamic acid and proline are the most abundant amino acids in durum and bread wheat grains. In contrast, the remaining amino acids demonstrated significantly lower concentrations: tyrosine recorded 2.72 g 100 g^{-1} protein, alanine 4.03 g 100 g^{-1} protein, glycine and serine both accounted for 4.13 g 100 g^{-1} protein, arginine 4.95 g 100 g^{-1} protein, and aspartic acid displayed a content of 5.55 g 100 g^{-1} protein. Quantitative analysis shows higher concentrations of tyrosine, alanine, glycine, and serine compared with the values obtained by Tsochatzis et al. [32]. The content of aspartic acid aligns closely with the observations reported by Siddiqi et al. [33], displaying only marginal divergence from the values established by Tsochatzis et al. [32]. Similarly, the arginine content is almost identical to that found by Tsochatzis et al. [32]. Cysteine exhibits the lowest abundance among the amino acids studied, with a content of 1.76 g 100 g^{-1} protein. This particular amino acid was not discernible in samples from the three wheat genotypes: Gružanka, Oplenka, and Agrounija (Figure 5a). This observation finds semblance with the findings of Laze et al. [61] in their scrutiny of amino acid profiles within Albanian wheat genotypes, wherein cysteine was absent in two out of the ten wheat genotypes analyzed. These results highlight the significance of cysteine scarcity in the examined wheat genotypes, which may have implications for their nutritional value and metabolic pathways.

The interrelationships of nonessential amino acids in the analyzed wheat genotypes were investigated using correlation matrix analysis with the principal component method (Figure 5b). This approach to the presentation of the relationship between the amino acids of cereals has been applied by many researchers [20,32,61,70]. The principal component analysis (PCA) aids in reducing the data's dimensionality while retaining crucial information about the underlying relationships and patterns among the nonessential amino acids in the wheat genotypes under investigation (Figure 5b). Two main PCA components with eigenvalues >1 are extracted from the input data, where the first PCA component explains 41.4% and the second one 19.0% of the total variance. Cysteine is positioned in a distinct quadrant of the biplot, exhibiting a negative correlation with serine and arginine and a particularly strong negative correlation with glutamic acid. The negative correlation observed between cysteine and glutamic acid in the wheat genotypes can be attributed to their intricate metabolic interactions, which are influenced by both genetic and environmental factors. Laze et al. [61] also reported similar findings, noting that the cysteine vector stood out distinctly from the vectors of other amino acids in the PCA biplot. This spatial separation emphasizes the unique behavior of cysteine in relation to the other amino acids within the dataset. The genotypes Jugoslavija, Proteinka, Zadruga, Rodna, and Tera, which exhibited above-average levels of cysteine content, had a positive correlation with the vector of cysteine in the PCA biplot (Figure 5a,b). This positive correlation implies that these genotypes tend to have higher levels of cysteine when compared with other amino acids within their metabolic profiles. In contrast, the genotypes Agrounija and Gružanka were found to be positioned closely to the glutamic acid vector in the biplot (Figure 5b). These genotypes displayed the highest values of glutamic acid and no detectable levels of cysteine (Figure 5a).

In the PCA biplot analysis, proline and tyrosine were observed to be positioned in close proximity to each other. Both amino acids are involved in stress responses, but they serve different functions. Proline acts as an osmoprotectant, protecting plant cells against damage incurred due to dehydration during stressful conditions [71]. On the other hand,

tyrosine and its derivatives, such as flavonoids, act as antioxidants and protect against oxidative stress [72]. The genotype Sloga, which exhibited high levels of proline and tyrosine, was also observed to be closely positioned to the vectors representing these two amino acids in the biplot. Additionally, the genotypes Balkan and Tanjugovka, which demonstrated the highest average content of the total nonessential amino acids (61.5 and 61.09 g 100 g^{-1} protein, respectively), were located within the first quadrant of the biplot. This quadrant accommodated the largest number of amino acid vectors, suggesting that these genotypes have more complex metabolic interactions and varied amino acid profiles compared with other genotypes. The different distribution of wheat genotypes on the biplot indicates substantial variability in the concentrations of nonessential amino acids among these genotypes. This suggests that genetic factors play a key role in the variation of amino acid content in wheat cultivars. Furthermore, the results imply that both the old genotype Balkan, approved in the 1970s, and the middle-old genotype Tanjugovka, approved in the 1980s, possess the potential to be valuable genetic resources for breeding programs focused on enhancing the content of nonessential amino acids. Also, Sułek et al. [70] found that the variation of nonessential amino acids in wheat grains is more influenced by genetic factors than by production technology (Figure 5b).

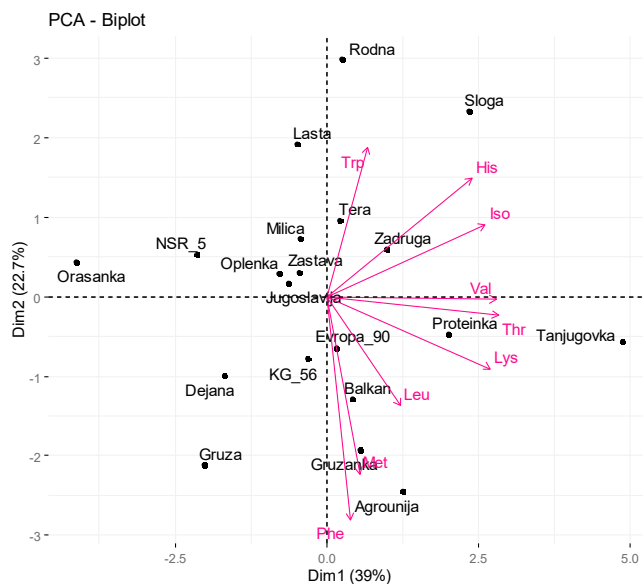### 3.4. Essential Amino Acid Content

The essential amino acids play a significant role in determining the nutritional value of protein [29,73,74]. The essential amino acids, which included threonine, histidine, valine, methionine, phenylalanine, isoleucine, leucine, and lysine, accounted for 34.34% of the total amino acids (Figure 6a). This is confirmed by the results established by Siddiqi et al. [33] and Tomičić et al. [64]. The highest content of total essential amino acids was found in genotypes approved in the 1980s, such as Agrounija (32.62 g 100 g^{-1} protein) and Tanjugovka (32.47 g 100 g^{-1} protein), whereas genotype Orašanka, approved in the 1970s, had the lowest content (28.54 g 100 g^{-1} protein). This variation in essential amino acid content underscores the importance of genotype selection for optimizing protein nutritional quality. A significant influence of genetic factors on the content of essential amino acids in wheat grain has been established by many authors [31,61,65,70].

In the analyzed wheat genotypes, leucine was identified as the most abundant amino acid, constituting 5.85 g 100 g^{-1} protein (Figure 6a). Studies conducted by Siddiqi et al. [33] and Khan et al. [35] revealed a slightly higher leucine content in wheat. However, Tsochatzis et al. [32] found significantly lower leucine content compared with our results. Duan et al. [74] emphasized that leucine is usually one of the most abundant amino acids in high-quality protein foods. Additionally, leucine emerged as a predominant essential amino acid within the albumin, glutenin-1, and glutenin-2 protein fractions of wheat flour [75]. In the analyzed wheat genotypes, the average content of phenylalanine was determined to be 4.59 g 100 g^{-1} protein, which is in line with the results obtained by Siddiqi et al. [33] and Khan et al. [35]. Similar to our results, Sułek et al. [70] also point out that phenylalanine is the second most abundant essential amino acid in wheat grain after leucine. Phenylalanine is a vital component of proteins in all living organisms, and in plants, it serves as a precursor for the synthesis of thousands of additional metabolites. A significant portion of photosynthetically fixed carbon is directed toward the synthesis of phenylalanine [76]. Following phenylalanine, the most abundant amino acid is valine, with a content of 4.57 g 100 g^{-1} protein, which is significantly higher than the content established by Tsochatzis et al. [32] and Siddiqi et al. [33]. Valine is part of the group of branched-chain amino acids (BCAAs), which also include isoleucine and leucine. BCAAs and their derivatives are involved in various plant processes, such as growth, stress response, and the production of food flavor components [77]. The mean isoleucine content among the examined wheat genotypes was 4.01 g 100 g^{-1} protein. This finding closely aligns with the result reported by Khan et al. [35]. The amino acids lysine (2.93 g 100 g^{-1} protein), threonine (2.87 g 100 g^{-1} protein), and histidine (2.80 g 100 g^{-1} protein) were less abundant in the analyzed wheat genotypes, but their concentration was higher compared with

values obtained by the previous research of abovementioned researchers. Lysine is a critical nutrient in plants, serving as a key component of proteins, playing a role in stress responses and contributing to plant growth and development [78]. On the other hand, threonine serves as a precursor for the synthesis of isoleucine, suggesting an interconnected metabolic pathway between these two amino acids in plants [79]. Histidine is intricately linked to several other metabolic pathways, including those involved in the synthesis of purines, pyrimidines, pyridine nucleotides, folates, and tryptophan [80]. The methionine content was measured as 1.38 g 100 g^{-1} protein, which aligns with the results reported by Siddiqi et al. [33], (Figure 6a). Higher levels of methionine have been associated with increased tolerance to abiotic stress in plants [81]. According to Song et al. [82], methionine is the main limiting sulfur essential amino acid in plants. It can be converted to cysteine in animals, fulfilling the requirements of both amino acids. The analyzed wheat genotypes exhibited the lowest tryptophan content (1.20 g 100 g^{-1} protein) (Figure 6a). These values were found to be similar to those reported by Khan et al. [35]. Sulek et al. [70] also found the lowest concentration of tryptophan in durum and common wheat samples. This essential amino acid contributes to the synthesis of indole acetic acid, the most abundant and biologically active auxin in plants, which regulates many aspects of plant growth and development [83].
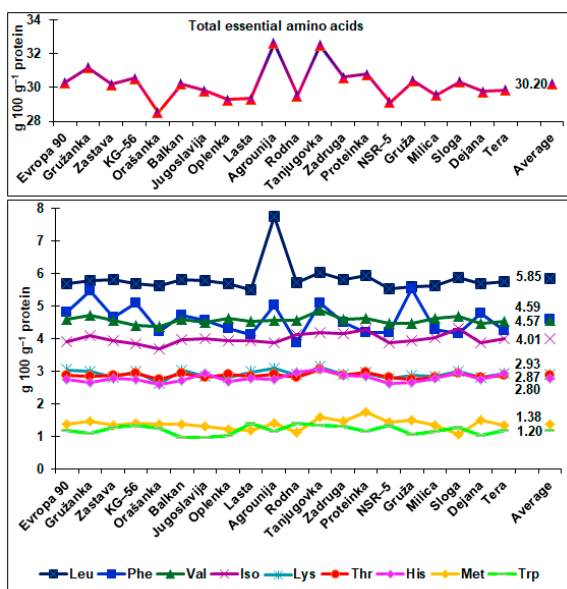
(a)　　　　　　　　　　　(b)

Figure 6. Mean values (g 100 g^{-1} protein) of essential amino acid content (**a**) and principal component analysis (**b**) for essential amino acids (Leu—leucine, Phe—Phenylalanine, Val—valine, Ile—isoleucine, Lys—lysine, Thr—threonine, His—histidine, Met—methionine, and Trp—tryptophan,) in the whole grain of analyzed bread wheat genotypes.

In the PCA biplot analysis of essential amino acids, the first interaction component (PCA_1) accounted for 39.0% of the total variation, while the second interaction component (PCA_2) explained 22.7% of the variation (Figure 6b). The essential amino acids were organized into three distinct groups based on their positions in the biplot. The first group was characterized by positive PCA_1 and PCA_2 amino acid vectors, specifically isoleucine, histidine, and tryptophan. These three amino acids formed a sharp angle of 90° with each other in the biplot (Figure 6b). Sulek et al. [70] observed a positive correlation between histidine and tryptophan, while Siddiqi et al. [33] demonstrated a similar positive correlation between histidine and isoleucine. The genotypes Sloga and Rodna were located in close proximity to the vector formed by tryptophan, isoleucine, and histidine in the biplot. Notably, these genotypes exhibited above-average values of these particular amino acids

(Figure 6a). The vectors representing the amino acids valine, threonine, and lysine are situated close to the abscissa, indicating their relatively low variation along the first interaction component (PCA$_1$). Genotypes Proteinka and Tanjugovka are positively correlated with threonine, lysine, and valine as they achieved above-average values of these specific amino acids (Figure 6a,b). Also, the vectors for leucine, methionine, and phenylalanine are characterized by positive values along the first interaction. Moreover, these vectors overlap to form a sharp angle (<90°) with each other. The genotypes Agrounija, which exhibited the highest value of leucine, and Gružanka, which achieved the highest value of phenylalanine, are positioned within the vectors representing these amino acids in the biplot. The most prominent negative association is observed between tryptophan and phenylalanine, as their vectors overlap to form an obtuse angle that is greater than 90° in the biplot (Figure 6b). Both amino acids are part of the aromatic group and use the same enzymes in their biosynthesis. Therefore, the significant inverse relationship between tryptophan and phenylalanine may be related to their shared biochemical pathways and the fact that tryptophan is the most complex and least represented aromatic amino acid [84].

3.5. Amino Acid Score

The biological value of a protein is primarily determined by its amino acid composition and the specific proportions of individual amino acids it contains. This is particularly important for essential amino acids, which human bodies cannot produce and which, as a result, must be obtained through dietary intake [30,85,86]. Amino acids play a crucial role in protein synthesis, regulating blood pressure, energy generation, enhancing the immune and nervous systems, and muscle repair and growth [30]. The Food and Agriculture Organization (FAO) underscores the importance of considering amino acids as distinct nutritional elements within our diet. This recommendation emphasizes the significance of not just protein quantity but also the specific amino acid composition [45]. The amino acid score (AAS) is used to predict the protein completeness of the analyzed wheat genotypes (Table 2). The AAS is a measure that indicates how the essential amino acid content in the wheat genotypes compares to the recommended intake levels for adults, as specified by the FAO/WHO standard (mg/g) [45]. Among the essential amino acids analyzed in all genotypes, tryptophan exhibited the highest AAS with an average value of 1.81. This indicates that the tryptophan content in the wheat genotypes is relatively high compared with the recommended intake for adults. In addition to affecting the growth of wheat [87], tryptophan also plays a role as a precursor for the synthesis of the neurotransmitter serotonin, which has implications for various physiological processes [88]. The amino acid with the lowest values of AAS is considered the limiting amino acid [63,69,89]. In the tested wheat genotypes, the lowest AAS was established for lysine (0.61) (Table 2). The main role of lysine is to participate in protein synthesis [90], so the study of this amino acid occupies a lot of attention among researchers. Siddiqi et al. [33], Anjum et al. [63], Jiang et al. [91], and, Hu et al. [92] also found that lysine is the most limited amino acid in wheat genotypes. A low concentration of lysine in wheat has been established by Tomičić et al. [13], Sulek et al. [70], and Laze et al. [61]. In addition to wheat, the grains most commonly used in developing-country diets are corn and rice, both of which have low lysine content [92,93]. These studies collectively reinforce the notion that lysine is a critical amino acid of concern in wheat and highlight the importance of addressing its deficiency to enhance the nutritional value of wheat-based products. Therefore, several approaches have been developed in recent decades to improve the lysine content in cereals [93–95]. Alternatively, combining cereals with foods that have a high lysine content (i.e., legumes and oilseeds) will produce a meal complete in lysine and all other limiting amino acids [61,96].

The genotype Tanjugovka stands out with the highest AAS value for total essential amino acids (0.45), indicating that its protein profile is relatively better in terms of essential amino acid content compared with other genotypes. Zadruga closely follows with a high AAS value of 0.44, indicating good protein quality as well. Furthermore, when specific essential amino acids are considered, genotype Tanjugovka also exhibits superior AAS

values. It has the highest AAS for valine (1.22), phenylalanine + tyrosine (2.04), histidine (1.92), and lysine (0.66) among all tested wheat genotypes (Table 2). This means that the genotype Tanjugovka has relatively higher levels of these essential amino acids, which are crucial for protein synthesis and various physiological processes in the human body. On the other hand, genotype Oplenka demonstrates the lowest AAS for total amino acids, with a value of 0.36, indicating that its protein profile may be less favorable for meeting essential amino acid requirements. Gružanka, while slightly better than Oplenka, still has a relatively low AAS value of 0.39. These findings underscore the considerable variation in lysine content and overall protein quality among different wheat genotypes.

Table 2. Amino acid score (AAS) in the whole grain of twenty bread wheat genotypes.

No.	Genotype	Thr	Val	Met + Cys	Ile	Leu	Phe + Tyr	His	Trp	Lys	Total
	FAO/WHO Standard (mg/g) for adults [45]	25	40	23	30	61	41	16	6.6	48	290.6
1.	Evropa 90	1.15	1.16	1.50	1.30	0.93	1.86	1.72	1.78	0.64	0.41
2.	Gružanka	1.13	1.18	0.63	1.36	0.95	2.04	1.67	1.66	0.63	0.39
3.	Zastava	1.16	1.15	1.49	1.31	0.96	1.84	1.74	1.95	0.59	0.42
4.	KG–56	1.18	1.10	1.52	1.28	0.93	1.93	1.72	2.02	0.63	0.42
5.	Orašanka	1.10	1.09	1.47	1.23	0.93	1.68	1.63	1.87	0.55	0.40
6.	Balkan	1.18	1.16	1.53	1.32	0.95	1.82	1.70	1.47	0.63	0.40
7.	Jugoslavija	1.13	1.12	1.46	1.34	0.95	1.75	1.84	1.48	0.60	0.40
8.	Oplenka	1.16	1.16	0.53	1.31	0.93	1.58	1.69	1.56	0.59	0.36
9.	Lasta	1.14	1.14	1.40	1.31	0.90	1.49	1.74	2.15	0.62	0.41
10.	Agrounija	1.16	1.15	0.61	1.30	1.27	1.91	1.72	1.77	0.65	0.40
11.	Rodna	1.13	1.14	1.41	1.37	0.94	1.53	1.85	2.14	0.60	0.42
12.	Tanjugovka	1.23	1.22	1.64	1.40	0.99	2.04	1.92	2.05	0.66	0.45
13.	Zadruga	1.15	1.16	1.75	1.39	0.96	1.90	1.79	1.98	0.61	0.44
14.	Proteinka	1.19	1.16	1.55	1.43	0.98	1.90	1.78	1.74	0.62	0.42
15.	NSR–5	1.12	1.12	1.46	1.29	0.91	1.67	1.64	2.02	0.58	0.41
16.	Gruža	1.10	1.12	1.53	1.31	0.92	2.07	1.66	1.59	0.60	0.41
17.	Milica	1.13	1.16	1.43	1.34	0.92	1.55	1.75	1.74	0.59	0.40
18.	Sloga	1.18	1.17	1.33	1.44	0.96	1.71	1.85	1.93	0.63	0.42
19.	Dejana	1.13	1.12	1.65	1.29	0.93	1.81	1.73	1.56	0.59	0.41
20.	Tera	1.15	1.13	1.57	1.33	0.94	1.67	1.84	1.80	0.61	0.41
	Average	1.15	1.14	1.37	1.33	0.96	1.79	1.75	1.81	0.61	–

Note: Thr—threonine, Val—valine, Met—methionine, Cys—cysteine, Ile—isoleucine, Leu—leucine, Phe—Phenylalanine, Tyr—tyrosine, His—histidine, Trp—tryptophan, Lys—lysine

4. Conclusions

This study provides valuable insights into the genetic diversity among wheat genotypes bred in the Serbian region, with the aim of establishing their influence on grain yield and nutritional characteristics, particularly in relation to the pedoclimatic conditions of this area. Our findings demonstrate that the genotypes Zadruga and Agrounija exhibit superior abilities for overall grain yield, with the Zadruga genotype standing out in a distinct cluster group due to high values of both thousand grain weight and grain yield per plant. It was established that there was a positive trend of increasing protein content over time, with the newer genotype Sloga achieving the highest protein content, making it a valuable genetic resource for breeding programs aimed at enhancing protein content in wheat varieties. However, in the case of most yield components, no continuous increase over time was observed, with middle-old genotypes showing the highest average values. This conclusion highlights the significant challenge of achieving improvements in grain quality while simultaneously increasing grain yield. The old genotype Balkan, with its notably high total nonessential amino acid content, stands out as a favorable candidate for utilization as a parent in breeding programs aimed at enhancing grain quality. Tanjugovka and Zadruga genotypes, with the highest AAS value, present good genetic resources for enhancing the nutritional value of wheat varieties. Also, the Tanjugovka genotype has

shown the highest AAS for most amino acids, including lysine, which further emphasizes its potential to address nutritional deficiencies. On the contrary, the Oplenka genotype had the lowest AAS, highlighting its limitations in meeting essential amino acid requirements and its potential as a target for improvement through breeding programs. Furthermore, our research highlights potential grain yield limitations for the Europa 90 and Dejana genotypes. The genotype Gružanka showed a low value of thousand grain weight and the lowest protein content, which makes it unsuitable for inclusion in breeding programs. The integration of high-yielding genotypes with a favorable amino acid profile, such as Zadruga, Tanjugovka, Agrounija, and Sloga, has a huge potential to positively affect human nutrition and health, especially in regions where wheat is the staple food.

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