Genetic diversity of Polish cultivars of common wheat (*Triticum aestivum* L.) based on molecular and protein markers

Ewa FILIP[1,2]*, Aleksandra STROŃSKA[1], Magdalena SZENEJKO[2,3], Waldemar PLUTA[1]

[1]*University of Szczecin, Institute of Biology, Waska 13, 71-415 Szczecin, Poland; ewa.filip@usz.edu.pl (*corresponding author); aa.stronska@gmail.com; wwaldekpluta@gmail.com*
[2]*University of Szczecin, The Centre for Molecular Biology and Biotechnology, 71-415 Szczecin, Poland*
[3]*University of Szczecin, Institute of Marine and Environmental Sciences, Adama Mickiewicza 16, 70-383 Szczecin, Poland; magdalena.szenejko@usz.edu.pl*

Abstract

Knowledge of the composition subunits of proteins glutenins makes it much easier to determine the quality and suitability of the final product obtained from gluten. It is important to remember that not only molecular processes model gluten protein polymorphism. In this study, the genetic diversity of Polish common wheat cultivars was examined at the level of DNA and glutenin's proteins HMW-GS. The SDS-PAGE and RAPD bands were evaluated in binary matrix, which was the basis for further analysis of results, using appropriate measures of variability: Q-Cochran test (Cochran, 1950), $p < 0.05$; I-Shannon index; Si-Similarity index; PIC-Polymorphism Information Content; (PCA-Principal component analysis). Both types of markers proved to be useful in the overall assessment of genetic variability between tested of common wheat cultivars. The general genetic diversity indicates that good candidates with unique composition of HMW-GS subunits were selected among the examined cultivars and three OPA-02, OPA-03, OPB-08 primers with the highest power differentiating for the studied genotypes were selected. Results of the research revealed the potentials of RAPD and SDS-PAGE technique in determining genetical diversity and make a suitable qualitative assessment of common wheat cultivars.

Keywords: HMW-GS; RAPD; wheat

Introduction

Common wheat (*Triticum aestivum* L.)*,* used in many foodstuffs, is of great importance to the global economy as it. Cultivating cultivars of high-quality wheat is a primary objective in wheat cultivation programmes since the demand for high-quality wheat flour products has increased. In Poland, wheat growers have begun to research, and develop methods, of screening and identification of grains with high-quality germ plasma, for the purpose of developing high-quality wheat cultivars. These types of studies enable evaluation of grain indices and potential industrial value and intended use of genotypes. Protein content, hardness, test weight of the wheat grain and sedimentation volume of the flours and dough strength, and extensibility are

some of the important quality parameters in wheat. Classical quality tests, such as farinograph and extensograph measurements, which test the properties of flour and dough made from it, are not suitable for early breeding programmes because they require more wheat grains and are time-consuming. Glutenin subunits (HMW and LMW) are more informative about the quality of wheat genotypes and can be easily identified via SDS-PAGE (a type of electrophoresis) using a small number of wheat grains. Several studies have reported the attempts of researchers to provide specific molecular and technological characteristics of wheat cultivars (Horvat *et al*., 2006). Produces are increasingly willing to suse molecular markers to examine genetic polymorphism as this method entails no environmental impact and no need for lineage information (Bohn *et al*., 1999; Ahmed *et al*., 2010). Among the molecular markers, random amplified polymorphic DNA (RAPD) is one of the most frequently used analyses for polymorphism evaluation because it is simple to conduct, efficient and requires no knowledge of a sequence. The applications of RAPD include the identification of genotypes in crop plants, the study of genetic variability within a species and the indication of relationships between populations (Freitas *et al*., 2000; Langridge *et al*., 2001). In this study cultivars of common wheat, with different genotypes, determined by the SDS-PAGE was extended to include assessment of RAPD polymorphisms. Knowledge of the genetic diversity of wheat genotypes, based on DNA polymorphisms and storage proteins, is essential for determining the potential value of breeding programmes.

Materials and Methods

Plant material

The research material consisted of 26 cultivars of common wheat. All the materials were obtained from the collection of the National Centre for Plant Genetic Resources: Polish Genebank; Plant Breeding and Acclimatization Institute - National Research Institute in Radzików (Poland) (Table 1).

Table 1. The list cultivars of common wheat with accession number

Cultivars	Accession number	Species
'Biała Kaszubska'	PL000093	*Triticum aestivum* L.
'Choryńska'	PL001401	*Triticum aestivum* L.
'Dańkowska Biała'	PL001426	*Triticum aestivum* L.
'Eka132'	PL001462	*Triticum aestivum* L.
'Finezja'	PL6776	*Triticum aestivum* L.
'Fregata'	PL6825	*Triticum aestivum* L.
'Kobiera'	PL6802	*Triticum aestivum* L.
'Magnatka Ragalińska'	PL000350	*Triticum aestivum* L.
'Murzynka Lipińskiego'	PL000433	*Triticum aestivum* L.
'Mydliczanka'	PL000434	*Triticum aestivum* L.
'Niewylegająca'	PL000458	*Triticum aestivum* L.
'Ostka Czerwona Łopustka'	PL00520	*Triticum aestivum* L.
'Ostka Kazimierska'	PL000524	*Triticum aestivum* L.
'Poznańska'	PL000577	*Triticum aestivum* L.
'Sielecka Genetyczna'	PL000755	*Triticum aestivum* L.
'Sobieszyńska'	PL000781	*Triticum aestivum* L.
'Sobótka'	PL000782	*Triticum aestivum* L.
'Squarehead Grodkowicka'	PL000797	*Triticum aestivum* L.
'Srebrzysta'	PL000799	*Triticum aestivum* L.

'Ślązaczka'	PL000778	*Triticum aestivum* L.
'Tonacja'	PL6712	*Triticum aestivum* L.
'Tryumf Mykulic'	PL000433	*Triticum aestivum* L.
'Udyczanka Czerwona'	PL000958	*Triticum aestivum* L.
'Wysokolitewska Antonińska'	PL001065	*Triticum aestivum* L.
'Wysokolitewska Sztywnosłoma'	PL001071	*Triticum aestivum* L.
'Żelazna'	PL001908	*Triticum aestivum* L.

Isolation of the genomic DNA

Genomic DNA was extracted from fresh (5 or 6 day-old) etiolated coleoptiles from 26 cultivars of common wheat. The isolation was performed using NucleoSpin® Tissue XS (Macherey Nagel)

RAPD analysis

The reaction was carried out by the RAPD-PCR analysis, based on random amplification of polymorphic DNA, was performed according to the modified method described by Williams *et al.* (1990). It was performed in a T100TM Thermal Cycler (Bio-Rad) in the final volume of 25 µl. The single PCR reaction mixture contained: 1x DreamTaq Buffer containing the optimized amount of $MgCl_2$, buffer (Thermo Scientific), 2 mM dNTP (0.2 mM dNTP per 25 µL), 10 µM each primer (0.4 µM per 25 µL) of primer, 50 ng genomic DNA (2 µL per 25 µL) and 0.25 units DreamTaq DNA Polymerase (Thermo Scientific DreamTaq DNA Polymerase No EP0702). The conditions and profiles of PCR reactions have been optimized accordingly. The primers used for amplification of RAPD are shown in the Table 2.

Table 2. Characteristics of the ten primers used in this study

Name of primer	Sequence 5' - 3'	G/C (%)
OPA-02	TGCCGAGCTG	70
OPA-03	AGTCAGCCAC	60
OPA-13	CAGCACCCAC	70
OPB-08	GTCCACACGG	70
OPD-02	GGACCCAACC	70
OPD-07	TTGGCACGGG	70
OPD-08	GTGTGCCCCA	70
RLZ6	GTGATCGCAG	60
RLZ8	GTCCCGACGA	70
SIGMA-D-P	TGGACCGGTG	70

The primers were synthesized in the Laboratory of DNA Sequencing and Synthesis of IBB PAN Genomed S.A. (Warsaw). To amplify products of RAPD-PCR The following thermal reaction profile was used: initial denaturation at 94 °C for 3 minutes followed by 39 cycles of denaturation at 94 °C for 30 seconds, annealing at 38 °C for 1 minute, extension of the primer at 72 °C for 2 minute and final extension of 72 °C for 10 minutes. The resulting amplification products were separated on 1.5% agarose gel with ethidium bromide (5 µg/ml, Sigma-Aldrich). To visualize, document and analyse the results obtained, a set of Gel Doc™ XR and Quantity One 4.6.5 software (Bio-Rad) were used.

Isolation of HMW storage proteins and separation by SDS-PAGE

Identification of HMW glutenin alleles and subunits was performed according to the methods described by Payne and Lawrence (1983) and McIntosh *et al.* (2017). The HMW-GS protein extraction method used

contained the following steps: Grains of individual wheat cultivars have been milled. The resulting flour was transferred to Eppendorf tubes with a spatula and poured into a full solution of 1.5 M dimethylformamide (DMF). Then a 10-minute centrifugation was carried out at 14.000 × g. The resulting supernatant was allowed to analyse gliadins at -20 °C. The sludge was treated with 1ml 0.08M TRIS-HCL buffer pH 8.5 with SDS (1% w/v). The resulting mixture was vortexed for 30 minutes. Then an 8-minute centrifugation was carried out at 14.000 × g. Step 3 and 4 were repeated and the supernatant was discarded in each case. The resulting pellet was suspended in 0.08M TRIS-HCL buffer pH 8.5 with DTT (1.5% w/v) and SDS (1% w/v) and then 30 minutes were incubated at 60 ℃ with vortex. After the incubation, the samples were subjected to 10-minute centrifugation at 14.000 × g. 200μl of each alkylating supernatant were taken by adding 2.8 μL 4-pyridine. The samples were incubated for 30 minutes at 60 ℃ with two vortexes. For the precipitation of glutenins, 1 ml of acetone was added to each test tube. The resulting samples were left overnight at -20 °C. The thawed material was centrifuged for 10 minutes at 14.000 × g and the supernatant were discarded. The left pellet was dissolved in 0.2 M TRIS-HCL buffer pH 6.8 containing (1.5% w/v) DTT. The volume of solvent for the test material was 5 μl: 1 mg.

Electrophoresis SDS-PAGE

The composition of HMW-GS for each cultivar was determined by SDS-PAGE electrophoresis using 11.5% (m/v) polyacrylamide gel in the presence of 1x TGS buffer. The gels used in the experiment came from the biotechnology company Bio-Rad, named Criterion TGX Stain-Free Gels. With the Stain-Free system a UV light was used, which allowed a faster carrying out of the experimental part of the experiment (exposure time max. 5 minutes / min. 2 minutes). Rapid detection was possible due to the presence of a trihalogen compound in the gel, which is combined with protein tryptophan residues. After UV irradiation, fluorescence occurred and thus the individual protein fractions were labelled. Identification of individual HMW-GS protein fractions was determined by comparison with the Precision Plus marker Protein [TM] Unstained Standards (Bio Rad). This standard contained recombinant proteins that were in the masses of 10 to 250 [kDa]. Precisely determined bands were determined on the scale: 10, 15, 20, 25, 37, 50, 75, 100, 150, 250 [kDa], where 50 [kDa] → 750 [ng] a 20 [kDa], 100 [kDa] → 150 [ng]. Designation of the HMW glutenin alleles and subunits was performed following Payne and Lawrence (1983) and McIntosh *et al.* (2017).

Interpretation of HMW bands

The protein bands were visualized using the ChemiDocXRS + Bio-Rad gel documentation system, using the program it was possible to light the polyacrylamide gels after electrophoresis. Electrophoresis was carried out with the Stain-Free system using UV light, which allowed for faster detection of protein bands in the gel (exposure time max. 5 minutes / min. 2 minutes). By means of the Image lab. 5.1 Beta program (Bio-Rad), it was possible to analyse the resulting zymograms of the HMW-GS protein subunits.

Statistical analysis
Data analysis

The SDS-PAGE and RAPD bands were evaluated in binary form as presence (1) or absentness (0), each of which was treated as an independent form regardless of its intensity.

SDS-PAGE data analysis

STATISTICA 12.0 PL was used to analyse variance with Cochran's Q test (Cochran, 1950) (p < 0.05) for dichotomous variables (Etlinger, 1949). The test was aimed to differentiate the studied cultivars of wheat in terms of composition and high-molecular-weight glutenin subunits (HMW-GS). The basis was a zero-one matrix, in which 1 was treated as the presence of a given subunit and 0 was treated as its absence. With this denotation, genotypes under analysis were grouped by the mean similarity or protein subunit systems to be

identified (Unweighted Pair Group Method with Arithmetic Mean, UPGMA). Software called FreeTree and TreeView 1.6.6. was used (Pavlícek, 1999; Hampl and Pavlıcek, 2001).

<u>RAPD data analysis</u>

The number, percentage of polymorphic loci and values of Shannon index and genetic similarity index were determined according to Dice's formula (Dice, 1945) in place of Nei and Li (Nei and Li, 1979).

Polymorphism information content (PIC) for dominant marker systems was determined (Ghislain *et al.*, 1999). The determined genetic similarity matrix was used to build the UPGMA dendrogram using FreeTree and TreeView 1.6.6. programs. The statistical analysis used also the principal component analysis (PCA), based on the volume of amplification products generated in the presence of 3 starters (OPA-02, OPA-03 and OPB-08). STATISTICA 12.0.PL was used.

Results

Electrophoresis SDS-PAGE

The Q Cochran's test (Cochran, 1950) (Q = 32.623, p < 0.0002) showed statistically significant differences in the level of detection of glutenin subunit systems among the investigated wheat cultivars. A total of 11 different protein subunits have been identified, including: 3 in genome A, 5 in genome B and only 2 in genome D (Table 3). The most frequently detectable subunit systems were: (5+10), identified in 54.8% of genotypes and (2+12) and N, present in 12 cultivars (46.2%). Genotypes with glutenin subunit (5+10) in genome D were evaluated as most favourable with regard to the baking quality criterion and scored on average 7 and 9 of 10 points on the Payne scale (Payne and Lawrence, 1983). Cultivars with subunits 1 or 2* identified in genome A were also found to be favourable in terms of the baking quality, whereas subunit 2* was present only in 3 wheat cultivars ('Fregata', 'Kobiera', 'Tonacja').

The UPGMA dendrogram divided the analysed genotypes into two main groups (I and II), which differed in the protein subunit system evaluated on the basis of baking quality criterion (Figure 1). Two clusters called 'a' and 'b' were isolated in group I. Cluster 'a' included 5 genotypes for whose genomes A and D, identical subunits, 1 and (2+12), were identified. The second subgroup, 'b', consisted of 6 wheat cultivars with the highest scores of baking quality reflected by 8.3 points on the Payne scale (Payne and Lawrence, 1983). In the main group 'II', three clusters were distinguished: a, b, and c, accounting for 57.7% of the investigated cultivars. The three cultivars that were the only ones with subunits 2* formed a common subgroup 'a'. Five genotypes with identical subunits, N and (2+12), in genome A and D were classified to cluster 'b' of group II. To the third cluster (c) as many as 7 wheat genotypes were assigned, for which identical subunits in genome A and D were N and (5+10). It should be noted that they included the 'Srebrzysta' cultivar, which stood out among others due to its unique glutenin subunit system: N/6+8/5+10, not found in any other genotype.

RAPD polymorphism and genetic diversity

The random amplification of polymorphic DNA with 10 RAPD starters resulted in a total of 62 amplicons, for which 100% polymorphism was proven (Tables 2-3). The starters selected for polymorphism evaluation generated bands sized in a wide range from 439 bp (OPB-08) to 3669 bp in the presence of the OPA-03 starter. The highest number, that is, 11 and 10 amplification products were obtained in the reaction with the OPB-08 and RLZ8 starters, respectively. The value of the polymorphism information content (PIC) was also in a significant range from 0.074 to 0.500. In this range, the following starters: RLZ8 (0.409), OPD-08 (0.389) and OPA-13 (0.348) turned out to be the most informative. As few as 4 unique amplification products were identified exclusively for the 'Biała Kaszubska' cultivar.
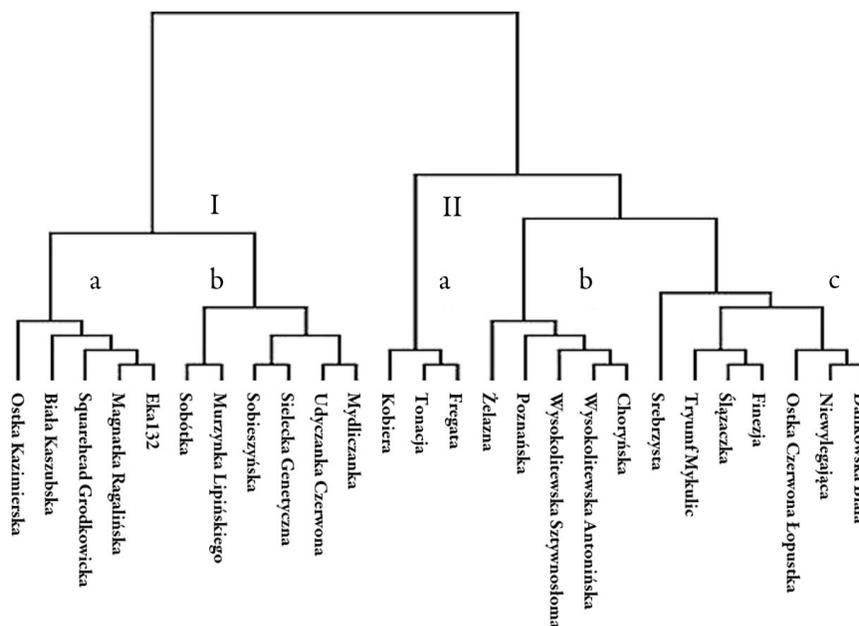
Figure 1. UPGMA dendrogram plotted in TreeView 1.6.6. based on the average similarity in the system of glutenin subunits of the wheat cultivars tested RAPD polymorphism and basis genetic diversity parameters

Table 3. RAPD primers used, total number of scored bands, number of polymorphic bands, their unique bands, percentage of polymorphism bands (P) for each primer and value of polymorphism information content (PIC)

Name of primer	Size range (bp)	Total of number products	Number of polymorphic products	P (%)	Number of unique products	PIC	
						Mean	Range
OPA-02	605-1034	5	5	100	0	0.185	0.074-0.311
OPA-03	703-3669	6	6	100	1	0.234	0.074-0.394
OPA-13	1101-2053	5	5	100	1	0.348	0.074-0.453
OPB-08	489-2394	11	11	100	0	0.322	0.074-0.497
OPD-02	907-1244	3	3	100	0	0.190	0.074-0.355
OPD-07	720-1234	3	3	100	1	0.212	0.074-0.488
OPD-08	909-2582	7	7	100	1	0.389	0.074-0.500
RLZ6	634-1217	4	4	100	0	0.240	0.142-0.473
RLZ8	850-2530	10	10	100	0	0.409	0.204-0.500
SIGMA-D-P	937-2596	8	8	100	0	0.244	0.142-0.394
Total	489-3669	62	62	-	4	-	0.074-0.500

On average, 28 polymorphic loci were identified for a single cultivar (45.1%) with the presence of 10 RAPD starters (Table 4). The most favourable in terms of this parameter was rated genotype 'Tryumf Mykulic' (42), while only 17 polymorphic loci (27.4%) were detected for 'Squarehead Grodkowicka'. The wheat cultivars were equalised in terms of the Shannon diversity index to fit in a small range from 0.264 (for 'Tryumf Mykulic') to 0.368 ('Biała Kaszubska', 'Fregata', 'Srebrzysta'). Taking into account the average values of I (0.352) and PIC (0.299), which allow determining the genetic diversity in a given locus, it should be stated that the genetic diversity among the analysed genotypes was not high (Table 4).

Table 4. Summary of genetic variation estimated using RAPD markers among the 26 cultivars

Cultivars	No. of polymorphic loci	Percentage of polymorphic loci	Shannon index
'Biała Kaszubska'	23	37.1	0.368
'Choryńska'	32	51.6	0.341
'Dańkowska Biała'	29	46.8	0.355
'Eka132'	28	45.2	0.359
'Finezja'	20	32.3	0.365
'Fregata'	22	35.5	0.368
'Kobiera'	28	45.2	0.359
''Magnatka Ragalińska'	25	40.3	0.366
'Murzynka Lipińskiego'	30	48.4	0.351
'Mydliczanka'	33	53.2	0.336
'Niewylegająca'	24	38.7	0.367
'Ostka Czerwona Łopustka'	33	53.2	0.336
'Ostka Kazimierska'	33	53.2	0.336
'Poznańska'	30	48.4	0.351
'Sielecka Genetyczna'	25	40.3	0.366
'Sobieszyńska'	30	48.4	0.351
'Sobótka'	28	45.2	0.359
'Squarehead Grodkowicka'	17	27.4	0.355
'Srebrzysta'	22	35.5	0.368
'Ślązaczka'	30	48.4	0.351
'Tonacja'	28	45.2	0.359
'Tryumf Mykulic'	42	67.7	0.264
'Udyczanka Czerwona'	31	50.0	0.347
'Wysokolitewska Antonińska'	29	46.8	0.355
'Wysokolitewska Sztywnosłoma'	31	50.0	0.347
'Żelazna'	24	38.7	0.367
Mean	28.0	45.1	0.3518
(Range)	(17-42)	(27.4-67.7)	(0.264-0.368)

The values of Polymorphism Information Content (PIC), Shannon Index (I) and Genetic Similarity (Si) are presented in the Table 5.

Table 5. Evaluation of RAPD polymorphism and genetic diversity

Parameter/Index	Values
PIC	0.2993
I	0.3518
Si	0.6477

The Dice's matrix of genetic similarity (Si) was determined for the wheat genotypes under study. A significant genetic similarity was found among cultivars, and the value of range for the Si index was very high as the difference between the maximum and minimum values was 0.795 (Table 4). The highest genetic affinity of 0.949 was found between two Polish wheat cultivars, 'Wysokolitewska Antonińska' and 'Eka 132', while the lowest Si of 0.154 was determined between genotypes 'Squarehead Grodkowicka' and 'Srebrzysta'. These genotypes not only differed from each other, but also from most other cultivars. Another cultivar, which represented a significant genetic distance from other analysed genotypes, was 'Biała Kaszubska'.

Cluster and PCA analysis

The applied measure of genetic similarity was used to plot the UPGMA dendrogram. This allowed to group the studied wheat genotypes by their average similarities between all possible pairs of objects and to assign most of them to two asymmetrical groups: I and II (Figure 2). The first main group consisted of 11 cultivars clustered in two subgroups, 'a' and 'b'. Except for 'Kobiera', these wheat cultivars were Polish and, based on the composition of protein subunits, they were rated on average at 6.7 points on the Payne scale. For comparison, in the cluster II, 9 genotypes showed better baking qualities and were rated at 7.6 points, i.e. almost 1 point more than genotypes in the group I. The UPGMA dendrogram also identified 6 genotypes that were not included in the two main groups. These showed a lower degree of genetic affinity compared to the remaining 22 wheat cultivars. These included two cultivars, 'Finezja' and 'Fregata', which were genetically similar - their genetic similarity index Si was 0.714. Two paired cultivars, 'Biała Kaszubska' and 'Squarehead Grodkowicka' and two genotypes, 'Srebrzysta' and 'Wysokolitewska Sztywnosłoma', turned out to be the furthest outliers in the set of other genotypes (Figure 2).

Table 6. Polish common wheat cultivars and its genotypes presenting HMW-GS glutenin subunits and bread-making quality scores (according to Payne and Lawrence, 1983)

| Cultivars	Genotype representing subunits HMW-GS	Quality scores 1-10°
'Biała Kaszubska'	1 / 7+9 / 2+12	7
"Choryńska'	N / 7+9 / 2+12	5
'Dańkowska Biała'	N / 7+9 / 5+10	7
'Eka 132'	1 / 6+8 / 2+12	6
'Finezja'	N / 7+8 / 5+10	8
'Fregata'	2* / 7+9 / 5+10	9
'Kobiera'	2* / 6+8 / 2+12	6
'Magnatka Ragalińska'	1 / 6+8 / 2+12	5
'Murzynka Lipińskiego'	1 / 20 / 5+10	8
'Mydliczanka'	1 / 22 / 5+10	8
'Niewylegająca'	N / 7+9 / 5+10	7
'Ostka czerwona'	N / 7+9 / 5+10	7
'Ostka Kazimierska'	1 / 7+8 / 2+12	8
'Poznańska'	N / 7+8 / 2+12	6
'Sielecka Genetyczna'	1 / 7+9 / 5+10	9
'Sobieszyńska'	1 / 7+9 / 5+10	9
'Sobótka'	1 / 20 / 5+10	8
'Squarehead Grodkowicka'	1 / 7+9 / 2+12	7
'Srebrzysta'	N / 6+8 / 5+10	6
'Ślązaczka'	N / 7+8 / 5+10	8
'Tonacja'	2* / 7+9 / 2+12	5
'Tryumf Mykulic'	N / 7+8 / 5+10	8
"Udyczanka Czerwona'	1 / 22 / 5+10	8
'Wysokolitewska Antonińska'	1 / 7+9 / 2+12	7
'Wysokolitewska Sztywnosłoma'	N / 7+9 / 2+12	5
'Żelazna'	N / 20 / 2+12	4
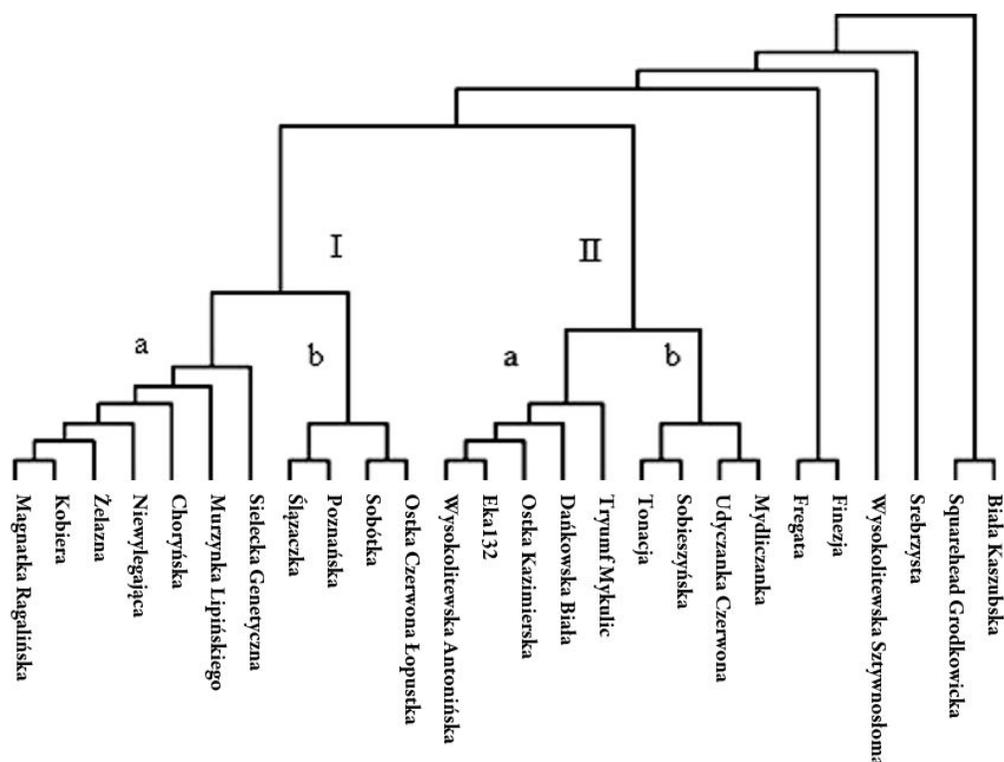
Figure 2. UPGMA dendrogram of genetic similarity of the studied cultivars of 26 obtained on the basis of RAPD markers constructed using the TreeView 1.6.6.

The location of wheat genotypes in the two-factor space was investigated through the Principal Components Analysis (Figure 3). The analysis was based on the volume of DNA amplification products produced in the presence of individual starters. From among 10 RAPD starters, OPA-02, OPA-03 and OPB-08 were selected as ones with the highest capacity of genotype differentiation and having the most consistent unweighted pair grouping with arithmetic mean (UPGMA). The cultivars were distributed on different sides of both axes of coordinates at a clear distance from the centre of the axis. They formed five separate clusters, the majority of which, 18 genotypes (69.2%) were assigned to two groups, lying close to each other, on the same side of the horizontal axis (abscissa).

For eleven of them, the consistency of grouping with the UPGMA dendrogram for glutenin subunits has been confirmed. The first cluster was assigned eleven cultivars, the majority of which had a common subunit 1, identified in genome A, while in the second group, subunit N was detected for more than half of cultivars. On the other side of the horizontal axis, three smaller clusters are located at a considerable distance from the others. In one of them there are two cultivars, 'Murzynka Lipińskiego' and 'Ślązaczka', which had a common subunit (5+10) in genome D. At a small distance from them, 'Dańkowska Biała', 'Poznańska' and 'Sielecka genetyczna' were located. Two genotypes, 'Biała Kaszubska' and 'Squarehead Grodkowicka', formed a distinct grouping which suggests that they were genetically distant from the other wheat samples. Thus, their grouping was confirmed to be consistent with the UPGMA dendrogram plotted for the RAPD markers. The analysis of the main components helped identify a separate genotype, 'Srebrzysta', which did not belong to any grouping. Although this cultivar was on the same side of the abscissa as the three smaller clusters, it was already on the other side of the vertical axis (ordinate). The distinctiveness of the 'Srebrzysta' cultivar was also shown in the UPGMA dendrogram drawn up for the RAPD.
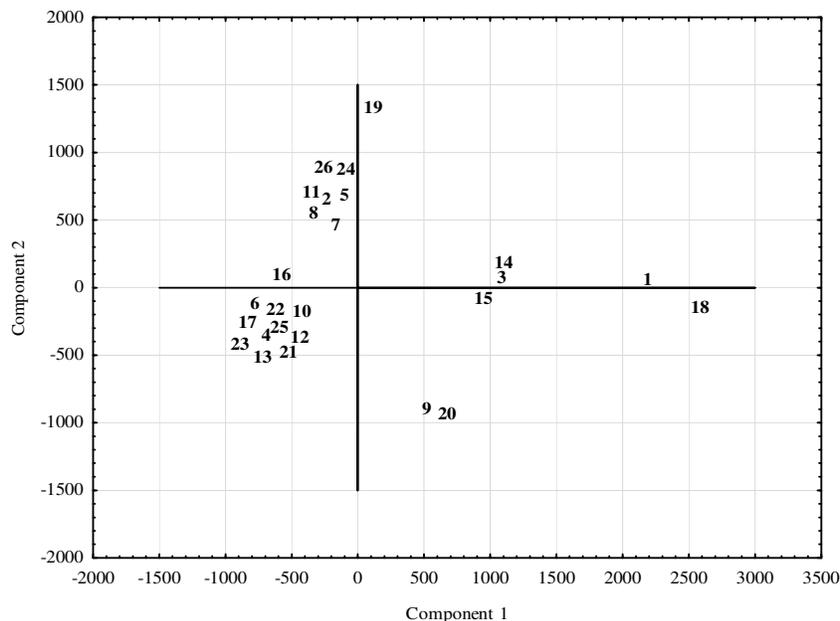
Figure 3. The distribution of the studied cultivars of 26 in two-dimensional space (PCA). 1 - 'Biała Kaszubska' 2 - 'Choryńska'; 3 - 'Dańkowska Biała'; 4 - 'Eka132'; 5 - 'Finezja'; 6 - 'Fregata'; 7 - 'Kobiera'; 8 - 'Magnatka Ragalińska'; 9 - 'Murzynka Lipińskiego'; 10 - 'Mydliczanka'; 11 - 'Niewylegająca'; 12 - 'Ostka Czerwona Łopustka'; 13 - 'Ostka Kazimierska'; 14 - 'Poznańska'; 15 - 'Sielecka Genetyczna'; 16 - 'Sobieszyńska 44'; 17 - 'Sobótka'; 18 - 'Squarehead Grodkowicka'; 19 - 'Srebrzysta'; 20 - 'Ślązaczka'; 21 - 'Tonacja'; 22 – 'Tryumf Mykulic'; 23 - 'Udyczanka Czerwona'; 24 - 'Wysokolitewska Antonińska'; 25 - 'Wysokolitewska Sztywnosłoma'; 26 - 'Żelazna'

Discussion

In our studies, we used two marker systems that differed in terms of assessing genetic variability and grouping of genotypes. The objective of this paper was to determine the variability of Polish common wheat cultivars in groups having high bread-making and poor bread-making quality of flour conditioned by the presence of subunits of HMW-GS in loci Glu-1, the background of genetic diversity determined with the use of RAPD markers. Knowledge of the genetic diversity of wheat genotypes based on DNA and protein polymorphisms is very important for breeding programmes (MAS), because common wheat (*Triticum aestivum* L.*)* is one of the most important cereals, followed by barley, maize and rice (Ortiz *et al.*, 2008; Ahmed *et al.*, 2010; Kuťka Hlozáková *et al.*, 2016).

For this reason, it is highly desirable to make biological progress in parallel with the introduction of new, high-yielding, disease-resistant cultivars showing also better parameters of grain quality. The rate and pace of the progress implementation depend primarily on the knowledge of the genetic fundamentals of processes and phenomena aimed to improve crop plants. Due to the complexity of the hexaploid wheat genome, the molecular markers for this species were created relatively late. However, the marker systems are being constantly improved and the techniques for identifying functional markers associated with useful features have developed significantly in recent years. In comparison to conventional variability assessment methods, molecular markers are more stable and do not affect the environment (El Siddig *et al.*, 2013). Molecular marker testing of the common wheat genome seems to be the most optimal option with respect to time and ability to analyse the results. In the fundamental and implementation research into cereal breeding systems, it is necessary to take

into account analyses based on both molecular tests and polypeptide polymorphism. The most commonly used molecular and biochemical markers include: RFLP - restriction fragments length polymorphism, RAPD - randomly amplified polymorphic, ISSR - inter simple sequence repeat, SSR - Simple Sequence Repeats, STS - Sequence Tagged Site (Kim and Ward, 2000; Varshney *et al.*, 2005; Iqbal *et al.*, 2009; Gorji *et al.*, 2010; Ahmad, 2014; Tékeu *et al.*, 2017; Huseynova *et al.*, 2018).

Two methods, SDS-PAGE and RAPD-PCR, were used to assess genetic diversity among 26 Polish common wheat cultivars. The obtained results allowed to hypothesize that there is a correlation between the genetic similarity of RAPD and variability of HMW-GS subunits that determine the technological value of flour, but further analysis excluded this relationship.

The results of HMW-GS subunit test were analysed using the Q Cochran's test (Cochran, 1950), based on the 0-1 matrix prepared beforehand for high molecular weight glutenin subunits (HMW-GS). The performed test showed that the wheat cultivars called 'Fregata', 'Kobiera', 'Tonacja' represent favourable baking quality (Table 6). In the next stage, the UPGMA analysis helped distinguish a unique system of subunits, i.e. N/6+8/5+10 occurring only in the cultivar 'Srebrzysta' and not found in any other genotype (Figure 1).

In the next part of the paper, a measure of genetic similarity was applied, by means of which the RAPD dendrogram showing appropriate groupings of wheat genotypes was plotted (Figure 2). To confirm this, we focused on the Principal Components Analysis (PCA) of wheat genotypes in the two-factor space. The applied methods confirmed the consistency of groups of the tested cultivars of common wheat and showed the distinctness of the "Srebrzysta" cultivar (Figure 2-3). In this study, it was found that RAPD analysis is an effective DNA marker system for assessing genetic diversity among the studied hexaploid wheat. Out of ten RAPD starters, three RAPD starters (OPA-02, OPA-03, OPB-08) were generated, which showed the highest differentiation level in the study. The polymorphism obtained in this study (Table 1, 4) is similar to the results obtained by Ahmed et al (Ahmed *et al.*, 2010). They reported a 61% polymorphism for 32 wheat genotypes based on the use of 15 RAPD starters. In this study, however, the RAPD polymorphism was at a higher level of 100% on the basis of 10 starters (Table 3).

Nowadays, molecular markers (DNA) biochemical markers (SDS-PAGE) are used simultaneously to study genetic variability of crop plants. Ahmed and co-authors (Ahmed *et al.*, 2010) used both of these markers in their research and compared them using various parameters, including the similarity matrix and percentage polymorphism. They attempted to superimpose the results of these two analyses and proved that similarity indexes of the combined RAPD and SDS-PAGE analyses were strongly correlated. In this paper, an attempt was also made to superimpose the results of these two analyses, but no satisfactory results were seen. However, as in Ahmed's paper (Ahmed *et al.*, 2010), the similarity between the results of RAPD and SDS-PAGE analyses was observed when generating UPGMA dendrograms. By comparing the subunits identified by the SDS-PAGE method with the dendrogram obtained as a result of the RAPD analysis, it can be noted that Polish common wheat cultivars are grouped on the tree consistently with HMW-GS subunits present in them (Figure 1-2).

In 2016, researchers (Kuťka Hlozáková *et al.*, 2016) have carried out similar studies on European common wheat cultivars to assess their genetic diversity. They have studied genetic variability of 24 cultivars of common wheat using RAPD-PCR and SDS-PAGE techniques. They also made a successful attempt to superimpose the results of these analyses. The results of their work confirmed the findings of Ahmed and co-authors (Ahmed *et al.*, 2010), who stated that RAPD PCR might be a cheaper alternative for testing the genetic variability of common wheat.

The absence of correlation between the identified similarity calculated from the composition of HMW glutenin subunits and RAPD-PCR data do show that each set of markers represents genetic variability in a different way. The index of the Cochran's Q test (Cochran, 1950) that analyses the variance of the composition of HMW glutenin subunits helped identify the most favourable 2 * subunit, which were present in three cultivars only 'Fregata', 'Kobiera','Tonacja' (Table 6). However, UPGMA analysis showed a distinct cultivar,

'Srebrzysta', which was characterized by a different genotype in the pool of 26 cultivars. In contrast, RAPD *loci* exhibited a polymorphism in the range of 27.4-67.7 and diversity with respect to *Shannon Index (I)* and the polymorphism information content (PIC) (Table 1,4). To continue, RAPD data - which generated the UPGMA dendrogram and then helped determine the genetic similarity index Si - were also used to group the cultivars into differentiating genotypes. In addition, the location of common wheat genotypes examined in the two-factor PCA (Figure 3) confirmed the consistency of genotype grouping with the UPGMA dendrogram for RAPD method.

Our results have shown that RAPD markers are useful for studying genetic diversity Polish common wheat cultivars in groups having high bread-making and poor bread-making quality of flour conditioned by the presence of subunits of HMW-GS. Although the value of the polymorphism information content (PIC) was not large, the analysed genotypes showed that 'Biała Kaszubska'- 1/7+9/2+12 had a significant genetic distance in relation to other analysed genotypes. Whereas the genotypes 'Squarehead Grodkowicka'- 1/7+9/2+12, 'Srebrzysta'- N/6+8/5+10 and 'Wysokolitewska Sztywnosłowa'- N/7+9/2+12 not only differed between themselves, but also in relation to most other common wheat cultivars. As you can see, genotypes from poor brad-making quality were separated from the entire research pool. It does not mean that there were no identical genotypes in the remaining research pool. Therefore, our studies suggest that two methods, SDS-PAGE and RAPD need to be applied together, as a complementary analysis, not one replacing the other. Genetic information obtained from data on HMW-GS and RAPD can be successfully used to differentiate cultivars of common wheat breeding.

Conclusions

In summary, two marker systems used in this study differed in terms of the assessment of genetic diversity and grouping of genotypes of common wheat cultivars. The lack of correlation between the results of identification of high-molecular-weight glutenin proteins by the SDS-PAGE method and with the RAP-PCR polymorphism suggests that both marker systems should be used as complementary analyses. Therefore, the genetic variability obtained from HMW-GS and RAPD-PCR can be used to differentiate common wheat cultivars in the course of traditional breeding programmes.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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