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Biometric Analyses of Yield, Oil and Protein Contents of Wheat (*Triticum aestivum* L.) Genotypes in Different Environments

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Abstract: The objectives of this study were to investigate: (1) the effects of genotype, environment, and their interactions on the oil content (OC), protein content (PC) and grain yield (GY) of 25 varieties of winter wheat, (2) the correlations among these traits in different environments, and (3) the effects of different climatic variables and their interactions with wheat genotypes for the examined traits. The field experiments were performed on three experimental sites in Serbia in 2009/10 and 2010/11. The most variable traits were GY and PC, while the variations of OC were lower. A significant positive correlation between wheat bran OC and GY was found in one, while highly significant negative correlations between PC and GY were found in three out of six environments. The partial least square regression (PLSR) triplots for protein content, oil content and grain yield enabled us to identify favorable and limiting climatic conditions for each trait and explained 31.9%, 32.6%, and 30.4% of the total variance, respectively. Cvs. Renesansa and Zvezdana were identified as genotypes with high average values for all traits, while cvs. Bankuty 1205 and Banatka were identified as potential sources of high protein content.

Keywords: G×E interaction; climatic variables; oil; proteins; yield; wheat

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the leading cultivated crops across the world, and is the most widely consumed cereal by humans [1,2]. Whole kernel of wheat is composed of endosperm (80–85%), bran (10–14%) and germ (2.5–3.0%) [3]. The solid outer layer of wheat kernel is wheat bran; it is composed of the connected pericarp and aleurone [2]. Wheat bran is regarded as a byproduct from flour milling process, and could be used for both animal and human feed [4–6].

The use of wheat bran for human consumption has increased progressively over the years, as well as the number of wheat bran-incorporated food products [7]. Wheat bran consists of proteins (25%), starch (9%) and dietary fibers (lignin, xylans, cellulose), bioactive components, phytic acid, antioxidants, vitamins and minerals [5,6,8–10]. On the other hand, it contains a large amount of dietary fibers ranging between 40 and 44% that can also be used as natural supplement for manufacturing high-fiber baked products [11]. The aleurone layer of bran contains significant amounts of proteins which have a higher nutritional value in comparison with proteins in wheat flour [6,11]. Likewise, wheat bran contains a significant amount of lipids (4%) which can be used as a source of profitable phytochemicals [12]. It is well-known that phytochemicals existing in grains have the potential to increase nutritional quality and health benefits [3]. In particular, wheat bran has been recognized as a cancer preventive agent, even though mechanism of the protection has not yet been explained completely [13].

The assessment of health-beneficial chemicals in wheat grain and bran, such as proteins and oil, is important for breeding due to increasing consumer demand for healthier diet. Previous studies have shown that the amounts of proteins in grains can be affected by different environmental parameters [14–16], cultivar [17], fertilization rate with nitrogen and their time of application [18,19], residual nitrogen content in the soil [20], crop rotation [21], and interactions of the mentioned factors [16,22]. It has broadly been confirmed that wheat cultivar, growing environment and cultivar-environment interactions may influence the chemical composition of wheat grain and bran as well as their yield [23,24]. Similar studies recorded the significant effects of cultivar, location and their interactions on protein and oil contents in other crops too [25–29].

The objectives of this study were: (1) to investigate the effects of genotype, environment, and their interactions on oil content, protein content and grain yield in 25 varieties of winter wheat (*Triticum aestivum* L.), (2) to estimate the correlations among these traits in different environments, and (3) to evaluate the effect of different climatic variables and their interactions with wheat genotypes for the examined traits. The results from this study could assist wheat breeders in creating new high-yielding wheat varieties with good quality parameters and for specific agro-climatic conditions.

2. Materials and Methods

2.1. Field Trials

The field trials were set on three experimental sites representing Serbia's most important wheat–growing areas: Rimski sancevi (RS 45°20′ N, 19°51′ E, 87 m altitude), Sremska Mitrovica (SM 46°06′ N, 19°33′ E, 83 m altitude) and Pancevo (PA 44°50′ N, 20°40′ E, 76 m altitude), during two growing seasons (2009/10 and 2010/11). The six analyzed environments were labeled as follows: E1, E2 and E3 represent locations RS, SM, and PA in the first growing season (2009/10), respectively, while E4, E5 and E6 represent the same locations in the second growing season, respectively. It means that for example E1 represents location of RS in the season 2009/10, while E4 represents the same location in the season 2010/11. The climate of Serbia is continental with hot springs and summers, and slightly cold winters. Soils of the experimental fields were Chernozem Chernic [30].

A randomized complete block design with three complete blocks was used on each location. The experimental plots consisted of 10 rows, 5 m long with spacing of 10 cm among rows. Wheat was sown at the optimum time (mid-October) and attained maturity in late June or early July. Plant spacing was achieved by implementing a density of 550 seeds per m^{-2}. Weed, disease, and pest control on all plots was performed by using standard cultivation practice.

The application of 110 kg N/ha nitrogen fertilizers was used on whole experiment as standard agronomical practice for wheat growing in Serbia. Experimental trials were fertilized before sowing with 45 kg N/ha (NPK, 15:15:15) during autumn. Additionally, 65 kg/ha of nitrogen fertilizers (calcium ammonium nitrate, N 27%) was applied in late February or early March for the improvement of wheat yield and quality. Kernel samples from the experimental trials were mechanically harvested while further analyses were done in laboratory.

2.2. Environmental Variables

The meteorological conditions during trials were obtained from automatic weather stations of the Republic Hydro-meteorological Service of Serbia, situated close to the experimental fields. In the wheat growing season (from October to July), the following climatic variables were collected and used as input for statistical modeling: minimum temperature (tmn, °C), maximum temperature (tmx, °C), temperature variation (tv, °C), and total precipitations (pr, mm). Variables were collected for each month of vegetation; therefore, the tmn10 corresponds to the minimum temperature in October (Figure 1).

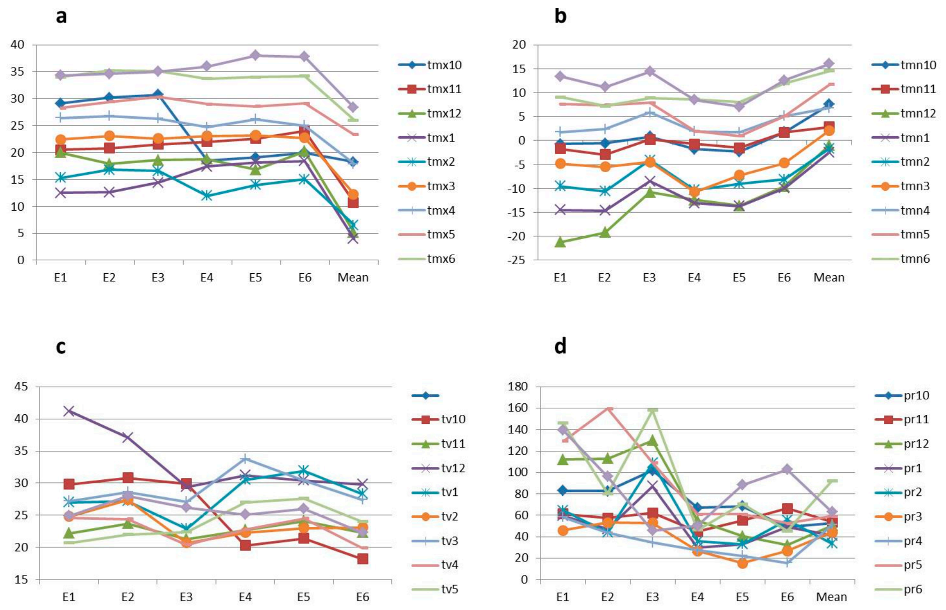

Figure 1. Climatic variables collected during wheat growing season (from October to July) for six environments: (**a**) maximum temperature (tmx, °C), (**b**) minimum temperature (tmn, °C), (**c**) temperature variation (tv, °C), and (**d**) total precipitations (pr, mm).

2.3. Wheat Cultivars

This study examined twenty-five cultivars of winter wheat which were registered and cultivated in Southeast Europe for the last 60 years (Table 1). For each decade, cultivars that were the most present in wheat production in the region were chosen to elucidate their contribution to breeding wheat cultivars with high nutrition content.

2.4. Agronomical and Technological Traits

The wheat grain was conditioned before milling by damping with water. After first conditioning during 24 h, moisture content of 13.5% in grain was achieved. Further damping before milling has increased moisture content in kernel to 15%. Thus, endosperm and grain coat textures were modified in order to obtain higher bran yields without endosperm and with lower energy consumption. The bran was obtained by milling wheat grains on MLU 202 laboratory flour mill (Buhler, Inc., Uzwil, Switzerland). After milling, brans were homogenized and put in cold storage for further analysis.

The protein content (PC–expressed as %) of the bran samples was determined using standard analytical method 46–16.01 [31]. The factor 6.25 was applied for converting nitrogen content to crude protein. In order to estimate the oil content (OC–expressed as %) in bran samples, approximately 1 g of bran of each cultivar were dried on filter paper during 2 h at 105 °C. Afterwards, the total oil content was determined by intensive extracting of bran samples using petroleum ether as the extractant and Soxhlet extractor. Grain yield data (GY–expressed in t/ha at 13% moisture) were obtained by harvesting each wheat cultivar plot (ten rows) separately.

Table 1. List of winter wheat cultivars with their year of release and country of origin.

Cultivar	Year of Release (YOR)	Country of Origin
Banatka	1955	Serbia
Bankuty 1205	1955	Hungary
San Pastore	1958	Italy
Bezostaja 1	1959	Russia
Libellula	1962	Italy
Zlatna Dolina	1970	Croatia
Sava	1970	Serbia
Partizanka	1973	Serbia
NS rana 2	1975	Serbia
KG 56	1975	Serbia
Balkan	1979	Serbia
Yugoslavia	1980	Serbia
Skopljanka	1982	Macedonia
Lasta	1987	Serbia
Evropa 90	1990	Serbia
NS rana 5	1992	Serbia
Pobeda	1990	Serbia
Renesansa	1994	Serbia
Pesma	1995	Serbia
Ljiljana	2000	Serbia
Cipovka	2002	Serbia
Dragana	2002	Serbia
Simonida	2003	Serbia
Zvezdana	2005	Serbia
NS 40S	2006	Serbia

2.5. Statistical Analyses

The basic variability indicators (maximum, minimum, mean, standard deviation–SD, coefficient of variation–CV, comparison of means, and Pearson correlation coefficients) were calculated for each trait in Excel program. The partial least squares regression (PLSR) model [32] was used for relating a two-way matrix of double centered tables for wheat OC, PC and GY, and matrix of centered environmental variables. The PLSR model relates effects of GE interaction (i.e., residual effects after adjusting for genotype and environmental effects) as a dependent variable matrix (Y) to an external climatic variable matrix (Z). The Y matrix contains data for grain yield, protein and oil contents, while the Z matrix contains climatic variables recorded during the experiments. In terms of the PLSR model both matrices can be expressed as: $Y = TQ' + F$ and $Z = TP' + E$, where matrix T contains Z scores, the P matrix contains Z loadings, matrix Q contains the Y loadings and F and E matrices are the residuals of the un-explained variation in Y and Z tables. The relationship among the Y and Z tables was derived through the latent variable T. The latent T variable represents the proportion of the explained interaction variance of in the Y matrix by the set of environmental variables from the Z matrix. The number of T variables that are requested to optimally predict the dimensionality of the Y matrix was determined by cross-validation procedure [33]. The estimates of the PLSR model can also be graphically presented by tri-plot since it contains the coordinates for genotypes, environments and environmental variables. The interaction of the specific genotype and environment combination is defined by the angle between them. The angle of 90° indicates a complete orthogonality in the interaction and angle larger than 90° a negative interaction. Similarly, for the environmental variables, a closer association of particular variable or group of the correlated variables to specific genotype and environment combination is an indication of the effect on the interaction. Additionally, the interaction residuals from the Y matrices for each dependent variable were visualized by the heat maps in order to elucidate the interaction pattern on simple and understandable way. More details about the model and its interpretation were given by Zoric et al. [34]. All data analyses were performed using R [35] computing environment.

3. Results

3.1. Genotypic Variations

In the experiment, the wheat bran oil content ranged between 2.4% and 5.6%, with average value of 3.85% (Table 2). The genotypes Zlatna Dolina and Balkan had very low oil content in almost all environments and cv. Zvezdana had the highest oil content in the three out of six environments (E3, E5 and E6). Consequently, these cultivars had also the lowest and the highest average oil content values for all environments (3.1 and 4.6%, respectively). The CV ranged from 10.9% in E4 to 19.8% in E2, with the average value of 15.7%.

Table 2. Oil content (%) in 25 winter wheat varieties and six environments and descriptive statistics.

OIL CONTENT (%)								
	E1	**E2**	**E3**	**E4**	**E5**	**E6**	**Avg**	**LSD (0.05)** [a]
Banatka	3.56	3.82	3.54	3.80	3.90	4.08	3.8	c [b]
Bankut 1205	3.47	3.31	3.86	4.29	3.70	3.85	3.7	cd
San Pastore	3.84	4.05	3.76	3.65	3.90	3.39	3.8	cd
Bezostaja 1	4.29	4.12	4.06	4.16	3.76	3.51	4.0	bc
Libelula	3.36	3.90	3.46	4.01	4.03	2.97	3.6	d
Zlatna dolina	2.49	2.98	2.40	3.49	3.84	3.55	3.1	cd
Sava	4.01	2.49	4.51	4.03	3.47	3.51	3.7	cd
Partizanka	3.82	3.59	4.37	3.88	3.73	3.53	3.8	c
NS rana 2	4.10	4.66	5.13	3.41	2.93	2.81	3.8	c
KG 56	5.00	5.04	3.62	3.69	3.10	2.63	3.8	c
Balkan	3.23	3.24	3.55	3.00	2.62	3.11	3.1	d
Jugoslavija	3.57	2.75	4.01	3.97	3.45	3.88	3.6	cd
Skopljanka	2.78	5.03	4.43	3.20	3.42	3.21	3.7	cd
Lasta	3.61	3.67	3.47	3.50	3.44	4.34	3.7	cd
Evropa 90	3.59	4.32	3.49	4.13	3.87	4.04	3.9	bc
NS rana 5	3.41	4.65	3.97	4.01	3.89	4.36	4.0	abc
Pobeda	3.43	4.44	3.74	4.38	4.39	3.59	4.0	abc
Renesansa	5.30	4.98	4.44	4.42	3.23	4.76	4.5	ab
Pesma	4.17	4.12	4.65	3.81	2.82	4.41	4.0	abc
Ljiljana	4.36	2.75	3.80	4.21	4.12	4.53	4.0	bc
Cipovka	4.33	3.60	3.31	4.39	3.40	4.43	3.9	bc
Dragana	3.57	2.72	4.11	4.62	4.39	4.50	4.0	bc
Simonida	3.80	3.24	4.16	4.72	4.07	4.48	4.1	abc
NS 40S	3.76	3.77	3.79	3.88	3.96	4.67	4.0	bc
Zvezdana	3.64	4.10	5.49	4.32	4.73	5.57	4.6	a
LSD (0.05)	a	a	a	a	a	a		
Max	5.3	5.0	5.5	4.7	4.7	5.6		
Min	2.5	2.5	2.4	3.0	2.6	2.6		
Avg	3.8	3.8	4.0	4.0	3.7	3.9		
StDev	0.60	0.76	0.63	0.43	0.50	0.70		
CV	15.97	19.82	15.77	10.87	13.69	18.02		

[a] Fisher's Least Significant Difference (LSD) Test, $p \leq 0.05$; [b] Different letters indicate significant difference between values at $p \leq 0.05$.

The average protein content in wheat grain varied among the environments from 12.6% in E4 to 15.1% in E1 (Table 3). The overall mean obtained in the experiment was 14.3% indicating high potential of the tested material for this trait. Coefficient of variation (CV) ranged from 3.0% in E2 to 8.3% in E4, with average value of 5.4% for the whole experiment. The lowest average protein content had cv. Simonida (13.4%) and the highest was found in cv. Banatka (15.9%). The minimal value for protein content (11.2%) was obtained in E4 for cv. Simonida, while maximum (17.8%) was determined in E3 environment for cv. Bankuty 1205.

Table 3. Protein content (%) in 25 winter wheat varieties and six environments and descriptive statistics.

PROTEIN CONTENT (%)								
	E1	**E2**	**E3**	**E4**	**E5**	**E6**	**Avg**	**LSD (0.05)** [a]
Banatka	16.7	15.3	17.4	15.4	14.2	16.5	15.9	a [b]
Bankut 1205	16.2	15.5	17.8	15.6	13.5	15.6	15.7	ab
San Pastore	14.0	14.9	14.2	12.0	13.5	14.2	13.8	cde
Bezostaja 1	15.3	14.8	16.0	13.6	13.8	15.2	14.8	abcd
Libelula	14.3	13.8	15.3	12.5	13.6	15.2	14.1	cde
Zlatna dolina	13.7	14.5	13.5	12.8	14.0	14.5	13.8	cde
Sava	14.8	15.4	15.6	13.3	14.8	15.9	15.0	abc
Partizanka	14.6	14.8	15.1	13.1	13.5	14.9	14.3	cde
NS rana 2	15.2	14.1	15.5	13.3	14.2	15.4	14.6	bcde
KG 56	15.8	14.8	15.1	12.2	14.9	14.1	14.5	bcde
Balkan	15.4	14.5	14.9	13.0	14.2	14.2	14.3	cde
Jugoslavija	15.9	14.4	15.2	12.5	14.5	14.4	14.5	bcde
Skopljanka	15.5	15.3	15.3	12.6	13.8	14.7	14.5	bcde
Lasta	14.7	15.0	14.4	11.7	13.9	14.0	14.0	cde
Evropa 90	14.5	14.3	14.5	12.3	13.3	14.1	13.9	cde
NS rana 5	15.4	15.1	14.6	11.8	14.3	14.5	14.3	cde
Pobeda	16.1	14.7	15.3	12.4	13.7	14.4	14.4	bcde
Renesansa	16.3	15.0	15.4	11.7	13.9	14.1	14.4	cde
Pesma	16.1	14.7	14.6	12.3	13.9	14.7	14.4	cde
Ljiljana	14.9	14.6	14.0	11.8	13.7	14.4	13.9	cde
Cipovka	15.6	14.4	14.3	12.3	14.4	15.0	14.4	cde
Dragana	13.7	15.4	14.1	11.5	14.3	14.1	13.8	cde
Simonida	14.9	14.0	13.1	11.2	14.0	13.3	13.4	e
NS 40S	13.7	14.7	13.3	12.0	13.8	14.3	13.6	de
Zvezdana	15.2	14.8	14.2	11.9	14.9	13.8	14.2	cde
LSD (0.05)	a	ab	ab	d	c	b		
Max	16.7	15.5	17.8	15.6	14.9	16.5		
Min	13.7	13.8	13.1	11.2	13.3	13.3		
Avg	15.1	14.8	14.9	12.6	14.0	14.6		
StDev	0.86	0.44	1.09	1.05	0.44	0.71		
CV	5.66	2.98	7.32	8.35	3.16	4.86		

[a] Fisher's Least Significant Difference (LSD) Test, $p \leq 0.05$; [b] Different letters indicate significant difference between values at $p \leq 0.05$.

Grain yield varied significantly among the genotypes and the environments (Table 4), with CV ranging from 13.7% (E4) to 21.2% (E2). The minimum value (2.3 t/ha) was obtained in E2 for cv. Banatka and maximum (9.8 t/ha) was determined in E4 for cv. Simonida. These environments also had the lowest and the highest average grain yield, 5.2 t/ha and 8.7 t/ha, respectively.

Significant positive correlation between wheat bran oil content and grain yield ($r = 0.441^*$) was found in the present study, but only in one (E6) out of six environments (Table 5). Highly significant negative correlations were found between protein content and grain yield in three (E3, E4, and E6) out of six environments. When the average values for all environments were analyzed, PC was negatively correlated with GY ($r = -0.74^{**}$), whereas the association between OC and GY ($r = 0.34$), and between oil and protein contents ($r = -0.09$) were not significant.

Table 4. Grain yield (t/ha) in 25 winter wheat varieties and six environments and descriptive statistics.

GRAIN YIELD (t/ha)								
	E1	**E2**	**E3**	**E4**	**E5**	**E6**	**Average**	**LSD (0.05)** [a]
Banatka	4.23	2.28	2.66	5.47	5.44	2.91	3.8	e [b]
Bankut 1205	4.31	2.36	2.81	5.49	5.40	2.87	3.9	e
San Pastore	5.26	3.58	4.99	7.46	5.94	4.46	5.3	de
Bezostaja 1	5.89	3.86	4.66	7.37	6.58	4.13	5.4	cde
Libelula	5.85	4.59	4.90	7.86	6.87	4.59	5.8	bcd
Zlatna dolina	5.79	5.18	6.15	8.39	7.30	5.09	6.3	abcd
Sava	5.85	5.01	5.81	8.42	7.41	5.01	6.3	abcd
Partizanka	6.23	5.27	5.69	8.33	7.25	5.35	6.4	abcd
NS rana 2	6.60	5.36	5.80	8.83	7.43	5.51	6.6	abcd
KG 56	6.80	5.31	5.42	8.83	7.81	5.41	6.6	abcd
Balkan	6.64	5.59	5.97	8.97	7.77	5.54	6.7	abcd
Jugoslavija	7.61	5.68	5.67	9.11	8.07	5.85	7.0	abc
Skopljanka	7.31	5.75	5.64	8.97	8.61	5.72	7.0	abc
Lasta	7.60	5.35	6.26	9.28	8.30	6.17	7.2	ab
Evropa 90	7.72	5.87	5.82	9.45	8.67	6.10	7.3	ab
NS rana 5	7.60	5.48	5.86	9.28	8.35	6.09	7.1	ab
Pobeda	7.27	5.79	5.66	9.76	8.48	6.23	7.2	ab
Renesansa	7.70	6.03	5.49	9.36	8.69	6.27	7.3	ab
Pesma	8.16	6.21	5.93	9.58	8.65	5.69	7.4	ab
Ljiljana	7.88	5.92	5.99	9.76	8.14	6.26	7.3	ab
Cipovka	8.07	5.73	6.14	9.36	8.65	6.50	7.4	ab
Dragana	8.03	6.03	6.34	9.24	8.33	6.20	7.4	ab
Simonida	8.38	6.35	6.76	9.81	9.45	6.62	7.9	a
NS 40S	8.58	6.19	6.35	9.76	9.74	6.70	7.9	a
Zvezdana	8.66	6.22	6.70	9.58	9.29	6.54	7.8	a
LSD (0.05)	c	d	d	a	b	d		
Max	8.7	6.4	6.8	9.8	9.7	6.7		
Min	4.2	2.3	2.7	5.5	5.4	2.9		
Avg	7.0	5.2	5.6	8.7	7.9	5.5		
StDev	1.26	1.11	0.99	1.19	1.15	1.04		
CV	18.05	21.18	17.73	13.66	14.62	18.95		

[a] Fisher's Least Significant Difference (LSD) Test, $p \leq 0.05$; [b] Different letters indicate significant difference between values at $p \leq 0.05$.

Table 5. Pearson correlations among OC, PC, GY and YOR in 25 winter wheat varieties and six environments.

	E1	**E2**	**E3**	**E4**	**E5**	**E6**	**Average**
OC-GY	0.19	0.05	0.17	0.13	0.08	0.44*	0.34
PC-GY	−0.14	−0.36	−0.88 **	−0.86 **	0.22	−0.74 **	−0.74 **
PC-OC	0.31	−0.03	0.11	−0.21	−0.11	−0.34	−0.09
OC-YOR							0.511 **
PC-YOR							−0.603 **
GY-YOR							0.914 **

Between the average PC and the year of release (YOR), a highly significant negative correlation (r = −0.603**) was found, while positive correlations were determined between OC and the YOR (r = 0.511**) and between the GY and the YOR (r = 0.914**).

3.2. GE Effect on Oil Content

PLSR triplot for oil content in wheat bran (Figure 2) explained 32.6% of the total variance. Grouping of the analyzed genotypes was not very clear and it was mostly based on the year of release.

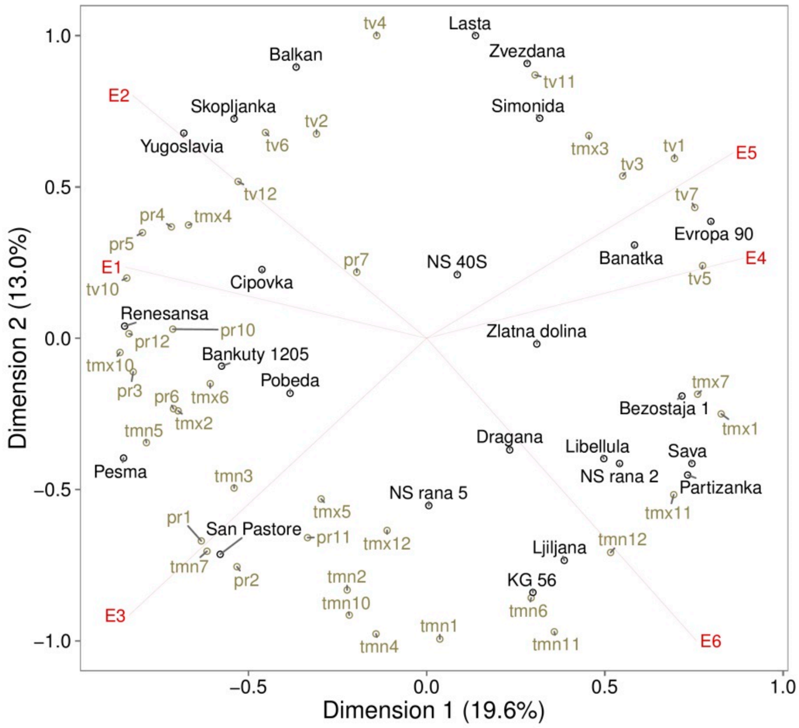

Figure 2. PLSR triplot for oil content of 25 winter wheat varieties in six environments. For variable and environment abbreviations see "Materials and methods" section.

The first group of genotypes (Evropa 90, Banatka, Bezostaja 1, Libellula, Sava, Zlatna dolina, Partizanka, NS rana 2) was placed between E5 and E6 and mainly consists of older varieties released from 1955 to 1975, with similar interactions to the environments in the second growing season (Figure 2). These environments were associated with climatic variables indicating high temperature variations (tv1, tv3, tv5 and tv7) and high maximum temperatures during the winter and in July (tmx11, tmx12, tmx1, and tmx7).

The second group consists of cultivars from different breeding cycles (Dragana, Ljiljana, KG 56, NS rana 5, San Pastore) but with similar average oil content (from 3.78% in cv. San Pastore to 4.04% in cv. NS rana 5) and they were placed between E6 and E3 (Figure 2). This part of the triplot was mainly characterized by high minimal temperatures for 8 out of 10 months (tmn10, tmn11, tmn12, tmn1, tmn2, tmn4, tmn6, and tmn7).

The group of climatic variables around E1 and E3 included high precipitation from October to June, high tmx in October and during the spring and high tmn in March and May (Figure 2). These variables affected the third group of cultivars (Pesma, Pobeda, Bankuty 1205, Renesansa and Cipovka), mostly released from 1990 to 2002. Cultivars Yugoslavia, Skopljanka and Balkan, from the same breeding period (1979–1982), were associated with E2 and climatic variables for high temperature variations (tv12, tv2, tv4, and tv6). The most recent cultivars Simonida, NS 40S and Zvezdana (released from 2003 to 2006) form the group with older cultivar Lasta (released in 1987). They were positioned along the positive part of Y axis, representing the Dimension 2 of PLSR triplot (Figure 2).

The heat map for oil content (Figure 3) shows that the interaction residuals of particular genotypes to different environments were more intensive for OC than in case of PC. A very strong negative interaction was determined for most of the genotypes with at least one environment.

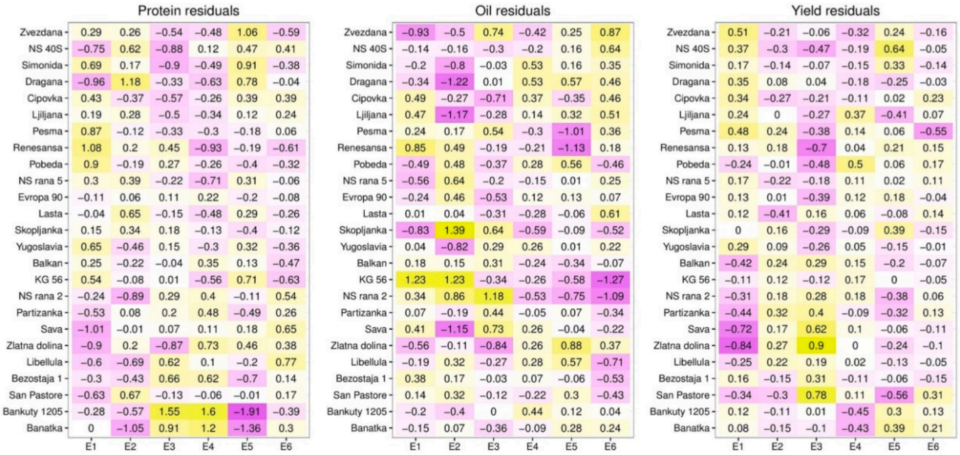

Figure 3. Heat maps with raw interaction residuals between genotypes and environments for PC, OC and GY.

3.3. GE Effects on Protein Content

For protein content in wheat grain, the PLSR triplot (Figure 4) explained 31.9% of the interaction variance and indicated that interaction was very important for this trait in all environments. The first triplot dimension clearly separates environments from different growing seasons (E1, E2 and E3 from the first growing season and E4, E5 and E6 from the second growing season), which indicates unpredictable year effects.

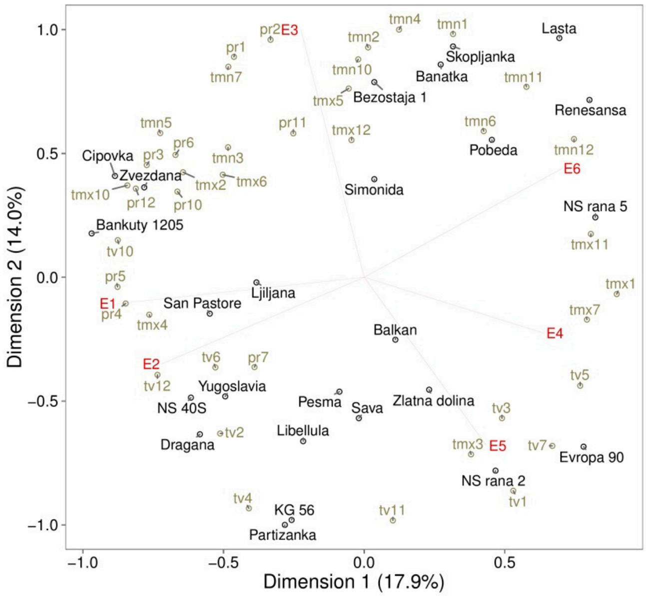

Figure 4. PLSR triplot for protein content of 25 winter wheat varieties in six environments. For variable abbreviations see "Materials and methods" section.

The precipitation was significantly higher during the first growing season with more variation among the environments, associating all precipitation variables in the same half of the triplot, around E1, E2, and E3. The cultivars which were well adapted to the certain conditions are Cipovka, Zvezdana, Bankuty 1205, San Pastore, Ljiljana, Yugoslavia, NS40S, and Dragana. E3 differed from E1 and E2 with higher level of precipitation during the winter (from October to February) and in June, while in July the precipitation was significantly lower than in the other two environments. Cultivars Skopljanka, Banatka, and Bezostaja 1 had positive reaction to high level of winter moisture reserves in the soil (Figure 4).

The highest maximum temperatures in November and January, together with highest minimal temperature in December made the winter in E6 warmer than in other environments (Figure 1). These conditions affected positive interaction of genotypes Pobeda, Renesansa, and NS rana 5 with E6. The group of genotypes between E5 and E2 contains mainly older, not very stable cultivars (Libellula, Sava, Zlatna Dolina, Partizanka, NS rana 2, KG 56 and Balkan), released during the 70s (Figure 4).

E4 had the lowest precipitation in the trial and the most of the varieties had their worst performance in this environment. Similarly, E5 had the second lowest precipitation in the trial and the lowest minimum temperatures in October, resulting in the second lowest average protein content (14.0%). Minimum temperatures in October are very important for soil conditions during the sowing and germination of wheat. E5 was also characterized with the highest temperature variations in January and July (Figure 4).

The heat map with raw interaction residuals between genotypes and environments (Figure 3) showed that with E4 and E5 most of the genotypes had negative values or interaction residuals around 0. A very small number of genotypes had positive values for interaction residuals with these environments. It is interesting that two oldest cultivars Banatka and Bankuty 1205 had positive interaction residuals with E4 and negative interaction residuals with E5. The opposite was found in new cultivars Dragana, Simonida, and Zvezdana.

3.4. GE Effects on Grain Yield

The PLSR triplot for grain yield (Figure 5) divided wheat genotypes into three large groups and few genotypes were positioned out of these clusters. Most of the genotypes had positive interactions with the environments E1, E4, and E5 and two clusters formed around these environments. The first cluster is on the right-hand side of the triplot and consists of genotypes Zvezdana, Yugoslavia, NS 40S, NS rana 2, Bankuty 1205, Simonida, and Cipovka. According to the triplot, they were mainly influenced by temperature variation in different months (tv11, tv1, tv3, and tv7). Also, all climatic variables indicating high precipitation together with high maximum and minimum temperatures during the season had a significant negative association with performance of this group. The second cluster was placed near the triplot centre and contained older genotypes with lower average yield (Banatka, Bezostaja 1, Zlatna Dolina, Libellula, and Partizanka).

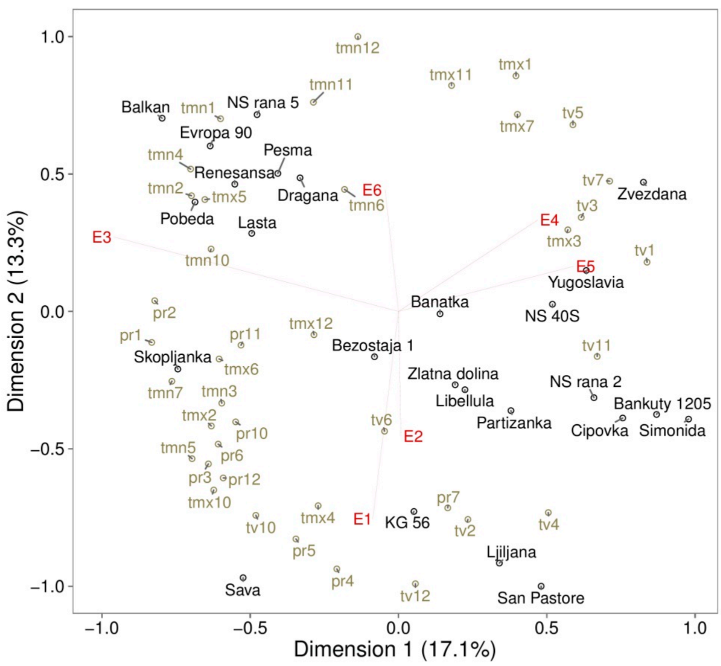

Figure 5. PLSR triplot for grain yield of 25 winter wheat varieties in six environments. For variable abbreviations see "Materials and methods" section.

The genotypes NS rana 5, Balkan, Evropa 90, Pesma, Renesansa, Pobeda, Lasta, and Dragana compose the third group (Figure 4). Most of these genotypes belong to the same breeding cycle and had very similar interaction with all environments and average grain yield around 7 t/ha. The cultivars Skopljanka, KG56, and Ljiljana were outside of these clusters and they had very small interaction residuals with all environments according to the heat map (Figure 3).

Heat map for grain yield (Figure 3) shows higher values for interaction residuals between genotypes and environments in the first growing season than in the second one resulting in lower average grain yield in the first (5.93 t/ha) then in the second year (7.36 t/ha).

4. Discussion

4.1. Genotypic Variations

In this study, 25 winter wheat varieties were used for investigations of OC, PC and GY in six different environments. Significant variations for all investigated traits were found among the genotypes, indicating the great genetic potential for further improvement. Oil content ranged from 2.4-5.6%, PC between 11.2% and 17.8% and GY varied from 2.3-9.8 t/ha, with average CV values of 15.7%, 5.4% and 17.2%, respectively. Moreover, the environments significantly affected PC and GY, while OC had very similar range of variations in all environments resulting in similar average values.

Moor et al. [36] assessed the diverse range of commercial and novel spring wheat cultivars in two years to evaluate the extent of genetic variation for lipid content. They found that the oil content in their collection varied from 4.27% to 5.32%, while in our present and earlier studies [37] wider ranges of variation were found. The reason for this disagreement could be in the fact that our collection contained old and new winter wheat genotypes, while their collection mainly included novel spring wheat genotypes. Moor et al. [36] also stated that increases in embryo size were associated with moderate increases in oil concentration ($r_g = 0.38$, $p < 0.01$). Moreover, they performed phenotyping of the mapping population CD87/Katepwa for grain oil and protein concentration during two years. Total grain lipid and protein concentrations varied significantly in this population ranging from 3.87% to 5.77% and from 11.3% to 15.6%, respectively. These variations are similar, but still smaller than those found in our experiment.

It is well known that sunflower, soybean and rapeseed are the main oil sources, and soybean is the main protein crop, globally. Plant species like castor, flax, chickpea, safflower, false flax, caper spurge, mary thistle, summer savory, coriander, dill, chard, pumpkin, oil gourd, okra and white mustard represent a possible source of oil and proteins of diverse quality. Marjanovic Jeromela et al. [38] investigated seed oil and protein content in 15 oil plant species represented with 1–5 cultivars or local populations. It was found that some species like castor (52.28%) and caper spurge (50.80%) have extremely high oil content. However, safflower had low oil content (8.71%) in comparison with data obtained in other agro ecological conditions. Oil gourd had both high oil (48.04%) and protein content (35.29%) and it could become one of the main cultivated species besides rapeseed and soybean. On the basis of seed protein content, white mustard, oil gourd and false flax could be classified in the same group with soybean as the most important protein plant. Among grain legumes, white lupin and chickpea had the highest protein and oil contents, and as such, could be excellent supplements to soybean.

Wheat oil could be extracted from bran and germ which are the by-products of wheat milling industry. These by-products contain valuable fat-soluble substances, such as tocopherols, sterols, carotenoids, steryl ferulates and others, having biological effects [39–41]. The oil content from wheat bran (3–5%) or germ (7–9%) is much lower than that from main oil crops, such as sunflower (46%), safflower (32%), rapeseed (39%), soybean (20%), and linseed (34%). Also, the protein content of wheat (11–18%) is lower but better comparable with those reported for some conventional oil and protein crops like safflower (29.3%), sunflower (19.5%), peanut (30.3%), and rapeseed (21.5%) [42–44]. Since wheat is widely present in human consumption on a daily bases, it could therefore serve as an alternative source of protein and oil. Also, the wheat bran and germ oils are rich in unsaturated fatty acids and minor components and it is expected to have number of beneficial health effects: reducing serum cholesterol levels [40,45], inhibiting tumoroigenesis [46,47], anti-diabetic properties [48], and others. Some of the nutrients, like carotenoids (β-carotene, pro vitamin A), tocopherols (vitamin E), cannot be synthesized de novo and hence have to be supplemented exogenously through foods. These are the reasons why wheat germ and bran oils have increasingly been investigated in the previous years. In order to improve these health beneficial effects of wheat, it is necessary to identify the sources for further simultaneous improvement of oil and protein contents in new wheat cultivars together with breeding for higher yield. In order to successfully achieve these breeding goals, it is also necessary to investigate the relationships between these traits, as well as the main factors influencing their expression.

In the present study, significant positive correlation between OC and GY (r = 0.441*) and highly significant negative correlation between PC and GY were found (Table 5). However, these correlations are environmentally dependent and could be modified by certain growing conditions. Since OC-GY association was found only in E6 with conditions which caused a significant decrease in grain yield, this correlation probably would not be very useful for the breeders, unless if it were possible in the future studies to distinguish climatic variables which stimulate the positive correlation from those which reduce the yield. The negative PC-GY correlation is also environmentally dependent, and it will be very valuable for breeding process to identify climatic variables and growing conditions that can reduce it, in order to allow successful breeding for improvement of both traits at the same time. When we analyze the growing environments and climatic variables, it can be concluded that the conditions which are very favorable for one of the traits stimulate a negative correlation, while in suboptimal conditions for both traits, the correlation is not significant.

On average for all six environments, PC was negatively correlated with GY (r = −0.74**), whereas the association between OC and GY (r = 0.34), and between oil and protein contents (r = −0.09) were not significant. These associations are present in wheat, but also in other crops [37,49–51] which makes it difficult to develop productive cultivars with high contents of protein and oil [52]. In our study, cvs. Renesansa and Zvezdana were identified as the genotypes with high yield and satisfactory average values for quality traits (protein content over 14%, oil content over 4%, and grain yield over 7 t/ha), while cvs. Bankuty 1205 and Banatka were identified as the potential sources of high protein content. These genotypes can serve as parents in wheat breeding for high quality and yield.

Between the average PC and the year of release (YOR) a highly significant negative correlation (r = −0.603**) was found, while positive correlations were determined between OC and the YOR (r = 0.511**) and between the GY and the YOR (r = 0.914**). It means that the older cultivars had higher protein content, but lower oil content and yield, than the new ones. These results also suggest presence of the negative correlation between PC and OC, which was not established in this study. This could be explained in two different ways. First, one should assume that this correlation is also environmentally dependent, as during our experiment, we did not have conditions which favor such a correlation, but during the 50-year-long breeding period, these conditions occurred in a significant number of years. The other explanation could be that OC and PC are not directly associated traits, but continuous long-term breeding process towards high-yield and simultaneously higher quality [53,54], resulted in an increase of OC in new cultivars, probably due to its positive correlation with some other quality trait that was forced during the selection. On the other hand, the established negative correlation between PC and GY had negative implications to protein content in new cultivars. This was also proven in the study of Hristov et al. [54], in which the annual decrease in protein content of 0.03% over the 40-year study period was reported. Even this value was not significant; it is obvious that the old cultivars had higher PC, but low yields and final quality. This is also in agreement with the results of many other studies [51,55,56].

4.2. GE Effects on OC, PC and GY

It can be clearly seen from Figure 1 that the maximum temperatures in all environments were significantly higher during the whole growing season, while the minimum temperatures were significantly lower than the 30-year (1981–2010) average values, causing very high temperature variations (mostly from 20 to 35 degrees in all environments). Also, minimum temperatures below 0 °C were recorded from October to March in E1, E2, E4 and E5, while according to the 30-year average values, this should occur from December to February. Generally, the minimum temperatures varied more among the environments than maximum temperatures, and more in the first than in the second growing season.

PLSR triplots for all the traits explained around 30% of the total variance (OC 32.6%, PC 31.9% and GY 30.4%) indicating the presence of other sources of variations, not included in the analyses. For larger percentage, it will be necessary to include in future studies other variables which were not used in this study.

The grouping of the genotypes, environments and climatic variables in PLSR triplots was different for analyzed traits. Since there was no significant effect of environment on oil content in wheat bran, grouping of the genotypes in this PLSR triplot (Figure 2) was mostly based on the year of release. In the other two triplots (Figures 4 and 5) the grouping according genotype's reaction to specific environment and climatic variables was more pronounced.

In the PLSR triplots for OC and PC, the environments from the first and second growing season (E1, E2 and E3 from the first growing season and E4, E5 and E6 from the second growing season) were clearly separated by the first triplot dimension, indicating unpredictable year effects. The first growing season was characterized by very high maximum temperatures in October (around 30 °C, 30-year mean is 18.2 °C), very low tmn in December (around −20 °C in E1 and E2, −10 °C in E3, 30-year mean is −1 °C) and very high temperature variations in these two months (Figure 1). Also, the precipitation was significantly higher during the first growing season with more variation among the environments, associating all precipitation variables in the same half of the triplots, around E1, E2, and E3. In the case of GY, this kind of separation was not clearly present.

The heat maps (Figure 3) showed that the interaction residuals of particular genotypes to different environments were more intensive for OC and GY than in case of PC. Regarding the OC very strong negative interaction was determined for most of the genotypes with at least one environment. E3 and E4 had less pronounced negative interactions with the genotypes and they had the highest average oil content. A small number of mostly older genotypes (Banatka, Bankuty 1205, San Pastore, Partizanka, Balkan) and NS 40S from the latest varieties had very small interaction with all environments and they can be considered stable genotypes for this trait. All other genotypes had stronger interactions, either positive or negative, with some of the environments. However, the heat map for PC (Figure 3) shows that in the environments with the highest average protein contents (E1, E2 and E3), most of the genotypes had very small positive or negative values for interaction residuals, indicating that high protein content is very rarely associated with positive interaction between genotype and the environment; more often, it is a result of genotype's ability to adapt and tolerate different conditions. On the same heat map, it is possible to identify a set of genotypes (from Skopljanka to Renesansa) which had positive or neutral interaction with the first growing season and stronger negative interaction with the second one. These cultivars were positioned on the right-hand side of the PLSR triplot (Figure 4). In contrast to this, there was a group of genotypes (from San Pastore to NS rana 2) with negative reactions to the first and neutral or positive reaction to the second growing season. On the PLSR triplot the grouping of these cultivars was not very obvious, but they were mostly positioned on the bottom of the triplot. Since the amount of precipitation is the main difference between the seasons, it could be an indicator how, in these genotypes, high or low amounts of water during the season affect the protein content.

Rodrigues et al. [52] reported that water stress and high temperatures reduce both protein and oil contents in soybean seeds, while Rotundo and Westgate [57] concluded that temperature has a more pronounced effect on oil content, but it also affects protein content. Barbosa et al. [58] found that higher temperatures during grain filling favored oil accumulation in soybean. In our study, the first growing season had higher precipitation and also higher temperatures in April, May and June, while in the second season water stress was present in all three environments, with slightly lower spring temperatures. The protein content was higher in the first growing season, while for oil content there was no difference between the seasons. It can be concluded that protein content in wheat was more affected by water stress than by high temperatures, while the oil content was less affected by both stresses.

The heat map for grain yield (Figure 3) shows higher values for interaction residuals between genotypes and environments in the first growing season than in the second one, resulting in lower average grain yield in the first (5.93 t/ha) then in the second year (7.36 t/ha). Even the first season had higher precipitation than the second one; the distribution of rainfall was not very good. A large amount of precipitation in May (from 109.2 mm to 159.6 mm, or 2 to 3 times more than the average values), when flowering occurs in our agro-ecological conditions, probably caused poor pollination, and consequently, a small number of formed grains and yield loss.

According to the results, the best and the worst environments for wheat grain yield were E4 and E2, respectively. The triplot for grain yield (Figure 5) showed that some climatic variables were positively or negatively associated with these environments. E4 was positively associated with high maximum temperature in March (tmx3) and with temperature variations in March and July (tv3 and tv7). This environment had a strong negative association with climatic variables indicating high precipitation and high temperatures in winter and spring. E2 was associated with high precipitation, especially in April and May, and negatively associated with high winter temperatures (tmn11, tmn 12, tmx11). All of this confirms our assumption that low yield in the second season was caused by very high precipitation during flowering and other important developmental stages in spring.

In the study on physiological factors associated with G×E interaction in wheat [59], it was suggested that bread wheat favors warmer night temperatures and lower photothermal quotient during the spike primordia stage when potential grain number is being determined. This is in agreement with our findings that high yielding environment was positively associated with high temperatures in March, when this stage usually occurs in our ecological conditions. Also, Fisher [60] suggested that yield in bread wheat is most sensitive to environmental conditions during the rapid spike growth stage (during April in the Southeast European conditions), grain number being increased by lower average temperature and higher solar radiation, which corresponds with our findings that high maximum temperature in April were negatively associated with the most productive environment (E4).

These results indicate that even in wheat, which is well adapted to a wide range of environments, extreme climatic conditions can cause a significant decrease in some important agronomical traits. Similar results were obtained in other studies with different crop species [34,61]. Also, variable genotype reactions to different environments confirm the importance of evaluating breeding collections in order to identify the optimal genotype for specific growing conditions [34,62].

5. Conclusions

The obtained results showed a large level of variation in the material for all investigated traits in different environments. The most variable traits were GY and OC, while the variations of PC were significantly lower. PC was negatively correlated with GY ($r = -0.74^{**}$), whereas the associations between OC and GY ($r = 0.34$), and between oil and protein contents ($r = -0.09$) were not significant. The established correlations between the traits and the year of release of the genotypes showed that the older cultivars had higher protein content, but lower oil content and grain yield compared to the new ones. The PLSR triplots showed specific associations of the traits, genotypes, environments and climatic variables, enabling us to identify favorable and limiting climatic conditions for each trait. It was possible to identify stable genotypes with high average values for each of the traits. The identified genotypes can serve as parents in wheat breeding for higher oil and protein content combined with high yield.

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