# Analysis on some qualitative traits of Bulgarian triticale cultivars

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#### Abstract

In order to identify the triticale genotypes, which combine high productivity with qualitative traits, eleven Bulgarian cultivars were studied during three contrasting periods. Protein content in grain, lysine content and protein yield were assessed. Based on the suggested biplot method, the genotypes were determined, which were characterized by values of protein and lysine content above the average of the studied set of cultivars. A 3D-method is suggested for determining of the best combinations of yield with protein and lysine content. The obtained results showed that the variation of the protein content, averaged for the period of study, was comparatively low – between 10.16 and 11.58 %. Between the separate growing periods, however, significant differences were observed – from 8.06 to 12.83 %. With regard to lysine content, a rather different tendency was observed. The world standard Presto was with the lowest values, and Atila – with the highest. No significant correlation was found between protein content and grain yield and between lysine content and grain yield with lysine content was registered only in cultivars Atila and Bumerang. A good combination of grain yield with lysine content and lysine content was found. Such results showed that cultivars Atila and Bumerang were valuable initial material for improvement of the protein and lysine content in the breeding program of triticale in Bulgaria.

Keywords: triticale; protein; lysine; yield; quality

#### INTRODUCTION

A main task of contemporary agricultural production in relation to the provision of quality plant raw materials is the diversification of the cultivated plants. Such a task is dictated by the constantly growing global population and by the increasing demand for food and forage. Triticale is one of the crops with most varied usage both as food and forage. Although the crop was developed by man as an amphidiploid between wheat and rye more than 100 years ago, in the last two decades it has become a universal plant raw material. High-quality forage grain with rich protein and amino acid composition can be produced from this crop, as well as green mass with very good fodder properties. These peculiarities of triticale determine the main task of its breeding – developing genotypes, which combine high productivity with high protein content of rich amino acid composition.

In triticale, as in other cereals crops, different researches (Gulmezoglu et al., 2010; Ozturk et al., 2019; Saed-Moucheshi et al., 2019) show that yield and protein content are often negatively correlated. Therefore, a thorough investigation of these parameters and their combination in optimal values is needed. Rapp et al. (2018) point out that especially efficient for combining yield with protein content are the parameters protein yield and deviation of protein in grain. At the same time, yield is largely a dynamic value and in triticale it often varies according to the environment (Stoyanov & Baychev, 2018). Although varied results have been reported with regard to yield from triticale, the protein content of this crop has been comparatively less studied.

Wojtkowiak et al. (2015) pointed out that the higher doses of nitrogen fertilization and the conditions of the environment had the highest effect on the protein yield from cultivar Milewo, which the authors studied. Lestingi et al. (2010) demonstrated that the protein content in triticale was influenced by both the soil tillage system and the amount of applied fertilizers. Gulmezoglu & Aytac (2010) reported that in the six triticale genotypes they investigated, the protein content varied from 10 to 18 %, depending on the genotype, the growing conditions and the fertilization regime. Similar results were reported also by Tababtabaei & Ranjbar (2012), Wojtkowiak et al. (2013), Burešova et al. (2010), Dumbravă et al. (2016), Wozniak & Soroka (2014). Kızılgeçi (2019), on his part, reported low variation of the location when studying three cultivars and two lines of triticale. On the other hand, Kendal & Sayar (2016) pointed out that the content of protein depended highly on the genotype, being also influenced by the conditions of the environment in a study on twenty cultivars and three lines of triticale. Salehi & Arzani (2013) also noted that the protein content was significantly affected by the genotype, the environmental conditions and their interaction, similar to the yield from this crop. At the same time, these authors registered negative correlation between protein content and yield. Goyal et al. (2011) also reported a negative correlation between the two traits. While investigating 1006 triticale accessions during 1982 - 2008, Ukalska & Kociuba (2013) found out that the mean protein content in triticale was 12%, varying between 6.3% and 15.7% according to the investigated sub-periods.

Even less studied is the lysine content of the contemporary triticale genotypes. Peña (2004) pointed out that higher lysine content was observed in the older triticale genotypes characterized by small and shriveled grain. The results of Jaśkiewicz & Szczepanek (2018) showed that the content of lysine was affected both by the triticale cultivar and by the conditions of the environment and the applied agronomy practices. These authors noticed that there were no significant correlations between lysine content, protein content and yield.

The negative correlations or the absence of correlations between yield and the content of protein and the content of lysine indicates that the three traits are very difficult to combine within a single genotype. At the same time, the above investigations show that similar to yield, both protein content and lysine content, although determined by the genotype, are significantly influenced by the conditions of the environment and the growing technology. Therefore, in breeding for simultaneous increasing of productivity and grain quality, it is necessary to study in detail these three parameters and their correlations.

The main goal of this study was to investigate Bulgarian triticale cultivars for their yield, protein content in grain and lysine content and to identify the genotypes, in which the most favorable combination of these traits is present.

#### **MATERIALS AND METHODS**

#### **Plant material**

To implement the above goal, eleven Bulgarian triticale cultivars, presented in Table 1, were involved. The studied cultivars were grown as a whole-area crop in experimental plots of 10 m<sup>2</sup>, in four replications according to a standard block design within a competitive varietal trial. Sowing was mechanized, within the standard dates, at density 550 seeds per m<sup>2</sup>. Besides the above cultivars, the competitive varietal trial also included the standard cultivars AD-7291, Vihren and Rakita, as well as the world standards Lasko and Presto. The plots were harvested at full maturity, reading the yield from each plot separately.

#### Growing conditions

The experiment was carried out in three successive harvest years - 2015/2016, 2016/2017, 2017/2018. The presented data on the average monthly temperature and the total monthly precipitation (Table 2) reveal the contrasting nature of the investigated periods. The highest differences according to the long-term tendency with regard to temperature were observed during December-March, and with regard to precipitation - in December and May. The differences in these periods were a sufficient reason to consider that the vegetative growth occurred in different ways during the separate years. Certain meteorological events and processes were clearly outlined, which were of single occurrence, were not repeated over periods and were capable of strongly influencing the physiological processes in the plant organism.

No	Name	Origin	Year of registration
1	Kolorit	BGL "S" – BGC / 568-343	2005
2	Atila	AD 8x(Ep 1034/79 x Harkovska 60) /F <sub>1</sub> [F <sub>1</sub> (Yuzhnaya zrya/ Harkovska 60) / 804-503]	2007
3	Akord	MT-3 / $F_2$ populations	2007
4	Respekt	1262-12-2-10 / Veleten	2008
5	Bumerang	LP 3090.91 / 2853-1044	2009
6	Irnik	5252 - 131 / 2853-1044	2011
7	Dobrudzhanets	Chrono / 2853-1044	2012
8	Lovchanets	F <sub>1</sub> (Tornado / 3493-699) / Zaryad	2013
9	Doni 52	5279-131 / 3370-190	2014
10	Blagovest	32/99 / Zaryad	2015
11	Borislav	46/95-96 / 129/98	2016

Table 1. Cultivars used during the period of study

Table 2. Average monthly temperature (AMT) and total monthly precipitation (TMP) during the period of study

Parameter	Year	Sep	Oct	Noe	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
	2015/2016	19.5	10.9	9.3	3.4	-0.8	7.3	6.8	13.2	14.7	20.9	22.8
AMT, °C	2016/2017	18.1	10.6	6.5	-0.6	-4.1	2.0	7.3	8.7	15.0	20.2	21.8
AMI, C	2017/2018	19.0	11.8	7.5	4.7	1.7	1.1	4.6	13.4	17.7	20.4	22.2
	1960/2019	16.9	11.7	6.8	2.0	-0.2	1.1	4.7	9.9	15.2	22.0	21.4
	2015/2016	20.8	78.3	55.1	0.4	86.3	40.7	52.7	20.8	117.1	55.7	2.8
TMD mm	2016/2017	35.8	72.2	43.3	12.5	48.4	27.4	48.9	38.4	29.0	87.7	66.3
TMP, mm	2017/2018	69.9	50.5	57.2	55.8	75.4	48.8	4.9	30.9	90.8	59.6	59.6
	1960/2019	46.3	42.1	43.4	41.7	36.9	34.2	35.6	40.5	52.1	58.7	52.2

Growing periods 2015/2016 and 2017/2018 are worth of special attention due to the extreme intensive and long-lasting rainfalls during May (2015/2016) and untypical daily intermittent rainfalls observed. Highly unfavorable for growing of triticale was 2015/2016 due to the long-lasting, uneven and intensive rainfalls during May-June. At the same time, most favorable for growing of triticale were the conditions of 2016/2017, when the lowest number of negative events during the vegetative growth of the plants occurred.

# Determining the content of protein and lysine in grain

The content of raw protein and lysine in the grain of triticale were determined by standard methods at the Biochemistry Laboratory of Dobrudzha Agricultural Institute – General Toshevo. The content of raw protein was established through the Kjeldahl method for determining the total nitrogen by using Keltek equipment (Cohen, 1910). The method of Kjeldahl is used to determine protein in the meal from the respective cereal crop. The proteins are nitrogen-containing compounds and the automated determining of the protein is reduced to determining the total nitrogen content in the sample. The mean coefficient of nitrogen transformation to protein is 5.7 for triticale. The content of the essential amino acid lysine was determined by the method of Museiko & Sysoev (1970). Lysine is a diaminocarbonic acid. The acid residue of lysine in the protein macro molecule contains an amino group, which undergoes a color reaction with ninhydrin. The intensity of coloration, determined by the photocolorimetry method, reflects the content of essential amino acid lysine in the protein of the sample, the content of which in the triticale meal is an important indicator of grain quality.

### Statistical analysis

The obtained data were summarized and averaged. An ANOVA was carried out over years and for the entire period of investigation. The significant differences for each of the studied parameters were established. In order to evaluate the combination of high values of protein and lysine content with high productivity of the same genotypes and periods, the yield over cultivars and years was evaluated. The protein yield was also determined according to a formula suggested by Neuweiler et al. (2021). A graphic biplot method was used to group the genotypes by comparing the values of yield and protein content, the yield and lysine content, and the protein and lysine content. To simultaneously determine the best combinations of yield, protein content and lysine content, a 3D-graphic method was used. A correlation analysis was performed between all studied traits. To summarize and average the data, Microsoft Office Excel 2003 was used. IBM SPSS Statistics 19 was applied for ANOVA and for the correlation analysis, and module 3d scatter plot for Excel was used for graphic interpretation of the results (Doka, 2013).

# RESULTS

# Protein content

During the three growing periods, as a result from the effect of the environmental conditions. the values of protein content in the grain of the investigated triticale cultivars differed significantly. The highest mean protein content was observed in 2016/2017, and the lowest - in 2017/2018. This tendency was noticed in all studied cultivars. The low protein content in 2017/20118 was related to the later harvesting in this year due to the frequent intermittent rainfalls in July of 2018. At the same time, the lower content of protein in 2015/2016 was due to the uneven intensive rainfalls in June of 2016, which, on the one hand, caused lodging in some cultivars, and on the other impeded grain filling due to the cooler weather and the reduced photosynthetic activity of the plants.

In 2015/2016, cultivars Lasko, Presto and Bumerang were with the highest protein content, and Akord, Irnik and Doni 52 – with the lowest. The rest of the cultivars, with the exception of Respekt and Dobrudzhanets, were at the level of the mean value of the investigated set of cultivars. Respekt was with respective significantly higher values, while Dobrudzhanets – with significantly lower ones. None of the cultivars exceeded significantly Lasko and Presto by this trait. The standards AD-7291, Vihren and Rakita did not differ significantly between themselves. In comparison to the standard with the higher protein content (Rakita), significantly higher values were read in Lasko, Presto, Respekt and Bumerang.

The standard cultivars differed significantly in 2016/2017. AD-7291 and Rakita significantly exceeded the values of Vihren. At the same time, the world standards Lasko and Presto were with the highest values in this growing period, too. None of the studied cultivars exceeded them by protein content. Only cultivar Kolorit exceeded significantly the standards AD-7291 and Rakita. At the level of Rakita were Bumerang and Lovchanets. With highest values were cultivars Dobrudzhanets and Doni 52, which were significantly below the results of the standard Vihren.

An entirely different tendency with regard to protein content was observed in 2017/2018. Cultivars Kolorit, Akord and Respekt were with the highest values, and Vihren, Rakita and Lovchanets – with the lowest, respectively. The world standards Lasko and Presto realized very low values during this period, 7.98 and 7.89, respectively. Cultivar Lovchanets exceeded all cultivars at the highest level of significance. Cultivar Irnik was also with high values.

Averaged for the three investigated periods, the three standard cultivars did not differ significantly between themselves. At their level were cultivars Kolorit, Atila and Bumerang. The world standards Lasko and Presto considerably exceeded the rest of the cultivars by protein content and only cultivar Lovchanets was at their level. All other cultivars were with lower protein content. The lowest values, averaged for the three studied periods, were registered in cultivars Akord, Dobrudzhanets and Doni 52.

### Lysine content

The lysine content was also highly influenced by the conditions of the environment. The data from the three studied periods showed that the highest val-

Cultinum		Protein co	ontent, %	Lysine content, mg/100g a.d.m					
Cultivar	2015/2016	2016/2017	2017/2018	Average	2015/2016	2016/2017	2017/2018	Average	
AD-7291	11,21	13,36	8,26	10,94	329	356	290	325	
Vihren	11,26	12,59	8,73	10,86	363	303	307	324	
Rakita	11,53	13,20	8,50	11,08	390	301	285	325	
Lasko	12,48	13,95	7,98	11,47	411	308	276	332	
Presto	12,70	14,16	7,89	11,58	390	240	278	303	
Kolorit	11,17	13,75	7,61	10,84	403	310	282	332	
Atila	11,53	13,10	8,11	10,91	471	310	297	359	
Akord	10,94	12,30	7,24	10,16	405	296	282	328	
Respekt	11,88	12,12	7,26	10,42	395	291	268	318	
Bumerang	12,29	13,06	7,91	11,09	421	303	282	335	
Irnik	10,43	12,78	8,31	10,51	411	305	297	338	
Dobrudzhanets	11,10	11,76	7,63	10,16	385	296	258	313	
Lovchanets	11,33	13,37	9,77	11,49	411	291	280	327	
Doni 52	10,84	11,78	7,94	10,19	400	287	290	326	
Blagovest	11,23	11,90	7,69	10,27	411	254	282	316	
Borislav	11,24	11,96	8,07	10,42	416	292	256	321	
Average	11,45	12,82	8,06	10,78	401	296	282	326	
LSD0.05	0,30	0,39	0,30	0,24	14,5	12,3	6,6	6,1	
LSD0.01	0,39	0,51	0,39	0,31	19,0	16,1	8,7	8,0	
LSD0.001	0,50	0,65	0,50	0,40	24,3	20,6	11,1	10,2	

Table 3. Protein and lysine content of Bulgarian triticale cultivars

ues of this trait were registered in 2015/2016. During the other two periods, although similar mean values were observed for all investigated cultivars, the individual genotypes reacted without following any particular tendency. This was an indication that certain cultivars responded in different ways with their lysine content to differing conditions of the environment. Noteworthy is the fact that the content of lysine in the grain was higher in the period, in which the productivity of triticale was considerably lower due to the unfavorable growing conditions.

In 2015/2016, the highest values of this trait were observed in cultivars Atila, Bumerang and Borislav. Respectively much lower were the results for AD-7291, Vihren and Dobrudzhanets. The standard cultivars differed significantly between themselves, Rakita being with the highest lysine content, followed by Vihren, and the standard AD-7291 was with the lowest values. Cultivars Lasko, Presto, Kolorit, Akord, Respekt, Irnik, Lovchsnets, Doni 52 and Blagovest were at the level of the mean value of the entire investigated set of cultivars. This revealed the considerable similarity of the studied accessions with regard to their response to the conditions of the environment.

A different tendency was observed with regard to 2016/2017. With highest values of lysine content were cultivars Lasko, Kolorit and Atila, while Presto, Doni 52 and Blagovest were with the lowest. The standard cultivars Vihren and Rakita were with equal values, while AD-7291 significantly exceeded both. At the level of the mean value of the studied set (respectively also at the level of the standards Vihren and Rakita) were Akord, Bumerang, Irnik, Dobrudzhanets, Lovchanets and Borislav. None of the cultivars exceeded the value of the standard AD-7291.

The highest values in 2017/2018 were determined in the standard Vihren and in cultivars Atila and Irnik. Cultivars AD-7291 and Rakita were with lysine content significantly lower than that of Vihren. Cultivars Lasko, Presto, Respekt, Dobrudzhanets and Borislav were with the lowest values, which were significantly lower than all studied cultivars. The values of the rest of the cultivars were rather similar, close to the average value of the entire investigated set.

Averaged for the investigated period, the highest lysine content was observed in cultivar Atila, this value being a result from a permanent tendency, regardless of the conditions of the environment during the studied period. High mean values were registered also in Bumerang and Irnik, but their variation according to the environmental conditions was considerably higher. The standard cultivars were equal both between themselves and in comparison to the average value of the entire investigated set of cultivars. Presto, Respekt, Dobrudzhanets and Blagovest were with the lowest values, and all other cultivars were at the level of the standards. A negative tendency was observed in cultivar Dobrudzhanets during two of the three studied periods towards realizing lower lysine content.

#### Grain yield and protein yield

The results on the yield from the studied cultivars revealed that the formed tendencies differed consid-

erably from the tendencies in protein content. At the same time, the productivity of the genotypes varied within a significantly wider range emphasizing the high impact of the environmental conditions on this trait. During the studied period, averaged for three years, only cultivar Doni 52 was with the highest productivity significantly exceeding the productivity of the better standard (Rakita). Cultivars Atila, Bumerang and Borislav were at the level of Rakita. With values significantly below the standard with the lower performance (AD-7291) were Lasko, Presto, Respekt, Irnik and Lovchanets, the lowest values being determined in cultivar Lovchantes. These results showed that often the high protein content did not imply high yields, and the combination of such values was comparatively rare.

The use of the cumulative value protein yield allowed following what part of the productivity of the studied genotype was related to its protein content. At the same time, the multiplicative combination of the two parameters allowed determining the effects of the environment simultaneously on the yield and the protein content. Thus, it was possible to identify

	Table 4. Yield and	protein viel	d of Bulgarian	triticale cultivars.
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Cultivar	tiver Yield, kg/dca Protein yield							
Cultivar	2015/2016	2016/2017	2017/2018	Average	2015/2016	2016/2017	2017/2018	Average
AD-7291	596	602	637	612	66,8	80,4	52,6	66,6
Vihren	540	631	745	639	60,8	79,4	65,0	68,4
Rakita	529	735	717	660	61,0	97,0	60,9	73,0
Lasko	455	614	631	567	56,8	85,7	50,4	64,3
Presto	436	681	624	580	55,4	96,4	49,2	67,0
Kolorit	543	609	661	604	60,7	83,7	50,3	64,9
Atila	632	757	609	666	72,9	99,2	49,4	73,8
Akord	552	692	584	609	60,4	85,1	42,3	62,6
Respekt	320	679	563	521	38,0	82,3	40,9	53,7
Bumerang	424	776	745	648	52,1	101,3	58,9	70,8
Irnik	521	603	634	586	54,3	77,1	52,7	61,4
Dobrudzhanets	469	770	664	634	52,1	90,6	50,7	64,4
Lovchanets	378	489	624	497	42,8	65,4	61,0	56,4
Doni 52	611	745	700	685	66,2	87,8	55,6	69,9
Blagovest	543	713	636	631	61,0	84,8	48,9	64,9
Borislav	605	711	602	639	68,0	85,0	48,6	67,2
Average	509	675	649	611	58,1	86,3	52,3	65,6
LSD0.05	43,4	38,5	26,4	25,2	4,43	4,49	3,23	2,65
LSD0.01	57,1	50,6	34,7	33,1	5,82	5,91	4,24	3,48
LSD0.001	72,9	64,7	44,3	42,3	7,44	7,55	5,42	4,44

the genotypes, which combined high productivity with high protein content in grain.

The results obtained for protein yield revealed the high effect of the environmental conditions on the mean value of all studied genotypes. The highest protein yield was measured in 2016/2017. At the same time, lower were the values in 2015/2016, and lowest - in 2017/2018. This parameter largely followed the tendency of the mean values of protein content with regard to the separate periods. Nevertheless, significant differences were observed in the individual genotypes according to both the yield and the protein content. This was related to the multiplicative effect of this parameter and the interaction between the different tendencies of the two parameters during the separate contrasting periods. The protein yield of the studied genotypes varied within a comparatively narrow range similar to protein content, but was influenced considerably by the conditions of the environment.

In 2015/2016, cultivars Atila and Borislav were with the highest protein yield. Only cultivar Atila exceeded significantly the better performing standard AD-7291 by this parameter. The other two standards (Vihren and Rakita) were at the level of the mean value of the entire investigated set. Cultivars Lasko, Presto, Kolorit, Akord and Blagovest were also at their level.

Respekt, Bumerang, Irnik, Dobrudzhanets, Lovchanets and Doni 52 were with significantly lower values than the standard with lower performance. Cultivars Respekt and Lovchanets were with the lowest values of protein yield.

Significantly, higher values of protein yield were read in 2016/2017 in all investigated cultivars according to the previous period. With highest values were Presto, Atila and Bumerang, and with lowest – Irnik and Lovchanets. In the studied standard cultivars, Rakita exceeded significantly the values of AD-7291 and Vihren. At the level of Rakita were Presto, Atila and Bumerang, but Atila and Bumerang were with higher values. None of the studied cultivars exceeded significantly Rakita. Cultivars Lasko, Kolorit, Akord, Respekt, Dobrudzhanets, Doni 52, Blagovest and Borislav were at the level of the mean value of the entire investigated set.

In the following growing period (2017/2018), considerable differences were observed between the standard cultivars, all three of them being significantly different between themselves. Vihren was

with the highest protein yield, followed by Rakita, and the standard AD-7291 was with the lowest values. Among the studied cultivars, Bumerang and Lovchanets were with the highest values, and Akord and Respekt – with the lowest. None of the cultivars exceeded significantly the better performing standard Vihren. At the level of the mean value of the entire investigated set were Lasko, Presto, Kolorit, Atila, Irnik, Dobrudzhanets and Doni 52. Significantly below the mean value were cultivars Akord, Respekt, Blagovest and Borislav.

Averaged for the investigated period, the highest values of the parameter protein yield were observed in cultivars Atila and Bumerang and in the standard Rakita. At the same time, the lowest results were read in cultivars Respekt and Lovchanets. A tendency was observed in Atila and Bumerang towards realizing higher values under differing conditions of the environment, while in Respekt and Lovchanets there was a tendency towards lower values. This indicated that protein yield within a single genotype was closer to grain yield than to protein content.

The results of the correlation analysis (Table 5) clearly prove the above statements. In the short term (individual harvest years) there is a higher correlation of protein yield with grain yield than protein yield with protein content. Such a correlation was emphasized also by the high value of the correlation coefficient between grain yield and protein yield (0.608) in long term. With regard to the protein content during the individual periods, there is practically a weak correlation with the yield, although insignificant in 2016/2017 and 2017/2018 but higher than with protein yield (except in 2017/2018). This, however, was valid within a given growing period. In a long-term perspective, the protein content was in higher correlation with the protein yield than with the grain yield. This was related to the fact that the mean values of the protein yield over growing periods followed the tendency more of the protein content than the tendency of the yield. At the same time, there was no correlation between protein content and yield. An exception from this tendency was only 2015/2016, when a negative significant correlation was observed of yield with protein content. Such an effect was related to the fact that due to the high precipitation norms in June of 2016 and the insufficient grain filling, the yields remained low but the protein content did not vary that much.

Trait	Protein content	Lysine content	Yield	Protein yield
		2015/2016		
Protein content	1	0,099	-0,515*	-0,258
Lysine content	0,099	1	0,051	0,095
Yield	-0,515*	0,051	1	0,961**
Protein yield	-0,258	0,095	0,961**	1
		2016/2017		
Protein content	1	0,140	-0,463	0,081
Lysine content	0,140	1	-0,225	-0,168
Yield	-0,463	-0,225	1	0,845**
Protein yield	0,081	-0,168	0,845**	1
		2017/2018		
Protein content	1	0,343	0,290	0,773**
Lysine content	0,343	1	0,406	0,480
Yield	0,290	0,406	1	0,830**
Protein yield	0,773**	0,480	0,830**	1
		2015-2018		
Protein content	1	0,320*	-0,127	0,707**
Lysine content	0,320*	1	-0,627**	-0,192
Yield	-0,127	-0,627**	1	0,608**
Protein yield	0,707**	-0,192	0,608**	1

#### Table 5. Correlations between the studied traits

# Combining of yield, protein content and lysine content

The protein content gives a comparatively good idea for the combining of protein content and grain yield in the studied genotypes. Nevertheless, in combinations such as low protein content with high yield, and high protein content with low yield, the values of protein yield were equal or rather similar and therefore the exact breeding value of a given investigated genotype could not be determined. One of the most simple and most efficient methods for determining the combination of the two traits, in which there were no correlation, was their graphic representation by a biplot.

Figure 1 presents a biplot combining the data on the protein content and the yield from the investigated triticale cultivars, averaged for the period of growing. The graph is divided into four quadrants based on the mean values of the two parameters. Their combinations resulted in varied grouping of the genotypes. The best combination of high protein content with high yield was observed in cultivars Atila and Bumerang, and in the standard Rakita. Similar was the behavior of the standards AD-7291 and Vihren and of cultivar Kolorit, although at lower values of yield. High protein content but low productivity was read in the world standards Lasko and Presto and in cultivar Lovchanets, the yield values of Lovchanets being rather low. On the other hand, high productivity but low values of protein content were combined in cultivars Dobrudzhanets, Doni 52, Blagovest and Borislav. The most unfavorable combination of the two investigated parameters was found in cultivars Akord, Respekt and Irnik.

The quality composition of protein is significant for the quality of the triticale grain in general. Therefore, it is important to determine to what extent the high-yielding triticale combines the content of the essential amino acid lysine. In this respect, the combination of lysine with yield considerably differed from the combination of protein content with yield (Figure 2). The distribution of the studied genotypes on the biplot was comparatively less scattered, and the greater part of the genotypes tended towards the mean value of lysine content. Regardless of yield, 14 out of the 16 investigated genotypes realized lysine content within the range 310-340 mg/100g, while 9 out of 16 were within 320-335 mg/100g. Nevertheless, a certain tendency may be observed on the graph of the more high-yielding cultivars, such as Dobrudzhanets and Doni 52, Blagovest and Borislav being characterized by lower lysine content, while the lower-yielding ones, such as Lasko, Kolorit, Akord, Irnik and Lovchanets having higher lysine content. Although this correlation was not strictly linear, such behavior was emphasized also by the data on the correlation coefficient, averaged for the studied period.

An exception from this tendency were cultivars Atila and Bumerang, in which the high productivity was combined with high lysine content. At the same time, the opposite correlation was observed in cultivars Presto and Respekt, in which low lysine content was combined also with low productivity.

Highly impressive is the fact that in cultivars Atila and Bumerang a good combination was observed both between protein content and yield and between lysine content and yield. This was an indication that there was a tendency in the two cultivars of combining the high protein content with high lysine. This can also be followed in Figure 3, which presents the combination of the values of protein with lysine content on a biplot. There was practically no tendency between the two parameters, confirmed also by the absence of a high correlation between them. A strong example in this respect were cultivars Akord and Lovchanets, which were with completely opposite values with regard to protein but their lysine content was practically identical. Similar was the situation with the lysine values of cultivars Lasko and Presto, which had almost the same protein content.

In practice, cultivars Lasko, Kolorit, Atila and Bumerang were with optimal combinations of the two parameters. On the other hand, with most unfavorable combinations were Respekt, Dobrudzhanets, Doni 52, Blagovest and Borislav. Such a tendency of the most recent and high-yielding cultivars to combine the lowest values of protein and lysine content demonstrated that there was a negative correlation between productivity and quality.

This tendency was the reason to search for those genotypes, which combined compromisingly high levels of yield, high protein content and high lysine content. The use of a 3D graph allowed determining such a correlation. Figure 4 clearly outlined those cultivars, which simultaneously realized high values with regard to the three investigated parameters. The best combination was observed in cultivar Atila, and a compromise combination of the three parameters was determined in Bumerang, Kolorit and Rakita. Extremely unfavorable combinations from a breeding point of view were observed in cultivars, Lovchanets, Akord, Doni 52, Presto, Blagovest and Borislav, in which at least one of the traits was with extreme

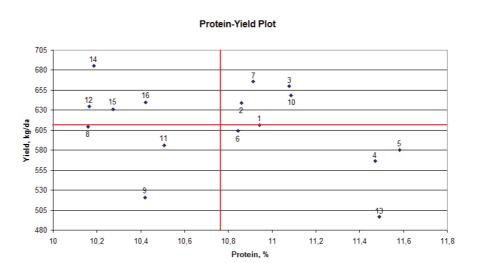
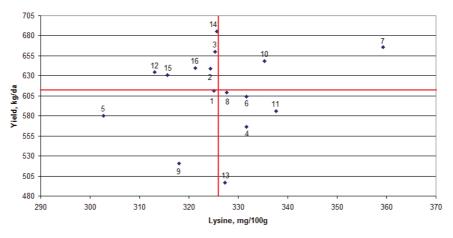


Figure 1. Biplot combination of protein content with yield in Bulgarian triticale cultivars, averaged for the period of study (1. AD-7291; 2. Vihren; 3. Rakita; 4. Lasko; 5. Presto; 6. Kolorit; 7. Atila; 8. Akord; 9. Respekt; 10. Bumerang; 11. Irnik; 12. Dobrudzhanets; 13. Lovchanets; 14. Doni 52; 15. Blagovest; 16. Borislav)

#### Lysine-Yield Plot



**Figure 2.** Biplot combination of lysine content with yield in Bulgarian triticale cultivars, averaged for the period of study (1. AD-7291; 2. Vihren; 3. Rakita; 4. Lasko; 5. Presto; 6. Kolorit; 7. Atila; 8. Akord; 9. Respekt; 10. Bumerang; 11. Irnik; 12. Dobrudzhanets; 13. Lovchanets; 14. Doni 52; 15. Blagovest; 16. Borislav)

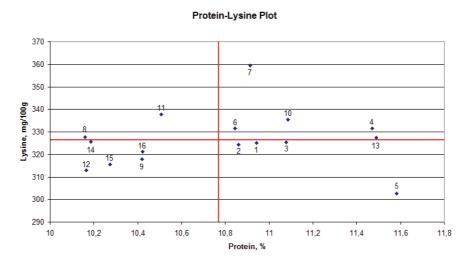


Figure 3. Biplot combination of lysine and protein content in Bulgarian triticale cultivars, averaged for the period of study (1. AD-7291; 2. Vihren; 3. Rakita; 4. Lasko; 5. Presto; 6. Kolorit; 7. Atila; 8. Akord; 9. Respekt; 10. Bumerang; 11. Irnik; 12. Dobrudzhanets; 13. Lovchanets; 14. Doni 52; 15. Blagovest; 16. Borislav)

low or extreme high values at the expense of the rest. Since the greater part of the cultivars were characterized by a productivity about and above the average, a tendency was noticed of their positioning farther back on the graph, where the lower values of protein and lysine content were. Practically, we did not observe a genotype combining simultaneously high productivity, high lysine content and high protein content.

#### DISCUSSION

#### **Protein content**

The results we obtained showed that protein content was largely dependent on both the conditions of the environment and the genotype. The values we determined were within the range other authors reported. Tohver et al. (2005) pointed out mean results of

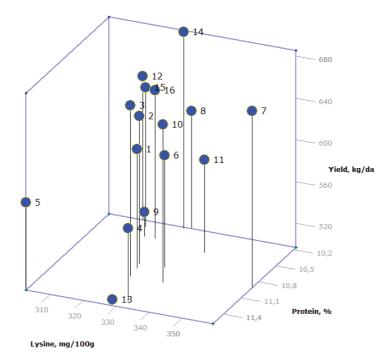


Figure 4. 3D plot combination of yield, lysine and protein content in Bulgarian triticale cultivars, averaged for the period of study (1. AD-7291; 2. Vihren; 3. Rakita; 4. Lasko; 5. Presto; 6. Kolorit; 7. Atila; 8. Akord; 9. Respekt; 10. Bumerang; 11. Irnik; 12. Dobrudzhanets; 13. Lovchanets; 14. Doni 52; 15. Blagovest; 16. Borislav)

11.83% of protein content, averaged for 13 genotypes during three growing periods. The values in our investigation were with about 1% lower, which can be explained by the rather low results in economic year 2017/2018. At the same time, the above authors reported that the values of Lasko and Presto were also comparatively higher than in the more recent cultivars, similar to the results we obtained. These authors, however, read considerably higher protein content in cultivar Tewo 2 – 14.5%, averaged for three years. We did not observe such results in the cultivars we studied. Results similar to ours, averaged for two growing periods in four investigated cultivars and two fertilization norms on the territory of the Czech Republic were reported by Burešová et al. (2010).

The researches of Goyal et al. (2011) and Lango et al. (2018) also demonstrated similar data. Significantly higher results than the ones we obtained (12.4 - 15.7%) were reported by Kendal & Sayar (2016) who investigated 25 genotypes under the conditions of four locations on the territory of Turkey. The authors observed a particularly high result in cultivar Karma 2000 – from 12.3 to 18.0%, the mean value for the four locations being 14.7%, which was a little above the durum wheat standard they used. Navarro-Contreras et al. (2014) and Salehi & Arzani (2011) also reported values of protein content higher than the values we obtained. Ukalska & Kociuba (2013) carried out a large-scale study on triticale during 1982-2008 investigating 1006 genotypes. The authors found out that protein content varied considerably during the individual sub-periods depending on the set of genotypes. In the early periods, the protein content of the more primitive triticale forms was significantly higher (about 15%), while in the contemporary cultivars in the later periods, the protein content dropped down considerably - to approximately 10 %. This tendency was also observed in the genotypes we studied. The most recent genotypes (Doni 52, Blagovest and Borislav) were characterized by lower protein content. Kızılgeçi (2019) obtained mean data between 12.4 and 14.3%, depending on the location, under the conditions of 2012/2013 at two locations in Turkey. In practice, the investigation showed that the conditions of the environment during the specific period and the location had significant impact on the values of protein content. This thesis was confirmed also by Kendal

& Sayar (2016) and Burešová et al. (2010). In the cultivars we studied, the environment had high effect on the protein content in grain, the late July rainfalls having particularly unfavorable effect, as well as the uneven rainfalls in June. Gulmezoglu et al. (2010), Kendal & Sayar (2016), Abdelkawi et al. (2020), Goyal et al. (2011), Gulmezoglu & Aytac (2010) demonstrated that both the conditions of the environment and the interaction between the genotype and the environment affected the protein content in the grain of triticale. It should be emphasized, however, that the conditions favorable for the development of the crop were not always related to higher protein content. Often, at high levels of some stress factors, the protein content was higher than at the absence of the stress factor. Salehi & Arzani (2011) observed higher protein content under conditions of higher saline concentration. At the locations with lower precipitation norms, higher protein content was obtained in comparison to the location with the highest precipitation during the vegetative growth of the crop, according to Kendal & Sayar (2016). Such data corresponded well with the results we obtained. On the other hand, Abdelkawi et al. (2020) observed different protein content in two of the studied periods, in which they read almost identical grain yields, the higher values being registered in the periods with higher temperatures.

Such data and results from other authors allow the assumption that the protein content in the grain of triticale is largely dependent on the conditions of the environment and the potential of the genotype. The greater part of the researches show that at higher amounts of rainfalls during grain filling and at lower temperatures, protein content is significantly lower. In this respect, the cultivars we studied were characterized by protein content above the average in comparison to the world breeding of this crop.

### Lysine content

The results presented on the content of lysine showed comparatively low variation between the separate genotypes but considerable variation between the periods of study. Such data on the genotypes are confirmed by different researches on triticale. Widodo et al. (2015) reported extremely low variation in the lysine content when studying 10 triticale cultivars on the territory of Australia during two growing periods. Between the two periods, significant differences were also not observed. Muhova & Dobreva (2020), too,

found out low variation in the three Bulgarian cultivars they investigated (Kolorit, Bumerang and Respekt), although using different predecessors and fertilization with Lombricompost. These authors demonstrated that the lysine content of cultivar Kolorit was close to the results we obtained, but in cultivars Bumerang and Respekt the values were lower than the data were had, the tendency between them remaining the same. Jaśkiewicz & Szczepanek (2018) confirmed the data we also obtained, reporting significant differences in the lysine content between the two periods they studied, and between the two investigated genotypes. Baboev et al. (2020) showed variation in the mean values of the genotypes they investigated. Brandt et al. (2000) pointed out mean data for lysine content in triticale amounting to 3.55% from the content of raw protein, approximating our results. Our data, however, were different and lower in comparison to the data obtained by Stankiewicz et al. (2003), Widodo et al. (2015) and Mikhailouskaya & Bogdevitch (2009).

The absence of significant variation in the studied cultivars revealed that although they had values close to the crop's mean, the genetic variability according to this trait was very low. Therefore, new sources are needed that can significantly contribute for higher lysine content in triticale. Stankiewicz et al. (2003) pointed out, that a possible way to increase the content of amino acids in triticale is to use octoploid primary forms and apply them to hybridization schemes with hexaploid ones.

On the other hand, Brandt et al. (2000) observed the highest mean content of lysine between wheat, triticale and barley in the investigated barley genotypes. The involvement, however, of this crop in hybridization with triticale would be a rather difficult task since the two plant species are phylogenetically considerably distant. Even the use of much closer species such as *Aegilops* gives contradictory results. Boros et al. (2010) demonstrated that in hybrids between triticale and *Aegilops*, the lysine content decreased significantly according to the parental triticale forms, which the authors explained by the increased content of protein. Therefore, a main task in this respect is to search for and develop secondary hexaploid triticale forms possessing higher values of this trait.

### Combining productivity and quality

The combination of productivity and quality is an extreme challenge since these traits are often in negative correlation, or there is no correlation between them at all. A considerable number of researches provide evidence for this with respect to triticale. Thus, for example, Gulmezoglu et al. (2010) demonstrated the absence of correlation between grain yield and protein content in triticale and the presence of a negative direct effect of protein content on yield. Kızılgeçi (2019) observed a very low and insignificant negative correlation between grain yield and protein content at the two locations, which the author investigated. Ozturk et al. (2019) reported a higher but insignificant negative correlation. A significant negative correlation, however, was reported by Goyal et al. (2011) (-0,65), Alaru et al. (2003) (-0.922) and Neuweiler et al. (2021). These data entirely confirmed the results we obtained with regard to the correlations between grain yield and the protein content in grain. Despite the absence of a correlation, or the presence of a negative one, among the genotypes we studied, cultivars Atila and Bumerang combined simultaneously high values of the two traits. Such a combination can be considered a significant breeding achievement in this crop, especially under the contrasting conditions of the experiment. Neuweiler et al. (2021), investigating 1218 triticale accessions, showed that the greater number of genotypes was characterized by yield and protein values below the ones the authors targeted for the two traits (12.5% protein and 9.5 t/ ha yield). Only six of all genotypes possessed grain yield and protein content in grain above these values. This indicated that the combining of the two traits was a rather difficult task. In this respect, the use of a biplot gives a considerable advantage because it allows identifying the genotypes, which would be appropriate for breeding. In our experiment, the biplot was based not on target values but on the means of the two traits, which allowed determining all optimal combinations. Among the 16 studied genotypes, only four fell within the group with values above the average - the standards Vihren and Rakita and cultivars Atila and Bumerang. Such results demonstrated that the breeding aimed at productivity is often related to lower qualitative traits, such as protein content.

The results from the biplot entirely corresponded to results of the parameter protein yield. Cultivars Atila and Bumerang, averaged for the studied period, realized the highest values, significantly above the greater part of the cultivars. In this respect, the possibility to use this parameter as a complex index for simultaneous breeding towards higher values of yield and protein content in triticale was confirmed by Neuweiler et al. (2021). Gulmezoglu & Aytac (2010) and Wojtkowiak et al. (2015) demonstrated that protein yield was largely determined by grain yield than by protein content. This is being also confirmed by our results on the correlation coefficients of protein yield with grain yield and protein yield with protein content. Such data reveal that the simultaneous improvement of the two parameters requires a complex approach. The combination of such a parameter as protein yield with a biplot between yield and protein content in grain allowed identifying the most valuable genotypes according to the specific breeding task.

The simultaneous improvement of yield and the lysine content is an aspect of triticale breeding for grain, which has been little investigated. There is hardly any information in practice on the correlation of productivity with the essential amino acid. Jaśkiewicz & Szczepanek (2018) reported a negative but insignificant correlation of grain yield with lysine content. Our results revealed a correlation between the two parameters but only in a long-term perspective with regard to the mean values of the investigated period. During the separate periods, a significant correlation was practically not observed. This indicated that there was no correlation between lysine content and yield, the obtained significant correlation being of a random nature. Similarly, there are no thorough studies on the correlation between lysine and protein content in triticale. Jaśkiewicz & Szczepanek (2018) observed a positive but insignificant correlation of the two traits, and Brandt et al. (2000) reported a low correlation in triticale. These data are a definite confirmation of our results. The absence of a correlation in this case, similarly to the correlation of grain yield with protein content, allowed using a biplot to identify the genotypes which combined high lysine content with high yield on the one hand, and high protein content with high lysine content, on the other. The results we obtained showed that such combinations with yield were observed only in Atila and Bumerang, and with regard to combinations of high values of protein with lysine - in Lasko, Kolorit, Atila and Bumerang. Although Muhova & Dobreva (2020) investigated the content of lysine and protein in cultivars Kolorit, Bumerang and Respekt, they did not indicate if there was any correlation with productivity.

The simultaneous improvement of all three traits – yield, protein content and lysine content in grain

and their correlations presented above – is an extremely labor-consuming task in practice. In the world literature there is no research investigating this topic at the current stage of the breeding process in the crop. One of the few researches on a simultaneous determining of the three traits we studied was that of Ruckman et al. (1973). The authors showed that all three traits were characterized by a very strong effect of the cultivar x location interaction. On the one hand, such researches on old genotypes demonstrated the initial idea of triticale as a high-quality fodder raw material. In practice, however, the results we obtained showed that breeding for quality parameters in triticale was at the expense of the higher productivity. The small number of researches in this direction both in Bulgaria and in different world breeding programs is a direct evidence for the necessity of a considerable improvement of the lysine and protein content in grain in combination with high productivity.

The data from the applied 3D-plot confirmed this thesis since only cultivar Atila combined high values of all three traits, and in Bumerang the combination was a compromising one. Regardless of this, such genotypes prove that a successful combination of high productivity with quality is possible in triticale. On the other hand, the high-quality and high-yielding triticale genotypes are valuable initial material for the breeding program of triticale in Bulgaria.

#### CONCLUSIONS

Based on the presented results, the following conclusions can be made:

1. The variation of protein content, averaged for the studied period, was comparatively low, the values of the separate genotypes being from 10.16 to 11.58 %. Between the separate growing periods, however, significant differences were observed, the lowest content being measured in 2017/2018 (8.06%), and the highest – in 2016/2017 (12.83%).

2. Lysine content, as compared to protein content, was affected in a different way, observing rather different tendencies between the two traits. The world standard Presto was with the lowest values, and Atila – with the highest.

3. There is a lack of significant interconnection between protein content and grain yield and between lysine content and grain yield, as confirmed by the low or insignificant correlation during the separate periods.

4. The best combination of protein content and grain yield was observed in cultivars Vihren, Rakita, Atila and Bumerang, which was supported by the applied biplot method and by the parameter protein yield. A good combination of grain yield and lysine content was registered only in cultivars Atila and Bumerang, and a good combination of protein and lysine – in Lasko, Kolorit, Atila and Bumerang.

5. In cultivar Atila, the best combination of yield, protein content and lysine content was noted. A compromise combination of the three traits was observed in cultivar Bumerang. Such results make cultivars Atila and Bumerang valuable initial material for improvement of the protein and lysine content in the breeding program of triticale in Bulgaria.

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