

Morphological markers to identify common winter wheat cultivars (*Tr. aestivum* L.) with variation analysis and homogeneity test

Zlatina Uhr, Bogdan Bonchev*, Evgeniy Dimitrov, Blagoy Andonov

Agricultural academy, Institute of plant genetic resources-Sadovo, Bulgaria

*E-mail: bogybontchev@yahoo.com

Citation

Uhr, Z., Bonchev, B., Dimitrov, E., & Andonov, B. (2022). Morphological markers to identify common winter wheat cultivars (*Tr. aestivum* L.) with variation analysis and homogeneity test. *Bulgarian Journal of Crop Science*, 60(3) 3-13

Abstract

The study was conducted at the experimental field of Institute of Plant Genetic Resources, Sadovo with comparative testing of progenies in two consecutive years (2016-2018) using the standard, local cultivation technology. Four common winter wheat cultivars Ginra, Gizda, Nikolay and Murgavetz are involved in the experiment. They are homogeneous in their progeny. The most suitable morphological marker is thousand kernels weight with low to middle variation during years and low variation among cultivars over the study period. Plant height is also low variable and can be used as a morphological marker. The accuracy of the study was high in both years of the study. The influence of genotype is highest a thousand kernels weight, productive tillering, and lowest on spike length. The influence of year is highest on spike length, kernel density and lowest a thousand kernels weight.

Key words: common winter wheat; morphological markers; cultivar; homogeneity; influence; genotype; year

INTRODUCTION

Extreme weather is characterized by phenomena such as prolonged droughts and floods (World Meteorological Organization, 2019). Most German farmers recognize the impact of climate change. Experience of German growers has shown that the choice of variety is the most important, with integrated protection and crop rotation being the main factors for a stable yield (Macholdt & Honermier, 2016, Macholdt & Honermier, 2017). Biomass dry weight and leaf area can be used as agronomic traits to screen waterlogging-tolerant cultivars in flooded areas (Jiang et al., 2022). The height of the plant, the number of spikes per unit area and the weight of thousand seeds have a direct and strong influence on the yield expression (Markova–Ruzdik, 2015). Fertilization is a modifying factor in agronomic traits.

An increase in the values from 6.6 to 9.2% was found for the characteristics of plant height, spike length, number of grains in a spike, grain weight, weight of thousand grains of research in triticale for organic and mineral fertilization (Muhova & Stefanova-Dobрева, 2021). Phenology has a profound effect on crop adaptation and productivity. Genes involved in the flowering signal have been identified. By alteration the variation of the environment, isolation of the expression of these genes from the expression of other traits has been achieved (Giunta et al., 2018).

Productive tillers numbers, plant height, spike length, grains number per spike, grains weight per spike, thousand kernels weight were considered as agronomic traits (Tshikunde et al., 2019). Wheat spike structure is a key determinant of multiple components of grain yield and the detailed study

of spike morphometric characters. It is useful for explaining wheat grain yield and studying the influence of different agronomic technologies and genotype. The difference between cultivars is in spike structure, so measurements of kernel density should be made in the middle part of the spike when looking for cultivar differentiation in morphological characters, (Zhou et al., 2021). Wide phenotypic variation in wheat was found with the best linear unbiased estimates at the thousand kernels weight with values from 35.9 to 58.2 g. Thousand kernel weight is determined by many markers with small effects, (Zanke et al., 2015). Seed thickness is strongly influenced by genotype and can serve as a trait in breeding programs and prediction of milling qualities (Ficco et al., 2020). It is associated with the thousand kernels weight (Kachakova & Desheva, 2013) found that the variation of the number of productivity tillers per plant and the thousand kernels weight had a slight variation. The plants height had a middle variation, while the spike length and the grains number per spike in 16 cultivars of wheat bordered on the big variation. High-throughput phenotyping platforms (HTPPs) have been created to replace the low-throughput manual work of biometric measurements (Khadka et al., 2020; Zhou et al., 2021). There are false positives in elite wheat germplasm that arise from population structure and multiple testing (Breseghello & Sorrells, 2006).

The aim of the research is to determine the traits the variation and which of them can be applied as morphological markers to identify the studied common winter wheat cultivars.

MATERIAL AND METHODS

The study was conducted at the experimental field of Institute of Plant Genetic Resources, Sadovo with comparative testing of progenies in two consecutive years (2016-2018) using the standard, local cultivation technology. The soil is a type of pellic vertisol according to FAO, medium strength (A+B horizon=60-80 cm), slightly clayey, with a high content of physical clay and silt fraction (Dimitrov, 2018). In order to establish the agronomic traits that may serve as morphological markers of cultivars of common winter wheat, the two-year progeny comparative test (TCT) was established: Nikolay, Gizda, Ginra and Murgavetz. The Ginra cultivar was

created by the method of intervarietal hybridization from ♀Sadovo 1 X ♂ Guinness /1322. In a cultivar test in 2014-2015, the thousand kernels weight varied from 33.40 to 49.55 g. The obtained results with a thousand kernels weight 42.51 g were established at 710 productive tillering/m². The stem height is 105-110 cm, lower than the parent forms; the number of productive tillering is greater than that of the cultivar Sadovo 1. It is forms a compacted crop. The spike length is 10 cm, the grains number per spike is 36, and the grains weight per spike is 1.9 g. The grain yield is 750-770 kg/da (APVTASC, 2016; Uhr, 2017). Cultivar Nikolay was created by intervarietal hybridization from Bulg.5052-p X VIII-F 5 cross 19a 2-10. A highly productive cultivar, from which 816 kg/da grain yield was obtained in the Sadovo region over a three-year trial period. The cultivar is 5-6 cm with a lower stem than Sadovo 1, with a thousand kernels weight 42 g. It belongs to group B - medium wheat with increased strength. On average, for the test period, 708 kg/da of grain were obtained, compared to 694 kg/da for the standard "Enola" cultivar. The cultivar is medium early. The Gizda cultivar was obtained from a mutant line by chemical mutagenesis from dry seeds of the Pobeda cultivar treated with sodium aside. The stem height is 95 cm, 15 cm lower than the Pobeda cultivar, with a pyramidal shape of the spike, length 8-12 cm, with an average intensity of the color of the spike from white to straw-yellow. The thousand kernels weight is 38.4 g, compared to 43.1 for Sadovo 1. The central spike weight is 2.45 g, with 60 grains of the central spike and a high productive tillering about 800/m². The cultivar showed an average yield of 747 kg/da (Uhr & Rachovska, 2016). Murgavetz is an intervarietal hybrid with parents Altimir 67 and Sadovo 1. Murgavetz is a medium-early cultivar, the plant height is about 90 to 95 cm, and it is the only cultivar with awns. Thousand kernels weight is 48 g, with 42-44 grains in spike and a spike length of 9-10 cm (Boyadjieva, 2007; Rachowska et al., 2012). The cultivar Murgavetz showed a thousand kernels weight 40.66 and 42.74 g, with the number of productive tillering 501 and 417 for the research years 2000-2001, in the region of Pazardzhik (Georgieva et al., 2004). During testing 2006 it was found that the Murgavetz cultivar gave 757 kg/da (<https://iasas.government.bg>). In order to establish the variation of the main morphological traits in the tested cultivars and to search for morphological markers for

their identification, a field experiments was made with elite progenies of cereal cultivars. There was used a scheme of a comparative test of progenies for the second year according to the methodology for varietal maintenance of cereal crops (Ministry of Agriculture & Foods Industry, 1977). In order to prevent the biological contamination of the cultivar as a result of crossing, a spatial isolation of 1.6 m was observed, which for basic seeds (B) common wheat is 1 m according to Ordinance 21 of December 10, 2007 (Ministry of Agriculture & Food, 2008). Sowing of the experiment, simulating the conditions of the second-year progeny comparative trial, was carried out by machine at an optimal time (October 20). In autumn, before sowing, NPK 15-15-15 fertilizer was applied in the amount of 20 kg/da. Crop care was carried out according to the technology adopted for wheat. Biometric measurements (Dimova & Marinkov, 1999) were performed on plants collected by measuring 50x50 cm from the inner parts of the plots. The spikes are hand-tipped to prevent seed mixing. Plant height without awns for wheat cultivars (cm), number of productive tillers per m², number of grains per spike, length of spike in (cm), weight of grain per spike (g), weight of thousand kernels (g) and kernel density were reported.

Thousand kernels weight is determined according to the methodology of BDS ISO 520:2003 in the air-dry state of the grains.

The following analyzes were performed: the arithmetic mean, coefficient of variation and accuracy indicator were calculated (Dimova & Marinkov, 1999). Dispersion analysis of the investigated characters and analysis of variance was performed, calculating the influence of the factors (η) - genotype, year, the interaction between them, and the influence of the error, presented in percentages, with the SPSS 19 and Excel 2010 software products. A Fit analysis was also performed with the JMP 5.0.1 product. (SAS Institute). The JMP 5.0.1 program groups values into a hierarchical structure. The method was applied to plant production in barley, presenting the least significant difference (LSD) and coefficient of variation (Vulchev & Vulcheva, 2019).

A test was performed for homogeneity of progeny across cultivars for the tested period, as they were assumed to be homogenous, (SPSS Inc.). At values of sig. (significance - degree of credibility), greater

than 0.05, it is assumed that the data are homogeneous. At a value of significance (sig.) smaller than 0.05, it is established that the studied groups are not homogeneous. Analysis of variance was performed on cultivar progeny to determine if there was a difference between the study groups. To determine morphological markers, a complex of mathematical treatments was used - analysis of variation with coefficient of variation (CV %), analysis of variance. The traits that can serve as morphological markers should meet the average characteristics: the studied groups should be homogeneous, have a slight coefficient of variation, not higher than the middle accuracy for the experiment, the strength of the genotype factor should be high (Lidanski, 2012).

RESULTS AND DISCUSSIONS

In terms of climate, the region is characterized by a transitional-continental climate, which manifests itself as a long and cool spring, a dry and hot summer, an extended dry and warm autumn, and a cold winter. The rainfall regime has a continental character with a summer maximum in June and a winter minimum in February. In August and September, a pronounced drought is observed, representing the second minimum rainfall. During the research period (2016/2017-2017/2018), a trend of increasing average monthly temperatures was observed. The trend is particularly noticeable in January with a 3.3°C deviation from the norm. Only in the winter of 2016/2017 in January was the average monthly temperature coinciding with the climatic norm (-4.4°C) reached (Figure 1).

Precipitation during the studied period shows a large difference by year. In the first year of the study, during the months of October to December in 2016, there was an accumulation of rainfall, and in 2017, the rainfall was below normal. In January of the first year of the study, a precipitation maximum was observed (103.1 mm), as well as in May-June (84-139.9 mm). The least rainfall was found in June 2017 - 11.7 mm and the highest rainfall maximum was observed in June 2018 (139.9 mm) (Figure 2).

The cultivars are in the process of cultivar maintenance. Off type progenies are discarded, and varietal cleaning was carry out of impurities from other cultivars, other types of cereal crops and rebounds was removed. Atypical forms in the cultivar de-

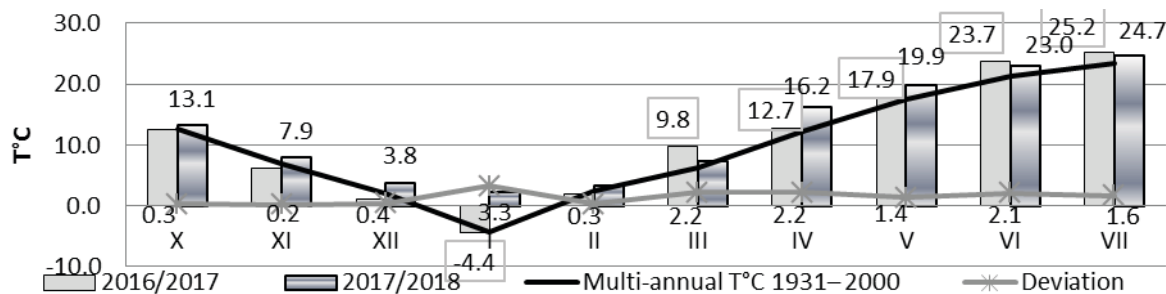


Figure 1. Average temperature T°C of months during two vegetation years 2016/2017 -2017/2018

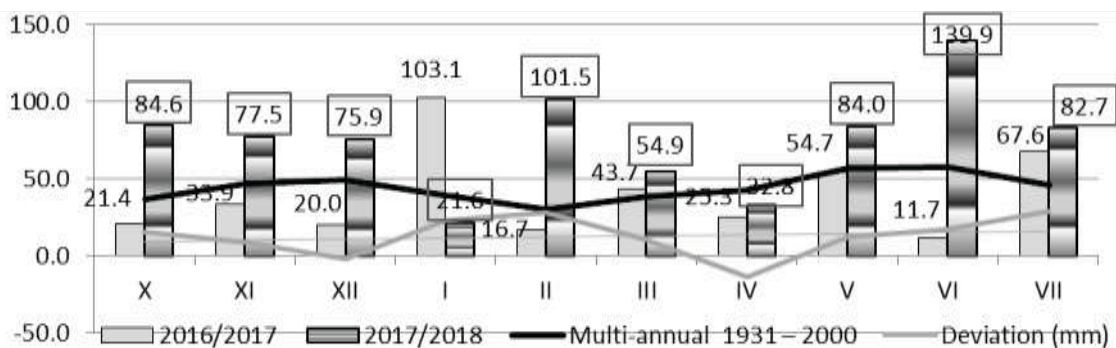


Figure 2. Sum of rainfall (mm) of months during two vegetation years 2016/2017 - 2017/2018

creased from the first to the second year of the comparative trial. The results will show greater homogeneity of the studied traits after the comparative study of progenies with a two-year follow-up period (Kolev, 1993). The expression of the marker traits of wheat cultivars can be different. It depends mainly on the genotype, but also on the environmental conditions, although within certain limits determining the rate of reaction. The nature of variability is mainly determined by the genetic characteristics of cultivars (Lovnyaeva, 2007). Since cultivars are assumed to be homogeneous at recognition, it would follow that their traits are homogeneous. With the help of the Levince test and dispersion analysis, it is established how homogeneous the data of the studied cultivars are.

According to the investigated traits, it is found that the number of productive brothers/m², plant height, spike length, number of grains per spike, density of the spike in all investigated cultivars are homogeneous at sig>0.05. The mass of one thousand grains is homogeneous in the cultivars Ginra, Gizda and Nikolay at sig>0.05. The Murgavetz cul-

tivar has lower homogeneity (sig=0.017) per mass of thousand grains (Table 1).

The dispersion analysis of the cultivars performed on the progenies of the studied traits shows that all traits are with significance (sign.) greater than 0.05. The cultivar Gizda has a value of sign.=0.073, therefore no difference is observed. This confirms the data from the Gizda cultivar homogeneity analysis. In the Murgavetz cultivar with a value of sign.=0.523, there is no significant difference between the progenies within the cultivar (table 2).

The accuracy of number of productive tillers/m² is high from 0.76 to 2.02% in the first year, in the second year it is from high to medium (0.46-2.79%). The plants height has a high accuracy of the data for the entire period of the study, with values from 1.4 to 1.92% in the first year, and from 1.11 to 1.84% in the second year. Spike length has an accuracy rate of 1.01 to 2.07% in 2016/2017 and 0.28 to 2.51% in 2017/2018. Spike length has high accuracy in the first year and high to good accuracy in the second year. Grain number per spike were highly accurate

Table 1. Test of homogeneity of variances of cultivar traits

Cultivars	Ginra		Gizda		Nikolay		Murgavetz	
	LS	Sig.	LS	Sig.	LS	Sig.	LS	Sig.
Number productive tillers /m ²	0.233	0.635	0.285	0.600	2.500	0.131	0.528	0.477
Plant hight (cm)	0.032	0.860	1.328	0.264	0.014	0.907	0.003	0.959
Spike length (cm)	0.003	0.957	0.147	0.706	0.574	0.458	2.384	0.140
Number of kernel/spike	0.153	0.700	0.35	0.561	0.145	0.708	0.162	0.692
Weight grain/spike (g)	0.335	0.570	0.002	0.961	1.159	0.296	0.105	0.749
Weight thousand kernel (g)	0.013	0.912	3.82	0.066	0.617	0.442	6.861	0.017
Kernel density	0.001	0.978	0.065	0.801	0.066	0.800	3.036	0.099

Sign. –significance, LS- Levine statistic

Table 2. Dispersion analysis of traits of common winter wheat cultivar

ANOVA of traits of cultivars			Ginra		Gizda		Nikolay		Murgavetz	
Traits	Interaction	DF	MS	Sig.	MS	Sig.	MS	Sig.	MS	Sig.
Number of productive tillers/ m ²	Betw. Gr.	1	105.80	0.939	8.45	0.953	369.80	0.414	204.80	0.580
	Within Gr.	18	17527.02		2393.12		528.44		643.29	
	Total	19								
Plant hight (cm)	Betw.Gr.	1	7.20	0.558	16.20	0.705	0.80	0.947	0.80	0.886
	Within Gr.	18	20.22		109.33		174.34		37.80	
	Total	19								
Spike length (cm)	Betw.Gr.	1	0.041	0.819	0.313	0.693	0.032	0.856	0.481	0.531
	Within Gr.	18	0.750		1.948		0.950		1.177	
	Total	19								
Kerner number of l/ spike	Betw.Gr.	1	9.80	0.663	16.20	0.837	7.20	0.810	198.45	0.230
	Within Gr.	18	49.84		373.64		121.16		128.69	
	Total	19								
Weight grain/spike (g)	Betw.Gr.	1	0.000	0.987	0.037	0.771	0.009	0.816	0.318	0.210
	Within Gr.	18	0.164		0.423		0.166		0.188	
	Total	19								
Weight thousand kernal (g)	Betw.Gr.	1	17.43	0.491	67.86	0.073	4.27	0.445	6.72	0.523
	Within Gr.	18	35.18		18.75		7.01		15.80	
	Total	19								
Kernel density / spike	Betw.Gr.	1	0.001	0.965	0.00	0.992	0.03	0.876	0.30	0.613
	Within Gr.	18	0.65		0.85		1.04		1.12	
	Total	19								

Sig. –significance

in the first year (1.00-1.93%) and good to poor in the second year (2.26-5.29%). The grain weight per spike ranged from high accuracy to satisfactory in the first year (1.88-3.67%) and from good to poor ac-

curacy in the second year of the study (0.87-5.41%). The thousand kernels weight was with an accuracy of 1.39 to 3.38 % in the first year and with an accuracy of 1.85 to 4.26 % in the second year of the

study. The thousand kernel weight was of high to satisfactory accuracy throughout the study period. Kernel density per spike has good to satisfactory accuracy in the first year (2.44-4.34%). In the second year, it has high to poor accuracy (0.60-5.72%) (Table 3).

The traits number productive tillers /m², grains number per spike, spike length, plant height, thousand kernels weight and grains weight per spike showed low variation in the first year of the study. The kernel density in the investigated wheat culti-

vars has an middle variation of 10.19 %. The trait with the highest variation is the kernel density/spike in the cultivar Murgavetz (13.73%). In the cultivar Ginra, there was a middle variation in the grains weight per spike (11.61%) and thousand kernels weight (10.69%) (Table 4).

In the second year of the study, the variation was middle for the traits grains weight per spike (14.80%), grains number of per the spike (14.34%), kernel density/spike (12.05%). The traits plant height, spike length, number of productive tillers/

Table 3. Accuracy indicator ($S\bar{x}$ %) and arithmetic mean of the traits

Traits	№	Year	2016/2017		2017/2018	
		Cultivars	\bar{x}	$S\bar{x}\%$ %	\bar{x}	$S\bar{x}\%$ %
Number of productive tillers/m ²	1	Ginra	575	1.66	328	1.85
	2	Gizda	555	0.76	481	2.61
	3	Nikolay	483	1.41	515	0.46
	4	Murgavetz	304	2.02	280	2.79
Plant height (cm)	1	Ginra	78	1.63	83	1.33
	2	Gizda	74	1.92	92	1.84
	3	Nikolay	82	1.51	106	1.11
	4	Murgavetz	73	1.43	82	1.64
Spike length (cm)	1	Ginra	10.5	1.01	9.0	1.88
	2	Gizda	11.2	1.77	8.9	2.30
	3	Nikolay	10.2	1.31	8.4	0.28
	4	Murgavetz	10.3	2.07	8.7	2.51
Number of grain per spike	1	Ginra	54	1.41	43	3.88
	2	Gizda	85	1.00	50	5.29
	3	Nikolay	53	1.50	33	2.26
	4	Murgavetz	69	1.93	49	4.74
Weight of grain per spike (g)	1	Ginra	2.8	3.67	2.42	4.95
	2	Gizda	3.02	3.28	1.9	3.84
	3	Nikolay	2.16	1.88	1.41	0.87
	4	Murgavetz	2.872	2.61	2.27	5.41
Weight thousand kernel (g)	1	Ginra	48.82	3.38	56.25	2.14
	2	Gizda	35.51	3.01	38.85	4.26
	3	Nikolay	40.11	1.37	43.14	1.85
	4	Murgavetz	42.57	2.57	45.82	2.64
Kernel density/ spike	1	Ginra	2.76	2.44	4.14	3.45
	2	Gizda	2.64	2.49	4.18	4.39
	3	Nikolay	3.94	2.86	5.81	0.60
	4	Murgavetz	3.2	4.34	4.76	5.72

\bar{x} -arithmetic mean, $S\bar{x}$ -Accuracy indicator

Table 4. Least significant difference (LSD) and variation coefficient of traits trough cultivars and years with JMP 5.0.1

Mean, LSD and variation of I year of study														
Cultivar	NPT/ m ²	CV %	Plant hight (cm)	CV %	Spike length (cm)	CV %	NGS	CV %	WGS	CV %	WTK	CV %	Kernel density	CV %
Ginra	575a	5.26	78.4a	5.14	10.45b	3.20	54.0c	4.45	2.80a	11.61	48.83a	10.69	2.76c	7.70
Gizda	555a	2.40	73.9b	6.07	11.24a	5.58	84.5a	3.17	3.02a	10.38	35.51c	9.53	2.67c	7.86
Nikolay	483b	4.47	81.5a	4.78	10.17b	4.15	53.2c	4.76	2.16a	5.94	40.11b	4.33	3.94a	9.04
Murgavetz	304c	6.39	72.6b	4.51	10.32b	6.55	68.7b	6.10	2.87a	8.24	42.59b	8.11	3.20b	13.73
RMSE*100/x ⁻	22.00	479.30	3.95	76.60	0.53	10.55	3.04	65.10	0.26	2.72	3.66	41.76	0.32	3.14
CV %		4.59		5.15		5.06		4.67		9.69		8.77		10.19
t*st.err.of diff	2.03	9.84	2.03	1.76	2.03	0.24	2.03	1.36	2.03	0.12	2.03	1.64	2.03	0.14
LSD		19.95		3.58		0.48		2.76		0.24		3.32		0.29
Mean, LSD and variation of II year of study														
Ginra	328c	5.84	83.2c	4.19	9.04a	5.95	43.0b	12.26	2.42a	15.66	56.25a	6.76	4.14c	10.92
Gizda	481b	8.25	91.5b	5.81	8.87ab	7.27	49.7a	16.73	1.90b	12.14	38.85c	13.47	4.18c	13.88
Nikolay	515a	1.45	105.5a	3.50	8.41b	0.88	32.8c	7.16	1.41c	2.76	43.14b	5.85	5.81a	1.91
Murgavetz	280d	8.83	81.8c	5.18	8.69ab	7.93	49.4a	15.00	2.27a	17.12	45.82b	8.34	4.76b	18.07
RMSE*100/x ⁻	25.55	401.2	4.24	90.50	0.54	8.75	6.27	43.73	0.30	2.00	3.96	46.01	0.57	4.72
CV %		6.37		4.69		6.22		14.34		14.80		8.61		12.05
t*st.err.of diff	2.03	11.43	2.03	1.90	2.03	0.24	2.03	2.81	2.03	0.13	2.03	1.77	2.03	0.25
LSD		23.17		3.85		0.49		5.69		0.27		3.59		0.52
Mean, LSD and variation of cultivars for the whole study period														
Ginra	452a	3.86	80.8a	0.42	9.77a	1.02	48.5a	1.60	2.61a	1.02	52.54a	1.00	3.45a	2.81
Gizda	518a	1.01	82.7a	1.50	10.05a	1.67	67.1a	3.67	2.46a	3.22	37.18b	0.63	3.41a	3.21
Nikolay	499a	0.46	93.5a	1.82	9.30a	1.34	43.0a	3.35	1.79a	2.98	41.63b	0.51	4.88a	2.71
Murgavetz	292a	0.58	77.2a	0.84	9.50a	1.21	59.1a	2.31	2.57a	1.67	44.20ab	0.52	3.98a	2.77
RMSE*100/x ⁻	92.35	440	11.14	83.55	1.29	9.66	16.28	54.41	0.54	2.36	3.28	43.89	1.13	3.93
CV %		20.99		13.34		13.32		29.92		22.83		7.47		28.68
t*st.err.of diff	2.78	92.35	2.78	11.14	2.78	1.29	2.78	16.28	2.78	0.54	2.78	3.28	2.78	1.13
LSD		256.47		30.95		3.57		45.22		1.49		9.10		3.13
Mean, LSD and variation of the years of study														
2016/2017	479a	25.74	76.6a	5.36	10.55a	4.53	65.1a	22.68	2.71a	13.96	41.76a	13.29	3.14b	18.76
2017/2018	400a	28.56	90.5a	12.02	8.76b	3.08	43.7b	18.09	2.00a	22.52	46.02a	16.08	4.72a	16.49
RMSE*100/x ⁻	118.90	440	8.22	83.55	0.37	9.66	11.85	54.41	0.42	2.36	6.54	43.89	0.69	3.93
CV %		27.02		9.84		3.87		21.77		17.69		14.91		17.60
t*st.err.of diff	2.45	440.00	2.45	5.81	2.45	0.27	2.45	8.38	2.45	0.30	2.45	4.63	2.45	0.49
LSD		84.08		14.22		0.65		20.50		0.72		11.32		1.20

RMSE- Root Mean Square Error, LSD- Least signigance difference, t- criteria of Student, st. err of diff.-standard error of difference, CV %-variation coefficient, NPT/m²-number of productive tillers/m², NGS-number of grain/spike, WGS-weight of grain/spike, WTK- weight thousand kernel.

Letter designations with the same letter are the same (a=a). Letter designations with different letters have proven different values: a>ab>b>c>d

m², weight of thousand kernels have low variation (table 5). By cultivars, the general variation for the whole period of the study was low by thousand kernels weight (7.47%), middle for spike length (13.32%) and plant height (13.34%). Variation by cultivars is significant in terms of productive tillers number / m² (20.99%), grains weight per spike (22.38%), kernel density/spike (28.68%), and grains number per spike (22.83%).

According to years, the lowest variation is spike length (3.87%) and plant height (9.84%). The variation of weight thousand kernels (14.91%), kernel density/spike (14.91%) and weight of grains per spike (17.69%) was medium. There is a high variation by year in the number of grains per spike (21.77%) and productive tillers number / m² (27.02%). With the fit analysis performed (JMP 5.0.1, SAS Institute), significant differences between trait values could be found. The significant differences can be used to recognize some typical traits and to identify cultivars by year. The cultivars Ginra and Gizda have the highest number of productive tillers per unit area in group "a", the cultivar Murgavetz has the smallest in the first year of the study in group "b". Cultivar Nikolay occupies an intermediate position (group c) between them. In the second year, the differences between all cultivars were significant, with cultivar Nikolay having the highest number of productive tillers/m² (group a), and the cultivar Murgavetz having the least number of productive tillers (group d). By years and cultivars, differences were not significant due to the high percentage of variation, (Table 4).

In the first year of the study, the cultivars Ginra and Nikolay (group a) were of equal height, while Gizda and Murgavetz (group b) were lower than them. In the second year, the cultivars are differentiated by height: the tallest is Nikolay (group a), the lowest are Murgavetz and Ginra (group c), and in intermediate group, "b" is the cultivar Gizda. There are no significant differences by cultivars and years. But, although there was no significant difference by year, in the second year the plants were taller, which was determined by most of the precipitation falling in February and March of the second year, leading to more vigorous vegetative growth. With the longest spike is the cultivar Gizda in the first year (group a), the other cultivars are shorter and are in one group "b" in terms of spike length. They are more distinct in the second year, when the cultivar Ginra (group

a) has the longest spike, the cultivar Nikolay (group b) has the shortest spike, and the cultivars Gizda and Murgavetz are in an intermediate group "ab" between the cultivars Ginra and Nikolay. There were no significant differences between the cultivars for the entire study period. When considering the year factor, the first year is characterized by longer spikes (group a) and the second year by shorter spikes (group b). In April of the second year, there is a lack of rainfall and higher temperatures, which aggravates the drought. Fewer spikes are staked, they remain short and with fewer spikelets staked. The significant differences by year are manifested due to the low variation by year (Table 4).

There was a difference in the grains number per spike in the first year, with the cultivar Gizda having the highest value of grains number per spike (group a). With the lowest value are the cultivar Nikolay and Ginra in one group "c". The cultivar Murgavetz differs from the two previous groups by occupying an intermediate position "b". In the second year, cultivars Gizda and Murgavetz are in one group "a" with the largest grains number per spike, cultivar Nikolay has the smallest grains number per spike (group c). The cultivar Ginra has a significant difference with them and occupies an intermediate group "b". According to cultivars of the trait grains number per spike, no difference was significant for the entire period of the study. The medium is the variation of the grains number per spike by cultivar separately and the total variation is medium. The trait grains number per spike is inappropriate for a selection in the first year of comparative testing of progenies, (Lovnyaeva, 2007). By years, the first year of the study (group a) is characterized by the highest number, the least grains number per spike was found in the second year (group b) due to the April drought.

Grain weight, which is a major component of yield, is under strong genetic control, but at the same time strongly influenced by the environment (Zanke et al., 2015). Grain weight per spike was the trait that varied the most in the second year of the study, by cultivar and overall for the year and for the entire study period. But the variation of grain weight per spike is within the medium variation. In the first year, the grains weight per spike of all studied cultivars is in one group "a". In the second year, greater differentiation occurs. Cultivar Ginra and Murgavetz fall into one group "a", cultivar Gizda is in group "b", cultivar Nikolay is in group "c". There

was no difference in cultivars for the entire period of the study, as well as in years (in group a).

During the studied period, the trait thousand kernels weight was dominated by a low variation by cultivars. By years, the variation is medium. The cultivar as a genetic system reacts specifically to external environmental factors. The dominant influence on the variability of the trait thousand kernels weight one is manifested by the factor year, the genotype is next in importance, the general influence between the two factors is proven, but it is weaker (Stanislavovna, 2017). In the first year of the study, the thousand kernels weight was the highest in the cultivar Ginra (group a), Nikolay and Murgavetz fall into group “b”, and cultivar Gizda into group “c”. In the second year of the study, cultivar Ginra is in group “a”, Nikolay and Murgavetz are in group “b”, cultivar Gizda is in group “c”. Separately by year, the trend of difference between the cultivars is preserved in terms of thousand grains weight. For the entire study period, the difference between the cultivars is preserved in a different configuration. The cultivar Ginra has the highest thousand kernels weight in group “a”, the Murgavetz cultivar falls in the intermediate group “ab”, the cultivars Gizda and Nikolay have the lowest thousand kernels weight in group “c”. A significant difference between the years of study was not observed. General trends between the cultivars differences in terms of the one thousand kernels weight are preserved, which confirms the study of Stoyanov (2018) in triticale.

The kernel density in spike by cultivar during the years of the study in the first year is predominantly slight variation, the variability increases in the fol-

lowing year following the trend of the number of grains per spike. The total variation in individual years is in the lower border of the medium variation (10.19-12.05%) (Table 3). The error increased in the second year of the investigation, leading to satisfactory and poor accuracy of the study. In the first year of the study, the most dense were the spikes of the cultivar Nikolay (group a), followed by the cultivar Murgavetz (group b), with a lower density of the clusters of the Ginra and Gizda cultivars (group c). The trend in terms of density is preserved in the following year of the study. No significant difference was observed by cultivars for the entire period. But, there is a significant difference between the years of the test, with spikes in the second year having a greater kernel density in spike, which is due to the deteriorated water regime during the spikelet setting period.

During the study period, it was found that spike length (85.41%) had the greatest impact on year, followed by kernel density per spike (61.84%). The influence of the environment on the weight of grains per spike (48.34%) and the height of the plants (48.44%) is the same. Similar to theirs is the influence of meteorological conditions on the grains number per spike (51.32%). With the weakest manifestation of the meteorological conditions per year is the thousand kernels weight (11.89%). Regarding the genotype with the greatest manifestation of the genotype is the thousand kernels weight (82.11%) and the productive tillers number /m² (64.97%). The possible reason for the more significant influence of the number of productive tillers/m² is the slight variation by year, as well as by cultivar, which made it possible to observe varietal differences, especially

Table 5. Analysis of the variance of morphological characters of wheat cultivars for the period 2017-2018

Traits	Influence of the year		Genotype		Year x Genotype		Error η %
	MS	η %	MS	η %	MS	η %	
productive tillers number /m ²	24414.063***	12.55	42142.563***	64.97	14474.729***	22.32	0.16
Plant hight (cm)	772.84***	48.44	196.813***	37.01	73.533***	13.83	0.72
Spike length (cm)	12.852***	85.41	0.432**	8.61	0.169	3.37	2.61
Kernel number /spike	1827.563***	51.32	463.576***	39.05	97.709***	8.23	1.40
Weight grain/spike (g)	2.042***	48.34	0.595***	42.28	0.098**	6.94	2.44
Thousand kernel Weight (g)	72.382***	11.89	166.652***	82.11	4.46	2.20	3.81
Kernel density per spike	10.053***	62.84	1.86***	34.87	0.043	0.80	1.49

*-prove for error $\alpha=0.05$, **- prove for error $\alpha=0.01$, ***- prove for error $\alpha=0.001$, η –power of the factor

in the second year. The variation of productivity tillers/m² of wheat cultivars for the whole period and by years is significant, giving no significant differences. Lovnyaeva (2007) recommends that in wheat, the selection of progeny in a comparative test of progeny in the first year should be based on morphological traits only, and in the second year of the test, it should be done by density of productive tillers and weight of grains in the spike. According to Kolev (1993), in triticale productive tillering has a significant variation and is not a reliable marker for breeding, as it is strongly influenced by environmental factors. According to him, the variation in plant height and thousand grains weight is low. He proves that they are more conservative traits, since they are hereditary in nature and can be used to carry out selection, (Table 5).

Plant height (37.01%) and kernel density/spike (34.87 %) were closely related to genotype. Grains number per (39.05%) and grains weight per spike (42.28%) had a higher but similar genotype influence to the above traits. The influence of year x genotype is greatest for the productive tillers number / m² (22.32 %). Of the listed traits for the cultivars Ginra, Gizda, Nikolay and Murgavetz, the most suitable morphological marker is thousand kernels weight with low to medium variation in the individual years and low variation by cultivar for the study period. The influence of genotype is the highest.

The plants height is also low variable during the individual years in the studied cultivars of common winter wheat. The accuracy of the study was high in both years of the investigation. For the entire period of the study, the variation is middle. The influence of genotype is weaker than that of weight per thousand kernels. The effect of genotype plus the combined effect of genotype and year on the trait plant height was similar in magnitude to the independent effect of year. The plant height may be used as a morphological marker due to the slightly variation of the trait. Kernel density is unsuitable as a marker due to the middle variation and satisfactory to poor accuracy of the study, found in the second year of the investigation.

CONCLUSIONS

Common winter wheat cultivars Ginra, Gizda, Nikolay and Murgavetz cultivars were homogeneous in progeny in a second year comparative study.

The most suitable morphological marker is thousand kernels weight with low to middle variation between years and low variation by cultivar over the study period.

Genotype influence is highest. Plant height is also low variable and may be used as a morphological marker.

The accuracy of the study was high in both years of the study.

The influence of genotype is highest on 1000 kernels weight, productive tillering, and lowest on spike length. The influence of year is highest on spike length, kernel density/spike and lowest on 1000 kernels weight.

REFERENCIAS

- Agency Plant Variety testing, Appr. & Seed Control** (2012). Results of varietal testing of varieties of field and vegetable crops recognized for inclusion in list "A", variety Nikolay, 12-13 (Bg). <https://iasas.government.bg>
- Agency Plant Variety testing, Appr. & Seed Control** (2016). Results of variety testing of varieties of plant species recognized for inclusion in lists "A" and "B" of the country's official variety list in 2016, Ginra cultivar. (Bg). <https://iasas.government.bg>
- Boydjjeva, D.** (2007). Cultivars of IPGR "K. Malkov"-town of Sadovo, cultivars of cereals crops, brochure. Institute of plant genetic resources "K. Malkov", Sadovo (Bg).
- Bresegghello, F., & Sorrells, M. E.** (2006). Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. *Genetics*, 172(2), 1165-1177. <https://academic.oup.com/genetics/article-pdf/172/2/1165/42070295/genetics1165.pdf>
- Dimitrov, G.** (2018). Establishment of genotypes of common winter wheat and peas suitable for organic farming, *Abstract of Dissertation*, Plovdiv, Bulgaria (Bg).
- Dimova, D., & Marinkov, E.** (1999). Experimental work with biometrics. *Academic print of AU*, 50, 93, 98 (Bg).
- Ficco, D., Beleggia, R., Pecorella, I., Giovanniello, V., Frenda, A. S., & De Vita, P.** (2020). Relationship between seed morphological traits and ash and mineral distribution along the kernel using debranning in durum wheats from different geographic sites. *Foods* (Basel, Switzerland), (11): E1523, pp 1-13. DOI: 10.3390/foods9111523.
- Georgieva, Hr., Tsankova, D., & Samodova, A.** (2004). Biological and economic qualities of some perspective wheat varieties. *Field crop studies*, vol. I, 1, 51-56 (Bg).
- Giunta, F., De Vita, P., Mastrangelo, A. M., Sanna, G., & Motzo, R.** (2018). Environmental and genetic variation for yield-related traits of durum wheat as affected by development. *Frontiers in plant science*, 9: 8.

- Jiang, X., Mao, D., Zhu, M., Wang, X., Li, C., Zhu, X., Guo, W., & Ding, J.** (2022). Evaluating the Waterlogging Tolerance of Wheat Cultivars during the Early Growth Stage Using the Comprehensive Evaluation Value and Digital Image Analysis. *Agriculture*, 12, 384. <https://doi.org/10.3390/agriculture12030384>
- Kachakova, Sv., & Desheva, G.** (2013). Correlations between the main structural elements of yield in common wheat cultivars, *Plant sci.*, 50, 5-8 (Bg).
- Khadka, K., Earl, H. J., Raizada, M. N., & Navabi, A.** (2020). Physio-morphological trait-based approach for breeding drought tolerant wheat, *Front. Plant Sci.* 11:715. doi: 10.3389/fpls.2020.00715.
- Kolev, K.** (1993). Studies on the methods and schemes for seed production of triticale. *Abstract of PhD Thesis*, IRGR, Sadovo (Bg).
- Lidanski, Tr.** (2012). Biostatistics: methods, schemes, analysis. Part I, Basic of biostatistics analysis. *Methods of biologicals traits*, 38-34 (Bg).
- Lovnyaeva, A. L.** (2007). Variability of spring soft wheat varieties and its use in primary seed production, (Izmenchivost' sortov yarovoy myagkoy pshenitsy i yeye ispol'zovaniye v pervichnom semenovodstve), *Abstract of PhD Thesis*, Voronezh, 9; 17-20 (Ru). <https://www.dissercat.com/content/selektionnaya-tsennost-sortov-i-gibridov-yarovoi-myagkoi-pshenitsy-v-usloviyakh-srednego-po/read>
- Macholdt, J., & Honermeier, B.** (2016). Variety choice in crop production for climate change adaptation: farmer evidence from Germany. *Outlook on Agriculture* 45(2):117–123. <https://journals.sagepub.com/doi/abs/10.1177/0030727016650770>
- Macholdt, J., & Honermeier B.** (2017). Importance of variety choice: Adapting to climate change in organic and conventional farming systems in Germany. *Outlook on Agriculture*. 46(3):178-184. <https://doi.org/10.1177%2F0030727017722420>
- Markova-Ruzdrik, N.** (2015). Characterization of autumn forms of barley (*Hordeum vulgare* L.) from different geographical origins. *Abstract of PhD Thesis*, Goce Delchev University. <http://eprints.ugd.edu.mk/13135/>
- Microsoft Excel-Microsoft Corporation, One Microsoft Way Redmond, WA 98052-6399
- Ministry of Agriculture and Food of Republic of Bulgaria** (2008). Ordinance No. 21 of 2007 on trade in cereal seed on the European Union market, State gazete №26/01.04.2022. Ordinance amending and supplementing Ordinance No. 21 of 2007 on trade in cereal seed on the European Union market (promulgated, SG No. 1 of 2008; amended, No. 49 of 2009, No. 38 of 2010, No. 40 of 2012, No. 74 of 2013, No. 58 of 2016, No. 25 of 2017, No. 6 and 30 of 2019, No. 57 of 2020 and No. 99 of 2021 (Bg). https://www.mzh.government.bg/media/filer_public/2022/06/09/naredba__21_ot_2007_g_za_trgovia_na_zrneni_kulturi_na_pazara_na_es.docx
- Ministry of Agriculture and Foods Industry** (1977). Instruction for production of super-elite and elite seeds and planting material from field, vegetable and perennial crops, NPO “Cultivar seeds and planting material”, direction “Cultivar maintenance”, Sofia, 10–12 (Bg).
- Muhova, A., & Stefanova-Dobрева, S.** (2021). *Triticale* (x *Triticosecale* Wittm.) grain quality under organic farming. *Journal of Mountain Agriculture on the Balkans*, 25 (1), 245-26 (Bg).
- Rachovska, G., Uhr, Zl., Andonov, Bl., Kamishev, K., & Tosheva, Sv.** (2012). Cultivars cereals crops, brochure. Agricultural academy, IPGR-Sadovo, 7 (Bg).
- SAS Institute Inc. (2002). A Business unit of SAS 1989 – 2002, JMP version 5.0.1.
- SPSS inc., IBM Corporation. Statistical package for the social sciences (SPSS 19).
- Stanislavovna, K. N.** (2017). Peculiarities of the formation of the qualities of winter soft wheat varieties in the conditions of the southern zone of the Rostov region. *Abstract of Dissertation*, Krasnodar, 1-26.
- Stoyanov, Hr.** (2018). Response of triticale (x *Triticosecale* Wittm.) to abiotic stress. Abstract of Dissertation, General Toshevo, 1-33 (Bg).
- Tshikunde, N. M., Mashilo, J., Shimelis, H. & Odindo, A.** (2019). Agronomic and Physiological Traits, and Associated Quantitative Trait Loci (QTL) Affecting Yield Response in Wheat (*Triticum aestivum* L.): A Review, *Front. Plant Sci.*, <https://doi.org/10.3389/fpls.2019.01428>
- Uhr, Zl., & Rachovska, G.** (2016). New Gizda variety of winter common wheat. *Phytologia Balcanica*, Sofia, 22(2), 205-207.
- Uhr, Zl.** (2017). Ginra variety - a combination of high yield and resistance to yellow rust. In *Jubilee scientific conference with international participation 135 years of science in Sadovo and 40 years of the Institute of Plant Genetic Resources-Sadovo*, 389-394.
- Vulchev, D., & Vulcheva, D.** (2019). Study on the influence of Panamin leaf fertilizer on plant development, resistance to abiotic stress, productivity and grain quality of wheat and barley. *Journal of Agriculture and Plant Sciences*, 17(1), 151 -157.
- World Meteorological Organisation** (2019). Global Climate in 2015-2019: Climate change accelerates.
- Zanke, C., Ling, J., Plieske, J., Kollers, S., Ebmeyer, E., Korzun, V., Argillier, O., Stiewe, G., Hinze, M., Neumann, F., Eichhorn, A., Polley, A., Jaenecke, C., Ganai, M.W., & Röder, M.** (2015). Analysis of main effect QTL for thousand-grain weight in European winter wheat (*Triticum aestivum* L.) by genome-wide association mapping. *Front. Plant Sci.* 6:644, doi: 10.3389/fpls.2015.00644
- Zhou, H., Riche, A. B., Hawkesford, M. J., Whalley, W. R., Atkinson, B. S., Sturrock, C. J., & Mooney, S. J.** (2021). Determination of wheat spike and spikelet architecture and grain traits using X-ray Computed Tomography imaging. *Plant Methods* 17 (26). <https://doi.org/10.1186/s13007-021-00726-5>