Evaluation of grain characteristics of domestic wheat (*Triticum aestivum* L.) obsolete cultivars and landraces

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Abstract

The research on wheat accessions was carried out in order to evaluate their grain characteristics. Experiment was set in the agro-ecological conditions of Banja Luka in two experimental years (2015/16 and 2016/17). Ten obsolete wheat cultivars (Brkulja, Ličanka, Partizanka, Vuka, Talijanka, Šiđanka, Orašanka, Lozničanka, Dokučajevskaja and Nemcitaevskaja) and two landraces (Podrašnica and Previja) were used. Following grain characteristics were evaluated: grain length (mm), grain width (mm), grain thickness (mm), grain volume (mm^3), grain surface area (mm^2), thousand grain weight (g) and grain protein content (%). Significant interactions were found for most of these traits. The relationship between traits was established by correlation analysis. In this study genotypes Brkulja and Šiđanka showed promising for most of the grain characteristics, while Vuka and Partizanka obtained the highest grain protein content.

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Key words: Genetic resources; grain morphology; grain protein; interaction.

Acknowledgements: the research was financially supported by Ministry for Scientific and Technological Development, Higher Education and Information Society of the Republic of Srpska, and examined wheat accessions are resources of Gene Bank of the Institute of Genetic Resources, University of Banja Luka. On this occasion, the authors would like to thank everyone.

Contributions: the authors contributed equally.

Conflict of interests: the authors declare no potential conflict of interests.

Received for publication: 16 October 2018.
Revision received: 5 November 2019.
Accepted for publication: 8 November 2019.

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Licensee PAGEPress, Italy
Italian Journal of Agronomy 2020; 15:1345
doi:10.4081/ija.2020.1345

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Introduction

Arable land in Bosnia and Herzegovina (BiH) is about 1 million hectares, and the wheat is present at about 66,000 hectares or about 7%, which is the main reason that we still import this basic cereal (Agency for Statistics of Bosnia and Herzegovina, 2018). In the wheat production areas in BiH obsolete cultivars were present earlier, and today they are replaced by more productive modern cultivars, while wheat landraces can be found in farms with extensive production conditions. Dotlacil *et al.* (2010) have found that the mean yield of modern cultivars was 51% higher than the examined obsolete cultivars and landraces. However, objectives in wheat breeding were improvement of crops and agronomic characteristics, as well as resistance against biotic and abiotic stress, but the most important goal was yield increase. Zeven (1999) claimed that wheat landraces are composed of traditional crop varieties developed through farmers and natural selection, adapted to local environmental conditions and management practices, and were often named after a farm or a locality. Landraces were largely cultivated until the first decades of the twentieth century, being progressively abandoned from the early 1970s and replaced with improved, genetically uniform semi-dwarf cultivars as a consequence of the Green Revolution (Ortiz *et al*., 2007). Landraces and obsolete cultivars can be considered as a valuable portion of the gene pool (Zou and Yang, 1995) due to their genetic diversity. Therefore, wheat genetic resources of each country should be preserved in gene banks, described, classified and evaluated, due to their potential significance to farmers and breeding programs. This research was performed in order to evaluate domestic winter wheat accessions by a quantification of grain morphological and qualitative traits, as well as their stability in two growing seasons. The question was raised whether the selected wheat genotypes have some valuable properties that make them interesting for reactivation in today's agroecological conditions.

Materials and methods

Plant material for this study consisted of 12 wheat accessions collected and stored by Gene Bank of the Institute of Genetic Resources, University of Banja Luka. Evaluation of morphological and qualitative traits was performed in two experimental years (2015/16 and 2016/17). Experiment was set in the agro-ecological conditions of Banja Luka (44°46' N; 17°11' E, and 164 m altitude). Study included 10 obsolete wheat cultivars (Brkulja, Ličanka, Partizanka, Vuka, Talijanka, Šiđanka, Orašanka, Lozničanka, Dokučaevskaja and Nemcitaevskaja) and two landraces (Podrašnica and Previja) that are still produced in a relatively poor households, as presented in Table 1.

Field experiments were based on a randomized complete block design with four replications, with 1×1 m plot size. All measurements were performed on 30 grains per replication. Standard production technology for winter wheat was applied, with seeding density of 504 grains m^{-2}. Sowing was performed on the 27th of October in 2015/16 and on the 2nd of November in 2016/17. Wheat harvest in both experimental years was at the end of the first decade of July. Plant samples were taken for the analysis just prior to harvest.

The average temperature in the vegetation period for winter wheat (from October to June) was 10.1°C in 2015/16 and 9.1°C in 2016/17, while the total precipitation in the same period was 855.8 mm in 2015/16 and 737.1 mm in 2016/17 (Table 2).

Dimensions of individual seed were calculated on the basis of following measurements: grain length (GL), grain width (GW) and grain thickness (GT), measured along the three principal axial dimensions by using a digital calliper with 0.01 mm accuracy (MIB, Germany).

Grain volume (GV) was calculated (Oručević *et al*., 2016) by using the following formula (Eq. 1):

$$GV(mm^3) = 0.52 \times GL \times GW \times GT \quad (1)$$

where 0.52 is a coefficient for wheat and barley seed shape. Grain surface area (GA) was also calculated (Eq. 2):

$$GA(mm^2)=12.56 \times R(GL+3R) \quad (2)$$

where R (seed diameter) was calculated as $R = (5GL + 5GW)/60$.

Grain protein content (PC) in % was calculated by multiplication of percentage of nitrogen content determined by Kjeldahl method with conversion factor for wheat (N×5.7). Grain protein content, as well as the thousand grain weight, were made in three replicates.

A two-factorial analysis of variance was conducted, with genotypes and years as factors. Significant differences were previously detected by calculating F test statistics with $P<0.05$ and $P<0.01$. Multiple comparisons between genotypes, years or genotype *x* year interactions were performed for significant F-values, by Fisher's *post-hoc* least significant difference (LSD) test at significance $P<0.05$ $P<0.01$, for different wheat seed characteristics. Relationship between analysed grain characteristics was evaluated by correlation analysis, using Pearson's correlation coefficient, at $P<0.05$ and $P<0.01$. All calculations were done in IBM SPSS Statistics 22.0 statistical software.

Results

Grain length, grain width and grain thickness

Obtained results (Table 3) indicate that the average GL ranged from 6.26 mm in genotype Podrašnica to 7.29 mm in genotype Brkulja in 2015/16 and from 5.70 mm in genotype Dokučaevskaja to 6.76 mm in genotype Brkulja in 2016/17. According to the analysis of variance the effects of genotype and year were both statistically significant at $P<0.001$. However, the interaction effect genotype × year was significant at $P<0.05$, therefore further analysis will be based on 24 genotype × year interaction means. According to the LSD-test, the highest GL of 7.29 mm in Brkulja

Table 1. General characteristics of analysed winter wheat genotypes.

	Genotype name	Genotype status	Genotype origin	Accession number
1.	Podrašnica	L	Mrkonjić Grad, Bosnia and Herzegovina	GB01054
2.	Brkulja	OC	Faculty of Agriculture and Institute for Agricultural Research Novi Sad, Serbia (YBP)	GB00107
3.	Dokučaevskaja	OC	Krasnodar Research Institute of Agriculture PP Lukyanenko, Russia	GB00213
4.	Ličanka	OC	Institute for Agricultural Research Novi Sad, Serbia (YBP)	GB00171
5.	Partizanka	OC	Institute for Agricultural Research Novi Sad, Serbia (YBP)	GB00089
6.	Vuka	OC	Institute for Plant Breeding and Plant Production Zagreb, Croatia (YBP)	GB00105
7.	Talijanka	OC	Obsolete cultivar from Italy (original name unknown)	GB00849
8.	Previja	L	Ribnik, Bosnia and Herzegovina	GB01060
9.	Nemcitaevskaja	OC	Obsolete cultivar from the Soviet Union	GB00193
10.	Šiđanka	OC	Institute for Agricultural Research Novi Sad, Serbia (YBP)	GB00146
11.	Orašanka	OC	Centre for Small Grains in Kragujevac, Serbia (YBP)	GB00208
12.	Lozničanka	OC	Centre for Small Grains in Kragujevac and Institute for Agricultural Research Novi Sad, Serbia (YBP)	GB00831

L, landrace; OC, obsolete cultivar; YBP, Yugoslav breeding program.

Table 2. The average monthly air temperatures (°C) and total monthly precipitation (mm) for the area of Banja Luka.

Year/Month		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2015	°C	3.4	2.4	7.3	11.8	17.4	20.9	25.2	24.0	18.3	11.5	7.1	3.2
	mm	111.2	91.1	79.0	54.1	117.6	60.5	20.5	22.8	75.0	142.7	85.7	8.1
2016	°C	2.3	7.6	8.0	13.5	16.2	21.5	23.3	20.5	17.8	10.6	7.4	0.4
	mm	109.7	108.5	112.2	70.5	100.6	117.8	125.9	100.2	63.3	76.0	68.9	5.0
2017	°C	–3.6	5.5	9.7	11.8	17.5	22.9	24.4	24.0	15.7	11.9	6.9	4.6
	mm	87.2	100.4	124.0	148.4	92.1	35.1	38.0	42.5	134.4	99.3	106.3	142.1

in 2015/16 was not statistically different from GL in Šiđanka in 2015/16 (7.08 mm), but was statistically significantly higher at P<0.01 in comparison to remaining genotype × year combinations.

The average GW ranged from 3.15 mm (Podrašnica) to 3.66 mm (Šiđanka) in 2015/16 and from 2.66 mm (Dokučaevskaja) to 3.08 mm (Šiđanka) in 2016/17. The effects of genotype and year were both statistically significant at P<0.001. The interaction effect was significant at P<0.01. LSD-test showed that the GW in Šiđanka in 2015/16 (3.66 mm) was statistically significantly higher at P<0.01 in comparison to remaining combinations. The average GT ranged from 2.57 mm (Dokučaevskaja) to 3.05 mm (Brkulja) in 2015/16 and from 2.08 mm (Nemcitaevskaja) to 2.32 mm (Šiđanka) in 2016/17. The effects of genotype and year were both statistically significant at P<0.001. The interaction effect was significant at P<0.001. According to the LSD-test, GT in Brkulja in 2015/16 (3.05 mm) was statistically significantly higher at P<0.01 in comparison to remaining combinations.

According to Figure 1 all genotypes showed decrease in the average GL in 2016/17. This decrease was less noticeable in Orašanka and Podrašnica, which expressed stability of GL during experiment. The largest decrease of GL was present in Dokučaevskaja in 2016/17.

The average GW and GT had similar tendency, with Brkulja and Šiđanka showing larger values among all tested genotypes, which is the reason why they can be marked out as genotypes with the largest grains in both seasons.

Grain volume and grain surface area

According to the results (Table 4) the average GV ranged from 27.27 mm^3 (Dokučaevskaja) to 39.73 mm^3 (Brkulja) in 2015/16 and from 16.77 mm^3 (Dokučaevskaja) to 24.38 mm^3 (Šiđanka) in 2016/17. The effects of genotype and year were both statistically significant at P<0.001. The interaction effect genotype × year was significant at P<0.01. According to the LSD-test, the highest GV of 39.73 mm^3 in Brkulja in 2015/16 was not statistically different from GV in Šiđanka in 2015/16 (38.67 mm), but was statistically significantly higher at P<0.01 in comparison to remaining genotype × year combinations.

The average GA ranged from 47.31 mm^2 (Dokučaevskaja) to 60.50 mm^2 in (Brkulja) in 2015/16 and from 34.57 mm^2 (Dokučaevskaja) to 44.94 mm^2 (Brkulja) in 2016/17. The effects of genotype and year were both statistically significant at P<0.001. The interaction effect was not statistically significant, therefore the analysis was done within genotype and year main effects, and did not need a graphical representation. According to the LSD-test, the highest GA of 60.50 mm^2 in Brkulja was not statistically different from GA in Šiđanka (59.53 mm^2), but was statistically significantly higher at P<0.01 in comparison to remaining genotypes. The average GA in 2015/16 (53.50 mm^2) was statistically significantly higher at P<0.01 in comparison to average GA in 2016/17 (40.17 mm^2).

According to Figure 1, in all genotypes GV decreased in 2016/17. The highest GV in Brkulja and Šiđanka in both experimental years was noticeable, which distinguishes them from other genotypes, as confirmed by LSD-test. When it comes to GA, based on the LSD-test, Brkulja and Šiđanka showed larger values among tested genotypes, with significantly higher values in 2015/16.

Thousand grain weight

The average thousand grain weight (TGW) ranged from 18.80 g (Dokučaevskaja) to 27.96 g (Brkulja) in 2015/16 and from 22.37 g (Dokučaevskaja) to 37.53 g (Brkulja) in 2016/17. The effects of genotype and year were both statistically significant at P<0.001. The interaction effect was significant at P<0.05. According to the LSD-test, the highest TGW of 37.53 g in Brkulja in 2016/17 was statistically significantly higher at P<0.01 in comparison to all other combinations (interaction means).

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Table 3. Grain length (mm), grain width (mm) and grain thickness (mm) of 12 wheat genotypes.

Genotypes/Trait	GL		GW		GT	
	2015/16 $\bar{X} \pm S_{\bar{x}}$ C_v	2016/17 $\bar{X} \pm S_{\bar{x}}$ C_v	2015/16 $\bar{X} \pm S_{\bar{x}}$ C_v	2016/17 $\bar{X} \pm S_{\bar{x}}$ C_v	2015/16 $\bar{X} \pm S_{\bar{x}}$ C_v	2016/17 $\bar{X} \pm S_{\bar{x}}$ C_v
Podrašnica	6.26±0.059 10.48	6.16±0.045 7.95	3.15±0.027 9.67	2.70±0.028 11.33	2.76±0.028 11.12	2.11±0.020 10.48
Brkulja	7.29±0.044 6.64	6.76±0.053 8.66	3.44±0.019 6.17	3.07±0.026 9.33	3.05±0.022 8.07	2.23±0.021 10.18
Dokučaevskaja	6.45±0.042 7.19	5.70±0.040 7.75	3.17±0.025 8.79	2.66±0.026 10.57	2.57±0.018 7.62	2.12±0.018 9.50
Ličanka	6.69±0.044 7.17	6.26±0.043 7.46	3.43±0.024 7.82	2.93±0.026 9.87	2.86±0.021 7.99	2.23±0.024 11.97
Partizanka	6.79±0.041 6.54	6.45±0.044 7.45	3.37±0.021 6.71	3.03±0.035 12.62	2.84±0.019 7.29	2.20±0.026 12.92
Vuka	6.33±0.044 7.56	5.89±0.062 11.71	3.22±0.022 7.34	2.78±0.029 11.27	2.76±0.019 7.70	2.24±0.026 12.99
Talijanka	6.57±0.047 7.87	6.24±0.056 9.88	3.41±0.028 9.13	2.82±0.030 11.56	2.86±0.023 8.83	2.22±0.024 12.09
Previja	6.89±0.043 7.07	6.41±0.053 9.03	3.44±0.021 6.63	2.88±0.028 10.67	2.90±0.017 6.48	2.19±0.019 10.12
Nemcitaevskaja	6.44±0.045 7.60	6.21±0.045 8.10	3.37±0.023 7.59	2.77±0.026 10.29	2.83±0.020 7.79	2.08±0.020 10.83
Šiđanka	7.08±0.046 7.13	6.58±0.051 8.62	3.66±0.025 7.34	3.08±0.029 10.48	2.87±0.018 7.22	2.32±0.029 13.79
Orašanka	6.67±0.045 7.54	6.59±0.044 7.25	3.30±0.020 6.66	2.84±0.024 9.29	2.93±0.018 6.77	2.18±0.019 9.61
Lozničanka	6.94±0.042 6.72	6.49±0.038 6.41	3.30±0.020 6.55	2.85±0.026 10.11	2.77±0.018 7.22	2.15±0.022 11.32
$F_{genotype}$	23.72***		23.48***		13.42***	
F_{year}	125.03***		982.00***		2668.62***	
$Fg_{enotype \times year}$	2.43*		2.58**		5.90***	
$LSD_{0.05}$	0.2391		0.1075		0.0860	
$LSD_{0.01}$	0.3173		0.1427		0.1142	

GL, grain length (mm); GW, grain width (mm); GT, grain thickness (height) (mm). *Significant at P<0.05; **significant at P<0.01; ***significant at P<0.001.

As opposed to other grain characteristics, the average TGW obtained an increase in 2016/17 (Figure 1). Brkulja had the highest values of TGW in both experimental years, followed by Previja and Šiđanka.

Grain protein content

The average PC ranged from 10.06 % (Nemcitaevskaja) to 12.76 % (Vuka) in 2015/16 and from 10.73 % (Lozničanka) to 13.43 % (Partizanka) in 2016/17 (Table 4). The effects of genotype and year were both statistically significant at P<0.001. The interaction effect was also significant at P<0.001. Performed LSD-test showed that there were no statistically significant difference between PC in Partizanka in 2016/17 (13.43 %) and PC in Brkulja in 2016/17 (12.86 %). Obtained PC in Vuka in 2015/16 (12.76 %) was statistically significantly lower at P<0.05 in comparison to Partizanka in 2016/17, while the remaining combinations obtained statistically significantly lower PC at P<0.01 in comparison to Partizanka in 2016/17.

According to Figure 1 genotypes reacted very differently considering PC in two seasons. Šiđanka expressed stability in PC during experiment, Vuka, Orašanka and Lozničanka obtained a decrease of PC in the 2016/17, while remaining genotypes

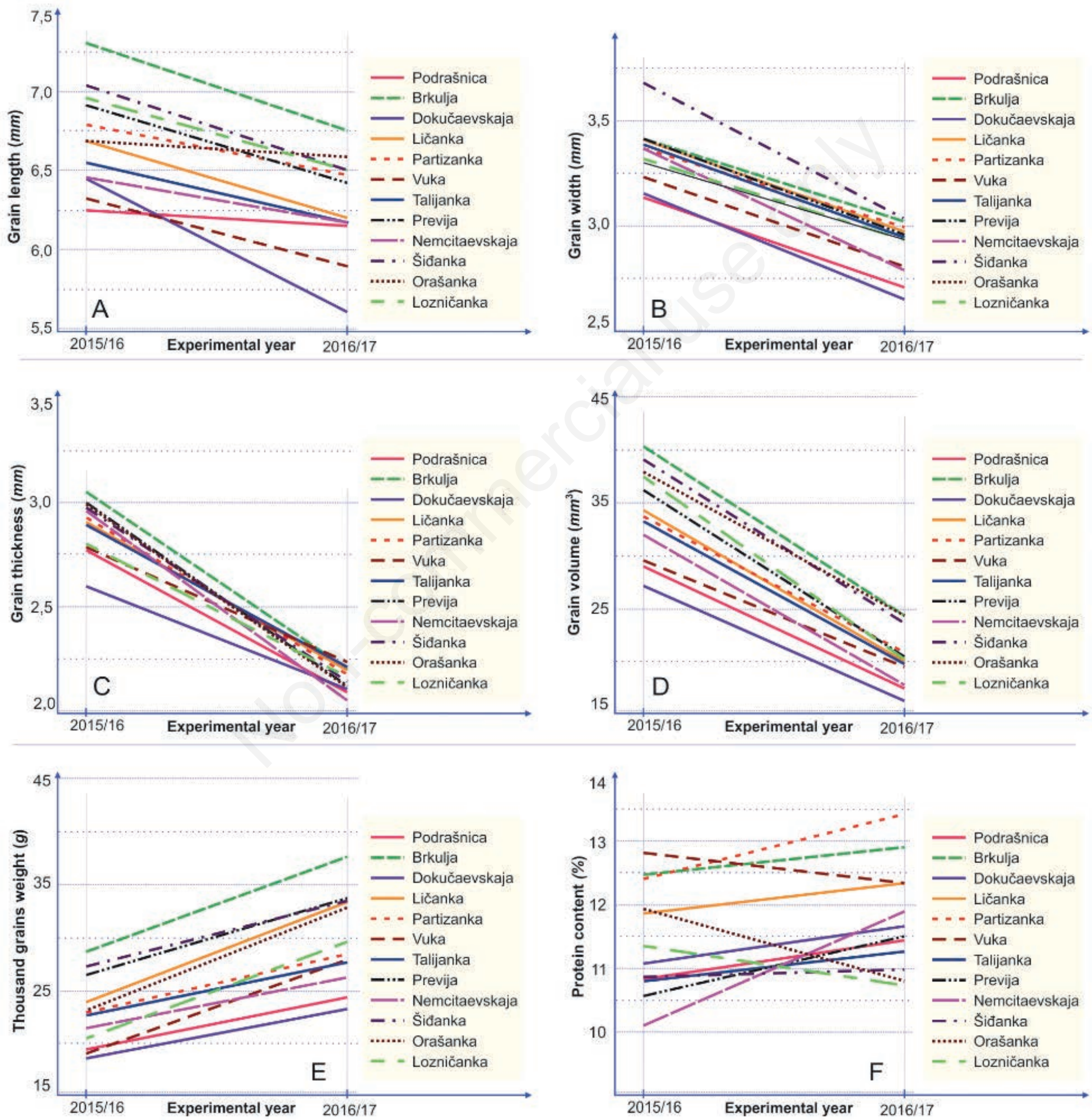

Figure 1. The analysis of interaction effects genotype × year. A) grain length; B) grain width; C) grain thickness; D) grain volume; E) thousand grain weight; F) grain protein content (%).

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obtained an increase. Partizanka and Brkulja, due to their increase, as well as the highest PC can be marked out as the most promising genotypes.

Relationships between analysed grain characteristics

According to Table 5, all correlations were positive. There were no significant correlations between PC (%) and remaining traits. GL correlated significantly (at $P<0.01$) with GW and GT while remaining correlations were highly significant (at $P<0.001$).

Discussion

The analyses of genetic diversity are very important in different breeding programs and management of plant genetic resources. Grain size and shape are two among main targets in wheat breeding programs (Gegas *et al*., 2010; Okamoto *et al*., 2013). Also, milling properties of wheat depend on the mechanical properties of the kernels, which are dependent on kernel size (Dziki and Laskowski, 2004). Grain morphology depends on accumulation of dry matter and water. The first dimension that reaches its maximum value is grain length, which happens about 15 days after anthesis, followed by grain width, height and volume (Hasan *et al*., 2011). Grain dimensions then slightly decrease and reach their final size at maturity (Liziana et al., 2010). In addition, the grain size is under a complex genetic control and at the same time is influenced by the environment (Cristina *et al*., 2016).

The average GL in our research was 6.70 mm in 2015/16 and 6.31 mm in 2016/17. This is similar to results by Varga *et al*. (2003) obtained on modern Croatian wheat cultivars and Xie *et al.* (2015). The average GW was 3.35 mm in 2015/16 and 2.87 mm in 2016/17, is in accordance with Gegas *et al.* (2010) and Abdipour *et al.* (2016) who studied 98 Iranian bread wheat landraces. The aver-

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Table 4. Grain volume (mm^3), grain surface area (mm^2), thousand grain weight (g) and grain protein content (%) of 12 wheat genotypes.

Genotypes/Trait	GV		GA		TGW		PC	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
	$\bar{X} \pm S_{\bar{x}}\ C_v$	$\bar{X} \pm S_{\bar{x}}\ C_v$	$\bar{X} \pm S_{\bar{x}}\ C_v$	$\bar{X} \pm S_{\bar{x}}\ C_v$	$\bar{X} \pm S_{\bar{x}}\ C_v$	$\bar{X} \pm S_{\bar{x}}\ C_v$	$X \pm S_x\ C_v$	$X \pm S_x\ C_v$
Podrašnica	28.30±0.48 18.47	18.29±0.32 19.22	47.92±0.54 12.38	37.11±0.43 12.62	19.51±0.94 8.32	23.78±1.77 12.91	10.83±0.47 7.56	11.44±1.04 15.73
Brkulja	39.73±0.46 1281	24.14±0.42 18.68	60.50±0.47 8.50	44.94±0.51 12.36	27.96±1.71 10.57	37.53±4.02 18.56	12.47±0.81 11.22	12.86±0.75 10.07
Dokučaevskaja	27.27±0.40 15.99	16.77±0.25 16.61	47.31±0.46 10.56	34.57±0.35 11.11	18.80±0.78 7.22	22.37±1.34 10.34	11.04±0.61 9.62	11.60±0.73 10.88
Ličanka	34.12±0.51 16.25	21.35±0.39 20.08	54.38±0.54 10.76	40.87±0.48 12.84	24.38±1.09 7.78	33.55±1.68 8.67	11.85±0.63 9.14	12.27±0.44 6.20
Partizanka	33.78±0.42 13.73	22.40±0.49 23.75	54.21±0.46 9.29	42.49±0.58 15.02	23.63±1.86 13.67	28.36±1.04 6.35	12.32±0.56 7.88	13.43±0.96 12.43
Vuka	29.32±0.41 15.44	19.09±0.47 26.91	49.04±0.47 10.49	37.54±0.60 17.43	19.98±1.31 11.33	27.51±1.46 9.18	12.76±0.75 10.12	12.38±0.64 8.93
Talijanka	33.42±0.54 17.52	20.25±0.40 21.71	53.49±0.57 11.67	39.51±0.52 14.26	23.53±1.28 9.42	27.91±2.15 13.36	10.82±0.42 6.67	11.26±0.52 8.03
Previja	35.76±0.43 13.38	20.99±0.38 19.78	56.25±0.47 9.25	40.71±0.49 13.18	25.88±1.46 9.76	33.94±3.38 17.23	10.61±0.50 8.18	11.50±0.45 6.70
Nemcitaevskaja	31.92±0.44 15.01	18.63±0.34 20.20	51.82±0.48 10.10	37.71±0.45 13.14	21.56±0.82 6.55	26.83±1.46 9.43	10.06±0.46 7.93	11.87±0.80 11.67
Šiđanka	38.67±0.54 15.25	24.38±0.48 21.64	59.53±0.57 10.54	44.71±0.56 13.72	26.35±1.07 7.03	33.88±3.09 15.81	10.85±0.57 9.10	10.91±0.69 11.03
Orašanka	33.61±0.43 13.99	21.29±0.36 18.61	53.72±0.47 9.57	41.30±0.46 12.05	23.43±1.64 12.09	33.10±3.05 15.96	11.87±0.65 9.52	10.84±0.46 7.33
Lozničanka	33.06±0.40 13.35	20.77±0.33 17.46	53.81±0.44 8.93	40.61±0.40 10.88	21.95±2.01 15.83	29.44±3.07 18.05	11.30±0.75 11.43	10.73±0.57 9.13
$F_{genotype}$	35.47***		35.44***		61.41***		16.66***	
F_{year}	1882.62***		1492.80***		637.30***		12.24***	
$F_{genotype}$ × year	3.23**		1.70ns		5.69*		4.16***	
$LSD_{0.05}$	1.9975		$LSD_{GENOTYPE}$ = 1.6842; LSD_{YEAR} = 0.6876		1.8521		0.6419	
$LSD_{0.01}$	2.6513		$LSD_{GENOTYPE}$ = 2.2354; LSD_{YEAR} = 0.9126		2.4582		0.8563	

GV, grain volume (mm^3); GA, grain surface area (mm^2); TGW, thousand grain weight (g); PC, grain protein content (%); ns, statistically not significant. *Significant at $P<0.05$; **significant at $P<0.01$; ***significant at $P<0.001$.

Table 5. Pearson's correlation coefficients between different traits (two year-average).

Variable	GL	GW	GT	GV	GA	TGW	PC
GW	0.8290**	1					
GT	0.7894**	0.8344***	1				
GV	0.9405***	0.9524***	0.9111***	1			
GA	0.9553***	0.9457***	0.8977***	0.9987***	1		
TGW	0.8862***	0.8608***	0.9254***	0.9471***	0.9419***	1	
PC	0.1075^{ns}	0.1649^{ns}	0.2995^{ns}	0.1826^{ns}	0.1768^{ns}	0.1946^{ns}	1

GL, grain length (mm); GW, grain width (mm); GT, grain thickness (height) (mm); GV, grain volume (mm^3); GA, grain surface area (mm^2); TGW, thousand grain weight (g); PC, grain protein content (%); ns, statistically not significant. *Significant at $P<0.05$; **significant at $P<0.01$; ***significant at $P<0.001$.

age GT was 2.83 mm in 2015/16 and 2.19 mm in 2016/17, similar to Xie *et al.* (2015).

Grain characteristics, such as grain volume, perimeter and area due to their larger heritability than grain yield, can be used as indirect indices in grain yield improvement (Abdipour *et al.*, 2016). The average GV in our research was 33.25 mm in 2015/16 and 20.70 in 2016/17. These results are in accordance with Nielsen *et al.* (2003) and Grabowski *et al.* (2012) who studied different wheat cultivars. The average GA was 53.50 mm^2 in 2015/16 and 40.17 mm^2 in 2016/17. Other researchers reported relatively less GA. In Abdipour *et al.* (2016) GA ranged from 8.1 to 26.5 mm^2 and in Gegas *et al.* (2010) from 14.69 to 33.08 mm^2 in different wheat genotypes. GA ranging from 11.44 to 24.71 mm^2 (Okamoto *et al.*, 2013) and from 8.32 to 14.93 mm^2 (Yan *et al.*, 2017) was previously obtained.

Calculated TGW, marked as one of the main grain size variables (Gegas *et al.*, 2010), is also one of the main target of wheat breeding activities (Abdipour *et al.*, 2016). The average TGW in our research was 23.08 g in 2015/16 and 29.85 g in 2016/17, similar to Yan *et al.* (2017). According to scale of characterization and evaluation descriptors of European Wheat Database, obtained TGW value of examined wheat genotypes belong to the category of very low to intermediate. However, TGW can be very variable trait, *i.e.* in bread wheat landraces it can range from 39.0 to 56.6 g (Piergiovanni, 2013) and from 26.2 to 68.7 g (Abdipour *et al.*, 2016). TGW of 55 Bulgarian wheat accessions was 40.59 g (Desheva, 2014). In Dotlacil *et al.* (2003) TGW ranged from 36.3 to 41.7 g in winter wheat landraces, obsolete cultivars and modern cultivars from different countries of origin.

In our study genotypes Brkulja and Šiđanka showed some promising characteristics regarding grain characteristics. In the 2015/16 Brkulja obtained the highest GL, GT, GV, GA and TGW, and Šiđanka had similar results, with the highest GW. In 2016/17 Brkulja achieved the highest GL, GA and TGW, similar to Šiđanka achieving the highest value for GT and GV.

It is well known that the economic value of wheat depends on the grain morphological and chemical characteristics. Grain protein content is important component of wheat grain quality due to its effect on milling yield, end-use quality and market price (Abdipour *et al.*, 2016). Wheat grain storage protein content and composition are main determinants of the end-use value of bread wheat grain (Plessis *et al.*, 2013). Baking potential of wheat flour is influenced by many factors, most notably protein content (Huebner *et al.*, 1999). According to Shevry (2009) grain wheat in the dry matter contains about 8 to 15% proteins. The average PC in our research was 11.39% in 2015/16 and 11.76% in 2017, with genotypes Partizanka and Vuka as dominant ones. However, according to the scale of IPGRI descriptor 6.3.3 (protein content) we must note that highest obtained protein content (13.43%) in genotypes Partizanka correspond to intermediate values. In Dotlacil *et al.* (2003) PC ranged from 13.2 to 13.3% in modern cultivars and from 15.3 to 16.2 % in landraces and obsolete cultivars. In Punia et al. (2017) PC ranged from 9.03 to 12.33% in different cultivars.

Based on the average temperatures and total precipitation (Table 2) in the vegetation period for winter wheat (from October to June) it is indicative that 2015/16 season was 1°C warmer than 2016/17 and 2.3°C warmer than reference period 1960-1991, with 118.7 mm more rainfall than 2016/17 and 95.2 mm more than 1960-1991. These obviously caused higher values for GL, GW, GT, GV and GA in 2015/16. However, as opposed to these traits, TGW and PC were lower.

Relationship between different grain characteristics were determined by a correlation. According to the results, there were no significant correlations between PC (%) and remaining traits. This is in accordance with Varga *et al.* (2003), while in Abdipour *et al.* (2016), PC and GT correlated negatively and significantly at P<0.01 (–0.256**). Five traits (GL, GW, GT, GV and GA) significantly correlated in our research, similar to Varga *et al*. (2003), Okamoto *et al.* (2013) and Yan *et al.* (2017).

Also, TGW positively and significantly correlated with GL, GW, GT, GV and GA as in Abdipour *et al.* (2016). In Varga *et al.* (2003) GL, GW and GT positively and significantly correlated with TGW. Also, TGW positively and significantly correlated with GL, GW and GA in Gegas *et al.* (2010) and Yan *et al.* (2017). Grain size and shape were positively correlated with TGW and they have affected wheat flour characteristics, yield and market price in Williams *et al.* (2013). Our results indicate that genotypes with larger grains obtained higher TGW.

Conclusions

Our results imply that genotypes Brkulja and Šiđanka bear some promising grain characteristics at the morphological level. Higher temperatures and precipitation increased grain width, thickness, volume and surface area, while thousand grain weight and grain protein content decreased at the same time. Also, genotypes Partizanka (12.88%) and Vuka (12.38%) obtained the highest protein content. Based on the protein content remaining genotypes can be categorized as genotypes with weak flour that can be used for the production of biscuits and cakes.

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