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, Šidanka, 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 (mm³), grain surface area (mm²), 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 Šidanka showed promising for most of the grain characteristics, while Vuka and Partizanka obtained the highest grain protein content.

**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, Šidanka, Orašanka, Lozničanka, Dokučajevskaja 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):

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

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

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

where R (seed diameter) was calculated as $R = (5GL + 3GW)/60$.

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 × 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 Dokućaevskaja 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
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 (Nemcicaevskaja) 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 Brkulja and Podrašnica, which expressed stability of GL during mental 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.

**Grain volume and grain size**

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 in Brkulja in 2015/16 was not statistically different from GV in Šiđanka in 2015/16 (38.67 mm\(^3\)), 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 50.50 mm\(^2\) (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 50.50 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 statistically 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).

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

| Genotypes/Trait | 2015/16 | 2016/17 | 2015/16 | 2016/17 | 2015/16 | 2016/17 | 2015/16 | 2016/17 |
|-----------------|---------|---------|---------|---------|---------|---------|---------|---------|
|                 | X ± SxC | X ± SxC | X ± SxC | X ± SxC | X ± SxC | X ± SxC | X ± SxC | X ± SxC |
| Podrašnica      | 6.26±0.059 | 7.54 | 6.59±0.044 | 7.25 | 3.30±0.020 | 6.66 | 2.84±0.024 | 9.29 |
| Brkulja         | 7.29±0.044 | 6.64 | 6.76±0.032 | 8.66 | 3.44±0.019 | 6.17 | 3.07±0.026 | 9.33 |
| Dokučaevskaja   | 6.45±0.042 | 7.19 | 5.70±0.040 | 7.75 | 3.17±0.025 | 6.55 | 2.85±0.026 | 10.11 |
| Ličanka         | 6.99±0.044 | 7.17 | 6.26±0.043 | 7.46 | 3.43±0.024 | 7.82 | 2.93±0.026 | 9.87 |
| 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 |
| Vaka            | 6.33±0.044 | 7.56 | 5.89±0.062 | 11.71 | 3.22±0.022 | 7.34 | 2.78±0.029 | 11.27 |
| Talijanka       | 6.57±0.047 | 6.87 | 6.24±0.056 | 9.88 | 3.41±0.028 | 9.13 | 2.82±0.030 | 11.56 |
| Prevlja         | 6.89±0.043 | 7.07 | 6.41±0.053 | 9.03 | 3.44±0.021 | 6.63 | 2.88±0.028 | 10.67 |
| Nemcicaevskaja  | 6.44±0.045 | 7.60 | 6.21±0.045 | 8.10 | 3.37±0.023 | 7.59 | 2.77±0.026 | 10.29 |
| 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 |
| 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 |
| Loznjačanka     | 6.94±0.042 | 6.72 | 6.49±0.038 | 6.41 | 3.30±0.020 | 6.55 | 2.85±0.026 | 10.11 |

**F**\(_{\text{genotype}}\) 23.72***

**F**\(_{\text{year}}\) 125.03***

**F**\(_{\text{genotype × year}}\) 2.43*

**LSD**\(_{\text{genotype}}\) 0.2391

**LSD**\(_{\text{year}}\) 0.2391

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 Šidanka.

**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. Šidanka expressed stability in PC during experiment, Vuka, Orašanka and Lozničanka obtained a decrease of PC in the 2016/17, while remaining genotypes expressed differences in PC in two seasons.
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). 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 average

**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).

Table 4. Grain volume (mm³), grain surface area (mm²), thousand grain weight (g) and grain protein content (%) of 12 wheat genotypes.

| Genotypes/ genotype × year | GV          | GA          | TGW         | PC          |
|----------------------------|-------------|-------------|-------------|-------------|
| Podrašnica                 | 28.30±0.48  | 18.47       | 18.20±0.32  | 19.63±0.42  |
| Brkulja                    | 39.73±0.46  | 23.86±0.52  | 22.50±0.48  | 23.92±0.53  |
| Dokučaevskaja              | 27.27±0.40  | 15.99±0.56  | 16.77±0.25  | 17.60±0.28  |
| Ličanka                    | 34.12±0.51  | 22.40±0.51  | 21.35±0.39  | 22.30±0.34  |
| Partizanka                 | 33.78±0.42  | 13.73±0.56  | 22.40±0.59  | 23.75±0.50  |
| Vuka                       | 29.32±0.41  | 15.44±0.45  | 20.25±0.35  | 20.71±0.30  |
| Talijanka                  | 33.42±0.54  | 17.52±0.40  | 20.25±0.40  | 21.71±0.33  |
| Prečja                     | 35.76±0.43  | 13.38±0.58  | 20.99±0.38  | 21.98±0.37  |
| Nemčitaevskaja             | 31.92±0.44  | 15.99±0.56  | 16.77±0.25  | 17.60±0.28  |
| Šidanka                    | 38.67±0.54  | 15.25±0.40  | 24.38±0.48  | 21.64±0.30  |
| Orašanka                   | 33.61±0.43  | 13.89±0.56  | 21.25±0.36  | 21.08±0.36  |
| Lounčanka                  | 33.06±0.40  | 13.35±0.56  | 20.77±0.33  | 17.46±0.30  |

GV: grain volume (mm³); GA: grain surface area (mm²); 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       | 0.829**     | 1           |             |             |             |             |             |
| GT       | 0.789**     | 0.834***    | 1           |             |             |             |             |
| GV       | 0.940**     | 0.925***    | 0.9111***   | 1           |             |             |             |
| GA       | 0.953***    | 0.947***    | 0.8977***   | 0.9058***   | 1           |             |             |
| TGW      | 0.886**     | 0.806***    | 0.9254***   | 0.9471***   | 0.9419***   | 1           |             |
| PC       | 0.187**     | 0.169**     | 0.299**     | 0.182**     | 0.178**     | 0.1946***   | 1           |

GV: grain length (mm); GW: grain width (mm); GT: grain thickness (height) (mm); GV: grain volume (mm³); GA: grain surface area (mm²); 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 (Abidipour 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 in 2015/16 and 40.17 mm in 2016/17. Other researchers reported relatively less GA. In Abidipour et al. (2016) GA ranged from 8.1 to 26.5 mm and in Gegas et al. (2010) from 14.69 to 33.08 mm in different wheat genotypes. GA ranging from 11.44 to 24.71 mm (Okamoto et al., 2013) and from 8.32 to 14.93 mm (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 (Abidipour 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 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 (Abidipour 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 Šidanka showed some promising characteristics regarding grain characteristics. In the 2015/16 Brkulja obtained the highest GL, GT, GV and TGW, and Šidanka had similar results, with the highest GW. In 2016/17 Brkulja achieved the highest GL, GA and TGW, similar to Šidanka 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 (Abidipour 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 the dry matter contains about 8 to 15% proteins. The average PC 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 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.

Conclusions

Our results imply that genotypes Brkulja and Šidanka 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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