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This issue is printed with the financial support by Contract No. DNP 06-41/20.12.2017, financed from Fund ‘Scientific Research’ grant Bulgarian scientific periodicals.

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Phenotypic diversity in six-rowed winter barley (*Hordeum sativum* L.) varieties

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(Manuscript received 24 July 2017; accepted for publication 19 January 2018)

**Abstract.** The objective of this investigation was to study the phenotypic diversity among six-rowed winter barley varieties. The study was conducted in the Institute of Agriculture – Karnobat, during the period 2014/2015-2015/2016. The diversity among 22 barley varieties was estimated using principal component analysis and cluster analysis. Maximum variation was found for grain weight per a spike and grain number per a spike. The presence of significant variation among the evaluated six-rowed winter barley varieties suggests an opportunity for improvement of grain yield through using promising genotypes as parents in the winter barley improvement program.

**Keywords:** barley, phenotypic diversity, cluster analysis, principal component analysis

**Introduction**

Barley is an important cereal crop in Bulgaria. One of the main objectives of winter barley breeding program is to produce high-yielding and better-quality lines for release as cultivars (Mihova, 2013; Valcheva et al., 2013; Gocheva and Vulchev, 2014; Dimova, 2015). The presence of genetic diversity among genotypes is a prerequisite and paramount important for a successful breeding programme. The study of genetic divergence can assist in the choice of genotypes to be used in breeding programs for the development of new populations as it estimate the extent of diversity existed among selected genotypes (Mondal, 2003). Genetically diverse parents are able to produce considerable variability, which can enhance the scope of selection. The more diverse the parents, greater are the chances of obtaining broad spectrums of variability in segregating generations (Arunachalam, 1981).

A number of methods like principal component analysis, factor analysis and cluster analysis are presently available for the selection of parent and detection of genetic and phenotypic variability (Bertan et al., 2007). Hierarchical clustering techniques with Ward's method and average linkage probably have long been the most popular clustering method (Milligan, 1980). The techniques of multivariate analysis are also often used in genetic improvement programs to predict the genetic distance among the accesses (Hair et al., 2006). With each approach having distinct advantages and disadvantages, it has been proposed to use both methods to gain the benefits of each (Milligan, 1980; Hair et al., 2006).

The aim of this study was to identify phenotypic diversity among six-rowed winter barley varieties using principal component analysis and cluster analysis and select varieties with desirable traits for including in Bulgarian winter barley breeding program.

**Material and methods**

This research was conducted in the 2014/2015 and 2015/2016 growing seasons in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria. The experiments were organized in a Randomized Complete Block Design with 3 replications. Each plot consisted of five 110 cm rows, 30 cm apart. Sowing was performed by hand in March. Standard agronomic and plant protection practices were used.

The materials used in present study included 22 varieties of six-rowed winter barley: Albacete - Spain, Asterix and Sympa - France, Bagova, Dura, Georgia, Gita, Hasso, Hauers and Structure - Germany, Elassona – Greece, Hongehon Mahrve and Zaizai Shiro - Republic of Korea, Jumbo – Netherlands, Kerajio and Takayama Sangatsu –Japan, Kujawiak – Poland, Manatou – Belgium, Puebla – Mexico, Rebekka – Austria, Rinker – Switzerland, Veslets - the Bulgarian standard for winter feed barley (used as check variety).

The traits spike length (cm), awn length (cm), spikelet number per a spike, grain number per a spike, grain weight per a spike (g) and 1000 grains weights (g) were measured in the laboratory on ten plants sampled from the middle of the plot of each genotype in each replication.

Two multivariate analyses - principal component and cluster analyses were utilized. Principal component analysis and ANOVA were obtained by SPSS 16.00 for Windows (SPSS Inc., 2007). The cluster analysis was performed using the program Statistica 7.0 (StatSoft Inc., 2004) that adopts Euclidean distance as a measure of dissimilarity and the Ward's method as the clustering algorithm (Ward, 1963) using the newly created variables after standardizing. Mean values of variables, calculated based on measurements on plants from replication and years for each genotype, were used in the cluster and principal component analysis.

**Results and discussion**

The mean values of studied traits of 22 six-rowed winter barley varieties are presented in Table 1. Spike length ranged from 6.66 cm to 10.16 cm with coefficient of variation of 11.16%. Variety Kujawiak had significantly longer spike compared to Veslets. Significantly shorter spike than the check variety was recorded in varieties...
Table 1. Mean values of studied traits of 22 six-rowed winter barley varieties (2014/2015-2015/2016)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Spike length, cm</th>
<th>Awn length, cm</th>
<th>Peduncle length, cm</th>
<th>Spikelet number per a spike</th>
<th>Grain number per a spike</th>
<th>Grain weight per a spike, g</th>
<th>1000 grains weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veslets</td>
<td>8.51</td>
<td>12.75</td>
<td>25.03</td>
<td>73.75</td>
<td>64.16</td>
<td>3.22</td>
<td>49.14</td>
</tr>
<tr>
<td>Albacete</td>
<td>7.85</td>
<td>12.56</td>
<td>28.68</td>
<td>69.06</td>
<td>60.88</td>
<td>2.86</td>
<td>46.83</td>
</tr>
<tr>
<td>Asterix</td>
<td>8.32</td>
<td>12.22</td>
<td>20.64</td>
<td>66.50</td>
<td>55.58</td>
<td>2.68</td>
<td>49.60</td>
</tr>
<tr>
<td>Bogova</td>
<td>8.44</td>
<td>12.11</td>
<td>25.63</td>
<td>69.54</td>
<td>60.74</td>
<td>2.91</td>
<td>48.18</td>
</tr>
<tr>
<td>Dura</td>
<td>9.01</td>
<td>12.38</td>
<td>24.13</td>
<td>79.17</td>
<td>65.36</td>
<td>3.04</td>
<td>45.98</td>
</tr>
<tr>
<td>Elassona</td>
<td>6.66</td>
<td>11.97</td>
<td>22.38</td>
<td>61.03</td>
<td>54.61</td>
<td>2.71</td>
<td>49.00</td>
</tr>
<tr>
<td>Georgia</td>
<td>8.28</td>
<td>11.92</td>
<td>25.36</td>
<td>74.17</td>
<td>62.51</td>
<td>3.26</td>
<td>51.08</td>
</tr>
<tr>
<td>Gita</td>
<td>8.81</td>
<td>11.43</td>
<td>27.39</td>
<td>76.09</td>
<td>62.38</td>
<td>3.04</td>
<td>49.88</td>
</tr>
<tr>
<td>Hasso</td>
<td>9.58</td>
<td>13.70</td>
<td>22.91</td>
<td>80.00</td>
<td>57.06</td>
<td>3.11</td>
<td>47.52</td>
</tr>
<tr>
<td>Hauters</td>
<td>9.57</td>
<td>13.15</td>
<td>25.34</td>
<td>76.92</td>
<td>63.83</td>
<td>3.38</td>
<td>49.48</td>
</tr>
<tr>
<td>HongehonMahrye</td>
<td>7.77</td>
<td>6.02</td>
<td>24.12</td>
<td>67.58</td>
<td>51.68</td>
<td>2.39</td>
<td>49.05</td>
</tr>
<tr>
<td>Jumbo</td>
<td>9.21</td>
<td>12.92</td>
<td>27.63</td>
<td>76.33</td>
<td>61.07</td>
<td>2.63</td>
<td>42.80</td>
</tr>
<tr>
<td>Kerajiro</td>
<td>8.56</td>
<td>13.81</td>
<td>23.33</td>
<td>70.00</td>
<td>48.03</td>
<td>2.33</td>
<td>44.05</td>
</tr>
<tr>
<td>Kujawiak</td>
<td>10.16</td>
<td>13.63</td>
<td>25.38</td>
<td>82.79</td>
<td>48.40</td>
<td>2.50</td>
<td>47.28</td>
</tr>
<tr>
<td>Manatou</td>
<td>8.38</td>
<td>13.69</td>
<td>24.97</td>
<td>68.70</td>
<td>57.93</td>
<td>2.84</td>
<td>47.70</td>
</tr>
<tr>
<td>Puebla</td>
<td>6.77</td>
<td>15.34</td>
<td>19.07</td>
<td>60.34</td>
<td>48.55</td>
<td>2.44</td>
<td>46.53</td>
</tr>
<tr>
<td>Rebekka</td>
<td>7.13</td>
<td>14.67</td>
<td>25.16</td>
<td>64.34</td>
<td>55.57</td>
<td>2.44</td>
<td>44.85</td>
</tr>
<tr>
<td>Rinker</td>
<td>9.30</td>
<td>13.34</td>
<td>26.27</td>
<td>79.00</td>
<td>58.44</td>
<td>2.70</td>
<td>46.28</td>
</tr>
<tr>
<td>Structura</td>
<td>8.57</td>
<td>12.55</td>
<td>22.19</td>
<td>71.59</td>
<td>64.89</td>
<td>3.48</td>
<td>52.30</td>
</tr>
<tr>
<td>Sympa</td>
<td>9.14</td>
<td>12.27</td>
<td>23.22</td>
<td>79.00</td>
<td>66.47</td>
<td>3.02</td>
<td>46.43</td>
</tr>
<tr>
<td>TakayamaSangatsu</td>
<td>9.10</td>
<td>13.08</td>
<td>25.07</td>
<td>70.72</td>
<td>54.39</td>
<td>2.60</td>
<td>42.51</td>
</tr>
<tr>
<td>ZaizaiShiro</td>
<td>7.05</td>
<td>16.15</td>
<td>28.03</td>
<td>62.00</td>
<td>55.67</td>
<td>2.45</td>
<td>44.33</td>
</tr>
<tr>
<td>Mean</td>
<td>8.46</td>
<td>12.89</td>
<td>24.63</td>
<td>71.76</td>
<td>58.10</td>
<td>2.83</td>
<td>47.45</td>
</tr>
<tr>
<td>Minimum</td>
<td>6.66</td>
<td>6.02</td>
<td>19.07</td>
<td>60.34</td>
<td>48.03</td>
<td>2.33</td>
<td>42.51</td>
</tr>
<tr>
<td>Maximum</td>
<td>10.16</td>
<td>16.15</td>
<td>28.68</td>
<td>82.79</td>
<td>66.47</td>
<td>3.48</td>
<td>52.30</td>
</tr>
<tr>
<td>CV. %</td>
<td>11.16</td>
<td>16.31</td>
<td>9.53</td>
<td>9.08</td>
<td>9.75</td>
<td>13.12</td>
<td>5.40</td>
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<tr>
<td>LSD 0.05%</td>
<td>1.29</td>
<td>1.35</td>
<td>2.11</td>
<td>4.16</td>
<td>3.97</td>
<td>0.33</td>
<td>2.50</td>
</tr>
</tbody>
</table>

Puebla, Rebekka, Elassona and Zaizai Shiro.

Awns are the most photosynthetically-active spike tissues and according Motzo and Giunta (2002) they play an important role in grain yield. Awn length in our study varied from 6.02 cm (Elassona) to 16.15 cm (Zaizai Shiro). Peduncle length in tested varieties ranged from 19.07 cm (Puebla) to 28.68 cm (Albacete). Positive correlation between peduncle length and grain yield in barley has been reported (Hosin babaiy et al., 2011; Jouyban et al., 2015; Singh et al., 2015).

Spikelet number per a spike varied from 60.34 (Puebla) to 82.79 (Kujawiak) with a CV 9.08%. Varieties Dura, Hasso, Kujawiak, Rinker and Sympa had a spikelet number per a spike significantly higher the Bulgarian check variety. Maximum grain number per a spike 66.47 was recorded in Sympa and the minimum 48.03 in Kerajiro.

Grain weight per spike is an important component of yield. In this study, the highest grain weight per spike was founded in Structura (3.48 g), and Hauters (3.38 g), whilst Kerajiro had the lowest grain weight per spike (2.33 g). The 1000 grain weight ranged from 42.51 g to 52.30 g. Variety Structura had significantly higher 1000 grain weight compared with check variety Veslets. Thousand grain weight is one of the major yield components having a direct effect on the yield in barley (Pasam et al., 2012; Ahmadi et al., 2016).

The dendrogram divided studied six-rowed barley into three cluster groups (Figure 1). The first cluster included genotypes: Zaizai Shiro, Rebekka, Takayama Sangatsu, Kerajiro, Puebla, Hongehon Mahnye, Elassona and Asterix characterized by the less spikelets and grains per spike and lower g 1000 grains weight per spike. The second cluster group the varieties: Kujawiak, Jumbo, Rinker, Hasso, Sympa and Dura, which were characterized by the highest values of spike length and spikelet number per spike. The third cluster consisted of: Manatou, Bogova, Albacete, Structura, Hauters, Gita, Georgia and Veslets which are characterized by the highest grain number per a spike, grain weight per a spike and 1000 grains weight. The superior varieties from second and third clusters may be involve in a crossing programme to recover transgressive segregants with high yield potential.

The first two principal components accounted for 66.5% of the overall variation among varieties (Table 3). The first principal component (PC1) was positively correlated with grain weight per a spike (0.863), grain number per a spike (0.800), spikelet number per a spike (0.774) and spike length (0.694). Maximum genetic variance to PC2 was contributed by 1000 grains weight (0.752) and peduncle length (0.571). Such results indicated that traits spikelet number per a spike, grain number per a spike, grain weight per a spike and spike length contributed the maximum towards diversity of genotypes.
Figure 1. Dendrogram base on 7 traits of 22 six-rowed winter barley varieties (2014/2015-2015/2016)

Table 2. Cluster mean of characters of 22 six-rowed winter barley varieties (2014/2015-2015/2016)

<table>
<thead>
<tr>
<th>Traits</th>
<th>Cluster I /8/*</th>
<th>Cluster II /6/</th>
<th>Cluster III /8/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike length (cm)</td>
<td>7.67</td>
<td>9.40</td>
<td>8.55</td>
</tr>
<tr>
<td>Awn length (cm)</td>
<td>12.91</td>
<td>13.04</td>
<td>12.52</td>
</tr>
<tr>
<td>Peduncle length (cm)</td>
<td>23.48</td>
<td>24.92</td>
<td>25.57</td>
</tr>
<tr>
<td>Spikelet number per a spike</td>
<td>65.31</td>
<td>79.38</td>
<td>72.48</td>
</tr>
<tr>
<td>Grain number per a spike</td>
<td>53.01</td>
<td>59.47</td>
<td>62.17</td>
</tr>
<tr>
<td>Grain weight per a spike (g)</td>
<td>2.51</td>
<td>2.83</td>
<td>3.16</td>
</tr>
<tr>
<td>1000 grains weight (g)</td>
<td>46.12</td>
<td>46.05</td>
<td>49.32</td>
</tr>
</tbody>
</table>

*Figures in parentheses indicate number genotypes included

Table 3. Principal Component analysis of 22 six-rowed winter barley varieties

<table>
<thead>
<tr>
<th>Characters</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues</td>
<td>2.905</td>
<td>1.749</td>
<td>1.074</td>
</tr>
<tr>
<td>% of variance</td>
<td>41.505</td>
<td>24.991</td>
<td>15.347</td>
</tr>
<tr>
<td>Cumulative %</td>
<td>41.505</td>
<td>66.496</td>
<td>81.843</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Factor loadings</th>
<th>PC1</th>
<th>PC2</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike length</td>
<td>0.694</td>
<td>0.552</td>
<td>-0.357</td>
</tr>
<tr>
<td>Awn length</td>
<td>-0.320</td>
<td>0.267</td>
<td>0.606</td>
</tr>
<tr>
<td>Peduncle length</td>
<td>0.208</td>
<td>0.616</td>
<td>0.490</td>
</tr>
<tr>
<td>Spikelet number per a spike</td>
<td>0.774</td>
<td>0.518</td>
<td>-0.279</td>
</tr>
<tr>
<td>Grain number per a spike</td>
<td>0.800</td>
<td>-0.106</td>
<td>0.451</td>
</tr>
<tr>
<td>Grain weight per a spike</td>
<td>0.883</td>
<td>-0.386</td>
<td>0.241</td>
</tr>
<tr>
<td>1000 grains weight</td>
<td>0.544</td>
<td>-0.752</td>
<td>-0.025</td>
</tr>
</tbody>
</table>
Ram and Singh (1989) found spike length, grain per spike and grain weight were the main characters contributing yield in barley. It may be concluded that the divergence of these traits could be used in breeding programme for improvement in six-rowed winter barley.

Figure 2 displays a biplot in the dimension of the first and second PCs. The accessions with positive values for PC1 and negative values for PC2 were of considerable breeding interest because of their good combination of the studied yield related traits – Structura, Bagova, Georgia and Veslets. Hybridization between the genotypes with high positive values for PC1 as Hauteres, Gita, Dura, Sympa, Hasso and varieties with high negative values for PC2 as Hongehon Mahrve, Elassona and Asterix is expected to give promising and desirable segregants in subsequent generations.

Conclusion

The multivariate methods applied revealed considerable phenotypic diversity among varieties for traits under study. Cluster analysis grouped the 22 test winter six-rowed varieties into three three cluster groups. Furthermore, the first two principal components with eigenvalues greater than unity extracted 66.5 % of the total variation. Maximum variation was found for traits grain number per a spike and grain weight per a spike. According mean performance varieties Structura, Hauters, Georgia, Gita, Hasso and Dura might be selected as promising genotypes for future hybridization program.

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Yield and coefficient of ecological valence of spring barley in the regions of Sadovo and Karnobat, Bulgaria
N. Neykov, T. Mokreva

Agronomic performance of mutant lines of winter two-rowed barley
B. Dyulgerova, D. Valcheva, N. Dyulgerov

Phenotypic diversity in six-rowed winter barley (Hordeum sativum L.) varieties
N. Dyulgerov, B. Dyulgerova

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M. Koleva, Y. Stanoeva, I. Kiryakov, A. Ivanova, P. Chamurlyiski

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A. Genchev, H. Lukanov, I. Penchev

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Ts. Hristov, R. Binev

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M. Oblakova, Y. Popova, P. Hristakieva, N. Mincheva, M. Lalev
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G. Zhelyazkov, S. Stoyanova, I. Sirakov, K. Velichkova, Y. Staykov

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R. Nastova, V. Kostov, N. Gjorgovska, V. Levkov

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V. Jankuloska, I. Karov, G. Pavlovska

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M. Ahmed, Y.B. Kiri, M.S. Abubakar

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Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal?

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Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists, the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusions: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

References: In the text, references should be cited as follows: single author: Sandberg (2002); two authors: Andersson and Georges (2004); more than two authors: Andersson et al. (2003). When several references are cited simultaneously, they should be ranked by chronological order e.g.: (Sandberg, 2002; Andersson et al., 2003; Andersson and Georges, 2004). References are arranged alphabetically by the name of the first author. If an author is cited more than once, first his individual publications are given ranked by year, then come publications with one co-author, two co-authors, etc. The names of authors, article and journal titles in the Cyrillic or alphabet different from Latin, should be transliterated into Latin and article titles should be translated into English. The original language of articles and books translated into English is indicated in parenthesis after the bibliographic reference (Bulgarian = Bg, Russian = Ru, Serbian = Sr, if in the Cyrillic, Mongolian = Mo, Greek = Gr, Georgian = Geor., Japanese = Ja, Chinese = Ch, Arabic = Ar, etc.)

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