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Agronomic performance of mutant lines of winter two-rowed barley

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Abstract. The aim of this investigation was to study agronomic and morphological traits of winter two-rowed barley mutant lines in the M6 and M7 generations. Eight mutant lines, their parent – breeding line 244D and national standard cultivars – Obzor and Emon were evaluated in Complete Block Design with four replications. The research was conducted in the 2013/2014, 2014/2015 and 2015/2016 growing seasons in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria. The characters studied included days to heading, plant height, lodging, peduncle length, spike length, awn length, spikelet number per spike, grain number per spike, grain weight per spike, 1000 grains weight, grain yield, protein content, extract content and grading (>2.5mm). Mutant lines M1/3, M1/5 and M1/217 produced a significantly greater grain yield than the parent and standard cultivars. The improvement of grain yield was associated with increasing of spike length and grain weight per spike. The studied mutant lines were characterized with a high yield ability combined with other valuable agronomic traits and can be used in a breeding program for developing winter malting barley varieties.

Keywords: barley, mutation, grain yield, agronomic traits

Introduction

Barley is one of the major grain crop. Its distribution is worldwide and is of considerable economic importance for animal feed and malt production. To improve yield and other traits in barley, many breeding techniques are being used successfully. Mutation breeding is one of the important techniques to induce variation. The mutant cultivars in different crops had a great economic impact on agriculture and food productions (Ahloowali et al., 2004). More than 300 varieties of barley have been officially released by mutation breeding technique (Joint FAO/IAEA Mutant Variety Database).

Mutation breeding is part of the winter barley breeding program at the Institute of Agriculture, Karnobat and it has led to improved barley cultivars with higher yield (Vulchev and Dyulgerova, 2011; Dyulgerova and Vulchev, 2012; Dyulgerov, 2017).

Natural or induced genetic diversity can be promoted for the improvement of all major crops and the use of mutagenesis to create novel variation in particularly valuable crops with limited genetic variability. The use of mutagenesis in breeding has involved the selection of individual mutants with improved traits and their incorporation into breeding programmes (Parry et al., 2009). The mutants developed in barley had great potential for direct release and to include them in cross breeding programme. Many barley cultivars possess tolerance to biotic and abiotic stress and improved traits developed in the world through induced mutagenesis (Ahloowali et al., 2004).

The aim of this investigation was to study agronomic and morphological traits of winter two-rowed barley mutant lines in the M6 and M7 generations.

Material and methods

The research work was conducted during 2013/2014, 2014/2015 and 2015/2016 growing seasons at the Institute of Agriculture, Karnobat. For mutation induction the seeds pre-soaked in water for 16 hours were treated with 2 mM sodium azide for 2 hours, prepared in a buffer solution (pH=3) at room temperature and washed for 6 hours after treatment. The M1 plants grown in field were harvested in bulk. In M2 generation one spike per selected plant was harvested and the seeds of each M2 spike were sown in the field as spike to row progeny for M3 generation. The parent variety was planted in every 10 rows as a check. The mutants were developed through selection for higher yield than the parental material by applying selection pressure from M2-M6. Finally, the selected lines were tested in yield trial in the present investigation.

The 8 mutants and their parent - breeding line 244D and national standard cultivars – Obzor and Emon were evaluated in yield trial in Complete Block Design with four replications.

The characters studied included days to heading, plant height (cm), lodging (scale 9-1, where 9=no lodging and 1=100% lodging), peduncle length (cm), spike length (cm), awn length (cm), spikelet number per spike, grain number per spike, grain weight per spike (g), 1000 grains weight (g), grain yield (kg/ha), protein content (%), extract content (%) and grading (>2.5 mm, %).

The data were recorded on a plant basis by randomly selecting 10 plants from each plot. Days for heading, lodging, 1000 kernel weight, grain yield, protein content, extract content and grading were estimated on plot basis.

The significance of differences among means was compared by using Least Significant Difference (LSD) test at the 0.05 level of probability and the correlations were analyzed by Pearson’s correlation coefficient. Analyses were performed using SPSS 16.00 for Windows 16.0 (SPSS Inc., 2007).

Results and discussion

Mean grain yield and yield related traits of the mutant lines, parent line and check varieties (2013/2014 - 2015/2016) are...
presented in Table 1.

From the pooled data of the two years, there were no significant differences between mutant lines and parent variety for plant height and lodging. Plant height of mutant lines varied from 97.60cm to 109.15cm. Minimal lodging was recorded in line M 1/3 and maximal lodging in line Obzor. Days to heading of mutant lines were between 195.67 (M1/511) and 197.17 (M1/25). There were no significant differences in days to heading among tested lines, parent and standard Emon. Spike length ranged from 7.24cm (Obzor) to 10.82cm (M1/5) Lines M 1/3, M 1/5 and M 1/27 had significantly longer spike compared with the parent line 244D. Awn length ranged from 9.63cm to 12.00cm among the studied lines. Peduncle length of the mutant lines varied from 28.31cm (M1/25) to 38.93cm (M1/511), while that for the parent line was 31.67cm. Two mutant lines M1/3 and M1/5 had a significantly higher spikelet number per spike compared to the parent line. The highest number of grain per spike (32.01) was observed in M3/11, followed by M1/217 (31.65), M1/3 (31.53) and M1/5 (31.31). The lowest number of grains per spike (28.22) was observed in M3/2, M1/217 (1.85g) and M1/3 (1.83g) had the highest grain weight per spike, which was significantly different from 244D. The value of 1000 grains weight ranged from 41.84g (M1/25) to 46.67g (M1/3). The grain yield of the mutant lines was from 8.22% to 18.02% higher than the grain yield of the parent line. Lines M1/3 (7068 kg/ha), M1/5 (7097 kg/ha) and M1/217 (7483 kg/ha) had a significantly higher grain yield over parent 244D (6013 kg/ha) and over checks Obzor (6303 kg/ha) and Emon (6491 kg/ha).

Table 1. Mean grain yield and yield related traits of mutant lines, parent line and check varieties (2013/2014 - 2015/2016)

<table>
<thead>
<tr>
<th>Mutant lines</th>
<th>PH</th>
<th>L</th>
<th>DH</th>
<th>SL</th>
<th>AL</th>
<th>PL</th>
<th>SNS</th>
<th>GNS</th>
<th>GWS</th>
<th>TGW</th>
<th>GY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obzor</td>
<td>102.90</td>
<td>5.50</td>
<td>199.83</td>
<td>7.24</td>
<td>9.44</td>
<td>28.85</td>
<td>33.38</td>
<td>31.84</td>
<td>1.62</td>
<td>43.59</td>
<td>6302.50</td>
</tr>
<tr>
<td>Emon</td>
<td>101.43</td>
<td>7.33</td>
<td>198.50</td>
<td>7.49</td>
<td>9.40</td>
<td>27.35</td>
<td>32.74</td>
<td>31.45</td>
<td>1.59</td>
<td>45.59</td>
<td>6490.83</td>
</tr>
<tr>
<td>244D</td>
<td>103.11</td>
<td>7.75</td>
<td>197.17</td>
<td>9.23</td>
<td>11.40</td>
<td>31.67</td>
<td>30.06</td>
<td>28.61</td>
<td>1.57</td>
<td>44.43</td>
<td>6013.33</td>
</tr>
<tr>
<td>M 1/3</td>
<td>107.31</td>
<td>8.33</td>
<td>197.00</td>
<td>10.62</td>
<td>12.00</td>
<td>31.29</td>
<td>35.27</td>
<td>31.53</td>
<td>1.83</td>
<td>46.67</td>
<td>7068.33</td>
</tr>
<tr>
<td>M 1/5</td>
<td>101.88</td>
<td>8.17</td>
<td>197.00</td>
<td>10.82</td>
<td>10.75</td>
<td>35.35</td>
<td>33.32</td>
<td>31.31</td>
<td>1.77</td>
<td>43.92</td>
<td>7096.67</td>
</tr>
<tr>
<td>M 1/25</td>
<td>99.69</td>
<td>8.25</td>
<td>197.17</td>
<td>9.67</td>
<td>10.43</td>
<td>28.31</td>
<td>31.35</td>
<td>30.16</td>
<td>1.68</td>
<td>41.84</td>
<td>6890.00</td>
</tr>
<tr>
<td>M 1/217</td>
<td>108.16</td>
<td>8.25</td>
<td>196.58</td>
<td>10.41</td>
<td>10.68</td>
<td>32.10</td>
<td>32.62</td>
<td>31.65</td>
<td>1.85</td>
<td>45.54</td>
<td>7483.33</td>
</tr>
<tr>
<td>M 1/511</td>
<td>109.15</td>
<td>7.67</td>
<td>195.67</td>
<td>10.12</td>
<td>10.64</td>
<td>38.93</td>
<td>31.53</td>
<td>30.18</td>
<td>1.80</td>
<td>46.03</td>
<td>6930.00</td>
</tr>
<tr>
<td>M 2/14</td>
<td>97.60</td>
<td>8.25</td>
<td>196.50</td>
<td>9.44</td>
<td>9.63</td>
<td>30.22</td>
<td>31.94</td>
<td>30.53</td>
<td>1.60</td>
<td>44.12</td>
<td>6507.50</td>
</tr>
<tr>
<td>M 3/2</td>
<td>102.58</td>
<td>8.17</td>
<td>196.08</td>
<td>9.40</td>
<td>10.99</td>
<td>31.51</td>
<td>29.62</td>
<td>28.22</td>
<td>1.65</td>
<td>45.97</td>
<td>6712.50</td>
</tr>
<tr>
<td>M 3/11</td>
<td>100.89</td>
<td>8.00</td>
<td>196.42</td>
<td>10.05</td>
<td>10.60</td>
<td>29.94</td>
<td>33.24</td>
<td>32.01</td>
<td>1.73</td>
<td>46.12</td>
<td>6886.67</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>6.9</td>
<td>0.84</td>
<td>2.24</td>
<td>1.12</td>
<td>0.91</td>
<td>2.52</td>
<td>3.19</td>
<td>2.18</td>
<td>0.25</td>
<td>1.71</td>
<td>562.44</td>
</tr>
</tbody>
</table>

PH - plant height (cm), L - lodging (scale 9-1), DH - days to heading, SL - spike length (cm), AL - awn length (cm), PL - peduncle length (cm), SNS - spikelet number per spike, GNS - grain number per spike, GWS - grain weight per spike (g), TGW - 1000 grains weight (g), GY - grain yield (kg/ha)

The results of linear correlation between grain yield and yield related traits are shown in Table 2. Grain yield was found to be positively and significantly associated with peduncle length, spike length, spikelet number per spike, grain number per spike and grain weight per spike, indicating the importance of those traits for yield improvement in mutant lines. These results are supported by Jouyan et al. (2015) and Ahmad et al. (2016) for peduncle length, by Hosin Babaiy et al. (2011) and Budakli Carpici and Celik (2012) for spike length, grain number per spike and grain weight per 1 pike.

Table 2. Correlation coefficients between grain yield and yield related traits

<table>
<thead>
<tr>
<th>Traits</th>
<th>L</th>
<th>DH</th>
<th>SL</th>
<th>AL</th>
<th>PL</th>
<th>SNS</th>
<th>GNS</th>
<th>GWS</th>
<th>TGW</th>
<th>GY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH</td>
<td>-0.667**</td>
<td>-0.021</td>
<td>-0.054</td>
<td>-0.291</td>
<td>0.471*</td>
<td>0.286</td>
<td>0.345</td>
<td>-0.088</td>
<td>-0.100</td>
<td>0.107</td>
</tr>
<tr>
<td>L</td>
<td>0.160</td>
<td>0.401</td>
<td>0.136</td>
<td>-0.331</td>
<td>0.135</td>
<td>0.037</td>
<td>0.209</td>
<td>-0.156</td>
<td>-0.202</td>
<td></td>
</tr>
<tr>
<td>DH</td>
<td>0.157</td>
<td>-0.387</td>
<td>-0.361</td>
<td>0.335</td>
<td>0.317</td>
<td>0.071</td>
<td>0.037</td>
<td>-0.107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL</td>
<td>-0.188</td>
<td>0.287</td>
<td>0.736**</td>
<td>0.734**</td>
<td>0.752**</td>
<td>0.228</td>
<td>0.429*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>-0.236</td>
<td>-0.444*</td>
<td>-0.414</td>
<td>-0.055</td>
<td>-0.069</td>
<td>-0.333</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL</td>
<td>0.352</td>
<td>0.439*</td>
<td>0.267</td>
<td>0.309</td>
<td>0.601**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNS</td>
<td>0.928**</td>
<td>0.645**</td>
<td>0.157</td>
<td>0.451*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GNS</td>
<td>0.673**</td>
<td>0.132</td>
<td>0.509*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GWS</td>
<td>0.581**</td>
<td>0.536*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGW</td>
<td>0.349</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PH - plant height (cm), L - lodging (scale 9-1), DH - days to heading, SL - spike length (cm), AL - awn length (cm), PL - peduncle length (cm), SNS - spikelet number per spike, GNS - grain number per spike, GWS - grain weight per spike (g), TGW - 1000 grains weight (g), GY - grain yield (kg/ha); *significant at the 5% level; ** significant at the 1% level.
The protein content in barley grains has been considered as an important trait affecting malt quality. According to European Brewing Association, the barley used for malting should have a protein content below 11.5%. Generally, malt extract is reduced with the increase of protein content in grains, and higher protein content is also a major factor attributed to deterioration of flavor stability and formation of turbidity in beer. It is well documented that the protein content in barley grains is genetically controlled, but easily affected by the environmental conditions (Smith, 1990; Kaczmarek et al., 1999; Zhang et al., 2001; Wang et al., 2001). In our study standard
varieties Obzor and Emon had a protein content of 11.84% and 11.95% (Figure 1). The lowest protein content was found in parent line 244D (10.81%). Protein content in mutant lines varied from 11.72% to 12.34%.

Grain grading is an important parameter for malt barley. The percentage of a plump grain on a 2.5×20mm sieve for malt barley must be no less than 90%. Weather conditions, especially during the grain filling period, had the strongest influence on the grain grading percentage (Schelling et al., 2003; Paynter and Young, 2004). The variety Emon (95.30%) and the lines M 1/511 (90.33%), M 2/12 (90.47%) met the requirements for malting barley grain grading (Figure 2).

Extract content is the most important economic trait of malting barley. The extract content of the standard varieties was 76.87% for Obzor and 77.07% for Emon (Figure 3). Only one of the mutant lines M3/2 (77.13%) showed higher extract content compared to parent line 244D (75.63%).

The results of the present study showed that by applying induced mutagenesis useful changes in important agronomic traits such as grain yield, yield and malt quality related traits were obtained. Similar findings were reported by Bughio et al. (2007), Deniz (2007), Singh and Balyan (2009), Laghari et al. (2012), Albokari (2014), Obare et al. (2014) and Dyulgerova and Dyulgerov (2017).

**Conclusion**

The studied barley lines showed grain yield higher than that of the parent line by 8.2-18.0%. Mutant lines M1/3, M1/5 and M 1/217 had a significantly greater grain yield compared to the parent line and the check varieties Obzor and Emon. Positive changes in spike length, spikelet number per spike, grain number per spike, grain weight per spike, 1000 grains weight and extract content were also observed. The improvement of grain yield was associated with increasing of spike length and grain weight per spike. This study showed positive effects in the use of experimental mutagenesis in inducing improvement for important agronomic traits in winter barley.

**References**


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The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

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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?

Material and methods: The objects of research, organization of experiments, chemical analyses, statistical and other methods and conditions applied for the experiments should be described in detail. A criterion of sufficient information is to be possible for others to repeat the experiment in order to verify results.

Results are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text. Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

Figures should be sharp with good contrast and rendition. Graphic materials should be preferred. Photographs to be appropriate for printing. Illustrations are supplied in colour as an exception after special agreement with the editorial board and possible payment of extra costs. The figures are to be each in a single file and their location should be given within the text.

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 study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: 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, in the Cyrillic, Mongolian = Mo, Greek = Gr, Georgian = Geor., Japanese = Ja, Chinese = Ch, Arabic = Ar, etc.)

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Studies performed on experimental animals should be carried out according to internationally recognized guidelines for animal welfare. That should be clearly described in the respective section “Material and methods”.

Table: The editors recommend up to 15 tables for full research paper. Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted. Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted. Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.