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Grain quality of mutant lines of six-rowed barley

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Abstract. *The aim of this study was to assess the variation in grain quality traits among mutant lines of winter feed barley. Nine mutant lines derived from the advanced breeding line Kt3029 and 10 mutant lines derived from variety GA-Luttrell along with their parent forms were evaluated. The study was conducted at the Institute of Agriculture – Karnobat during the period 2015-2017. Test weight, 1000-grain weight, the content of protein, starch, lysine, fat, ash, and fibre were determined. Significant differences between the mutant lines and the parent forms for the studied traits were observed. Significantly higher protein content compared to parent was found in the grain of mutant lines 22/1-5, 22/2-10 and 22/1-12 from line Kt3029 and 26/1-1, 26/1-3 and 26/2-11 from variety GA-Luttrell. The grain of mutant line 26/2-12 had the best combination of protein and starch. Mutant lines 22/1-11 and 22/1-12 from Kt3029 and 26/1-1, 26/2-1 and 26/2-12 from variety GA-Luttrell showed a good combination between protein content and 1000-grain weight. Those mutant lines can be included in the breeding program for the development of varieties with improved feed quality*.

Kaywords: chemical composition, feed quality, grain weight, mutants, winter barley

Introduction

Barley is grown worldwide because of its adaptability to different environmental conditions and diverse end-use of barley grain. In Bulgaria, barley grain is used mainly for livestock feed. The quality of feed grain depends on its chemical composition and physical parameters. The most important components in barley for animal feeding are carbohydrates and protein (Blake et al., 2010).

Starch is the primary component of the carbohydrates contained in barley grain and normally its concentration ranges from 40 to 65% (Ullrich, 2002). Due to the relatively high starch content in barley grain, it is considered mainly as an energy source in livestock feed.

Protein is one of the main grain attributes with a direct impact on feed value. The content of protein in barley grain varied in a wide range and is influenced strongly by environmental factors and production practices (Ingvordsen et al., 2016).

The high content of lysine is also valuable for the identification of lysine as one of the most limiting amino acids in cereal protein. Normally whole grain of hulled barley contains about 0.45% lysine while the nutritional requirements for growing pigs vary from 0.6 to 1.0% (Ullrich, 2002). Considerable attempts for improving barley lysine content have been made since the discovery of the first high-lysine mutant „Hiproly“. Numerous studies demonstrate the nutritional superiority of high-lysine barley (Newman and McGuire, 1985), but pleiotropic effects of high-lysine genes as shrunken endosperm have been difficult to overcome in breeding work (Ullrich, 2002).

Fat content is relatively low in barley grain and usually varies between 1.9 and 2.8% (Griffey et al., 2010). Although several high-fat mutants had been isolated, the efforts to improve this trait have been limited in barley (Newman and Newman, 1992b).

From the physical parameters, important for the quality of feed barley are grain weight and size (Bleidere and Gaile, 2012). These parameters were found to be associated with the concentration of many nutrients in the barley grain. Grain with high 1000-grain weight and test weight usually is characterized with higher starch content and lower fibre content and therefore has high digestible and metabolizable energy (Fairbairn et al., 1999; Baik, 2014).

The grain hull is the primary source of fibre in barley grain. The hull is 10-15% of the dry weight of the barley grain and consists mainly of cellulose, hemicellulose, lignin, and pectins (Ullrich, 2002). High fibre content is particularly undesirable when the grain is used for feeding poultry and no ruminant livestock (Bleidere and Gaile, 2012). Removal of grain hull decreases barley fibre content to that of wheat and maize (Bhatty, 1993).

There are various plant breeding methods for obtaining increased grain yield and quality. Mutation breeding has been used successfully for improving many crops, including barley (Kharkwal and Shu, 2009). The major advantage of the application of experimental mutagenesis in plant breeding is the possibility of improving a single trait while preserving the complexity of valuable traits in elite breeding material (Shu, 2009).

Our previous study showed significant variation in some traits related to the malting quality of mutant lines selected for higher grain yield of four malting winter barley varieties (Dyulgerova and Dyulgerov, 2020).

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The aim of the present study was to assess the variation in grain quality traits among mutant lines of winter feed barley selected based on grain yield.

Material and methods

The materials used in the present study were 9 mutant lines derived from the Kt3029 line and 10 mutant lines derived from the GA-Luttrell variety. The Kt3029 is a high-yielding advanced line obtained at the Institute of Agriculture (IA) - Karnobat, after the treatment of seeds from variety Balaki with 300 Gy gamma rays. GA-Luttrell is a medium-high winter six-row variety resistant to *Rhynchosporium secalis* and BYDV, selected at the University of Georgia, USA (Johnson et al., 1998). All mutant lines were obtained after treatment with 1 mM or 2 mM sodium azide of seeds presoaked for 16h in water. From M_2 to M_5 selection for grain yield was applied and selected mutant lines were used in the present study. The mutant lines along with parents were grown in the experimental field of IA – Karnobat, Southeast Bulgaria by block method in 4 replications, on plots of 10 m^2, for three growing seasons - from 2015 to 2017.

Test weight, kg/hl; 1000-grains weight, g; protein content, % (ISO 20483:2006); starch content, % (ISO 10520:1997); lysine content,% (Lie,1973); fat content,% (ISO 6492:1999); ash content,% (ISO 2171:2007) and fibre content,% (ISO 5498:1981) were analysed.

The means were compared by Duncan's multiple range test at a 5% level of probability. Hierarchical cluster analysis using Between-groups linkage method with squared Euclidean distance and standardized mean variables was performed. All data were processed with the program SPSS 16.00 for Windows.

Results and discussion

Table 1 presents the mean values of quality traits of mutant lines derived from line Kt3029. The analysis of these lines showed significant ($p<0.05$) variation for the total protein content. It varied between 11.34% and 13.20%. Mutant lines 22/1-5, 22/1-12, and 22/2-10 had significantly higher total protein contents compared to the corresponding parent line Kt3029.

Table 1. Mean values and variation of quality traits of mutant lines derived from line Kt3029 (2015-2017)

Mutant line/Parent	Protein, %	Lysine, %	Starch, %	Ash, %	Fat, %	Fibre, %	Test weight, kg/hl	1000-grain weight, g
Kt3029	11.68[de*]	2.98[ab]	55.89[bc]	2.53[a]	1.87[cde]	4.31[d]	69.05[b]	47.21[ab]
22/1-2	11.63[de]	3.13[a]	55.91[bc]	2.54[a]	1.64[de]	6.01[abc]	68.27[c]	46.01[bc]
22/1-5	13.20[a]	2.53[c]	54.69[cd]	2.49[a]	1.43[e]	5.12[cd]	68.10[c]	45.05[c]
22/1-11	12.24[bcd]	2.74[bcd]	54.06[c]	2.64[a]	2.59[ab]	5.62[abc]	69.80[a]	48.31[a]
22/1-12	12.63[abc]	3.09[a]	53.45[c]	2.49[a]	2.34[abc]	5.66[abc]	68.18[c]	47.85[a]
22/1-14	11.34[e]	2.96[abc]	57.61[a]	2.44[a]	3.02[a]	6.16[ab]	68.88[b]	44.83[c]
22/2-1	11.68[de]	3.01[ab]	56.22[b]	2.51[a]	2.25[bcd]	5.61[abc]	67.53[d]	45.28[c]
22/2-4	11.37[e]	2.92[abc]	57.69[a]	2.39[a]	2.37[abc]	6.49[a]	69.83[a]	48.47[a]
22/2-10	12.80[ab]	2.47[c]	54.41[cd]	2.64[a]	2.63[ab]	5.33[abc]	68.26[c]	46.23[bc]
22/2-11	12.02[cde]	2.67[cd]	54.73[cd]	2.50[a]	2.35[abc]	5.56[abc]	69.21[b]	47.06[ab]
Mean	12.06	2.85	55.47	2.52	2.25	5.59	68.71	46.63
Min	11.34	2.47	53.45	2.39	1.43	4.31	67.53	44.83
Max	13.20	3.13	57.69	2.64	3.02	6.49	69.83	48.47
CV, %	5.03	7.74	2.47	2.94	20.25	10.20	1.06	2.74

*means with the same letter are not significantly different

Significant differences between mutant lines for lysine content were observed. The highest percentage of lysine content was found in the grains of 22/1-2 (3.13%), whereas the lowest one was demonstrated in the grain of 22/2-10 (2.47%). The starch content of mutant lines varied from 53.45% to 57.69% while it was 55.89% for the parent line. The mean content of starch for the studied period was higher in lines 22/1-14 and 22/2-4 compared to the parent line. No significant ($p<0.05$) differences were observed in ash content between the mutants from Kt3029.

The content of fat was the trait with the highest variation among mutant lines (CV=20.25%). Maximum fat content was observed in the grain of line 22/1-14 - 3.02% and minimum in line 22/1-5 - 1.43%.

The fibre content also varied significantly among mutant lines. All studied lines contain a higher percentage of fibre than the respective parent line. Two mutant lines (22/1-11 and 22/2-4) with higher test weight and 1000-grain weight compared to advanced line Kt3029 were found.

The studied grain quality traits of mutant lines developed from variety GA-Luttrell are presented in Table 2. Higher amount of protein than that in the parent variety was found in lines 26/1-1 (12.68%), 26/1-3 (12.63%), and 26/2-11 (12.84%). The lysine content varied from 2.29% (26/1-1) to 3.09% (26/2-16) while in the parent it was 2.72%. A significant variation in starch content among mutant lines was observed. Most of the mutants from GA-Luttrell were found to possess lower starch content than the parent. The maximum concentration of ash in grain was found in line 26/2-4 followed by line 26/1-10, whereas the lowest concentration was obtained in the grain of line 26/1-1. The highest fat content was recorded in mutant line 26/2-4 (2.64%) and the lowest in mutant line 26/1-6 (1.47%). One of the mutant lines (26/1-1) showed a significantly lower percentage of fibre in the grain. Significantly higher test weight was found in line 26/1-6. Mutant lines differing for 1000-grain weight were also observed. Lines 26/1-1, 26/1-6, 26/1-10, 26/2-1, and 26/2-12 had higher mean 1000-grain weight compared to variety GA-Luttrell.

Table 2. Mean values and variation of traits, related to grain feed quality (2015-2017)

Mutant line/Parent	Protein, %	Lysine, %	Starch, %	Ash, %	Fat, %	Fibre, %	Test weight, kg/hl	1000-grain weight, g
GA-Luttrell	11.74[bcd*]	2.72[ab]	56.03[abc]	2.60[bcd]	2.14[bc]	5.69[a-d]	69.22[b]	37.18[d]
26/1-1	12.68[a]	2.29[c]	53.07[ef]	2.51[d]	1.85[d]	4.64[f]	69.15[b]	39.97[b]
26/1-3	12.63[a]	2.63[bc]	54.01[de]	2.73[ab]	2.00[c]	5.09[ef]	69.96[a]	38.40[cd]
26/1-6	11.17[ef]	3.01[ab]	53.08[ef]	2.60[bcd]	1.47[e]	5.40[cde]	67.73[c]	39.59[bc]
26/1-10	11.48[de]	2.80[ab]	53.11[ef]	2.64[a]	2.39[a]	6.11[a]	67.51[cd]	39.92[b]
26/2-1	12.10[b]	2.62[bc]	55.43[bc]	2.56[cd]	1.76[d]	5.21[de]	67.15[d]	41.65[a]
26/2-4	11.54[cde]	2.75[ab]	52.56[fg]	2.86[a]	1.51[e]	5.53[b-e]	63.31[f]	38.19[cd]
26/2-8	11.09[ef]	3.01[ab]	57.10[a]	2.62[bcd]	2.19[b]	6.21[a]	67.20[d]	37.78[d]
26/2-11	12.84[a]	2.79[ab]	51.50[g]	2.75[ab]	2.04[bc]	5.99[ab]	64.30[e]	35.44[e]
26/2-12	12.07[bc]	2.64[bc]	56.29[ab]	2.64[bcd]	2.15[b]	6.21[a]	69.22[b]	40.18[b]
26/2-16	10.81[f]	3.09[a]	55.06[cd]	2.72[abc]	1.48[e]	5.81[abc]	69.41[b]	35.57[e]
Mean	11.83	2.76	54.30	2.66	1.91	5.63	67.65	38.53
Min	10.81	2.29	51.5	2.51	1.47	4.64	63.31	35.44
Max	12.84	3.09	57.1	2.86	2.39	6.21	69.96	41.65
CV, %	5.83	8.18	3.30	3.74	16.65	9.03	3.18	5.09

*means with the same letter are not significantly different

The increased protein and lysine percentage in protein are especially important for the improvement of the nutritional quality of feed barley (Newman and Newman, 1992a; Munck, 1992; Jorgensen et al., 1997, 1999). In our study, six mutant lines with significantly higher protein content compared to parents (22/1-5, 22/2-10, and 22/2-12 from Kt3029 and 26/1-1, 26/1-3, and 26/2-1 from GA-Luttrell) were found. Hadjichristodoulou (1989) and Ramesh et al. (2021) also reported the selection of mutants with higher protein content in barley.

Among the studied lines, mutants with remarkably high lysine content were not identified. Although a number of high-lysine mutants were selected in early mutation studies of barley, it was found that those mutants are usually characterized with lower grain yield, grain weight, and starch content (Belgravia et al., 1976; Oram and Doll, 1981). Because in the present study only mutant lines with high yield potential were included, probably even if there were mutants with high lysine content, they were discarded in earlier mutant generations.

The starch content of barley grain largely determines its energy value (Newman and Newman, 1992b). Griffey et al. (2010) stated that breeding a high-starch barley variety will improve barley feed quality. Two mutant lines (22/1-14 and 22/2-4 from Kt3029) with significantly higher starch content in grain than that of the parent were found. While line 22/1-14 had a lower 1000-grain weight, line 22/2-4 was also characterized by a significantly higher test weight and 1000-grain weight compared to the parent.

The high amount of fibre in the feed grain is undesirable particularly when it is used in the feeding of monogastric animals. Only two mutants (26/1-1 and 26/1-3 from GA-Luttrell) showed lower fibre content compared to the parent.

Higher 1000-grain weight and test weight is associated with higher starch and lower fibre amount in barley grain. Three lines (22/1-11 and 22/2-4 from Kt3029 and 26/1-3 from GA-Luttrell) with higher test weight than the respective parent were observed. Two parents differed considerably in terms of 1000-grain weight - 47.21 g for Kt3029 and 37.18 g for GA-Luttrell. Mutants with higher 1000-grain weight compared to parent were observed only in lines from GA-Luttrell where half of the studied lines had higher values for this trait (26/1-1, 26/1-6, 26/1-10, 26/2-1, and 26/2-12). The improved yield of these lines is probably due to higher grain weight. Selection of mutants with the improvement of 1000-grain weight and test weight was also reported in barley and wheat (Albokari, 2014; Gómez et al., 2017).

Negative correlations between some of the quality parameters strongly obstructed breeding progress for the development of high-quality feed barley varieties. Therefore, for breeding purposes of particular interest are mutants with a favourable combination between these traits. Negative association between protein content and grain weight has been reported (Pasam et al., 2012; Pržulj et al., 2013). In this study combination of high percentage of protein and high 1000-grain weight was observed in mutant lines 22/1-11 and 22/1-12 from Kt3029 and 26/1-1, 26/2-1 and 26/2-1 from variety GA-Luttrell (Figure 1). Because of the inverse relation between protein and starch in barley (Pasam et al., 2012; Benková et al., 2012), genotypes with high content of both components also have practical breeding value. From the studied mutant lines, the best combination of protein and starch was found in line 26/2-12 (Figure 2).

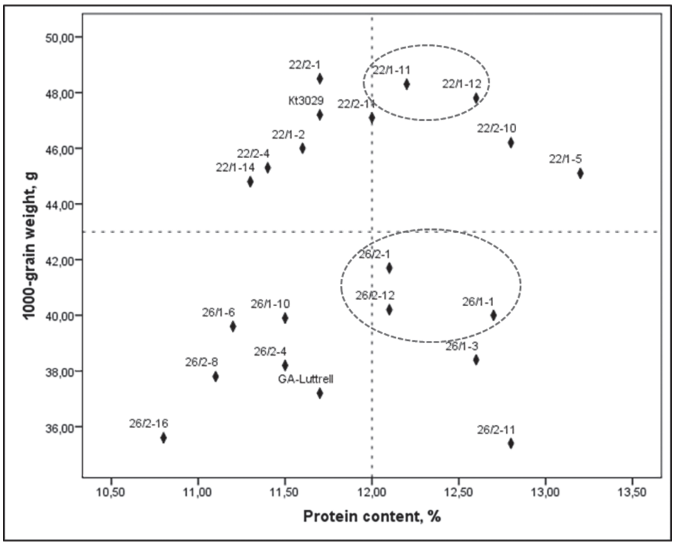

Figure 1. Scatter-plot of protein content (%) against the 1000-grain weight (g) of mutant lines and their parents

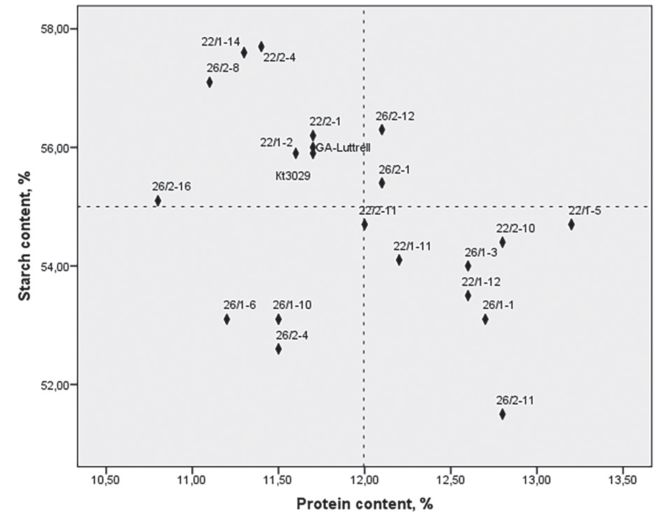

Figure 2. Scatter-plot of protein content (%) against the starch content (%) of mutant lines and their parents

Cluster analysis grouped genotypes into two main clusters (Figure 3). Cluster 1 comprised parent line Kt3029 and all mutant lines developed from this parent. Regarding the studied quality traits, most similar to line Kt3029 were lines 22/1-2 and 22/2-1 and the most diverse were 22/1-14 and 22/2-4. The dendrogram showed greater genetic diversity for feed quality traits in cluster 2 consisting of GA-Luttrell mutants than those derived from Kt3029. Lines 26/2-4 and 26/2-11 were separated from the other mutant lines in cluster 2 and were characterized with the lowest test weight. The most similar to parent variety GA-Luttrell was line 26/2-6. Many authors point out that the more diverse the parents used in hybridization, the greater the spectrum of variability and chances to produced transgressive segregants (Kuczyńska et al., 2007; Krystkowiak et al., 2009; Subhashchandra et al., 2009). Therefore, the present grouping of mutant lines allowed the selection of divergent ones for use in breeding work with feed barley.

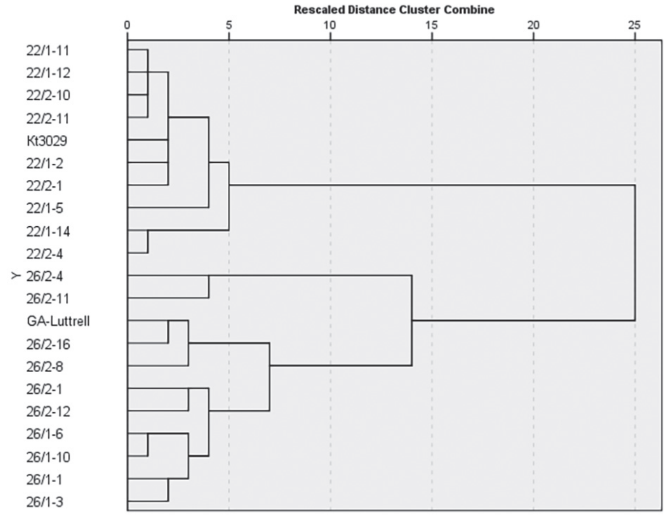

Figure 3. Dendrogram of mutant lines for traits related to grain feed quality (2015-2017)

Conclusion

Considerable differences between the mutant lines selected based on grain yield and their parent forms for traits related to the feed quality of grain were observed. Significantly higher protein content was found in the grain of mutant lines 22/1-5, 22/2-10, and 22/1-12 from line Kt3029, and 26/1-1, 26/1-3, and 26/2-11 from variety GA-Luttrell. The grain of mutant line 26/2-12 had the best combination of protein and starch. Mutant lines 22/1-11 and 22/1-12 from Kt3029 and 26/1-1, 26/2-1, and 26/2-12 from variety GA-Luttrell showed a good combination between protein content and 1000-grain weight. Those mutant lines can be used in the breeding program for the development of varieties with high feeding quality.

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