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Assessment of grain quality traits in mutant lines of winter barley

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Abstract: This study aimed to evaluate the variation among mutant lines derived from the winter barley variety Gigga, focusing on crucial feed quality traits such as protein, lysine, ash, fat, fiber content, and 1000-grain weight. Significant differences were identified in specific traits among the mutant lines, revealing promising candidates for further breeding programs. Mutant lines Gigga-25, Gigga-26, and Gigga-29 exhibited significantly higher protein content in their grains compared to the parent variety Gigga. In addition, Gigga-10 demonstrated elevated lysine content, while Gigga-4, Gigga-12, and Gigga-20 displayed increased 1000-grain weight. Of particular interest was Gigga-20, standing out for its exceptional combination of high protein content and favorable 1000-grain weight. This dual advantage positions Gigga-20 as a prime candidate for further investigation and potential integration into breeding programs, aiming to develop barley varieties with superior feed quality. Furthermore, a promising balance was observed in mutant lines Gigga-25 and Gigga-28, showcasing favorable proportions of protein and lysine content. These mutants present additional options for incorporation into breeding initiatives directed at enhancing the overall nutritional quality of feed grains.

Key words: feed barley; sodium azide mutagenesis; feed quality traits

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a significant crop globally, serving as a valuable source of food, feed, and malt. Feed barley, specifically, plays a crucial role in animal nutrition, providing essential nutrients for livestock growth and production. Enhancing the grain quality of feed barley is a paramount objective in barley breeding programs, as it directly influences animal performance and feed efficiency.

Grain quality encompasses various attributes that contribute to the nutritional value and overall suitability of barley for animal feed. Key grain quality traits influence aspects such as feed digestibility, nutrient absorption, and animal health (Kincheloe et al., 2003; Bleidere & Gaile, 2012).

Protein, a vital nutrient for animal growth, tissue repair, and hormone production, is present in barley as a valuable source of essential amino acids. Higher protein content in barley feed can positively impact animal growth performance (LaFrance & Watts, 1986).

Lysine, an essential amino acid often limited in plant-based diets, is at a relatively high content in barley compared to other cereal grains. Increased lysine content in barley feed ensures that animals meet their lysine requirements for optimal growth and development (Badea & Wijekoon, 2021).

Fat, providing a concentrated source of energy, is essential for various physiological processes and it contributes to improved feed intake, digestibility, and overall animal growth performance (Bleidere & Gaile, 2012; Badea & Wijekoon, 2021).

Ash content represents the mineral composition of barley grain, with essential minerals such as potassium, phosphorus, calcium, and magnesium playing crucial roles in animal nutrition, growth, and reproduction (Kincheloe et al., 2003). However, high ash content may indicate excessive hull content, potentially reducing feed digestibility and energy value (Bleidere & Gaile, 2012).

Fiber, or non-starch polysaccharides, is important for maintaining gastrointestinal health and promoting digestive function in animals, excessive fiber content can diminish feed digestibility and energy density (Bleidere & Gaile, 2012; Badea & Wijekoon, 2021).

The 1000-grain weight (TKW) serves as a significant indicator of barley yield and grain quality. Heavier grains generally contain more endosperm, the starchy portion of the grain providing energy for animals. Larger grains also tend to have higher starch content and lower hull content, leading to improved feed digestibility and nutrient utilization (Bleidere & Gaile, 2012).

Mutant breeding techniques have emerged as a powerful tool for generating genetic diversity and improving crop traits. Sodium azide is a mutagenic agent that can induce random mutations in plant genomes, leading to the creation of novel genetic variants. Sodium azide-induced mutagenesis has been successfully employed in various crops to enhance grain quality and yield.

Enhanced levels of protein, essential micronutrients, such as iron and zinc, and improved grain size and shape as a result of treatment with sodium azide have been reported in different cereal crops (Rao et al., 1986; Jeng et al., 2012).

The objective of this study was to assess the variation among mutant lines derived from winter barley variety Gigga, which is important for the feed quality traits as protein, lysine, ash, fat, fiber content and 1000-grain weight.

MATERIAL AND METHODS

The materials used in the present study consisted of 20 mutant lines derived from the winter six-rowed variety Gigga. The variety Veslets was also included in the trial as the national standard for feed barley. All mutant lines were obtained after treatment with 2 mM sodium azide on seeds presoaked for 16 hours in water. Selection for grain yield was applied from M2 to M5, and the selected mutant lines were used in the present study.

The mutant lines, along with the parents, were grown in the experimental field of Institute of Agriculture – Karnobat in Southeast Bulgaria using a block method with 4 replications, on plots of 10 m^2, for three growing seasons from 2019/2020 to 2021/2022.

The following parameters were analyzed: 1000-grain weight (g), protein content (% as per ISO 20483:2006), lysine content (% according to Lie, 1973), fat content (% as per ISO 6492:1999), ash content (% following ISO 2171:2007), and fiber content (% as per ISO 5498:1981).

To compare means, the least significant difference (LSD) test at the 5% probability level was used. Hierarchical cluster analysis was performed using the Between-groups linkage method with squared Euclidean distance and standardized mean variables. All data were processed using the SPSS Statistics, version 20 (IBM Corp., 2011).

RESULTS AND DISCUSSION

The results for mean of studied grain quality traits of mutant, along with the parent variety Gigga and the standard Veslets are presented in Table 1. Mutant lines exhibited variability in protein content, ranging from 12.20% to 13.73%. Gigga-25, Gigga-26, and Gigga-29 had a statistically significantly higher protein content compared to the parent and the standard variety. Our previous study also identified mutants with improved protein content in barley (Dyulgerova & Dyulgerov, 2020)

The lysine content varied among the mutant lines, with Gigga-10 showing a significant increase (2.70%) compared to the parent variety Gigga (2.40%). Gigga-4 and Gigga-8 also exhibited slightly higher protein content, showing the effectiveness of mutagenesis in generating genetic variants with improved lysine levels.

The ash and fat content among the mutant lines did not exhibit significant variations compared to the parent variety.

While Gigga-8 exhibited a substantially higher fiber content compared to the other lines, no lines with lower fiber content were found.

Gigga-4, Gigga-12, and Gigga-20 showed significantly higher 1000-grain weights, suggesting the potential for increased yield and improved grain quality. Heavier grains generally contain more endosperm, contributing to enhanced energy provision for animals, and lower hull content, indicating improved feed digestibility (Bleidere & Gaile, 2012). Barley and wheat mutants with enhanced 1000-grain weight have been reported in previous studies (Laghari et al., 2012; Gómez et al., 2017; Kenzhebayeva et al., 2017).

The mutant lines derived from the winter barley variety Gigga exhibited diverse grain quality traits, indicating the effectiveness of sodium azide-induced mutagenesis in generating genetic variation. Further research and field trials will be necessary to validate the performance of these mutant lines under different environmental conditions.

Figure 1 presents a scatter plot illustrating the relationship between 1000-grain weight (g) and protein content (%) among the mutant lines. Understanding this relationship is crucial as it unveils potential trade-offs or synergies between grain size and nutritional composition. Lines with heavier grains and higher protein content may indicate the possibility of selecting mutants with both increased yield and improved nutritional quality. Conversely, a lack of correlation

Table 1. Grain quality of mutant lines, parent variety and standard Veslets

Genotype	P	L	A	F	Fb	TGW
Vaslets	12.96	2.42	2.63	1.86	4.65	43.83
Gigga	*12.56*	*2.40*	*2.56*	*2.18*	*5.07*	*41.00*
Gigga-1	12.96	2.66	2.44	2.19	5.09	40.83
Gigga-4	12.72	2.41	2.48	1.86	5.18	43.00*
Gigga-8	12.77	2.66	2.40	2.23	5.71*	40.67
Gigga-9	12.94	2.39	2.63	1.99	5.59	41.67
Gigga-10	12.20	2.70*	2.54	2.09	5.14	41.30
Gigga-11	12.28	2.52	2.51	2.13	5.11	42.33
Gigga-12	12.63	2.30	2.30	1.82	5.16	42.67*
Gigga-13	12.73	2.58	2.30	1.94	5.31	42.33
Gigga-15	12.67	2.29	2.51	1.93	5.37	41.67
Gigga-16	13.03	2.50	2.42	1.74	5.00	41.17
Gigga-17	12.90	2.39	2.46	1.70	4.99	42.33
Gigga-18	13.15	2.29	2.39	1.99	5.03	41.00
Gigga-19	13.25	2.16	2.52	2.11	4.79	41.17
Gigga-20	13.56	2.16	2.49	1.78	4.95	42.67*
Gigga-22	12.83	2.26	2.41	2.03	4.52	40.83
Gigga-25	13.66*	2.46	2.45	2.16	5.48	41.17
Gigga-26	13.71*	2.30	2.58	1.88	5.18	41.33
Gigga-27	12.67	2.33	2.63	2.17	5.26	42.00
Gigga-28	13.54	2.56	2.41	2.04	5.49	41.80
Gigga-29	13.73*	2.25	2.65	1.91	5.21	41.67
LSD 0.05	1.02	0.29	ns	ns	0.53	1.58

**P - protein, L - lysine, A - ash, F - fat, Fb - fiber, TGW - 1000-grain weight*

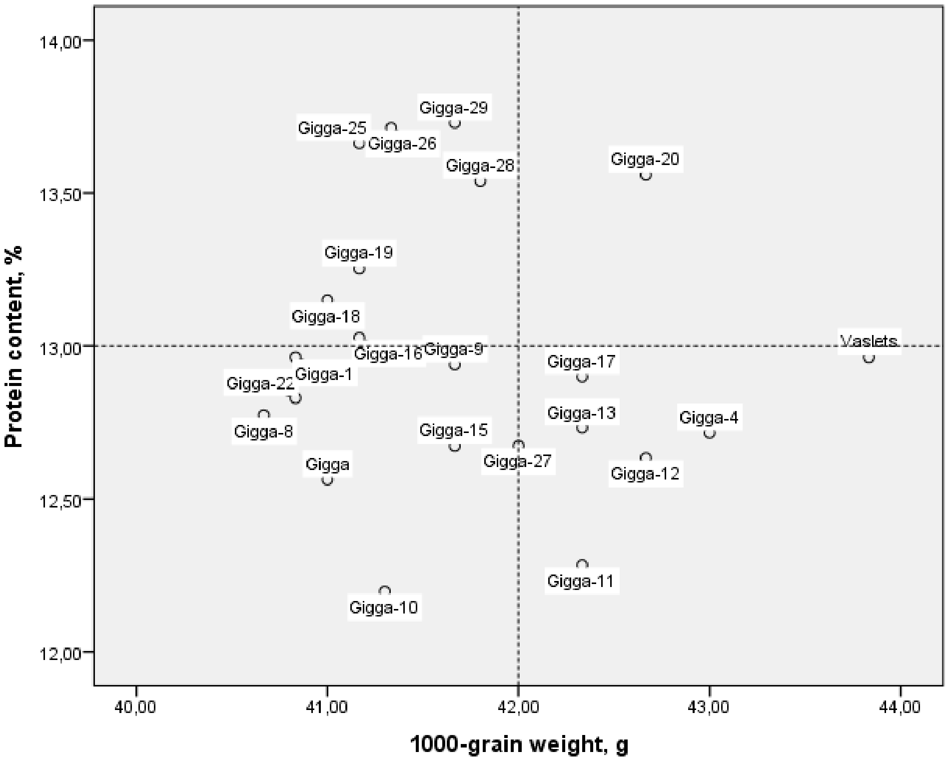

Figure 1. Scatter-plot of the 1000-grain weight (g) against protein content (%) of mutant lines

or a negative relationship could suggest that certain lines achieve higher grain weights without a proportional increase in protein content. In this study, Gigga-20 emerged as the line with the most favorable combination, showing the potential to enhance both grain yield and nutritional quality simultaneously.

Lines Gigga-17, Gigga-12, Gigga-13, and Gigga-4 are of particular interest for breeding purposes due to their higher 1000-grain weight coupled with protein content levels that are either on a par with or surpass those of the parent variety. The identification of these lines underscores their significance as promising candidates for further breeding efforts, seeking to optimize both yield and nutritional quality.

Figure 2 depicts a scatter plot illustrating the distribution of mutant lines based on their protein and lysine content. The assessment of protein and lysine content is pivotal for evaluating the overall nutritional quality of barley grains, particularly in the context of animal feed. The correlation between higher protein content and increased lysine content suggests the potential for selecting mutant lines that could significantly enhance the nutritional value of barley for animal feed applications. Lines Gigga-18, Gigga-19, Gigga-20, Gigga-26, and Gigga-29, exhibit higher protein content but lower lysine content, or vice versa, as observed in Gigga-10, Gigga-8, Gigga-1, Gigga-11, and Gigga-13. This variability in the distribution highlights the diversity in the nutritional profiles of the mutant lines. The optimal balance between these two crucial nutritional components is observed in lines Gigga-28 and Gigga-25. These lines stand out as they are presenting a promising combination of elevated protein and lysine content. The identification of such lines is of considerable significance for breeding programs aiming to enhance the nutritional quality of barley, providing valuable insights for the development of varieties with a well-balanced and improved nutritional profile for animal feed applications.

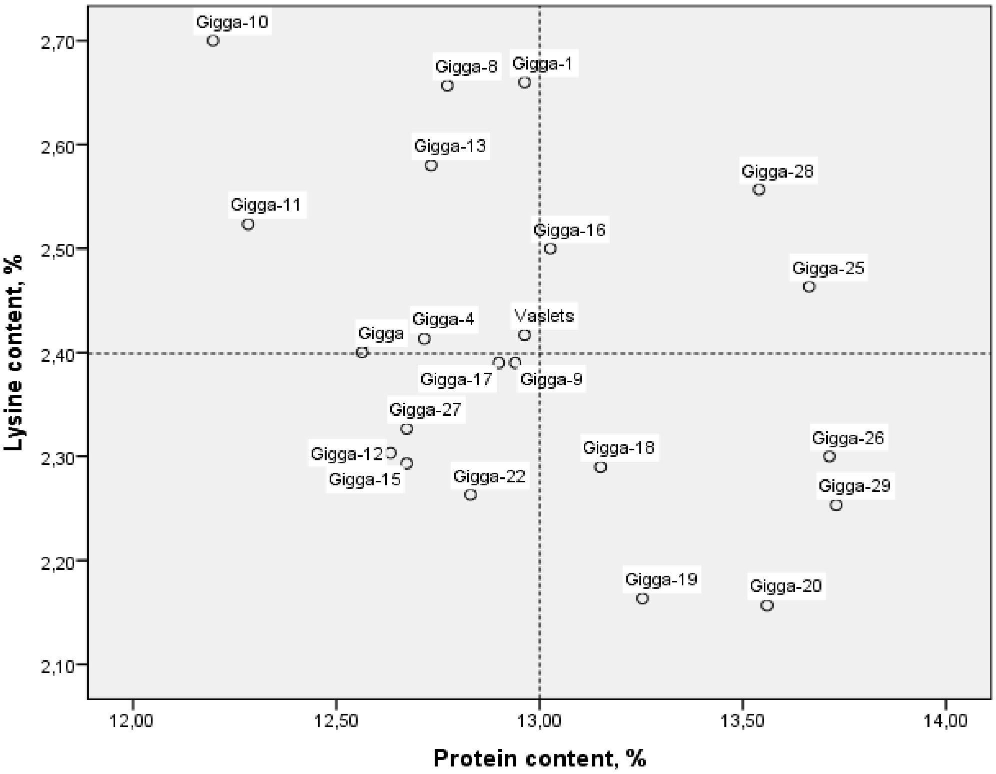

Figure 2. Scatter-plot of the protein content (%) against lysine content (%) of mutant lines

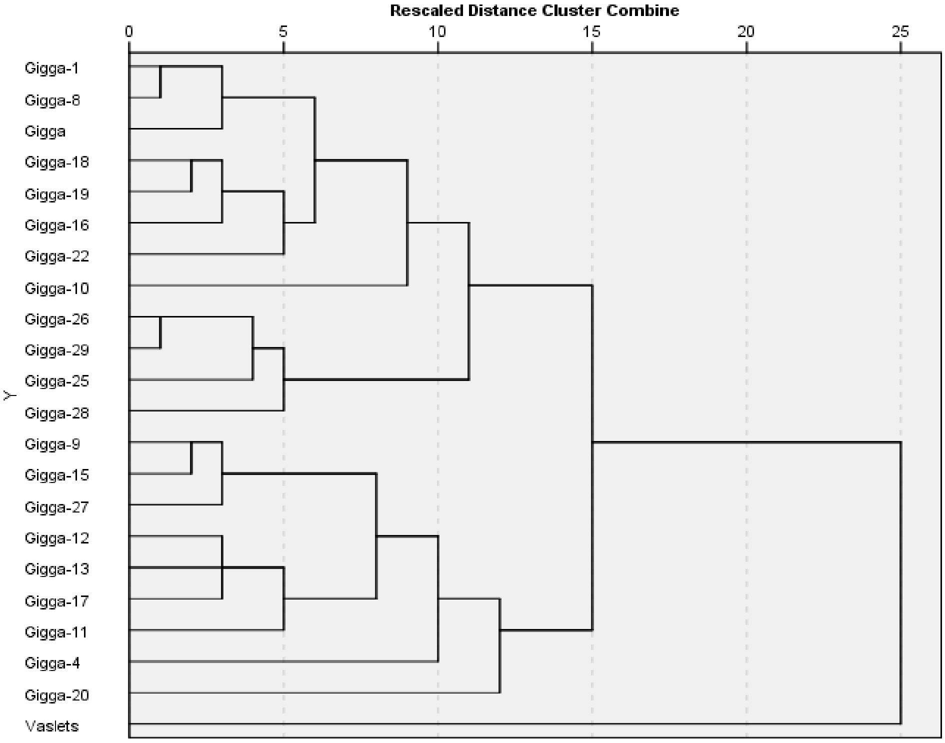

Figure 3. Dendrogram of mutant lines for grain quality traits

Figure 3 presents a dendrogram illustrating the relationships among mutant lines concerning grain quality traits. At a rescaling distance of 15, the dendrogram reveals the formation of two distinct clusters, alongside a singular variety Veslets. Within the first cluster, 11 mutant lines, along with the parent variety Gigga, are grouped together. A subcluster emerges within this group, encompassing lines characterized by elevated protein content, namely Gigga-25, Gigga-26, Gigga-28, and Gigga-29. Gigga-1 and Gigga-8 emerge as the most closely related to the parent variety, indicating a higher similarity in grain quality. The second cluster comprises 9 mutant lines distinguished by higher 1000-grain weight. Mutant line Gigga-20, also characterized by high protein content, was separated individually in this cluster.

The presence of distinct clusters and subclusters in the dendrogram suggests that the sodium azide mutation treatment has led to a wide range of genetic variations in the studied grain quality traits.

CONCLUSIONS

Mutant lines that showed significant differences in protein, lysine, fiber content, and 1000-grain weight were found. Mutant lines Gigga-25, Gigga-26, and Gigga-29 exhibited significantly higher protein content in their grain compared to the parent variety Gigga. Furthermore, Gigga-10 displayed elevated lysine content, while mutant lines Gigga-4, Gigga-12, and Gigga-20 demonstrated increased 1000-grain weight.

Among the mutant lines, Gigga-20 stood out as having the most favorable combination of protein content and 1000-grain weight. Additionally, mutant lines Gigga-25 and Gigga-28 showcased a promising balance between protein and lysine content. These mutants hold great potential for incorporation into breeding programs aimed at developing high-quality feed grain varieties.

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