



Genetics and Breeding

Assessment in mutant lines of winter barley using BLUPs

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Abstract. *This study aimed to assess genetic variability for grain yield and yield-related traits of mutant lines of winter barley, employing BLUP methodology. Traits spike length, spikelet number in spike, grain number in spike, grain weight per spike, 1000-grain weight, and grain yield, were evaluated at the Institute of Agriculture - Karnobat, Bulgaria, during 2021/2022 cropping year. The experimental design was a Complete Block Design with four replications, involving 35 genotypes. This included 32 mutant lines, the parent variety Ahil, and two standard varieties, Obzor and Emon. The results showed that mutant lines exhibited superior performance across multiple traits, including grain yield and yield-related traits. The observed high heritability and accuracy of the traits studied suggest a strong possibility for genetic gain through selection. Genetic diversity among the mutant lines, based on BLUP values, revealed substantial genetic differentiation associated with their yield potential. The MGIDI selection strategy was utilized to identify promising mutant lines with high genetic potential. Mutant lines 54/30, 54/49, 54/54, 54/10, and 55/22 were selected based on their predicted genotypic values and favorable characteristics. These lines hold potential for further development as candidate varieties or parents in breeding programs. The use of BLUPs in the selection process proved to be an effective tool for identifying superior mutant lines, further highlighting the usefulness of this approach in barley breeding. The findings of this study also demonstrate the efficacy of sodium azide mutagenesis in generating mutant lines of winter barley with improved yield and yield-related traits.*

Keywords: *Hordeum vulgare* L., mutant lines, Best Linear Unbiased Predictors, multi-trait genotype-ideotype distance index

Introduction

In the context of quantitative genetics, a mixed model incorporates both fixed and random effects (Gelman, 2005). Fixed effects are factors that are constant and often of primary interest (e.g., genetic factors), while random effects account for variability that cannot be precisely measured or controlled (e.g., environmental effects). Restricted Maximum Likelihood (REML) is a

statistical method used for estimating the variance components of a mixed model (Robinson, 1987). REML is often preferred over traditional maximum likelihood methods in the context of mixed models because it provides unbiased estimates of variance components. Once the variance components are estimated using REML, BLUPs (Best Linear Unbiased Predictors) are employed to predict the genetic values of individuals, considering both fixed and random effects (Robinson, 1987). BLUP

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produces estimates that are unbiased and have the minimum mean squared error among all linear unbiased predictors. This is crucial for obtaining accurate predictions, especially in the presence of complex genetic and environmental interactions. Mixed models facilitate the estimation of genetic parameters such as heritability, which quantifies the proportion of phenotypic variation attributable to genetic factors (Piepho et al., 2008). These parameters provide valuable insights into the genetic architecture of traits and guide breeding decisions. By employing BLUP, one can predict the genetic values of individuals, isolating the genetic component from the influence of environmental factors. This is crucial in breeding programs as it allows for the selection of individuals with desirable genetic traits, leading to the improvement of those traits in subsequent generations.

The use of BLUPs can be particularly appropriate when comparing the traits of mutant lines derived from one parent because BLUPs provide a reliable estimation of the genetic values of individuals, accounting for both the genetic and environmental components of variation (Piepho et al., 2008). When comparing mutant lines of one parent, it is important to account for the fact that the lines share a common genetic background. However, they may still exhibit variation due to the influence of other factors such as environmental conditions. BLUPs help address this by estimating the true breeding values (genetic values) of individuals while accounting for environmental effects. By utilizing BLUPs, researchers can account for the genetic similarities among mutant lines derived from one parent and accurately compare the traits of these lines. This approach helps reduce bias caused by shared genetic background and allows for a more reliable assessment of the genetic effects on the traits under investigation (Ganapathy et al., 2021).

This research aimed to assess genetic variability for grain yield and yield-related traits in mutant lines of winter barley by using BLUP values.

Material and methods

The field trial was conducted at the Institute of Agriculture – Karnobat, Southeastern Bulgaria during 2021/2022 cropping season. The

experimental region has a transitional continental climate characterized by an extended and relatively cool spring, a dry and hot summer, a prolonged and rainy autumn, and minimal snowfall in winter, showcasing significant temperature fluctuations. The soil in the experimental field is leached vertisol, slightly acidic, with a pH level of 6.2.

The study included thirty-two mutant lines, their parent variety Ahil, and two standard varieties, Obzor and Emon. The mutant lines were developed after treatment with 2 mM sodium azide. Mutants with higher yield potential were selected from M2 to M6, and in the present study, stable M7 mutant lines were included.

The trial was organized in a complete block design with 4 replications on plots of 10 m². Standard agronomic and plant protection practices were used.

The traits: spike length (cm), spikelet number per spike, grain number per spike, grain weight per spike (g) were recorded on 25 randomly selected plants in each replication for each genotype. Grain yield (t/ha) and 1000-grain weight (g) were estimated on plot basis.

Data analyses were performed using the *metan* package v1.18.0, in R software version 4.2.0 (R Core Team, 2022), employing the function “*gamem()*” (Olivoto and Lúcio, 2020). Model information, such as variance components, genetic parameters and BLUP for genotypes, was extracted.

The BLUPs were utilized for the hierarchical heatmap clustering based on Ward’s method, implemented with R package “*pheatmap*” (Kolde and Kolde, 2015).

The selection of the best mutant lines was executed using a multi-trait genotype–ideotype distance index based on BLUPs (MGIDI) (Olivoto and Nardino, 2021) calculated with R Package ‘*metan*’. Predicted genetic gains using MGIDI were calculated for each trait, considering a selection intensity of 10%. To prioritize grain yield over yield-related traits in the selection process, specific weights were assigned to different traits. The assigned weights were as follows: spike length (SL): 0.2, spike number per spikelet (SNS): 0.2, grains per spike (GNS): 0.2, grain weight per spike (GWS): 0.2, thousand grain weight (TGW): 0.5 and

grain yield (GY): 1.0.

The selection gain (SG) was estimated by the expression: $SG = (SD * h^2) / X_0$, where SD is the selection differential, given by difference between the average of selected (Xs) and the general average observed (X_0) and h^2 is heritability.

Results and discussion

Variance components, genetic parameters and BLUPs for genotypes

Higher phenotypic variance was observed for spikelet number in the spike and grain number in the spike compared to the other traits (Table 1). Notably, 1000-grain weight exhibited the highest genotypic variance, constituting 95.10% of the total variance.

Individual heritability values were high, particularly for 1000-grain weight (0.95) and grain weight per spike (0.92). These findings indicate a favorable condition for genetic gains through selection, especially concerning grain weight, a crucial factor linked to overall grain yield enhancement.

Heritability estimates based on genotype average (h^2 mg) were consistently high across all traits, ranging from 0.87 for grain yield to 0.99 for 1000-grain weight. These values tended to exceed heritability estimates at the individual level (h^2 g), suggesting that the assessed traits hold potential for evaluating genetic variation in the set of genotypes. These results are in agreement with previous studies that reported high values of heritability in the broad sense for 1000-grain weight in barley (Yadav et al., 2014; Wang et al., 2019).

Selective accuracy, assuming no loss of plots (Accuracy), serves as an indicator of results quality, reflecting the correlation between predicted genetic values and genetic values of individuals. The selective precision ranged from 0.93 for grain yield to 0.99 for grain weight per spike and 1000-grain weight, which are considered high values and attest to the high quality of these results.

Furthermore, traits such as grain weight per spike and 1000-grain weight exhibited the highest coefficients of genetic variance (CVg) - 12.20 and 10.88, respectively. These high CVg

values are desirable, since this variable quantifies the magnitude of genetic variation available for selection.

Ten mutant lines exhibited higher BLUP values for spike lengths than the parent variety Ahil (Figure 1). Lines 54/20, 54/32, 54/42, and 54/36 stood out with significantly higher spike lengths.

Four mutant lines (54/42, 54/32, 54/52, and 54/20) and the standard variety Emon displayed increased spikelet numbers per spike compared to parent variety, as indicated by BLUPs (Figure 1). The spikelet count may contribute to overall yield by influencing the number of grains per spike - a key factor in barley yield.

Mutant lines 54/42, 54/52, and 54/32, along with the standard variety Emon, exhibited higher genetic values for grain numbers in the spike than variety Ahil (Figure 2). This finding suggests the presence of genetic factors in these lines contributing to increased grain production per spike, which is a critical determinant of overall yield.

Line 54/42 stood out as a top performer, showing the highest grain weight per spike (Figure 2). Additionally, lines 54/52, 54/32, and 54/20, along with the variety Emon, showed higher BLUPs for grain weights per spike compared to Ahil. These lines hold promise for enhancing the yield by contributing to heavier grains.

The BLUPs for 1000-grain weight revealed variability among lines, ranging from 47.19 g to 52.19 g (Figure 3). Mutant lines 54/53, 54/4, 54/3, 54/18, 54/55, 54/26, and 54/42 exhibited significantly higher 1000-grain weight. While Ahil had a value close to the mean, standard varieties Obsor and Emon showed the highest 1000-grain weight.

Lines 54/31, 54/29, 54/55, 54/26, 54/53, 54/42, 54/54, 54/49, 54/10, and 54/30 showed considerably higher BLUPs values for grain yields compared to the parent variety Ahil (Figure 3). No significant differences were observed between these lines, as their confidence intervals overlapped.

These findings are consistent with our expectations, as the outcomes reflect the deliberate selection for high grain yield in previous mutant generations. The emphasis on enhancing specific traits related to spike length, spikelet number,

grain number, grain weight, and overall grain yield has evidently contributed to the identification of promising lines with superior performance in these key agronomic characteristics. These high-yielding lines present promising candidates for further breeding efforts to develop improved barley varieties with enhanced yield potential.

The analysis of BLUPs provides valuable information for selecting promising mutant lines with desirable traits for barley breeding programs. The identification of lines with superior performance in spike length, spikelet number, grain number, grain weight, 1000-grain weight, and overall grain

yield is crucial for advancing the breeding objectives and developing improved varieties with enhanced agronomic characteristics. Moreover, according to previous studies, genetic values are more important for breeders as they represent true values in predicting and representing the genetic potential of individuals (Piepho et al., 2008). Ganapathy et al., 2021 found that BLUPs were more accurate than traditional regression methods at predicting grain yield, and that they were particularly effective when used to compare mutant lines derived from one parent.

Table 1. Estimates of variance components and genetic parameters for spike length (SL), spikelet number in spike (SNS), grain number in spike (GNS), grain weight per spike (GWS), 1000-grain weight (TGW), and grain yield (GY) for 35 barley genotypes

Parameters	SL	SNS	GNS	GWS	TGW	GY
Vg	0.82	7.18	7.44	0.04	0.45	2.78
Gen (%)	67.09	78.00	78.02	91.72	95.10	62.64
Vr	0.40	2.03	2.10	0.01	0.02	1.66
Res (%)	32.91	22.00	21.98	8.28	4.90	37.36
Vph	1.23	9.21	9.53	0.04	0.47	4.44
h ²	0.67	0.78	0.78	0.92	0.95	0.63
h ² _{mg}	0.89	0.93	0.93	0.98	0.99	0.87
Accuracy	0.94	0.97	0.97	0.99	0.99	0.93
CVg	9.50	9.01	9.62	12.20	10.88	3.33
CVr	6.65	4.78	5.11	3.67	2.47	2.57
CV ratio	1.43	1.88	1.88	3.33	4.41	1.29

Legend: Vg- genotypic variance; Gen (%)- genotypic variance percentage; Vr- residual variance; Res (%)- residual variance percentage; Vph- phenotypic variance; h²- heritability of individual plots in the broad sense; h²_{mg}- heritability of genotype mean, assuming complete survival; Accuracy- selective accuracy assuming no loss of plots; CVg- genotypic coefficient of variation; CVr- residual coefficient of variation

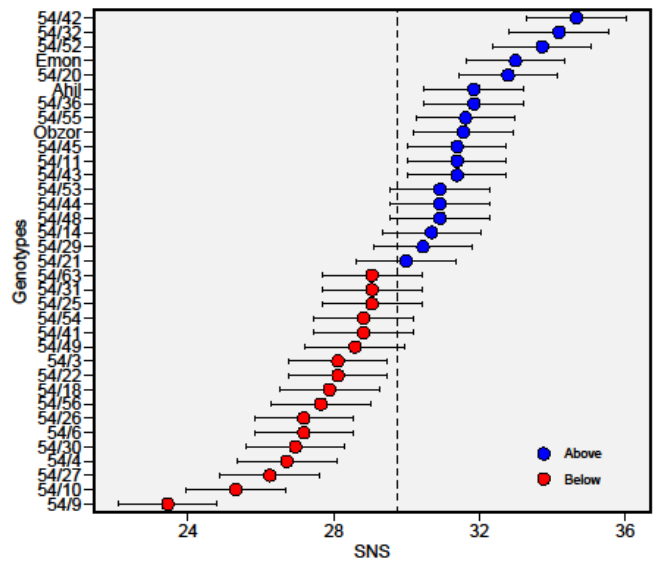
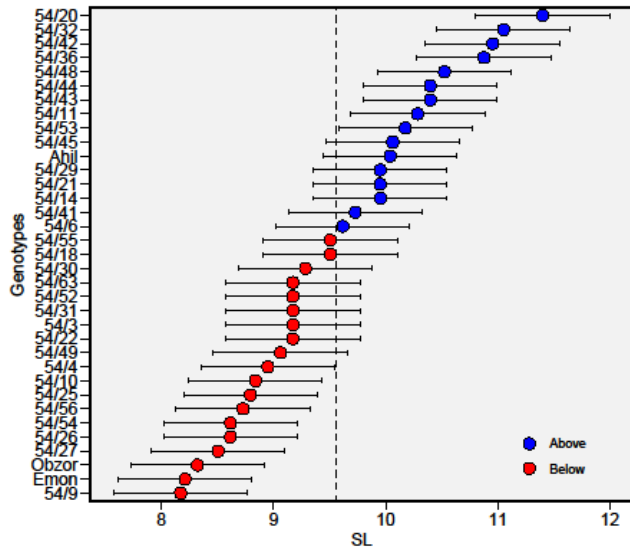


Figure 1. Best linear unbiased prediction (BLUP) of 35 barley genotypes for the traits spike length (SL) and spikelet number per spike (SNS)

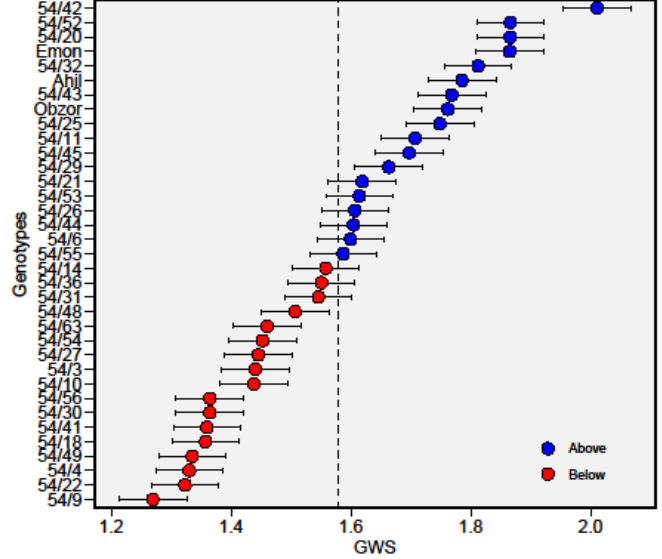
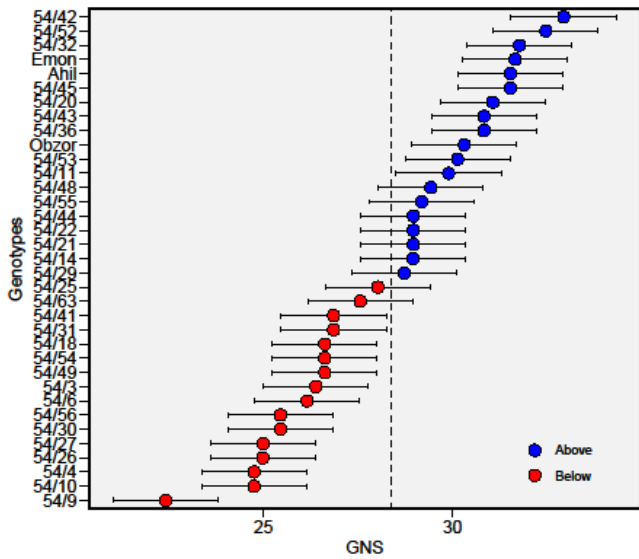


Figure 2. Best linear unbiased prediction (BLUP) of 35 barley genotypes for the traits grain number per spike (GNS) and grain weight per spike (GWS, g)

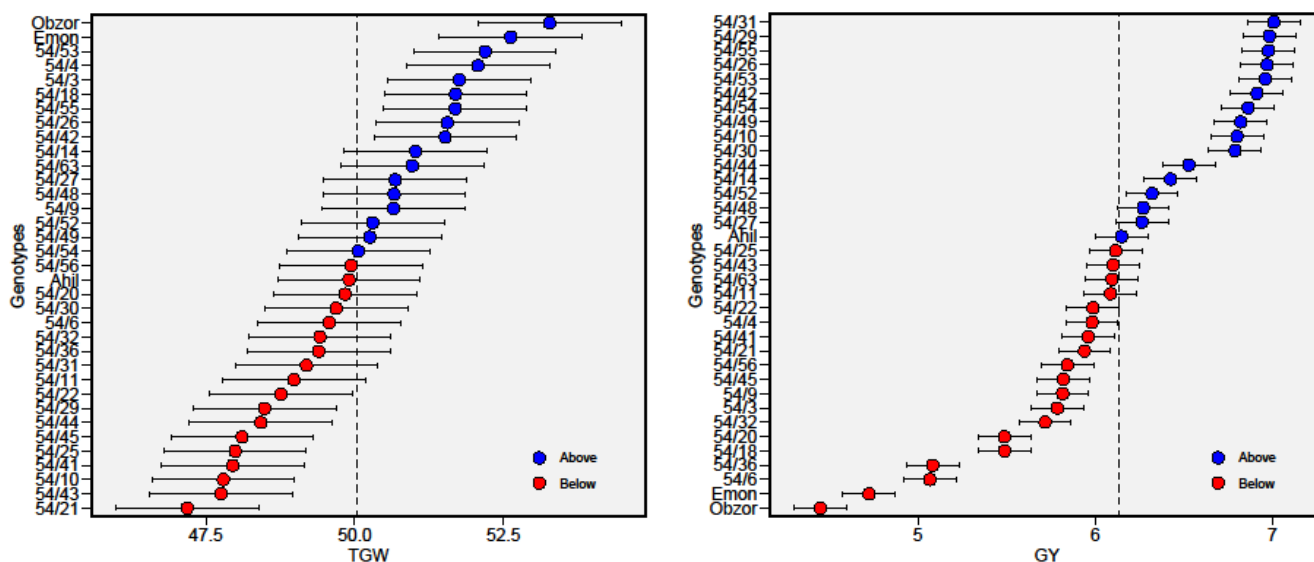


Figure 3. Best linear unbiased prediction (BLUP) of 35 barley genotypes for the traits 1000-grain weight (TGW, g) and grain yield (GY, t/ha)

Hierarchical clustering analysis

The hierarchical heat map of genotypic values reveals the presence of four distinct genetically divergent clusters among the 35 studied genotypes, each exhibiting unique yield potential. The first cluster comprises 16 mutant lines, characterized by relatively low to average values for spike length, number of spikes, grains per spike, and grain weight per spike. Within this cluster, a subcluster emerges, including lines 54/10, 54/26, 54/31, 54/30, 54/49, and 54/54, which exhibit notably high grain yield, with most displaying an approximately averaged 1000-grain weight.

The second cluster is composed of two standard varieties, Obzor and Emon. These varieties are

distinguished by short spikes with numerous grains and exceptionally high grain weight.

The third cluster consists of the parent variety Ahil and mutant lines 54/14, 54/48, 54/53, 54/55, 54/42, and 54/52, the majority of which demonstrate average or above-average values across all studied traits.

The fourth cluster is formed by the majority of mutant lines, which exhibit around or below-average grain yield and 1000-grain weight.

This hierarchical clustering analysis provides valuable insights into the genetic diversity and yield potential of the studied mutant lines, laying the foundation for further exploration and selection of promising lines for a future breeding program.

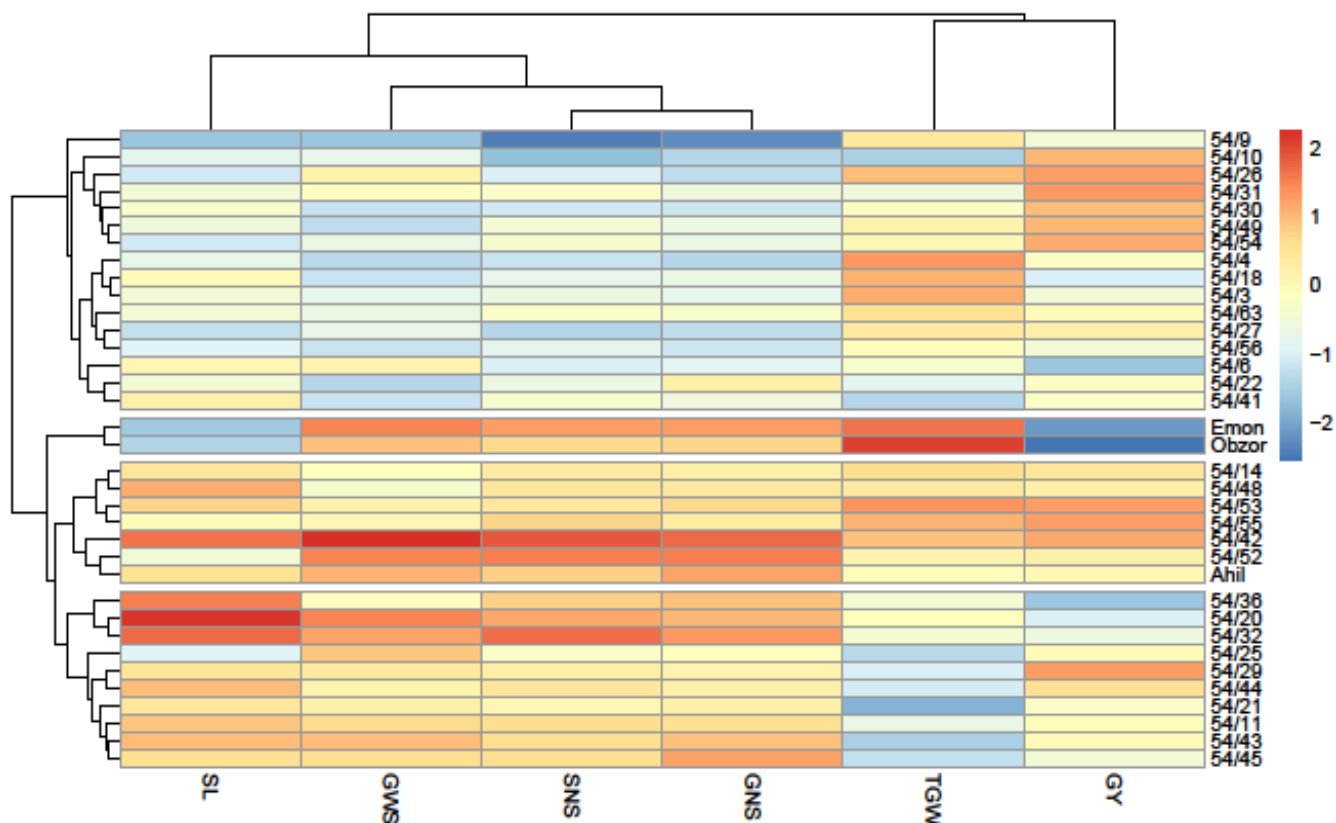


Figure 4. Heat map and hierarchical clustering analysis by Euclidian distance using Ward’s method generated from BLUPs value for spike length (SL), spikelet number in spike (SNS), grain number in spike (GNS), grain weight per spike (GWS), 1000-grain weight (TGW), and grain yield (GY) for 35 barley genotypes

Multi-Trait Genotype–Ideotype Distance Index (MGIDI)

Breeders commonly consider a combination of traits that, when integrated into a single genotype, would exhibit outstanding performance. This genotype is known as an ideotype (Saini et al., 2020). The objective of ideotype design is to enhance crop performance by selecting genotypes that excel in multiple traits simultaneously. Selection indexes are used in plant breeding to assist the multi-trait selection. Nevertheless, challenges such as multicollinearity and the complexities associated with assigning economic weights to the traits can impact the genetic gains achieved through these selection indexes. In response to these limitations, a multivariate selection index called the multi-trait genotype–ideotype distance index (MGIDI) has been developed recently (Olivoto and Nardino, 2021).

In the present study, the MGIDI, based on BLUPs and prioritizing grain yield, identified mutant lines

54/30, 54/49, 54/54, 54/10, and 55/22 as high-performing genotypes for multiple traits (Figure 5). However, the selection differential and selection gain observed in the MGIDI were negative for the studied yield-related traits (Table 2). These findings may be attributed to the prioritization of grain yield, suggesting a potential negative relationship between the studied yield-related traits and actual grain yield.

The obtained results not only confirm the superiority of mutant lines over their parent variety but also over standard varieties, emphasizing the effectiveness of mutagenic treatments in improving key yield-related traits.

To our knowledge, this is the first report on the application of MGIDI for selecting barley mutants that demonstrate superior performance across multiple quantitative traits. Previously, Mamun et al. (2022) employed MGIDI to select EMS-induced rice mutants.

MGIDI stands out by recognizing the unique

importance of different traits, unlike traditional methods that focus on single traits. This multi-trait selection approach allows breeders to conduct a comprehensive evaluation of a genotype's potential, leading to informed decisions about future breeding programs. By using the concept of an ideotype,

which represents the ideal combination of traits for a specific crop, MGIDI helps breeders adapt their efforts for maximum effectiveness. However, the accuracy of this ideotype remains crucial for optimizing MGIDI's performance.

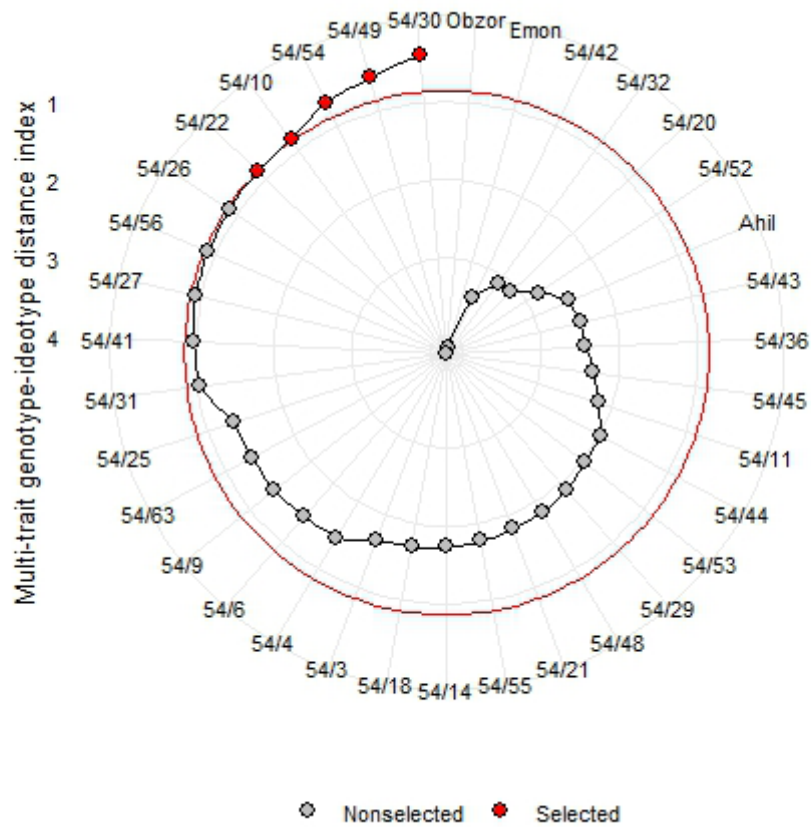


Figure 5. The MGIDI ranks genotypes in descending order. The non-selected and selected genotypes are shown in gray and red circles, respectively, and depending on the intensity (10%), the red circle depicts the cut point.

Table 2. Original value (X_o), selected value (X_s), selection differential (SD), selection differential in percentage (SDperc), and selection gain (SG) for the MGIDI in 35 barley genotypes

Trait	Factor	X _o	X _s	SD	SDperc	SG
SL	FA1	9.55	8.95	-0.60	-6.32	-0.06
SNS	FA1	29.75	27.40	-2.34	-7.86	-0.07
GNS	FA1	28.36	25.90	-2.50	-8.81	-0.08
GWS	FA1	1.58	1.40	-0.18	-11.60	-0.11
TGW	FA2	50.04	49.40	-0.59	-1.18	-0.01
GY	FA2	6.13	6.82	0.69	11.20	0.10

Legend: SL- spike length, cm; SNS- spikelet number in spike; GNS- grain number in spike; GWS- grain weight per spike; TGW- 1000-grain weight, g; GY- grain yield, t/ha

Conclusion

Mutant lines outperformed not only the parent variety Ahil but also the standard varieties Obzor and Emon for predicted genotypic values for yield-related traits and grain yield were found. This suggests that sodium azide mutagenesis can be a useful tool for improving yield in winter barley. The high heritability and accuracy of the traits studied indicate a favorable condition for genetic improvement through selection. The analysis of genetic diversity among mutant lines based on predicted genotypic values identified several promising lines with distinct yield potential. Mutant lines 54/30, 54/49, 54/54, 54/10, and 55/22 were selected through multi-trait genotype–ideotype distance index (MGIDI) as high-performing genotypes. These lines are suitable for further studies and could be used as candidate varieties or parents in crossing programs after their yield potential is confirmed. The use of BLUPs proved to be a reliable tool for selecting mutants with desirable traits, further emphasizing the robustness of this approach in identifying promising lines for barley breeding programs.

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