

## Genotype-by-year interaction and simultaneous selection for grain yield and stability in winter barley

Boryana Dyulgerova\* and Nikolay Dyulgerov

*Agricultural Academy, Institute of Agriculture, 8400 Karnobat, Bulgaria*

\*Corresponding author: bdyulgerova@abv.bg

### Abstract

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The objective of this study was to evaluate genotype-by-year interaction for grain yield of 20 winter barley genotypes. The study was conducted during six growing years in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria. Various stability models were employed to identify high-yield and stable genotypes. The AMMI and BLUP methods demonstrated that the grain yield of the winter barley genotypes was significantly affected by genotype, growing season, and their interaction. Most of the non-parametric stability statistics used showed a significant positive correlation with grain yield, suggesting that they can be used as an alternative to parametric methods in identifying stable genotypes. Based on several statistical measures, genotypes G17 (K16/3-12), G20 (A8/2), and G15 (K16/1-21) were found to be more high-yielding and stable than the national standard cultivars G1 (Obzor) and G2 (Emon). This result was confirmed with the WAASBY index, indicating the efficiency of the WAASBY statistics in selecting superior barley genotypes. The genotype G17 (K16/3-12), with a consistently high yield performance, could be recommended as a new cultivar and genetic resource for improving the grain yield of winter barley in Southeastern Bulgaria.

*Keywords:* *Hordeum vulgare* L.; multi-year trials; stability; grain yield

### Introduction

The main aim of any breeding program is the developing of high-yielding and stable cultivars. This also applies to barley, which usually is grown in highly variable rain-fed conditions. In general, barley breeders are rather focused on the development of stable high-yielding cultivars specific to a target environment instead of across environments. Hence, the importance of growing year as one of the main factors affected barley production. Highly variable conditions during growing seasons cause different rank ordering of tested genotypes as a result of genotype-by-environment interaction (GEI). GEI complicates the selection of promising advanced breeding lines by declining the association between genotypic and phenotypic values (Yan & Tinker, 2006).

Numerous approaches have been applied to analyze genotype stability and to understand and explain GEI. Different parametric procedures including Wricke's ecovalence  $W^2i$  (Wricke, 1962), regression coefficient  $b_i$  (Finlay & Wilkinson, 1963), deviation from regression  $S^2di$  (Eberhart & Russell, 1966), Shukla's stability variance  $\sigma^2i$  (Shukla, 1972), average of the squared eigenvector values EV (Sneller et al., 1997) as well AMMI-based stability parameters (Zali et al., 2012; Purchase et al., 2000) were widely used for the selection of better performing and higher yielding genotypes across environments.

Similarly, non-parametric methods such as Kang's rank-sum,  $S^{(1)}$ ,  $S^{(2)}$ ,  $S^{(3)}$ ,  $S^{(6)}$  and  $NP^{(1-4)}$  based on genotypic ranking across the environment were proposed (Kang, 1988; Huehn, 1990; Nassar & Huehn, 1987; Thennarasu, 1995). Non-parametric indices are a useful alternative to existing

parametric measurements because their performance is not limited by the need for normal distribution of model residuals and homogeneity of variances (Nassar & Huehn, 1987). Both parametric and nonparametric methods have strengths and weaknesses and can be used together to provide more complete information about genotypic responses to different conditions (van Eeuwijk et al., 2016).

This study aimed to evaluate genotype-by-year interaction for grain yield of 20 genotypes of winter barley using different stability models during six growing years in Southeastern Bulgaria and to select high-yielding and stable breeding lines.

## Materials and Methods

A set of 20 genotypes of winter 2-rowed barley, including six cultivars, two of which (Obzor and Emon) are national standards and 14 advanced breeding lines were evaluated (Table 1). Thirteen of this advanced breeding lines were developed after mutagenic treatment of pre-soaked seeds with 2 or 3 mM sodium azide and one double haploid line, obtained via anther culture from the cross Ahil x Kuber. All tested breeding lines and cultivars were developed at the Institute of Agriculture – Karnobat, Bulgaria.

The study was conducted during six the growing years (2015/16, 2016/17, 2018/19, 2019/20, 2020/21, and 2021/22) in the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria (42°39' N, 26°59' E). The soil of the

experimental field was slightly acid (pH is 6.2) *Pellic Vertisol*.

The experiments were organized in a Complete Block Design with 4 replications on plots of 10 m<sup>2</sup> with sowing rate 450 germinated seeds/m<sup>2</sup>. All necessary crop management practices were followed as recommended for the region. Grain yield was determined by weight of grains per plot and converted to t/ha.

Average air temperatures for the studied growing years were higher compared to the long-term air temperatures (Table 2). The sums of precipitations for two of the growing years (2016/17 and 2019/20) were lower than the long-term sum for barley vegetation. For the rest of the growing years, the precipitation sums were higher than the long-term sum but rainfalls were unevenly distributed throughout the vegetation period.

The combined and AMMI ANOVA was performed to determine the effects of genotype (G), environment (E) and genotype by environment interaction (GEI). Variance components were estimated in a linear mixed-effect model using restricted maximum likelihood (REML) considering genotype and genotype-vs-environment as random effects. The prediction accuracy of BLUP model and the AMMI family model were compared by their root mean square prediction difference (RMSPD) estimates (Piepho, 1994).

The weighted average of absolute scores from the singular value decomposition of the matrix of best linear unbiased predictions for the GEI effects generated by a linear mixed-effect model (WAASB) statistic was used to analyze

**Table 1. Information for the barley genotypes tested in this study**

Genotype code	Name	Breeding status
G1	Obzor	cultivar, national standard
G2	Emon	cultivar, national standard
G3	Zagorets	cultivar
G4	Imeon	cultivar
G5	Kuber	cultivar
G6	Ahil	cultivar, developed by mutation breeding
G7	KT3033	mutant breeding line, developed from line 3227
G8	KT3037	mutant breeding line, developed from line 3788
G9	KT3039	mutant breeding line, developed from line 8663
G10	Z18/1-5	mutant breeding line, developed from cultivar Zagorets
G11	Z1/1-17	mutant breeding line, developed from cultivar Zagorets
G12	I15/1-12	mutant breeding line, developed from cultivar Imeon
G13	I15/3-14	mutant breeding line, developed from cultivar Imeon
G14	K16/1-14	mutant breeding line, developed from cultivar Kuber
G15	K16/1-21	mutant breeding line, developed from cultivar Kuber
G16	K16/2-11	mutant breeding line, developed from cultivar Kuber
G17	K16/3-12	mutant breeding line, developed from cultivar Kuber
G18	244D-5	mutant breeding line, developed from line 224D
G19	244D-6	mutant breeding line, developed from line 224D
G20	A8/2	double haploid line, obtained via anther culture from Ahil x Kuber

**Table 2. Average air temperature, monthly sums of precipitation and long-term average data for Karnobat, Southeastern Bulgaria across 6 growing seasons**

Growing years		Months									Total	
		X	XI	XII	I	II	III	IV	V	VI	T, °C	P, mm
2015/16	T, °C	12.2	7.0	4.6	2.4	3.7	6.4	10.8	17.5	19.9	9.4	
	P, mm	41.8	45.6	0.2	133.2	43.7	56.1	55.2	130.8	42.9		549.5
2016/17	T, °C	12.1	7.5	0.6	-2.5	3.7	8.3	10.0	16.1	21.7	8.6	
	P, mm	67.0	36.9	5.7	28.9	32.9	24.1	35.4	36.6	55.0		322.5
2018/19	T, °C	12.5	8.4	5.2	2.5	3.5	6.4	19.3	17.9	20.8	10.7	
	P, mm	270.0	38.8	93.1	49.0	81.1	121.2	6.0	68.6	98.6		826.4
2019/20	T, °C	14.0	7.4	2.4	2.5	4.3	8.6	10.3	17.1	22.6	9.9	
	P, mm	15.4	68.3	27.3	38.9	15.6	8.9	52.9	44.9	95.6		367.8
2020/21	T, °C	15.8	7.4	6.6	3.7	4.9	4.9	9.5	16.6	19.3	9.9	
	P, mm	70.7	25.5	94.5	142.8	22.1	47.4	86.0	15.6	117.4		622.0
2021/22	T, °C	11.1	8.7	4.5	2.4	4.4	3.5	11.2	15.9	21.0	9.2	
	P, mm	79.1	31.7	105.7	8.0	39.7	12.3	48.2	36.3	86.8		447.8
LT	T, °C	12.5	7.1	2.6	0.6	2.2	5.3	10.5	15.6	19.6	8.4	
	P, mm	44.3	53.7	51.2	36.5	35.8	34.1	45.3	58.5	65.2		424.6

T, °C – average air temperature; P, mm – sums of precipitation; LT – long-term average air temperature and sums of precipitation (1931–2022)

the stability (Olivoto et al., 2019). The superiority index WAASBY for simultaneous selection for yield and stability by weighting the WAASB stability value and mean yield was calculated (Olivoto et al., 2019). All these analyses were performed in R software 4.2.1 (R Core Team, 2022) using the package “metan” (Olivoto & Lucio, 2020).

Totally, nine parametric and nine non-parametric stability statistics were estimated (Table 3), and the tested genotypes were ranked based on each statistic. The stability statistics was computed with the help of online program STABILITYSOFT (Pour-Aboughadareh et al., 2019) and the “metan” package in R. Spearman’s rank correlations to detect the as-

sociation between the stability statistics using the “corrplot” package in R were estimated (Wei et al., 2017). Hierarchical cluster analysis based on average sum of ranks (ASR) of all stability measures and mean grain yield through Ward’s method and Euclidean distance as a dissimilarity measure using the “stats” package in R was performed.

## Results

### *AMMI analysis of variance*

The AMMI analysis for grain yield of the 20 winter barley genotypes tested across 6 growing years is presented in Table

**Table 3. List of parametric and non-parametric stability measures used in this study**

Stability measure	Abbreviation	Reference
Parametric		
Wricke’s ecovalence	$W^2_i$	Wricke (1962)
Regression coefficient	$b_i$	Finlay & Wilkinson (1963)
Deviation from regression	$S^2d_i$	Eberhart & Russell (1966)
Shukla’s stability variance	$\sigma^2_i$	Shukla (1972)
AMMI stability value	ASV	Purchase et al. (2000)
Sum of the absolute value of the IPCA scores	SIPC	Purchase et al. (2000)
Absolute value of the relative contribution of IPCAs to the interaction	Za	Zali et al. (2012)
Harmonic mean of relative performance of genotypic values	HMRPGV	Resende (2007)
Weighted average of absolute scores	WAASB	Olivoto et al. (2019)
Non-parametric		
Huehn’s and Nassar and Huehn’s statistics	$S^{(1, 2, 3, 6)}$	Huehn (1990); Nassar & Huehn (1987)
Thennarasu’s statistics	$NP^{(1-4)}$	Thennarasu (1995)
Kang’s rank-sum	KR	Kang (1988)

4. The analysis showed that the grain yield was significantly affected by growing year, genotype by year interaction and genotypic effects. The variance explained by the effect of genotype by growing year interaction was almost two and a half times greater than the genotype effect. This implies that the genotypes responded differently in different growing years and that additional stability analysis is required to fully understand the effect of genotype by growing year interaction. The interaction effect was partitioned into 5 interaction principal components (IPCs) which explained 38.6, 23.7, 15.8, 14.9, and 7.0% respectively, of genotype by growing year interaction.

In AMMI analysis usually, the first two IPCs are used for the interpretation of GEI but in the present study, IPC1

and IPC2 were able to explain only 62.3% of genotype by growing year interaction indicating that this approach could be deceptive.

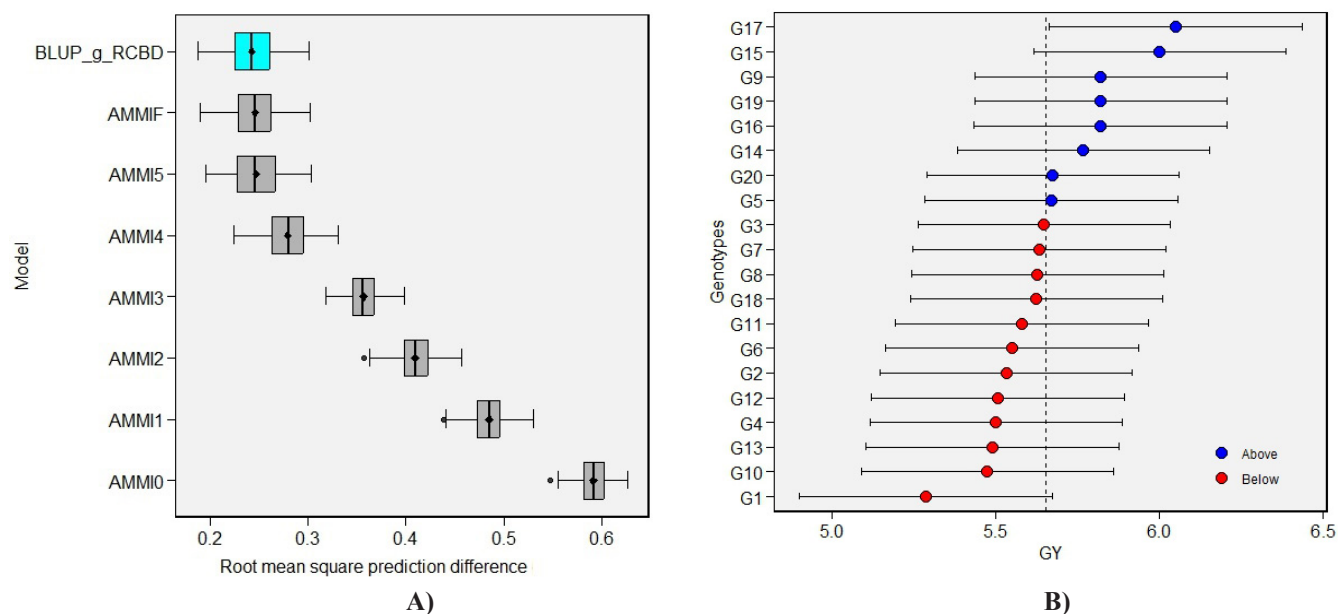
#### Model accuracy and predicted means

In result of test of the grain yield prediction accuracy of the BLUP and AMMI family models, the BLUP was found to be the most accurate predictive model for winter barley grain yield (Figure 1A). Eight barley genotypes had a BLUP mean greater than the grand mean, and the remaining twelve genotypes scored below the BLUP mean (Figure 1B). The lowest predicted mean had G1 and the highest predicted mean was calculated for G17.

**Table 4.** AMMI analysis for grain yield of 20 genotypes of winter barley across 6 growing seasons

Sources of variation	DF	Sum square	Mean square	TSS, %	GEI expl., %	Cumulative, %
Environment (E)	5	1612.06	322.41*	87.75		
Replication / E	18	1.23	0.07	0.07		
Genotype (G)	19	60.28	3.17*	3.28		
GE interaction	95	148.34	1.56*	8.07		
IPC1	23	57.20	2.49*		38.6	38.6
IPC2	21	35.19	1.68*		23.7	62.3
IPC3	19	23.48	1.24*		15.8	78.1
IPC4	17	22.08	1.30*		14.9	93.0
IPC5	15	10.38	0.69*		7.0	100
Residuals	342	15.18	0.04			

\* significant at the 0.05 probability levels, respectively; TSS, total sum of squares; GEI expl., genotype  $\times$  environment interaction explained;



**Fig. 1.** RMSPD estimates used to assess the predictive accuracy of the AMMI family and BLUP (A) and the BLUPs for grain yield (B) of 20 genotypes of winter barley across 6 growing seasons

### Variance components and genetic parameters

Based on the mixed model likelihood ratio test, both genotype and genotype by environment interactions had highly significant ( $P < 0.001$ ) effects (Table 5). The highest proportion of the phenotypic variance was due to the genotype by growing year interaction variance – 77.31%. The contributions of the genotypic and the growing year variances were only 13.64% and 9.05%, respectively. Low value of broad-sense heritability ( $h^2 = 0.137$ ) was found. The correlation between predicted and observed genotypic values represented by the accuracy of selection ( $As$ ) was high (0.713) according to the classification of Resende & Duarte (2007). The coefficient of relative variation ( $CVg/CVr$  ratio) was higher than 1, indicating the possibility of select genotypes with superior performance for grain yield despite the low heritability of the trait.

### Combining of AMMI Analysis and BLUP Techniques

WAASB method integrates features from AMMI and BLUP models for selecting high-yielding and stable genotypes. The stability of the genotypes can be presented graphically using biplots of the WAASB scores and grain yield.

**Table 5. Variance components and genetic parameters for grain yield of barley genotypes evaluated across 6 growing seasons**

Likelihood ratio test	
	G
$\chi^2$	4.62*
	GEI
	593.44*
Variance components	
$\sigma^2g$	0.067(13.64%)
$\sigma^2gei$	0.379 (77.31%)
$\sigma^2r$	0.044 (9.05%)
$\sigma^2p$	0.491
$h^2$	0.137
$R^2gei$	0.773
$h^2mg$	0.508
$As$	0.713
$rge$	0.895
$CVg\%$	4.582
$CVr\%$	3.726
$CV$ ratio	1.230

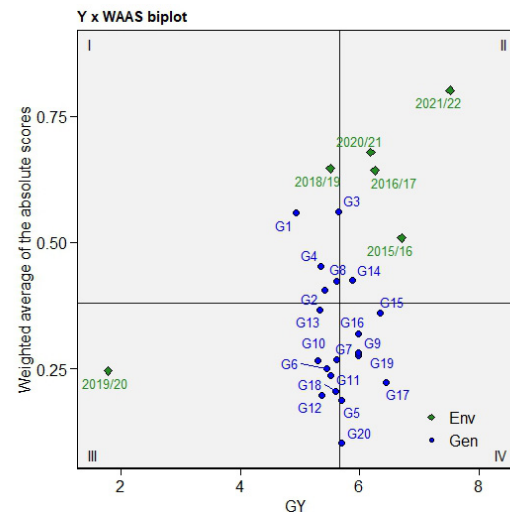
Note: G- genotype; GEI – genotype by environment interaction; significant effects ( $p < 0.001$ ); REML – restricted maximum likelihood;  $\sigma^2g$  – genotypic variance;  $\sigma^2gei$  – genotype by environment interaction variance;  $\sigma^2_r$  – residual variance;  $\sigma^2p$  – phenotypic variance;  $h^2$  – broad-sense heritability;  $R^2gei$  – coefficient of determination of the interaction effects;  $h^2mg$  – heritability of the genotypic mean;  $As$  – accuracy of selection;  $rge$  – genotype-environment correlation;  $CVg\%$  – genotypic coefficient of variation;  $CVr\%$  – residual coefficient of variation;  $CV$  ratio – ratio between genotypic and residual coefficient of variation; Parenthetical values indicate the percentage of the observed phenotypic variance ( $\sigma^2p$ )

The abscissa representing the grain yield and the ordinate representing the WAASB value, the biplot can be divided into four quadrants (Figure 2). The first quadrant includes the unstable genotypes with grain yield below the grand mean and environments with high discrimination ability. Genotypes G1, G2, G3, G4, and G8 are located in this quadrant. They had lower grain yield than the mean grain yield and high WAASB values. The conditions of the 2018/19 growing year were the most appropriate for distinguishing genotypes by their yield potential.

In the second quadrant, genotypes G14 and 2015/16, 2016/2017, 2020/21, and 2021/22 growing years were present. The genotypes in this quadrant have a higher grain yield, but similar to the first quadrant, they are unstable. The growing years contained in this quadrant had good discrimination abilities for the genotypes.

Genotypes G6, G7, G10, G11, G12, G13 and G18 and 2019/20 growing year were located in the third quadrant. These genotypes have a lower grain yield and higher stability than the average. The localization of the 2019/20 growing year here could be explained by the very lower precipitation less than 170 mm than the long-term precipitation sum for the barley vegetation period, which was the cause not only low grain yield but also not allowing distinguish the productive abilities of the genotypes.

The remaining genotypes G5, G9, G15, G16, G17, G19, and G20 fall into the fourth quadrant. The genotypes in this quadrant have more than the mean grain yield and better stability (lower WAASB value).



**Fig. 2. Biplot of mean grain yield and weighted average of absolute scores for the best linear unbiased predictions of the genotype vs. environment interaction (WAASB)**

### Parametric measures of stability

Based on grain yield, genotypes G17, G15, and G9 had the highest, while G13, G10, and G1 had the lowest mean values (Table 6).

In the joint regression model the genotypes G5, G11, and G17 with  $b_i = 1$  and low  $S^2d_i$  scores are classified as highly stable genotypes (Table 6). Genotypes G9, G20, and G15 with  $b_i$  values  $> 1$  and grain yield greater than the overall mean were adapted to the favorable environments. Genotypes G1 and G8 with  $b_i$  values  $< 1$  and yield performance lower than the overall mean have low stability or may have a specific adaptation to low-yielding environments.

Based on  $W_2i$  and  $\sigma^2i$ , genotypes G20, G5, G12, and G18 had the lowest values and were identified as the most stable. Using ASV, the four best-ranked genotypes were G20, G17, G11, and G7. The SIPC and Za also identified G20 as the most stable genotype and ranked the other genotypes in almost equal order. Genotype G20 was found to be the most stable and by the WAASB score, followed by G6 and G11. The best-ranked genotypes by the HMRPGV were among high-yielding genotypes.

### Non-parametric measures of stability

According to stability statistics  $S^{(1)}$ ,  $S^{(2)}$ ,  $S^{(3)}$ , and  $S^{(6)}$ , genotypes G17 and G15 were the most stable genotypes

(Table 7). The  $NP^{(1)}$  recognized G12 as the most stable genotype, followed by G20 and G18. While  $NP^{(2)}$  identified genotypes G20, G8, and G9 as the most stable.  $NP^{(3)}$  and  $NP^{(4)}$  considered genotypes G15 and G17 as most stable. The KR stability index identified genotypes G20, G17, and G19 as the most stable. Overall, the results of non-parametric statistics identified genotypes G17, G15, and G20 as stable genotypes.

### Association among stability statistics

A heatmap of the Spearman's rank correlation coefficients between mean grain yield (GY) and stability parameters is presented in Figure 3. The results showed that GY was significantly and positively correlated with HMRPGV,  $S^{(3)}$ ,  $S^{(6)}$ ,  $NNP^{(2)}$ ,  $NNP^{(3)}$ , and  $NP^{(4)}$  and KR. The  $s^2d_i$ ,  $W_1^2$ ,  $\sigma^2i$  were significantly associated with all other estimated stability measures, with exception of HMRPGV and  $NP^{(2)}$ . Positive associations were found between ASV and all parametric indexes except HMRPGV, and lack of associations with all non-parametric indexes except with KR. No significant correlations were found between HMRPGV and the rest estimated parametric indexes, while WAASB displayed a strong positive association with all parametric indexes except HMRPGV. The most of non-parametric stability measures had a strong positive correlation with each other.

**Table 6. The mean grain yield (GY) and parametric stability statistics values for 20 genotypes of winter barley across 6 growing seasons**

Genotype	Y	$W_i^2$	$s^2d_i$	$b_i$	$\sigma^2i$	ASV	SIPC	ZA	WAASB	HMRPGV
G1	4.93	4.56	0.60	0.87	0.99	1.44	0.31	0.31	0.61	0.87
G2	5.42	2.24	0.32	1.00	0.48	0.81	0.24	0.24	0.38	0.94
G3	5.64	4.52	0.64	1.03	0.98	1.44	0.31	0.31	0.52	0.98
G4	5.35	2.78	0.40	0.98	0.60	1.16	0.25	0.25	0.43	0.94
G5	5.69	0.54	0.07	0.96	0.10	0.52	0.1	0.11	0.17	1.02
G6	5.45	1.09	0.14	0.93	0.22	0.58	0.15	0.15	0.20	0.97
G7	5.61	1.66	0.23	1.04	0.35	0.45	0.16	0.16	0.26	0.95
G8	5.60	2.70	0.36	0.90	0.58	1.11	0.24	0.24	0.39	0.98
G9	5.98	1.82	0.22	1.11	0.38	0.67	0.17	0.17	0.26	1.04
G10	5.30	1.65	0.22	0.93	0.34	0.63	0.16	0.16	0.26	0.94
G11	5.51	0.80	0.11	1.01	0.16	0.39	0.14	0.14	0.19	0.95
G12	5.36	0.56	0.07	0.94	0.10	0.51	0.11	0.11	0.19	0.94
G13	5.33	2.43	0.35	1.02	0.52	0.73	0.22	0.22	0.39	0.92
G14	5.88	2.39	0.33	0.93	0.51	1.01	0.24	0.24	0.40	1.05
G15	6.34	1.95	0.28	1.00	0.41	0.92	0.2	0.2	0.40	1.13
G16	5.98	1.92	0.24	0.89	0.40	0.57	0.19	0.19	0.33	1.07
G17	6.44	1.29	0.10	1.17	0.26	0.29	0.14	0.14	0.34	1.12
G18	5.60	0.67	0.09	1.01	0.13	0.64	0.11	0.11	0.19	0.97
G19	5.98	1.07	0.09	1.15	0.22	0.53	0.16	0.16	0.24	1.04
G20	5.70	0.43	0.01	1.13	0.07	0.10	0.07	0.07	0.09	0.94

Table 7. Non-parametric stability statistics values for 20 genotypes of winter barley across 6 growing seasons

Genotype	S <sup>(1)</sup>	S <sup>(2)</sup>	S <sup>(3)</sup>	S <sup>(6)</sup>	NP <sup>(1)</sup>	NP <sup>(2)</sup>	NP <sup>(3)</sup>	NP <sup>(4)</sup>	KR
G1	6.60	31.37	25.43	4.43	6.33	1.88	1.21	1.07	40
G2	5.47	20.67	12.40	2.40	4.00	0.61	0.64	0.66	29
G3	7.93	42.97	21.85	3.15	5.17	0.52	0.63	0.81	28
G4	8.27	47.47	24.55	3.72	6.50	0.57	0.72	0.86	35
G5	5.60	22.27	10.44	2.19	4.17	0.43	0.44	0.53	10
G6	5.93	24.17	13.68	2.64	4.00	0.58	0.57	0.67	21
G7	7.67	39.77	20.22	3.36	6.50	0.58	0.67	0.78	20
G8	6.60	30.57	15.03	2.43	4.00	0.35	0.54	0.65	28
G9	7.00	36.17	14.09	2.42	6.33	0.37	0.50	0.55	14
G10	5.53	20.70	13.80	2.67	4.00	0.83	0.75	0.74	28
G11	6.60	29.90	17.59	3.18	4.17	0.60	0.55	0.78	18
G12	4.60	18.17	15.57	2.86	2.33	1.10	0.51	0.79	19
G13	6.87	30.97	18.96	3.31	5.67	0.73	0.79	0.84	34
G14	8.67	53.47	20.05	2.65	5.67	0.39	0.47	0.65	21
G15	4.40	15.20	4.47	1.06	4.00	0.54	0.33	0.26	15
G16	7.40	40.17	14.52	2.27	5.17	0.38	0.46	0.53	17
G17	2.93	8.27	2.38	0.77	5.17	0.41	0.34	0.17	9
G18	5.47	19.87	10.64	2.21	3.50	0.39	0.46	0.59	16
G19	4.87	18.30	7.32	1.60	4.00	0.45	0.39	0.39	10
G20	5.93	26.17	12.87	2.23	3.33	0.32	0.39	0.58	8

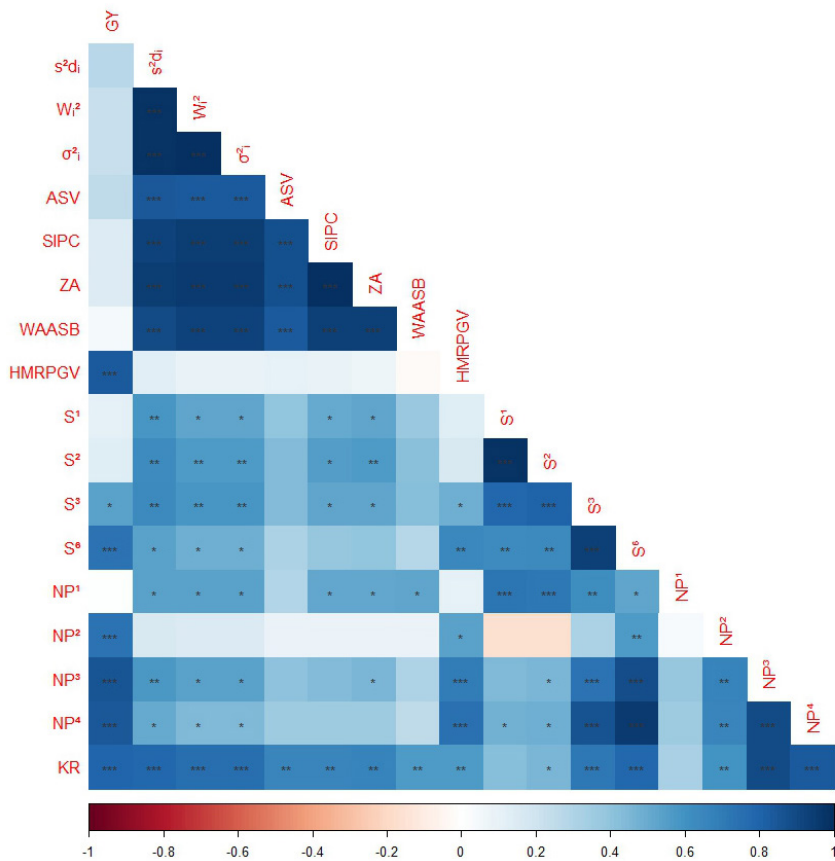
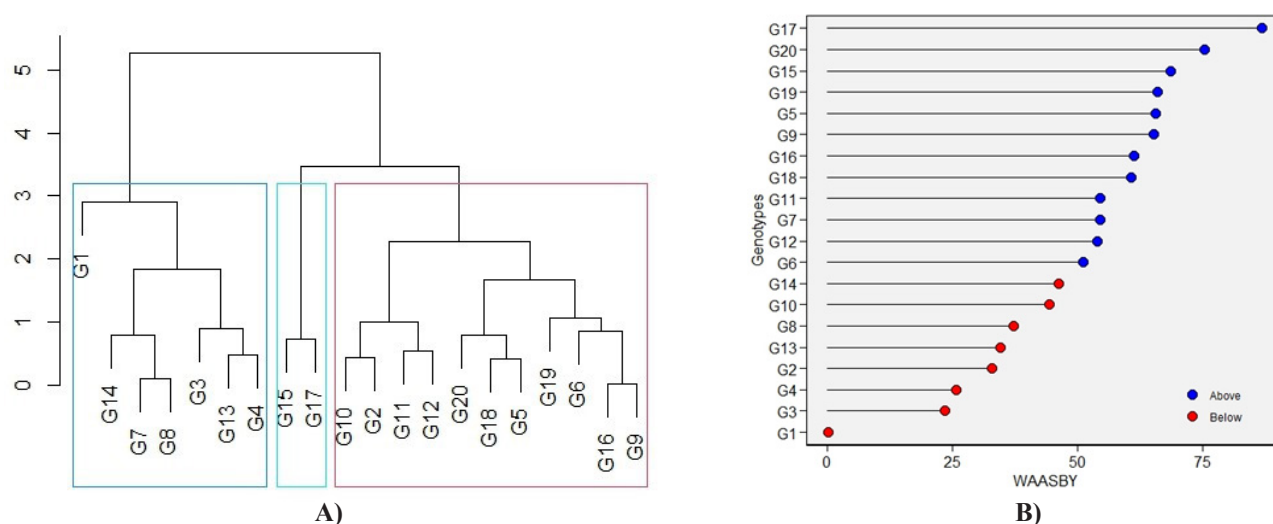


Fig. 3. Heatmap of Spearman's rank correlation of mean yield (GY) and stability statistics. \*, \*\*, and \*\*\* indicate significant at 0.05, 0.01, and 0.001 probability levels, respectively



**Fig. 4.** Hierarchical classification of barley genotypes based on ranks of mean grain yield and the average sum of ranks of stability statistics (A), and the weighted average of the stability and mean grain yield (WAASBY) (B)

### Clustering and ranking of genotypes

Hierarchical cluster analysis based on mean grain yield and average sum rank of stability statistics grouped barley genotypes into three main clusters (Figure 4A). The first cluster was subdivided into two subclusters, including genotypes G9, G16, G6, G19, G5, G18, and G20 in the first subcluster. These genotypes had grain yield around average grain yield and lower ASR scores than the average ASR values.

The second subcluster comprised four genotypes G11, G12, G2, and G10, and had grain yield lower than the average grain yield and ASR values around the overall mean for stability parameters.

The other main cluster contains only two genotypes G15 and G17, which had a highest grain yield and the low ASR values for stability parameters.

The third cluster included genotypes G4, G13, G3, G8, G7, G14, and G1, which had a lower average grain yield than the overall mean and the highest ASR values for stability parameters.

The genotypes were ranked and with the WAASBY superiority index (Figure 4B). The genotype with the highest WAASBY score was G17, followed by G20, G15, G19, and G5. The genotype with the lowest WAASBY score was G1, followed by G3, G4, G2, and G13. These genotypes had the highest ASR values and the lowest average mean grain yield.

### Discussion

In the present study, multiple statistical models to investigate the grain yield performance and stability of winter bar-

ley genotypes were employed. The present study found highly significant differences between genotypes, environments, and GEI effect. The results are in agreement with previously reported studies showing that the environment has a significant impact on barley grain yield (Mansour et al., 2018; Vaezi et al., 2019; Ahakpaz et al., 2021). This impact can be attributed to the combined influence of different abiotic and biotic factors, which can vary due to fluctuations in meteorological conditions throughout the crop-growing season.

The GEI had the largest effect on phenotypic variance of grain yield, resulting in lower heritability. GEI effect was considerably greater than the genotype effect also implied that genotype responses differ across growing years, which suggests a significant difference in genotype rank order. Significant GEI and large crossover GEI in barley METs for barley grain yield have been reported in many studies (Ahmadi et al., 2012; Mortazavian et al., 2014; Mansour et al., 2018; Vaezi et al., 2019). The genotype by environment interaction (GEI) is one of the main constraints in selecting desirable genotypes which prolongs and complicates the breeding process.

The magnitude of the GEI effect is primarily due to changes in environmental conditions from one location to another and from year to year. The differences in precipitation over the years can challenge the reproducibility patterns of the results of multi-environment trials. In addition to the amount, the distribution of precipitation during the growing year is also important (Ahakpaz et al., 2021). Additionally, part of the GEI is result of genetic differences between the studied genotypes. Therefore, using accurate prediction



models is critical for the analyze stability and yield performance of genotypes (Gauch & Zobel, 1988).

The AMMI is the one of the most widely used model to study the GEI. In the current study, the AMMI analysis revealed that the first five IPCs were significant, and the first two IPCs accounted for over half of the total GEI.

The interpretation of GEI by AMMI analysis based on the first two components could be unpunctual if only half of the variation was captured. Therefore, the use of model diagnosis has the greatest importance in choosing the best model (Gauch, 2013).

The comparison of the AMMI family and BLUP models found that the BLUP was the most predictively accurate model for the particular dataset. Similar findings were previously reported in other crops (van Eeuwijk et al., 2016; Olivoto et al., 2019; Huang et al., 2021; Nataraj et al., 2021).

The advantages of AMMI and BLUP models were used the WAASB a new stability index designated as the weighted average of absolute scores based on singular value decomposition (SVD) on BLUP-interaction effects (Olivoto et al., 2019). Superiority index WAASBY, which allows weighting between mean performance and stability (WAASB) (Olivoto et al., 2019) were also calculated. The WAASB was used in various crops to identify genotypes that are stable and highly productive (Huang et al., 2021; Nataraj et al., 2021; Koundinya et al., 2021; Pour-Aboughadareh et al., 2022).

Several stability models and statistics have been proposed to improve the assessment of genotype stability. In present study, different parametric and non-parametric stability indices were applied. The results revealed that GY was strongly and positively correlated with most of the non-parametric stability measures and only with HMRPGV from parametric statistics. These findings suggested that non-parametric statistics could be a valuable alternative to parametric approaches for identifying stable genotypes.

Part of studied genotypes were ranked as highly stable by some stability metrics and as unstable by others. This is a common problem in similar studies, and to address it, the use of ASR values from the stability statistics was suggested (Alizadeh et al., 2022; Pour-Aboughadareh et al., 2022). As low ASR value indicates a high stability, genotypes G20, G17, G19, G18, and G5 were identified as the most stable genotypes in this study. A similar outcome was observed by the grouping of the genotypes by average sum rank of stability statistics and by ranking by the WAASBY index. This showed the efficiency of the WAASB statistic for identifying the superior barley genotypes. The remarkable advantage of WAASBY superiority index is that it allows weighting between performance of response variables and the WAASB stability score for simultaneous selection of stability and

productivity under a mixed effect model (Olivoto et al., 2019). This allows, breeders to prioritize weights for grain yield and stability according breeding objectives and cultivar recommendations. Therefore, the genotype G17 that was found to have the WAASBY highest superiority index could be used in winter barley breeding programs in development of breeding lines with high adaptation to local conditions.

## Conclusions

The grain yield of the studied winter barley genotypes was highly affected by genotype, growing season, and their interaction, as demonstrated by the AMMI and BLUP methods. The majority of non-parametric stability statistics employed in the study displayed a significant positive correlation with grain yield, which suggests that non-parametric statistics may be a useful alternative to parametric methods for identifying the most stable genotypes.

Based on most of statistical measures, genotypes G17 (K16/3-12), G20 (A8/2) and G15 (K16/1-21) were identified as more high-yielding and stable genotypes than the national standard cultivars G1 (Obzor) and G2 (Emon). This result was confirmed with the WAASBY index, indicating the efficiency of the WAASBY statistics in selecting superior barley genotypes.

Overall, the genotype G17 (K16/3-12) with a high and stable yield performance could be recommended for testing as a new cultivar as well as a genetic resource for improving the grain yield of winter barley in Southeastern Bulgaria.

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