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## Assessing yield performance of winter barley genotypes

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### Abstract

The aim of this study was to evaluate the grain yield performance and stability of 33 winter barley (*Hordeum vulgare* L.) genotypes over a three-year period and to identify the stable and high-yielding breeding lines. The study was conducted at the experimental field of the Institute of Agriculture – Karnobat, in Southeastern Bulgaria, and was organized using a Complete Block Design with four replications on plots of 10 m<sup>2</sup>. Significant effects of environmental conditions on grain yield and substantial genotype-by-year interactions were observed. Parametric and non-parametric stability statistics were applied to identify genotypes with stable and high grain yields. Genotypes Q-28, Q-21, and Q-6 were identified as highly stable. Genotype Q-28 has exhibited superior performance in both stability and mean grain yield, establishing itself as a valuable candidate for breeding programs. The study highlighted the significance of integrating multiple parameters for the effective selection of genotypes that achieve both high yield and stability. The observed negative correlation between grain yield and stability statistics underlines the importance of applying targeted breeding strategies to simultaneously improve grain yield and stability.

**Keywords:** winter barley, grain yield, parametric stability, non-parametric stability

### INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest cereal crops, valued worldwide for its adaptability to diverse climates and its versatile applications in food, feed, and brewing industries. However, grain yield stability remains one of the main challenges for barley breeders (Costa & Bollero, 2001; Mohammadi & Nader Mahmoodi, 2008). Variation in grain yield across different growing years is due to the interactions between genetic, environmental, and management factors. As variation in temperature and precipitation, significantly impact barley grain yield, unpredictable weather patterns and increased frequency of extreme events further complicate the breeding of high-yielding and stable varieties (Costa & Bollero, 2001). Assessment of how genotypes respond to environmental factors over multiple years is essential for the selection of breeding lines

combining high grain yield with stability. Numerous statistics have been developed to assess the genotype performance across varying environments. These statistical methods are essential tools for identifying genotypes that exhibit a balanced combination of high yield potential and stability. Different statistical models focus on various aspects of genotype performance across environments. Regression analysis, introduced by Finlay & Wilkinson (1963) and Eberhart & Russell (1966), remains one of the most widely used approaches. This method provides reliable estimation of parameters, especially when a sufficiently large number of genotypes and environments are included in the analysis, but extreme environments that could affect regression slopes are excluded (Fernandez, 1991).

In addition to regression analysis, numerous other parametric stability parameters have been proposed, including stability variance

( $\sigma_i^2$ ) (Shukla, 1972), and coefficient of variability (CVi) (Francis & Kanenberg, 1978). These parametric methods rely on assumptions about data distribution and variance homogeneity.

In contrast, non-parametric stability methods are less affected by data distribution. Since they rely on ranks rather than absolute values, a genotype is considered stable if its ranking remains relatively consistent across environments (Paul et al., 2015). Several non-parametric methods have been developed to assess genotype responses to environmental variation (Nassar & Huhn, 1987; Huhn, 1990; Thennarasu, 1995).

The aim of this study is to evaluate the grain yield performance and stability of 33 winter barley genotypes over a three-year period and to identify stable and high-yielding breeding lines.

## MATERIALS AND METHODS

The plant material was consisted of 33 genotypes of winter 6-rowed barley, including the standard variety Veslets (Q-1) and the

varieties Izgrev (Q-2), IZ Bori (Q-3), Bojin (Q-4), and Zemela (Q-5), along with 28 advanced breeding lines. All tested breeding lines and varieties were developed at the Institute of Agriculture - Karnobat, Bulgaria.

The study was conducted during three growing years 2021-2022, 2022-2023, and 2023-2024 at the experimental field of the Institute of Agriculture – Karnobat, Southeastern Bulgaria. The soil of the experimental field was slightly acid (pH is 6.2) and classified as 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 of 450 germinated seeds/m<sup>2</sup>. All recommended crop management practices for the region were properly followed. Grain yield was determined by weight of grains per plot and converted to tones per ha.

Average monthly air temperatures for the studied years were higher compared to the long-term air temperatures (Table 1). The sums of precipitations for two of the growing periods (2022-2023 and 2023-2024) were lower than the long-term sum for barley vegetation.

**Table 1.** Average monthly air temperature, monthly sums of precipitation and long-term average data for the experimental area (Karnobat, Southeastern Bulgaria) across three growing years

Months	2021-2022		2022-2023		2023-2024		LT	
	T, °C	P, mm	T, °C	P, mm	T, °C	P, mm	T, °C	P, mm
October	11.1	79.1	13.7	2.0	12.6	1.2	12.5	44.3
November	8.7	31.7	9.9	59.5	7.2	120.1	7.1	53.7
December	4.5	105.7	5.7	49.3	2.8	26.4	2.6	51.2
January	2.4	8.0	6.0	21.0	0.5	28.9	0.6	36.5
February	4.4	39.7	4.7	4.1	2.4	0.5	2.2	35.8
March	3.5	12.3	7.6	24.6	5.6	20.1	5.3	34.1
April	11.2	48.2	10.5	86.0	10.7	45.6	10.5	45.3
May	15.9	36.3	14.5	105.3	15.7	58.4	15.6	58.5
June	21.0	86.8	20.4	23.5	19.8	41.1	19.6	65.2
T, °C	9.2		10.3		8.6		8.4	
P, mm		447.8		375.3		342.3		424.6

*Legend:* T, °C – average monthly air temperature; P, mm – monthly sums of precipitation; LT – long-term average monthly air temperature and sums of precipitation (1931–2024)

The weather data from the experimental site over the three growing years shows considerable variations in temperature and precipitation compared to the long-term averages (Table 1). The average air temperatures during the study period were higher than the long-term average monthly temperatures. Precipitation varied significantly between the years. The 2021–2022 year had a higher precipitation sum. Conversely, 2023–2024 had the lowest precipitation sum at 342.3 mm. These deviations from long-term norms indicate variable growing conditions, characterized with higher temperatures and an uneven precipitation pattern.

The estimated parametric stability statistics were as follows: regression coefficient ( $b_i$ ; Finlay & Wilkinson, 1963), variance of deviations from the regression ( $S_{di}^2$ ; Eberhart and Russell, 1966), Wricke's ecovalence stability index ( $W_i^2$ ; Wricke, 1962), Shukla's stability variance ( $\sigma_i^2$ ; Shukla, 1972), environmental coefficient of variance ( $CV_i$ ; Francis & Kannenberg, 1978), Plaisted and Peterson's mean variance component ( $\theta_i$ ; Plaisted & Peterson, 1959), Plaisted's GE variance component ( $\theta_{(i)}$ ; Plaisted, 1960), and the yield-stability index ( $YS_i$ ; Kang, 1991). For non-parametric methods, the following statistics were calculated: Nassar and Huhn's statistics ( $S^{(1)}$ ,  $S^{(2)}$ ; Nassar & Huhn, 1987), Huhn's equation ( $S^{(3)}$  and  $S^{(6)}$ ; Huhn, 1990), Thennarasu's statistics ( $NP^{(i)}$ ; Thennarasu, 1995), and Kang's rank-sum ( $KR$ ; Kang, 1988).

The studied genotypes were ranked per each statistic. The stability statistics was computed with the online program STABILITYSOFT (Pour-Aboughadareh et al.,

2019). STABILITYSOFT was also used to generate a heat map plot based on Pearson's correlation coefficients (Pearson, 1895) to display the relationships between stability statistics and yield performance.

## RESULTS AND DISCUSSION

The combined ANOVA revealed that the effects of genotype, growing year, and their interaction were statistically significant ( $p < 0.001$ ) (Table 2). The year accounted for 73.14% of the total sum of squares (SS), indicating that environmental factors had a dominant role in determining grain yield variability. The high percentage of variance explained by the year underlines the critical influence of environmental fluctuations, including temperature, precipitation, and other climatic variables, on barley grain yield. These findings are consistent with previous studies, which have reported that environmental factors typically contributed to yield variability in multi-year trials (Nissilä, 1992; Costa & Bollero, 2001). The genotype-by-year interaction accounted for 14.47%, highlighting the differential responses of genotypes to varying environmental conditions. Its high statistical significance indicates that genotype performance fluctuated across years, necessitating the identification of stable genotypes with minimal yield variability. Genotypic effects accounted for 11.58% of the total SS. Its high statistical significance indicates that genotype performance fluctuated across years, necessitating the identification of stable genotypes with minimal yield variability.

**Table 2.** Combined analysis of variance (ANOVA) for grain yield in three growing years and the proportion of the total variance attributable to the source of variation

Source	SS	DF	MS	F	SS (%)
Genotype	92.17	32	2.88	133.41*	11.58
Year	582.14	2	291.07	13482.49*	73.14
Genotype * Year	115.16	64	1.80	83.35*	14.47
Error	6.41	297	0.02		0.81

Legend: \* $p < 0.001$

During the 2021-2022 growing year, 18 out of the 33 tested barley genotypes demonstrated a grain yield higher than the average of 5.611 t/ha (Table 3). The highest yield was recorded in genotype Q-15 at 7.000 t/ha, followed by Q-26 with 6.593 t/ha and Q-23 with 6.575 t/ha. The highest mean grain yield, 7.379 t/ha, was recorded in 2022-2023, ranging from 5.024 t/ha for genotype Q-14 to 8.759 t/ha for genotype Q-9.

The lowest mean grain yield - 4.429 t/ha was observed in 2023-2024. The highest-

yielding genotypes during this period were Q-15 (5.498 t/ha), followed by Q-5 with 5.475 t/ha, and Q-29 with 5.210 t/ha.

Across the three-year study period, 18 genotypes had higher mean grain yield than the average for the period (5.806 t/ha). The standard variety Veslets (Q-1) ranked 13<sup>th</sup>, with 11 breeding lines and two varieties - Zemela (Q-5), and IZ Bori (Q-3) - ranking higher than the standard.

**Table 3.** Mean gain yield (t/ha) of 33 barley genotypes tested across the three years

Genotype	2021-2022	2022-2023	2023-2024	Mean
Q-1	5.458	7.834	4.581	5.958
Q-2	4.690	6.098	4.551	5.113
Q-3	5.525	8.578	3.879	5.994
Q-4	4.525	6.066	5.136	5.243
Q-5	5.493	8.601	5.475	6.523
Q-6	5.270	6.631	3.994	5.298
Q-7	4.740	7.813	4.284	5.612
Q-8	5.228	6.050	4.495	5.257
Q-9	5.973	8.759	4.806	6.513
Q-10	4.855	7.631	3.876	5.454
Q-11	5.293	7.076	4.820	5.730
Q-12	4.765	7.051	5.198	5.671
Q-13	5.633	6.656	5.201	5.830
Q-14	4.718	5.024	4.476	4.739
Q-15	7.000	8.060	5.498	6.853
Q-17	5.925	6.908	5.081	5.971
Q-18	5.475	8.208	3.947	5.877
Q-19	5.275	7.640	4.589	5.835
Q-20	6.125	8.188	3.909	6.074
Q-21	5.325	7.020	4.235	5.527
Q-22	6.018	7.935	4.413	6.122
Q-23	6.575	8.338	4.603	6.505
Q-24	5.643	7.358	4.050	5.683
Q-25	6.318	6.895	4.838	6.017
Q-26	6.593	7.565	3.999	6.052
Q-28	6.465	8.285	4.955	6.568
Q-29	6.220	6.516	5.210	5.982
Q-30	5.625	8.230	3.894	5.916
Q-31	6.150	6.445	2.325	4.973
Q-32	6.300	8.048	4.343	6.230
Q-33	4.610	7.405	3.616	5.210
Q-34	5.300	7.548	3.678	5.508
Q-35	6.050	7.060	4.198	5.769
Mean	5.611	7.379	4.429	5.806

The ranks of the studied genotypes for different parametric and non-parametric stability statistics are shown in Table 4. The genotypes Q-21, Q-28, and Q-6 showed highest stability according to Wricke's ecovalence ( $W_i^2$ ) and Shukla's stability variance ( $\sigma_i^2$ ), indicating stable grain yield performance across various growing years. The least stable were the

genotypes Q-4 and Q-31, ranked 32nd and 33rd, respectively.

According to the variance of deviations from the regression ( $S^2d_i$ ), genotypes Q-30, Q-14, and Q-21 ranked the highest. The genotypes Q-30, Q-14, and Q-29 were the most stable based on Francis and Kannenberg's coefficient of variation (CVi).

**Table 4.** The ranks of the studied genotypes for different parametric and non-parametric stability statistics

Genotype	Y	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>	$W_i^2$	$\sigma_i^2$	$s^2d_i$	CVi	KR	$\theta_{(i)}$	$\theta_i$
Q-1	14	5	5	7	7	7	6	7	8	6	6	15	18	6	6	28
Q-2	31	17	21	28	29	25	30	28	29	26	26	20	7	30	26	8
Q-3	11	32	30	29	25	32	19	24	26	30	30	12	32	23	30	4
Q-4	29	33	33	33	33	33	26	31	33	31	31	29	5	31	31	3
Q-5	3	17	22	14	11	10	8	13	11	25	25	32	17	11	25	9
Q-6	27	1	1	3	3	5	33	21	3	3	3	11	12	17	3	31
Q-7	23	17	16	17	20	21	24	26	22	19	19	28	25	25	19	15
Q-8	28	17	17	24	28	17	29	29	28	24	24	5	4	29	24	10
Q-9	4	9	9	9	8	26	5	9	7	17	17	14	24	7	17	17
Q-10	26	16	15	22	27	18	28	27	27	14	14	24	28	22	14	20
Q-11	20	12	13	13	13	14	16	11	14	9	9	16	10	13	9	25
Q-12	22	29	29	27	26	30	20	25	25	28	28	31	11	28	28	6
Q-13	18	26	26	23	17	21	15	16	20	27	27	7	3	26	27	7
Q-14	33	17	19	31	30	24	32	33	31	32	32	2	1	32	32	2
Q-15	1	5	7	4	4	15	2	5	4	8	8	18	9	2	8	26
Q-17	13	22	20	15	15	10	10	12	15	16	16	6	6	13	16	18
Q-18	16	23	23	19	19	29	21	23	19	20	20	9	29	19	20	14
Q-19	17	9	9	11	12	6	7	10	12	7	7	25	16	8	7	27
Q-20	8	23	25	20	21	10	9	17	17	21	21	22	26	13	21	13
Q-21	24	1	1	1	2	2	23	3	2	1	1	3	15	9	1	33
Q-22	7	4	4	5	6	3	3	2	6	5	5	10	20	3	5	29
Q-23	5	7	8	6	5	10	1	6	5	12	12	19	19	4	12	22
Q-24	21	7	6	8	9	4	11	4	10	4	4	13	21	9	4	30
Q-25	10	25	24	18	16	18	14	14	16	18	18	26	8	11	18	16
Q-26	9	26	28	21	18	28	17	18	18	23	23	30	23	18	23	11
Q-28	2	3	3	2	1	1	13	1	1	2	2	8	14	1	2	32
Q-29	12	30	32	26	23	15	12	19	21	29	29	21	2	23	29	5
Q-30	15	26	27	25	22	27	18	22	23	22	22	1	30	20	22	12
Q-31	32	30	31	32	32	31	27	30	32	33	33	33	33	32	33	1
Q-32	6	12	11	10	10	8	4	8	9	11	11	17	22	4	11	23
Q-33	30	14	17	30	31	23	31	32	30	15	15	23	31	26	15	19
Q-34	25	14	14	16	23	9	25	20	24	13	13	4	27	21	13	21
Q-35	19	11	12	12	13	20	22	15	13	10	10	27	13	13	10	24

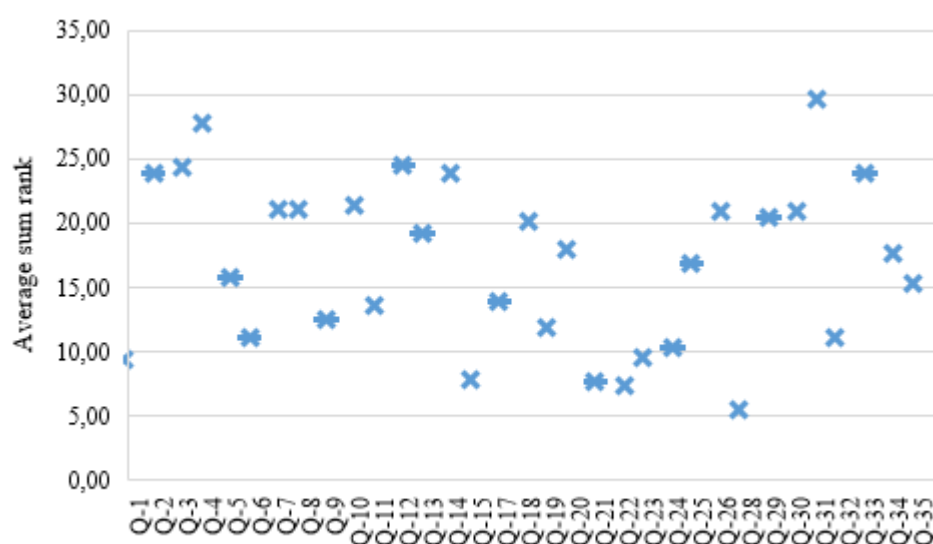
Based on the GE variance component ( $\theta_{ii}$ ), genotypes Q-21, Q-28, and Q-6 were identified as highly stable genotypes, while according to the mean variance component ( $\theta_i$ ), genotypes Q-31, Q-14, and Q-4 demonstrated the highest stability.

Based on  $Si^{(1)}$ ,  $Si^{(2)}$ ,  $Si^{(3)}$ , and  $Si^{(6)}$  statistics, as well as Kang's rank-sum (KR), the most stable genotypes were Q-6, Q-21, and Q-28. Similarly, the  $NPi^{(1)}$ ,  $NPi^{(2)}$ ,  $NPi^{(3)}$ , and  $NPi^{(4)}$  measures identified Q-21, Q-22, and Q-28 as the most stable genotypes.

Ahmadi et al. (2015) suggested that selecting stable genotypes based solely on a single stability measurement may be less effective and accurate. Similar findings were reported by several researchers who applied multiple stability measurements to identify stable and high-yielding genotypes in various crops, including barley (Vaezi et al., 2019), maize (Ruswandi et al., 2022), and soybeans (Wijaya et al., 2022). Integrating parametric and non-parametric stability assessments can enhance the accuracy of genotype selection. This approach aids in identifying high-yielding

and stable genotypes across diverse environments using a single measurement. Some researchers employ the average sum rank (AR) method to evaluate stability, where genotypes with the lowest AR values are considered the most stable (Ahmadi et al., 2015; Vaezi et al., 2019). In the present study, Q-28 was identified as the most stable breeding line based on the smallest AR value, followed by the lines Q-22, Q-21, and Q-15 (Fig. 1). Line Q-28 also demonstrated high mean grain yield. On the contrary, lines Q-4 and Q-31, with the highest rank values in multiple stability statistics, demonstrated poor stability and yield consistency.

Figure 2 presents Pearson's correlation coefficients between stability statistics and grain yield. Grain yield had positive correlation with  $b_i$  and  $\theta_{II}$  and negative correlation with most of the other of estimated stability statistics. Between most of the non-parametric measurements there are high positive correlations, while between non-parametric and parametric measurements exists a negative correlation.



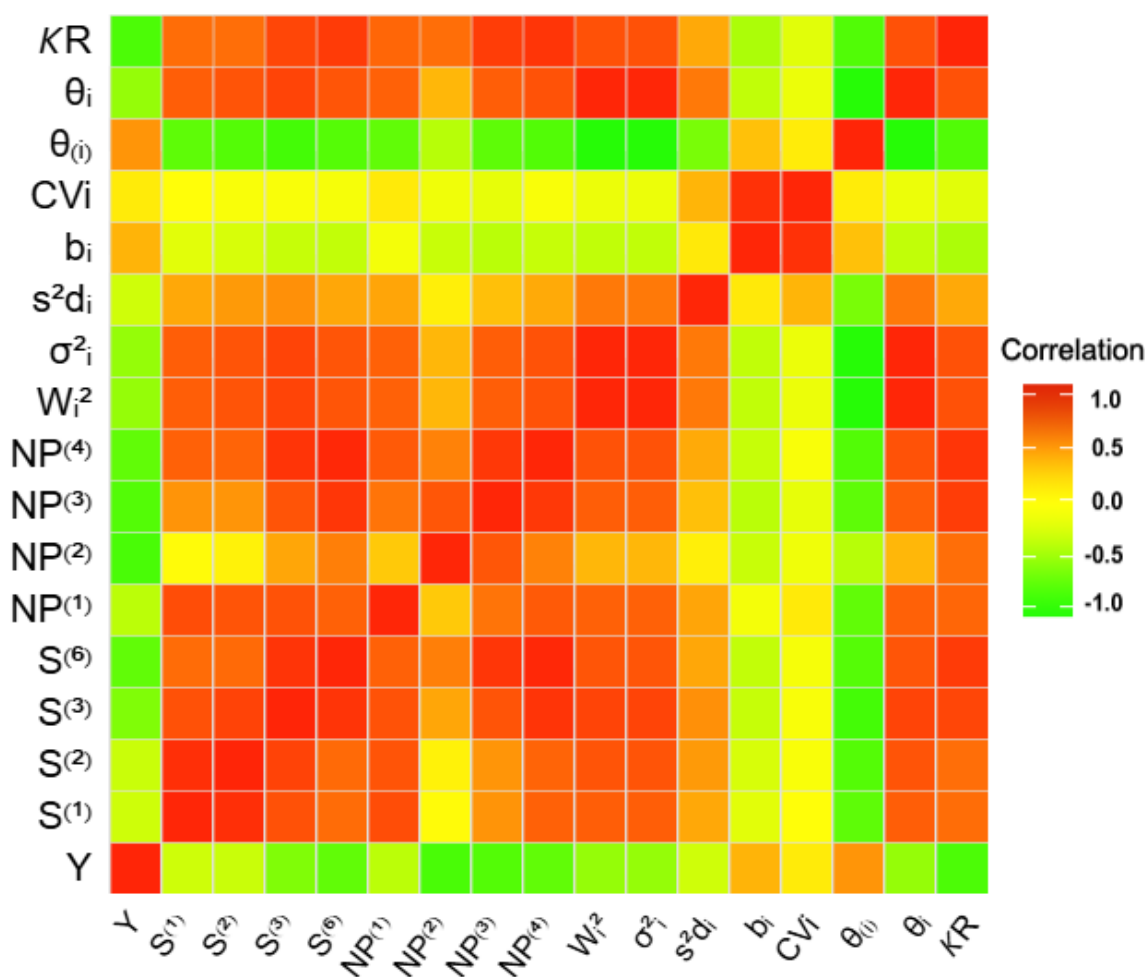
**Figure 1.** Average sum ranks of barley genotypes



In Figure 2 Pearson's correlation coefficients among stability statistics and their relationship with grain yield are presented. Grain yield showed a positive association with the regression coefficient ( $b_i$ ) and the genotype  $\times$  environment (GE) variance component ( $\theta_i$ ), while exhibiting negative correlations with most other stability statistics. The positive association between grain yield and  $b_i$  suggested that high-yielding genotypes usually have regression coefficients greater than one, making them better adapted to favorable environments. In contrast, the negative correlations between grain yield and other stability statistics, such as Wricke's ecovalence ( $W_i^2$ ) and Shukla's

stability variance ( $\sigma^2_i$ ), indicated that these measures were more sensitive to yield variability across environments. This suggested that genotypes with higher yields may exhibit lower stability.

Strong positive correlations were observed among non-parametric measurements, confirming their consistency in stability assessment. Conversely, negative correlations between most non-parametric and parametric measurements suggest that these approaches capture different aspects of stability. Their combined application enables the identification of both high-yielding and stable genotypes across diverse testing conditions.



**Figure 2.** Pearson's correlation coefficients among stability statistics and their relationship with grain yield

## CONCLUSIONS

Growing-year conditions had a dominant influence on barley grain yield, with significant genotype-by-year interactions underscoring the need for breeding programs to develop genotypes with stable performance across diverse environments. Based on multiple parametric and non-parametric stability statistics, genotypes Q-28, Q-21, and Q-6 were identified as highly stable. The superior performance of Q-28 in both stability and mean grain yield suggests its particular value for breeding programs aiming to achieve both high and stable yields. This study demonstrated that integrating parametric and non-parametric stability measurements enables the selection of genotypes that balance high yield and stability. The observed negative correlations between grain yield and stability statistics highlight the challenge of simultaneously optimizing both traits.

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