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YIELD STABILITY ANALYSIS OF BARLEY MUTANTS USING PARAMETRIC AND NONPARAMETRIC STATISTICS

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SUMMARY

Yield stability analysis is important in barley (*Hordeum vulgare* L.) breeding to produce the highest and most stable yields. This study used parametric and nonparametric statistical methods to assess the barley genotypes' stability. It aimed to assess the 40 barley mutants belonging to the subspecies of two-rowed and six-rowed barley obtained after mutagenic treatment with phosphemide in two concentrations. The study transpired in 2020–2022 in Russia's South Moscow and Tyumen regions. The results revealed that environment (46.6%), genotypes (9.1%), and the interaction of environment by study location (26.2%) and genotype by environment (9.5%) contributed the most to grain yield in barley. The highest correlation appeared among the variables. i.e., W_i^2 и σ^2_i, θ_i, S^2d_i; θ_i и σ^2_i, S^2d_i; $NP^{(4)}$ и $S^{(3)}$, $S^{(6)}$; $S^{(1)}$ и $S^{(2)}$; $S^{(2)}$ и $S^{(3)}$; KR; $NP^{(2)}$ и $NP^{(3)}$ (r = 0.80-1.00); $\theta_{(i)}$ и W_i^2, σ^2_i, S^2d_i; and θ_i и $\theta_{(i)}$ (r = - 0.92-1.00). Higher correlation with grain yield emerged with b_i (r = 0.52); $S^{(6)}$ (r = - 0.77); $NP^{(2)}$ (r = - 0.78); $NP^{(3)}$ (r = - 0.79); $NP^{(4)}$ (r = - 0.78); and KR (r = - 0.65). The most stable yields characterized by six-rowed mutants are G20, G22, and G28, derived from the hooded cultivar. The mutants G1, G2, and G40, belonging to the two-rowed barley subspecies, had the highest grain yield potential with less stability.

Keywords: Two-rowed and six-rowed barley, *Hordeum vulgare* L., phosphemide concentrations, chemical mutagenesis, genotype by environment interaction, stability parameters, correlation, grain yield

Key findings: The article discussed the results of yield and stability analyses in two-rowed and six-rowed barley (*Hordeum vulgare* L.) mutants of M5-M7 generations in different ecological areas using parametric and nonparametric statistical methods.

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INTRODUCTION

Stress factors accompany the growth and development of crop plants throughout the vegetation period, often adversely affecting their productivity (Ceccarelli *et al*., 1991). Along with enhancing grain yields, special attention is necessary to acquire crop cultivars adapted to a wide range of environmental variations in diverse ecological regions.

Mutational breeding has succeeded in improving the genetic diversity of various crops and enhancing grain yield levels and plant resistance to environmental stress conditions (Bado *et al*., 2023). According to the FAO/IAEA Mutant Cultivar Database (2023), the cultivated plant species' total number of registered cultivars created by different types of mutagens is 3,402 from top countries like China, Japan, India, Russia, and the Netherlands. The total number of mutant cultivars in cereal crops exceeds 1,500, leading over *Oryza sativa* L. (873 cultivars). Among bread cereals, barley (*Hordeum vulgare* L.) is leading with a maximum number and accounts for 307 cultivars, *Triticum aestivum* L. (265 cultivars), *Zea mays* L. (89 cultivars), *Triticum turgidum* ssp. durum Desf. (31 cultivars), *Avena sativa* L. (23 cultivars), and *Secale cereale* L. (four cultivars) (FAO, 2023). Currently, one of the new mutagens proposed for use in expanding the genetic diversity of crop plants and obtaining the diverse plant types with new valuable traits is phosphemide (Sin. phosphasin), an alkylating compound derived from ethylenimine (EI). The first cytogenetic studies with phosphemide commenced in 1963–1967 on primary embryonic tissue culture. In plants, the first studies to determine mutagen mutagenicity occurred on seeds of *Crepis capillaris* (L.) Wallr. In addition, studies using this mutagen on winter wheat flax have existed; however, not in barley (Weisfeld, 1965; Bome *et al*., 2021; Tetyannikov and Bome, 2022).

In barley breeding programs, along with the quality of the resulting products and resistance to biotic and abiotic stress factors (Kumar *et al*., 2020), grain yield has become one of the vital indicators of success and stipulate for the promising cultivar of barley (Friedt and Ordon, 2013). Grain yield is a highly complex, multifaceted phenomenon of genotype-environment interactions (Roostaei *et al*., 2022). This imperative interaction elucidates the different degrees of variations in a single trait in different genotypes in response to identical variations in environmental conditions (Oral *et al*., 2019). Yield variation seemed to manifest the genotype by environment interaction (GEI), the effect of which can reduce the association between phenotypic and genotypic traits, making it hard to select the best barley genotypes (Vaezi *et al*., 2019).

One of the crucial characteristics of an ideal cultivar is the combination of high yield with stability (Zali *et al*., 2023). In practice, however, high stability often exhibited no correlation with average high yields (Finlay and Wilkinson, 1963). Several statistical methods are available for assessing the stability and genotype-environment interactions to interpret long-term trial data, highlight promising cultivars, or discard unstable genotypes (Khalili and Pour-Aboughadareh, 2016; Kebede *et al*., 2023). Breeding programs use different parametric statistical methods based on absolute data and nonparametric statistics based on rank orders to evaluate the stability of yields, which allows the interpretation of data from multi-year trials of crop varieties. In a statistical evaluation of the studied cultivars, finding an optimal balance between yield and stability is mandatory. The presented study sought to evaluate the barley mutants belonging to the subspecies of two-rowed and six-rowed obtained through mutagenic treatment phosphemide using parametric and nonparametric statistical methods on grain yield stability in different ecological areas.

MATERIALS AND METHODS

Plant material

The object of the study was barley mutants of M5–M7 generations belonging to the subspecies of two-rowed and six-rowed (Table 1), selected for valuable traits (early ripening and late-ripening forms, large spikes, varying

Table 1. Mutants and control genotypes used in the study.

Code	Mutant	Spike rows	Mutagen concentration	Code	Mutant	Spike rows	Mutagen concentration
G1	$R_{II}2(39)$	2 row awned	$2\cdot10^{-3}M$	G23	$P_{IV}19(44)$	6 row awned	$1\cdot10^{-2}M$
G2	$R_{I}9(37)$	2 row awned	$2\cdot10^{-3}M$	G24	$P_{IV}19(45)$	6 row awned	$1\cdot10^{-2}M$
G3	$E_{III}6(54)$	6 row awned	$2\cdot10^{-3}M$	G25	$P_{1}16(69)$	6 row awned	$1\cdot10^{-2}M$
G4	$E_{III}9(61)$	6 row awned	$2\cdot10^{-3}M$	G26	$P_{1}17(71)$	6 row awned	$1\cdot10^{-2}M$
G5	$E_{III}11(62)$	6 row awned	$2\cdot10-^{3}M$	G27	$P_{II}5(73)$	6 row awned	$1\cdot10^{-2}M$
G6	$E_{III}11(63)$	6 row awned	$2\cdot10^{-3}M$	G28	$P_{II}5(74)$	6 row awned	$1\cdot10^{-2}M$
G7	$E_{III}11(64)$	6 row awned	$2\cdot10-^{3}M$	G29	$P_{II}6(75)$	6 row awned	$1\cdot10^{-2}M$
G8	$E_{1}6(1)$	6 row awned	$1\cdot10^{-2}M$	G30	$P_{II}6(76)$	6 row awned	$1\cdot10^{-2}M$
G9	$E_{II}9(2)$	6 row awned	$1\cdot10^{-2}M$	G31	$P_{II}6(77)$	6 row awned	$1\cdot10^{-2}M$
G10	$E_{II}9(3)$	6 row awned	$1\cdot10^{-2}M$	G32	$P_{II}7(78)$	6 row awned	$1\cdot10^{-2}M$
G11	$P_{1}17(7)$	6 row hooded	$2\cdot10^{-3}M$	G33	$P_{II}7(79)$	6 row awned	$1\cdot10^{-2}M$
G12	$P_{II}11(8)$	6 row hooded	$2\cdot10^{-3}M$	G34	$P_{II}7(80)$	6 row awned	$1\cdot10^{-2}M$
G13	$P_{III}11(15)$	6 row hooded	$2\cdot10^{-3}M$	G35	$P_{II}7(81)$	6 row awned	$1\cdot10^{-2}M$
G14	$P_{IV}2(18)$	6 row hooded	$2\cdot10^{-3}M$	G36	$P_{III}3(83)$	6 row awned	$1\cdot10^{-2}M$
G15	$P_{1}12(76)$	6 row awned	$2\cdot10^{-3}M$	G37	$P_{III}6(84)$	6 row awned	$1\cdot10^{-2}M$
G16	$P_{II}3(78)$	6 row awned	$2\cdot10^{-3}M$	G38	$P_{IV}19(86)$	6 row awned	$1\cdot10^{-2}M$
G17	$P_{II}15(88)$	6 row hooded	$2\cdot10^{-3}M$	G39	$P_{1}14(108)$	6 row hooded	$1\cdot10^{-2}M$
G18	$P_{II}15(89)$	6 row hooded	$2\cdot10^{-3}M$	G40	$P_{II}11(117)$	2 row awned	$1\cdot10^{-2}M$
G19	$P_{1}17(12)$	6 row awned	$1\cdot10^{-2}M$	C1	Zernogradsky 813	2 row awned	control 1
G20	$P_{II}5(15)$	6 row awned	$1\cdot10^{-2}M$	C2	Dz02-129	6 row awned	control 2
G21	$P_{III}8(23)$	6 row hooded	$1\cdot10^{-2}M$	C3	C.I.10995	6 row hooded	control 3
G22	$P_{IV}19(42)$	6 row awned	$1\cdot10^{-2}M$				

variety, differing spikes and awn coloration, highlighted plant), obtained by mutagen phosphemide seed treatment of the initial barley samples Zernogradsky 813, Russia, var. *erectum* (control 1); Dz02-129, Ethiopia, var. *nigripallidum* (control 2); C.I.10995, Peru, var. *sinicum* (control 3), with aqueous solution of mutagen phosphemide in two concentrations. The methods of seed treatment and obtaining mutants of the first generations had descriptions in a previous publication (Tetyannikov and Bome, 2022). The original samples served as a control.

Field experiment

The study happened in 2020–2022 in two geographical areas: Location 1 – on the experimental field of FSBSO Federal Horticultural Center for Breeding, Agrotechnology and Nursery (Moscow region) on sod-podzolic medium loamy soils (55.13552″N, 37.95932″E) and Location 2 – at the experimental site of the biostation “Lake Kuchak” of the University of Tyumen (Tyumen region) on sod-podzolic sandy loam soils (57.351684″N, 66.058883″E) (Figure 1). The field experiments, biometric surveys, and phenological observations of the barley plants applied the method according to Dospekhov (2011).

Statistical analyses

Statistical compilation of experimental data employed the analysis of variance (ANOVA) and correlation analysis. Calculating the index of environmental conditions (*Ij*) employed the technique of Eberhart and Russell (1966). Ranking and evaluation of yield stability proceeded using parametric: regression coefficient (b_i; Finlay and Wilkinson, 1963), deviation from regression (S^2d_i; Eberhart and Russell, 1966), Plaisted and Peterson's mean-variance component (θ_i; Plaisted and Peterson, 1959), Plaisted's GE variance component ($\theta_{(i)}$; Plaisted, 1960), Wricke's ecovalence stability index (W_i^2, Wricke, 1962), Shukla's stability variance (σ_i^2, Shukla, 1972), coefficient of variance (*CV*, Francis and Kannenberg, 1978), and non-parametric statistics, i.e., Nassar and Huhn statistics ($S^{(1)}$, $S^{(2)}$), Huhn's equation

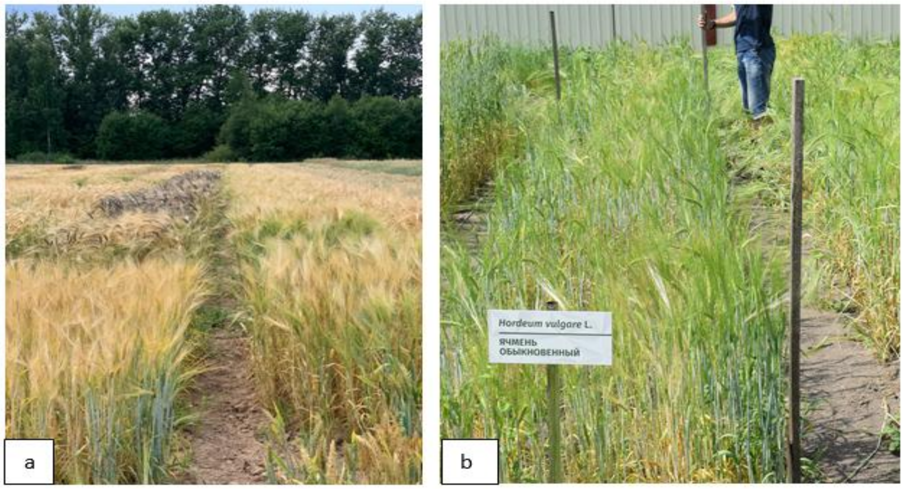

Figure 1. General view of mutants M7 in different areas: a) Moscow region; b) Tyumen region.

($S^{(3)}$, $S^{(6)}$, Nassar and Huhn, 1987; Huhn 1990), Thennarasu's non-parametric statistics ($NP^{(1\text{-}4)}$, Thennarasu, 1995), Kang's rank-sum (*KR*) (Kang, 1988), using the online-program STABILITYSOFT (Pour-Abaoughadareh *et al*., 2019).

RESULTS AND DISCUSSION

Climatic conditions of vegetation periods in the years of study (2020 and 2022) were contrasting and differed in temperature and moisture availability. It was apparent that the growing seasons of 2020 and 2021 were less favorable for barley plants' growth and development and yield formation in the South Moscow region, as also confirmed by the negative values of the index of environmental conditions (*Ij*). However, the most favorable ecological conditions occurred in 2022 for plant growth. A similar trend ensued in the settings of the Tyumen region (Table 2).

The ANOVA determined the estimation of the significance and percent influence of various factors in the barley grain yield formation. In the pertinent study, the first factor, 'genotype,' means the genotypic features of the mutants, the second factor, 'environment,' reflects hydrothermal characteristics (average daily air temperature, amount of precipitation) of vegetation periods, and the third factor, 'location,' showed the areas of the study. The highest contribution to the grain yield formation came from the environment (46.6%). Also, significant impacts ($P < 0.05$) emerged from the environment by location interaction (26.2%), genotype by environment interaction (9.5%), genotype (9.1%), and location (0.7%) (Table 3). The trait different degrees of variations in diverse genotypes can be observed in response to environmental variations (Oral *et al*., 2019; Roostaei *et al*., 2022).

According to locations used for the study of barley mutants, significant differences were evident for the obtained grain yield. The barley mutants grown under the environmental conditions of Moscow region, Russia, had a greater variability of grain yield over years, as evidenced by the highest values of the coefficient of variation (CV = 71.01% to 152.71%), compared with barley mutants grown under the climatic conditions of the Tyumen region, Russia (CV = 5.33% to 78.76%). In the Moscow region, Russia, the highest average grain yields sprang from the mutants, i.e., G1 (317.9 g/m^2), G2 (282.0 g/m^2), exceeding the control by 4.0%-17.3%, as well as mutants derived from the barley sample C.I.10995 (Table 4). On average, the grain yield excess observed ranged from 1.9% (G24) to 101.2% (G23), except the mutants G17 and G18 which were lower than the

Table 2. Climatic conditions in the years of research.

Months	$\bar{x}$ (°C)	Σ (mm)	$\bar{x}$ (°C)	Σ (mm)	$\bar{x}$ (°C)	Σ (mm)	$\bar{x}$ (°C)	Σ (mm)
	Moscow region							
	n (1968–2021)		2020 (E1)		2021 (E2)		2022 (E3)	
May	13.2	42.2	11.4	135.4	13.6	90.4	10.2	62.5
June	17.2	60.8	18.3	159.7	19.7	85.5	18.2	28.7
July	19.2	70.1	18.6	106.8	21.6	51.0	20.3	48.6
August	17.4	57.1	16.9	23.8	19.5	66.0	21.3	15.9
$\bar{x}$ (°C)	16.8		16.3		18.6		17.5	
Σ (mm)		230.2		425.7		292.9		155.7
Ij			-11.45		-13.97		25.43	
Months	Tyumen region							
	n (1968–2021)		2020 (E4)		2021 (E5)		2022 (E6)	
May	11.3	45.3	14.9	50.1	17.6	4.6	12.1	93.9
June	17.1	58.5	14.6	66.8	18.0	22.9	15.8	59.4
July	18.8	86.0	21.5	18.5	18.6	49.6	19.7	65.5
August	15.8	60.0	18.3	54.3	19.5	20.0	18.1	56.0
$\bar{x}$ (°C)	15.8		17.3		18.4		16.4	
Σ (mm)		249.8		189.7		97.1		274.8
Ij			-4.97		0.46		4.51	

Note: n – long-term average (according to state funds of the Hydrometeorological Center); $\bar{x}$ – average daily temperature; Σ – sum of precipitation.

Table 3. Contribution of factors to barley grain yield.

Source	d.f.	MS	F	Contribution to variation (%)
Genotypes (Gen.)	39	13644.96	3.17*	9.1
Environments (Env.)	2	1366263.68	317.50*	46.6
Locations (Loc.)	1	39428.68	9.16*	0.7
Gen. × Env.	78	7138.78	1.66*	9.5
Gen. × Loc.	39	3329.37	0.77	2.2
Env. × Loc.	2	767798.20	178.42*	26.2
Error	78	4303.24	-	5.7

Note: d.f. – degrees of freedom; MS – mean squares; * $P < 0.05$.

control treatments. In the environmental conditions of the Tyumen region, a similar trend also manifested in the studied barley mutants. The highest value of grain yield came from mutant G2 (274.4 g/m^2), whose yield was 23.5% higher than the control treatment, as well as all the awned mutants derived from the hooded cultivar (5.7% to 230.9%). It is worth mentioning that in the two different ecological areas, the mutants observed with changed spikelet coloration obtained from the basic sample Dz02-129 were also lower by 17.3%-43.7% in grain yield than the initial sample. Yield variation seemed to manifest the genotype by environment interaction, which can be reduced by the association between phenotypic and genotypic traits in barley genotypes (Vaezi *et al*., 2019).

Applying the GGE biplot analysis based on the principal component analysis (PCA) helped visualize the effects of genotype by environment interaction (GEI) and graphically demonstrated the distribution of barley genotypes and test environments in principal component space (Yan and Kang, 2003; Kendal *et al*., 2019). The barley mutants also differed in average grain yield, with the two mutants, G28 and G30, having the maximum values, with the minimum average grain yield characterized by the mutant G18. In the relevant study, the first two components explained 41.04% and 20.36% of the total yield variability caused by the genotype by environment interaction (Figure 2). All the test environments on GGE biplot had long vectors, detecting variations in the response of genotypes and the discriminating power of these environments was quite high. Environments E1 and E4 had the longest vectors and the GEI made the highest contribution. The mutant genotypes G1, G2, G19, G22, G26, and G2 positively correlated with these test environments. Using such environments was difficult for selecting the

Table 4. Grain yield of barley mutants grown under different environmental conditions (g/m^2).

Mutant genotypes	Moscow region					Tyumen region				
	min	max	$\bar{x}$	CV (%)	Control (%)	min	max	$\bar{x}$	CV (%)	Control (%)
G1	57.3	454.1	317.9	71.01	117.3	208.8	231.2	218.2	5.33	98.2
G2	45.8	407.8	282.0	72.58	104.0	222.8	354.6	274.4	25.65	123.5
G3	53.2	379.1	192.4	87.32	78.9	211.5	263.1	231.2	12.07	82.7
G4	31.2	454.6	176.9	135.96	72.6	119.8	280.2	187.1	44.49	66.9
G5	27.8	273.7	137.2	91.26	56.3	89.68	361.3	191.4	77.37	68.4
G6	22.5	374.1	142.4	140.88	58.4	196.0	230.1	213.8	7.99	76.4
G7	57.5	318.1	163.5	83.72	67.1	185.9	282.0	221.3	23.87	79.1
G8	80.1	364.6	198.2	74.78	81.3	52.0	286.0	175.2	67.07	62.6
G9	76.3	380.6	201.5	79.00	82.7	163.3	242.4	190.4	23.68	68.1
G10	60.0	395.9	176.6	107.61	72.5	123.3	155.0	138.2	11.54	49.4
G11	15.0	380.7	144.3	142.13	102.7	61.6	214.1	122.8	65.65	130.4
G12	10.0	484.2	176.5	151.10	125.6	108.3	341.3	192.8	66.92	204.7
G13	15.0	468.4	169.7	152.42	120.8	197.0	385.4	278.6	34.71	295.8
G14	15.0	412.1	149.1	152.71	106.1	85.2	216.1	146.8	44.82	155.8
G15	29.7	513.0	191.7	145.11	136.4	120.0	272.8	171.9	50.78	182.5
G16	40.0	648.1	273.1	120.08	194.4	145.0	351.5	236.7	44.43	251.3
G17	15.0	307.4	125.2	127.00	89.1	84.8	182.2	130.2	37.68	138.2
G18	29.8	190.6	90.9	95.67	64.7	19.3	176.0	99.6	78.76	105.7
G19	91.1	428.9	222.5	81.32	158.4	171.6	299.9	224.3	29.92	238.1
G20	130.7	442.7	236.1	75.76	168.0	206.7	298.9	255.6	18.13	271.3
G21	8.9	441.3	210.1	103.66	149.5	123.4	236.8	187.7	31.01	199.3
G22	28.0	558.8	233.9	121.69	166.5	204.7	297.5	258.9	18.66	274.8
G23	47.8	675.4	282.7	121.05	201.2	199.5	320.5	275.9	24.08	292.9
G24	0.0	346.9	143.1	126.61	101.9	189.6	296.1	254.7	22.40	270.4
G25	22.2	559.4	217.2	136.88	154.6	178.9	478.9	290.8	56.37	308.7
G26	44.8	571.3	250.4	112.46	178.2	287.6	337.1	311.7	7.95	330.9
G27	80.0	581.7	247.3	117.13	176.0	224.3	284.8	256.0	11.86	271.8
G28	62.6	570.2	245.1	115.15	174.4	287.1	373.9	330.7	13.12	351.1
G29	82.5	541.9	237.4	111.11	169.0	178.3	405.5	296.1	38.44	314.3
G30	40.0	612.2	238.4	135.87	169.7	201.2	476.1	340.9	40.32	361.9
G31	25.0	483.5	192.9	130.95	137.3	158.5	316.7	257.1	33.45	272.9
G32	32.5	557.1	223.5	129.74	159.1	180.7	268.0	229.1	19.39	243.2
G33	35.0	380.1	162.3	116.80	115.5	198.6	288.0	239.8	18.81	254.6
G34	67.5	569.4	241.2	117.90	171.7	187.4	355.0	273.2	30.70	290.0
G35	27.5	409.5	165.3	128.30	117.7	230.6	320.2	281.1	16.31	298.4
G36	27.5	623.2	247.5	132.12	176.2	154.0	416.6	262.6	52.18	278.8
G37	25.0	513.3	212.7	123.65	151.4	198.1	304.6	267.3	22.45	283.8
G38	25.0	586.2	230.9	133.79	164.3	201.0	295.7	254.8	19.10	270.5
G39	15.0	467.5	186.2	131.89	132.5	93.4	259.8	174.6	47.70	185.4
G40	25.0	414.0	173.1	121.54	123.2	103.2	240.9	190.3	43.73	202.0
Control (C1)	45.7	447.6	271.1	75.75	-	168.5	265.2	222.1	22.15	-
Control (C2)	57.8	511.5	243.7	97.50	-	290.5	267.0	279.7	4.23	-
Control (C3)	23.1	371.0	140.5	142.14	-	38.1	186.5	94.2	85.51	-

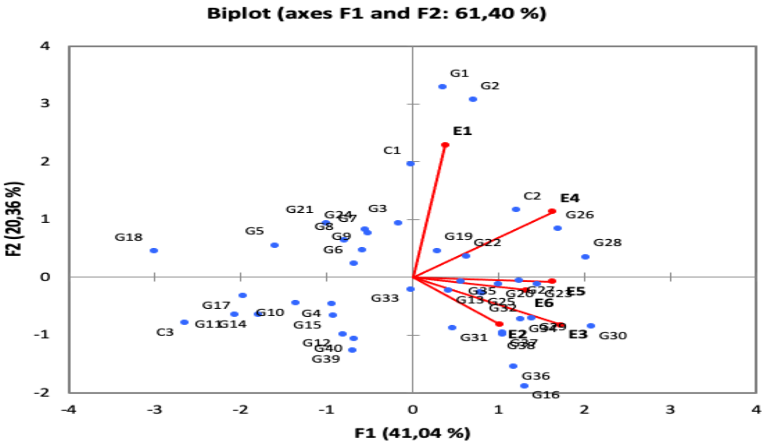

Figure 2. The grain yield variation of investigated 40 barley mutants under diverse environmental conditions during 2020–2022.

best genotypes; however, it can help identify the unstable mutants. Environments E5 and E6 had a smaller angle to the biplot mid-axis, indicating these environments gave better representation, interacting positively with the five other mutants, i.e., G23, G25, G26, G27, and G35.

For stability analysis, the most commonly used parametric statistical method is the regression coefficient characterizing plasticity (b_i) and deviation from regression (S^2d_i), reflecting the change in the dependent variable or stability (Pour-Aboughadareh *et al*., 2023). Allegedly, genotypes with value $b > 1.0$ are more responsive to high-yielding environments, while those with $b < 1.0$, are adaptable to low-yielding environments, and genotypes with a value close to unity are considered stable (Guendouz and Bendada, 2022). Values with minimum deviation from regression ($S^2d_i = 0$) are characterized as more stable genotypes (Yadav *et al.,* 2019). In the obtained values, the limits of variation were as follows: $b_i = 0.35$ (G18) to 1.51 (G23) and $S^2d_i = 0.65$ (G20) and 126.83 (G1). The majority of the mutants characteristically had high responsiveness ($b_i > 1.0$) to environmental changes. i.e., G12, G13, G15, G16, G22, G23, G25, G26, G27, G28, G29, G30, G31, G32, G34, G36, G37, and G38 (b_i = 1.15–1.51), among which the mutant G22, G23, and G28 had S^2d_i = 1.81–7.80. The barley genotypes belonging to the neutral type of group were found less responsive to environmental variations ($b_i < 1.0$), and included 15 mutants. High ecological plasticity, wherein the grain yield of a mutant genotype corresponds to variations in environmental factors (b_i = 0.90–1.09), was evident in seven mutants, namely, G4, G11, G14, G21, G35, G39, and G40. In barley, the resulting products and resistance to biotic and abiotic stress factors (Kumar *et al*., 2020), grain yield has become one of the vital indicators (Friedt and Ordon, 2013).

One parameter of stability is the mean-variance component (θ_i) allowing the average of the estimate for all combinations with a common genotype to be a measure of stability. Genotypes with lower values of θ_i become more stable, whereas according to $\theta_{(i)}$, a variety that shows higher values is considerably more stable (Pour-Abaoughadareh *et al*., 2019). In calculating the mean-variance components (θ_i), the mutants G4 ($\theta_i = 32.03$), G14 ($\theta_i = 29.67$), and G20 ($\theta_i = 31.24$) emerged more stable. On the variance component of GEI ($\theta_{(i)}$), the barley mutants, G4, G14, and G20 exhibited the higher stability. Barley mutants G1 and G2 were notably the most unstable as per the considered indicators of the dispersion components. Wricke's ecovalence stability index (W_i^2) serves as one of the indices evaluating the contribution of each genotype to the sum of squares of GEI, and the lower the value obtained, the more stable the genotypes are (Ferreira *et al.* 2015). Barley mutants G4 (W_i^2 = 52.41), G14 (W_i^2 = 29.41), and G20 (W_i^2 = 44.72) also stood out by this parameter. Among the mutants, G1 signified as unstable, which had the highest value W_i^2 = 1132.30. Shukla's stability model is better for estimating yield stability, where genotypes with minimum values are markedly stable (Bantayehu, 2009). According to stability variance (σ_i^2), six-row mutants G4 (σ_i^2 = 9.72), G14 (σ_i^2 = 4.90), and G20 (σ_i^2 = 8.11) had the lowest values indicating they are stable. Two-rowed mutants of variety Zernogradsky 813 had the highest values, characterizing them as unstable (Table 5a, Table 5b).

Using nonparametric statistical methods can be an additional tool in evaluating the grain yield stability of genotypes under different environmental conditions (Vaezi *et al.,* 2022). Among nonparametric methods of stability, the ranked parameters proposed by Nassar and Huhn statistics ($S^{(1)}, S^{(2)}$) and Huhn equation ($S^{(3)}, S^{(6)}$) look at mean values of absolute rank differences, variance between ranks in the tested environments, sums of absolute deviations, and sums of squares of ranks for each genotype relative to the mean. The calculated $S^{(1)}, S^{(2)}$ statistics showed that mutant genotypes G4, G11, G14, G17, G20, and G28 identified to be the most stable. The statistical parameters $S^{(3)}$ and $S^{(6)}$ highlighted the genotypes G20 and G28 with minimum values indicating same grain yield ranks of these mutants in different environments. Alternative methods include nonparametric statistics ($NP^{(1\text{-}4)}$) based on ranks of adjusted mean genotype values in each environment. On the $NP^{(1)}$ statistic, the genotypes G20 and G14 had lower values (4.83 and 5.50, respectively). The mutant genotypes G20, G22, G28, G17, and G18 occurred more stable under $NP^{(2)}$, $NP^{(3)}$, and $NP^{(4)}$. Rank-sum (*KR*) uses grain yield and σ_i^2 values as selection criteria. However, the genotypes G20, G22, G26, and G28 had the lowest *KR* values. The obtained data indicate greater stability of grain yield formation of these mutants.

Based on the used indicators, variations were visible in the ranks of the studied barley mutants. Of all the mutants, a lower sum of ranks (SR) and average sum of ranks (ASR), combined with relatively high average grain yield, characterize the six-row awned three mutants, i.e., G20, G22, and G28. Two-rowed mutants, i.e., G1, G2, and G40, were less stable; however, they had higher grain yield than the control treatment. Among the mutant genotypes with altered spikelet coloration, genotype G3 showed relatively high (192.4 g/m^2) and stable yields in all environments.

The correlation analysis revealed strong positive relationships among the parameters, W_i^2 and σ^2_i, θ_i, S^2d_i; θ_i and σ^2_i, S^2d_i; $NP^{(4)}$ and $S^{(3)}$, $S^{(6)}$; $S^{(1)}$ and $S^{(2)}$; $S^{(2)}$ and $S^{(3)}$; *KR*; $NP^{(2)}$, and $NP^{(3)}$ (r = 0.80–1.00). The parameters $\theta_{(i)}$ and W_i^2, σ^2_i, S^2d_i; θ_i and $\theta_{(i)}$ indicated a negative correlation (r = - 0.92–1.00) with each other. Most of the parameters showed weak correlations with grain yield, and the highest values were evident with the b_i (r = 0.52), $S^{(6)}$ (r = - 0.77), $NP^{(2)}$ (r = - 0.78), $NP^{(3)}$ (r = - 0.79), $NP^{(4)}$ (r = - 0.78), and *KR* (r = - 0.65) (Figure 3). Therefore, the established relationship between parametric and nonparametric statistics requires accounting for evaluating and selecting adaptive, stable, and high-yielding genotypes (Lodhi *et al*., 2015; Sabra *et al.*, 2023).

CONCLUSIONS

The study of barley in diverse ecological areas made it possible to assess the stability of grain yield formation and observe the differences in the response of genotypes to environmental factors. Significant effects of environments (46.6%), genotypes (9.1%), interactions of environment by location (26.2%), and genotype by environment (9.5%) were evident in the total variability of grain yield. The stability indices helped evaluate the response of mutant genotypes to environmental conditions in relatively favorable and stressful years. The observed weak correlation of yield

Table 5a. Mean yield, parametric and nonparametric statistics of stability and their ranks (in parentheses) for 40 barley mutants and three control genotypes during 2020–2022.

Mutant genotypes	GY	W_i^2	σ^2_i	S^2d_i	b_i	*CV*	$\theta_{(i)}$	θ_i	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	$NP^{(1)}$	$NP^{(2)}$	$NP^{(3)}$	$NP^{(4)}$	*KR*	SR	ASR	SD
G1	268.1 (6)	1132.30 (43)	236.24 (43)	126.83 (43)	0.52 (2)	57.11 (8)	47.67 (43)	142.59 (1)	15.60 (35)	165.87 (34)	33.62 (30)	2.49 (21)	10.00 (24)	0.71 (32)	0.56 (23)	0.63 (24)	49 (28)	440	25.88	14.41
G2	278.2 (5)	824.06 (42)	171.58 (42)	87.73 (42)	0.55 (3)	49.24 (2)	49.21 (42)	111.03 (2)	14.73 (33)	155.37 (31)	27.58 (22)	2.38 (20)	14.33 (41)	0.43 (18)	0.54 (21)	0.52 (18)	47 (26)	410	24.12	14.98
G3	211.8 (24)	112.02 (10)	22.23 (10)	5.21 (5)	0.73 (10)	51.84 (4)	52.76 (10)	38.13 (34)	11.73 (20)	100.27 (22)	22.12 (20)	2.09 (17)	7.67 (10)	0.38 (11)	0.43 (13)	0.52 (17)	34 (10)	247	14.53	7.65
G4	182.0 (32)	52.41 (3)	9.72 (3)	6.27 (7)	1.09 (25)	88.51 (37)	53.06 (3)	32.03 (41)	8.67 (6)	51.60 (6)	16.13 (10)	2.38 (19)	5.50 (3)	0.46 (20)	0.47 (15)	0.54 (19)	35 (11)	260	15.29	12.37
G5	164.3 (37)	541.93 (39)	112.40 (39)	55.68 (41)	0.62 (5)	76.81 (24)	50.61 (39)	82.14 (5)	17.53 (41)	230.17 (43)	75.88 (43)	4.97 (43)	13.50 (38)	1.84 (38)	1.02 (39)	1.16 (43)	76 (42)	599	35.24	12.20
G6	178.1 (36)	93.99 (7)	18.45 (7)	11.10 (12)	0.88 (16)	74.81 (22)	52.85 (7)	36.28 (37)	10.67 (16)	106.67 (23)	36.36 (35)	2.77 (27)	6.00 (6)	1.00 (36)	0.61 (29)	0.73 (29)	43 (18)	363	21.35	11.24
G7	192.4 (29)	273.67 (30)	56.13 (30)	14.73 (20)	0.60 (4)	50.96 (3)	51.95 (30)	54.68 (14)	13.20 (28)	124.67 (29)	32.24 (27)	2.86 (29)	11.17 (33)	0.51 (23)	0.71 (32)	0.68 (27)	59 (38)	426	25.06	9.67
G8	186.7 (30)	425.92 (35)	88.07 (35)	41.41 (37)	0.64 (6)	64.43 (10)	51.19 (35)	70.27 (9)	17.80 (42)	212.17 (41)	50.92 (39)	3.33 (34)	11.67 (34)	0.65 (31)	0.75 (33)	0.85 (35)	65 (40)	526	30.94	11.29
G9	195.9 (28)	138.12 (17)	27.70 (17)	5.67 (6)	0.69 (8)	53.49 (5)	52.63 (17)	40.80 (27)	12.67 (25)	115.07 (26)	28.30 (24)	2.59 (23)	9.33 (18)	0.58 (29)	0.50 (19)	0.62 (23)	45 (20)	332	19.53	7.43
G10	157.4 (38)	108.40 (8)	21.47 (8)	9.67 (9)	0.80 (12)	77.80 (25)	52.78 (8)	37.76 (36)	11.80 (22)	93.77 (18)	33.09 (29)	3.20 (33)	9.50 (20)	0.83 (35)	0.77 (35)	0.83 (34)	46 (23)	393	23.12	11.10
G11	133.5 (40)	129.80 (14)	25.96 (14)	17.08 (23)	0.90 (18)	104.74 (42)	52.67 (14)	39.95 (30)	6.27 (2)	27.87 (2)	19.00 (15)	3.82 (37)	8.50 (12)	2.56 (39)	1.52 (41)	0.85 (36)	54 (34)	413	24.29	13.86
G12	184.7 (31)	235.88 (29)	48.21 (29)	26.28 (32)	1.22 (31)	101.60 (41)	52.14 (29)	50.81 (15)	15.93 (36)	177.77 (37)	59.92 (42)	4.36 (41)	11.00 (32)	1.22 (37)	0.82 (36)	1.07 (42)	60 (39)	579	34.06	6.80
G13	224.2 (21)	296.18 (32)	60.86 (32)	38.13 (36)	1.17 (28)	82.34 (32)	51.84 (32)	56.98 (12)	17.80 (42)	217.10 (42)	55.67 (41)	3.44 (36)	9.67 (21)	0.53 (26)	0.61 (30)	0.91 (37)	53 (33)	533	31.35	8.02
G14	148.0 (39)	29.41 (1)	4.90 (1)	4.15 (3)	1.02 (23)	101.33 (39)	53.17 (1)	29.67 (43)	6.80 (3)	32.80 (3)	23.43 (21)	4.00 (38)	4.83 (1)	3.18 (42)	0.85 (37)	0.97 (38)	40 (15)	348	20.47	17.70
G15	181.9 (33)	163.91 (21)	33.11 (21)	15.39 (22)	1.23 (33)	101.60 (40)	52.50 (21)	43.44 (23)	11.00 (18)	83.37 (15)	28.10 (23)	2.90 (32)	8.83 (16)	0.78 (33)	0.77 (34)	0.74 (30)	54 (34)	449	26.41	7.56
G16	254.9 (11)	416.82 (34)	86.16 (34)	30.94 (34)	1.44 (40)	85.81 (34)	51.24 (34)	69.33 (10)	16.33 (38)	193.37 (39)	34.33 (33)	2.66 (25)	15.83 (42)	0.45 (19)	0.58 (25)	0.58 (21)	45 (20)	493	29.00	9.92
G17	127.7 (41)	228.05 (28)	46.57 (28)	14.56 (19)	0.65 (7)	82.44 (33)	52.18 (28)	50.01 (16)	8.47 (5)	51.50 (5)	30.29 (25)	4.35 (40)	10.00 (24)	2.67 (40)	1.52 (40)	1.00 (39)	69 (41)	459	27.00	12.86
G18	95.3 (43)	601.60 (41)	124.92 (41)	21.36 (28)	0.35 (1)	77.93 (26)	50.32 (41)	88.25 (3)	8.93 (7)	56.67 (7)	34.00 (32)	4.08 (39)	13.83 (39)	3.58 (43)	2.18 (42)	1.07 (41)	84 (43)	517	30.41	15.70
G19	223.4 (22)	188.43 (26)	38.25 (26)	18.49 (26)	0.76 (11)	54.64 (6)	52.38 (26)	45.95 (18)	15.13 (34)	170.97 (36)	31.47 (26)	2.31 (18)	8.17 (11)	0.30 (4)	0.41 (11)	0.56 (20)	48 (27)	348	20.47	9.30
G20	245.9 (16)	44.72 (2)	8.11 (2)	0.65 (1)	0.81 (13)	47.74 (1)	53.10 (2)	31.24 (42)	9.07 (9)	57.07 (8)	9.30 (3)	1.07 (2)	5.00 (2)	0.26 (3)	0.21 (1)	0.30 (2)	18 (4)	113	6.65	10.14
G21	198.9 (27)	139.16 (18)	27.92 (18)	18.89 (27)	0.92 (19)	71.94 (17)	52.63 (18)	40.91 (26)	14.20 (31)	158.57 (32)	46.18 (38)	2.87 (30)	5.83 (4)	0.54 (27)	0.56 (22)	0.83 (33)	45 (20)	407	23.94	8.14
G22	246.4 (15)	81.61 (5)	15.85 (5)	1.81 (2)	1.26 (35)	74.32 (21)	52.91 (5)	35.02 (39)	9.33 (10)	87.87 (17)	16.48 (11)	1.40 (8)	5.83 (4)	0.31 (6)	0.31 (3)	0.35 (8)	20 (5)	199	11.71	10.86
G23	279.3 (4)	305.94 (33)	62.90 (33)	4.98 (4)	1.51 (43)	78.96 (28)	51.79 (33)	57.98 (11)	10.07 (13)	70.57 (11)	11.08 (5)	1.11 (3)	9.83 (23)	0.40 (16)	0.40 (10)	0.32 (5)	37 (12)	287	16.88	12.71

Table 5b. Mean yield, parametric and nonparametric statistics of stability and their ranks (in parentheses) for 40 barley mutants and three control genotypes during 2020–2022.

Mutant genotypes	GY	W_i^2	σ^2_i	S^2d_i	b_i	CV	$\theta_{(i)}$	θ_i	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	$NP^{(1)}$	$NP^{(2)}$	$NP^{(3)}$	$NP^{(4)}$	*KR*	SR	ASR	SD
G24	198.9	180.49	36.59	22.16	0.84	67.77	52.42	45.14	14.13	153.07	41.00	3.36	9.67	0.41	0.63	0.76	51	444	26.12	6.78
	(26)	(25)	(25)	(30)	(15)	(15)	(25)	(19)	(30)	(30)	(37)	(35)	(21)	(17)	(31)	(32)	(31)			
G25	254.0	474.45	98.25	42.98	1.41	86.02	50.95	75.24	16.87	187.90	39.98	2.60	10.67	0.63	0.60	0.72	50	526	30.94	9.66
	(12)	(38)	(38)	(38)	(39)	(35)	(38)	(6)	(40)	(38)	(36)	(24)	(29)	(30)	(28)	(28)	(29)			
G26	281.0	124.48	24.84	10.18	1.22	64.73	52.70	39.41	10.67	93.87	14.08	1.14	10.00	0.31	0.36	0.32	15	216	12.71	9.25
	(3)	(12)	(12)	(10)	(32)	(11)	(12)	(32)	(16)	(19)	(9)	(4)	(24)	(7)	(5)	(6)	(2)			
G27	251.6	179.18	36.31	18.37	1.22	73.22	52.43	45.00	11.73	99.07	16.89	1.48	10.67	0.39	0.40	0.40	37	315	18.53	6.93
	(13)	(24)	(24)	(25)	(30)	(19)	(24)	(20)	(20)	(21)	(13)	(9)	(29)	(12)	(9)	(11)	(12)			
G28	287.9	128.10	25.60	7.80	1.26	64.81	52.68	39.78	7.93	45.37	6.51	0.96	8.50	0.21	0.31	0.23	15	157	9.24	10.57
	(2)	(13)	(13)	(8)	(37)	(12)	(13)	(31)	(4)	(4)	(1)	(1)	(12)	(1)	(2)	(1)	(2)			
G29	266.8	153.28	30.88	13.67	1.23	69.17	52.56	42.35	10.00	66.27	10.46	1.20	10.50	0.36	0.37	0.32	26	240	14.12	8.97
	(7)	(19)	(19)	(16)	(34)	(16)	(19)	(25)	(12)	(9)	(4)	(5)	(28)	(10)	(7)	(4)	(6)			
G30	289.7	566.68	117.59	46.30	1.48	79.24	50.49	84.68	11.53	87.37	13.58	1.40	15.83	0.52	0.52	0.36	41	396	23.29	14.74
	(1)	(40)	(40)	(39)	(41)	(29)	(40)	(4)	(19)	(16)	(8)	(7)	(42)	(24)	(20)	(9)	(17)			
G31	225.0	122.77	24.48	14.33	1.15	76.63	52.71	39.23	11.80	97.37	20.43	1.90	8.50	0.30	0.44	0.50	31	280	16.47	7.04
	(20)	(11)	(11)	(18)	(26)	(23)	(11)	(33)	(22)	(20)	(17)	(14)	(12)	(5)	(14)	(15)	(8)			
G32	226.3	280.15	57.49	35.00	1.18	81.99	51.92	55.34	16.00	168.80	33.76	2.56	12.33	0.40	0.57	0.64	50	474	27.88	7.37
	(19)	(31)	(31)	(35)	(29)	(31)	(31)	(13)	(37)	(35)	(31)	(22)	(37)	(14)	(24)	(25)	(29)			
G33	201.0	133.69	26.77	15.19	0.84	64.83	52.65	40.35	10.27	71.47	16.75	2.06	10.00	0.33	0.49	0.48	40	279	16.41	5.35
	(25)	(15)	(15)	(21)	(14)	(13)	(15)	(29)	(14)	(12)	(12)	(16)	(24)	(8)	(17)	(14)	(15)			
G34	257.2	168.64	34.10	13.89	1.26	73.23	52.48	43.93	10.27	74.80	12.47	1.53	9.33	0.53	0.40	0.34	31	280	16.47	8.02
	(9)	(22)	(22)	(17)	(36)	(20)	(22)	(22)	(14)	(13)	(7)	(10)	(18)	(25)	(8)	(7)	(8)			
G35	223.2	172.56	34.93	24.41	0.96	67.74	52.46	44.33	12.73	107.50	21.08	1.92	11.83	0.33	0.49	0.50	46	362	21.29	6.35
	(23)	(23)	(23)	(31)	(21)	(14)	(23)	(21)	(26)	(24)	(18)	(15)	(35)	(8)	(18)	(16)	(23)			
G36	255.1	447.92	92.68	29.23	1.48	87.97	51.08	72.52	16.33	200.57	35.60	2.73	14.17	0.49	0.58	0.58	46	506	29.76	10.32
	(10)	(36)	(36)	(33)	(42)	(36)	(36)	(8)	(38)	(40)	(34)	(26)	(40)	(21)	(26)	(21)	(23)			
G37	240.0	110.40	21.89	12.00	1.18	72.17	52.77	37.96	12.20	115.50	20.26	1.54	6.33	0.26	0.32	0.43	27	249	14.65	9.14
	(18)	(9)	(9)	(14)	(27)	(18)	(9)	(35)	(24)	(27)	(16)	(11)	(7)	(2)	(4)	(12)	(7)			
G38	242.9	199.79	40.64	11.51	1.36	81.62	52.32	47.12	13.20	122.27	22.10	1.83	8.67	0.39	0.42	0.48	44	356	20.94	7.86
	(17)	(27)	(27)	(13)	(38)	(30)	(27)	(17)	(28)	(28)	(19)	(13)	(15)	(13)	(12)	(13)	(19)			
G39	180.4	158.56	31.99	22.06	1.09	90.98	52.53	42.89	14.67	159.47	54.36	4.64	12.00	0.81	0.87	1.00	55	541	31.82	7.56
	(35)	(20)	(20)	(29)	(24)	(38)	(20)	(24)	(32)	(33)	(40)	(42)	(36)	(34)	(38)	(40)	(36)			
G40	181.7	76.36	14.75	10.36	0.95	78.02	52.94	34.48	12.73	109.77	32.60	2.79	7.50	0.56	0.59	0.76	38	360	21.18	11.32
	(34)	(4)	(4)	(11)	(20)	(27)	(4)	(40)	(26)	(25)	(28)	(28)	(9)	(28)	(27)	(31)	(14)			
C1	246.6	467.19	96.73	54.76	0.72	55.24	50.99	74.49	9.00	82.97	18.17	1.59	7.00	0.50	0.49	0.39	51	323	19.00	12.27
	(14)	(37)	(37)	(40)	(9)	(7)	(37)	(7)	(8)	(14)	(14)	(12)	(8)	(22)	(16)	(10)	(31)			
C2	261.7	85.02	16.56	12.14	1.02	58.00	52.90	35.36	9.60	68.27	11.13	1.35	9.17	0.40	0.36	0.31	14	184	10.82	8.80
	(8)	(6)	(6)	(15)	(22)	(9)	(6)	(38)	(11)	(10)	(6)	(6)	(17)	(14)	(6)	(3)	(1)			
C3	117.3	136.76	27.42	17.69	0.91	118.04	52.64	40.66	3.60	9.47	8.88	2.88	10.83	3.10	2.39	0.68	58	415	24.41	14.58
	(42)	(16)	(16)	(24)	(17)	(43)	(16)	(28)	(1)	(1)	(2)	(31)	(31)	(41)	(43)	(26)	(37)			

Note: GY – grain yield (g/m^2); W_i^2 – Wricke's ecovalence stability index; σ^2_i – Shukla's stability variance; S^2d_i – deviation from regression; b_i – regression coefficient; *CV* – coefficient of variance; θ_i – Plaisted and Peterson's mean variance component; $\theta_{(i)}$ – Plaisted's GE variance component; $S^{(1)}$. $S^{(2)}$ – Nassar and Huhn's statistics; $S^{(4)}$, $S^{(6)}$ – Huhn's equation; $NP^{(1\text{-}4)}$ – Thennarasu's non-parametric statistics; *KR* – Kang's rank-sum; SR – sum of ranks, ASR – average sum of ranks; SD – standard deviation for the sum of ranks (on parametric and nonparametric statistics).

Figure 3. Coefficients of pair correlations among the barley mutant yields, parametric, and non-parametric stability parameters.

with $S^{(1)}$, $S^{(2)}$, $S^{(3)}$, $NP^{(1)}$, W_i^2, σ^2_i, S^2d_i, CV, θ_i, and $\theta_{(i)}$ suggests that these parameters can benefit in selecting stable barley genotypes without considering their grain yield. Highly correlated with yield were parameters b_i. The statistical evaluation further showed that stable yields were characteristics of mutants G20, G22, and G28 derived from the hooded cultivar. Genotypes G1, G2, and G40, belonging to the subspecies two-rowed barley, had the highest grain yield potential but lesser stability. These barley mutants exceeded the parental (initial) samples for the studied parameters. Further complex evaluation of other valuable morphological and physiological traits can contribute to isolating high-yielding, adaptive, and promising barley mutant lines.

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