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Assessing Genotype × Environment Interactions for Yield Stability and Grain Quality in Spring Barley Under Contrasting Agro-Ecological Conditions for Sustainable Agriculture

Ioana Crișan [1], Florin Russu [1], Raluca Rezi [2], Liliana Vasilescu [3,*], Eugen I. Petcu [3], Ana-Maria Vălean [4], Laura Șopterean [4] and Andreea D. Ona [5,*]

[1] Department of Spring Barley Breeding, Agricultural Research and Development Station Turda, 401100 Turda, Romania; ioana.crisan@scdaturda.ro (I.C.); florin.russu@scdaturda.ro (F.R.)
[2] Department of Soybean Breeding, Agricultural Research and Development Station Turda, 401100 Turda, Romania; raluca.rezi@scdaturda.ro
[3] Department of Breeding Small Cereals, National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Romania; eugen_petcu12@yahoo.com
[4] Department of Plant Protection, Agricultural Research and Development Station Turda, 401100 Turda, Romania; anamaria.valean@scdaturda.ro (A.-M.V.); laura.sopterean@scdaturda.ro (L.Ș.)
[5] Department of Plant Breeding, Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 400372 Cluj-Napoca, Romania
* Correspondence: vasilesculiliana09@gmail.com (L.V.); andreea.ona@usamvcluj.ro (A.D.O.)

Abstract

Increasing climate variability is placing growing pressure on cereal production systems, highlighting the need to identify stable and resilient genotypes for sustainable crop production. This study evaluated phenotypic variability, grain yield, yield stability and grain-quality traits in 25 spring barley (*Hordeum vulgare* subsp. *distichon* (L.) Körn.) genotypes, including varieties and advanced breeding lines, under contrasting agro-ecological conditions in Romania. Field experiments were conducted over two growing seasons (2024–2025) at Turda and Fundulea using a randomized complete block design with three replications. The evaluated agronomic, phenological, and quality traits included plant height, heading and flowering time, thousand-kernel weight, grain yield, and grain protein and starch contents. The effects of year, location, genotype and their interactions were analyzed, and GGE biplot analysis was used to examine genotype performance and stability across environments. Across the 300 plot-level observations, grain yield ranged from 1631 to 7730 kg ha^{-1}, with an overall mean of 5283 kg ha^{-1} and a descriptive CV of 21.99%, reflecting overall variability across the dataset rather than experimental error. Year and genotype significantly affected all evaluated traits, while location significantly affected all traits except plant height. For grain yield, protein content, starch content, thousand-kernel weight, heading time, and flowering time, all main effects and interactions were significant ($p < 0.001$). For plant height, the location effect was not significant, whereas year, genotype, and the interaction terms were significant. Mean grain yield ranged from 4149 kg ha^{-1} at Fundulea in 2024 to 6364 kg ha^{-1} at Turda in 2025. Grain yield was strongly negatively correlated with protein content ($r = -0.832$, $p < 0.001$) and positively correlated with thousand-kernel weight ($r = 0.767$, $p < 0.001$). Ioana had the highest grain yield in three of the four environments and the highest thousand-kernel weight in all four environments, while also showing early heading and flowering in three environments. In the AEC analysis, To 2112-21 showed a more stable yield response across the four tested environments, whereas Ioana was characterized primarily by high yield performance. These findings identify promising barley germplasm for further multi-environment evaluation and breeding

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for productive, adaptable spring barley under variable growing conditions in central and southeastern Romania.

Keywords: spring barley; yield stability; genotype × environment interaction; climate variability; grain quality; sustainable agriculture

1. Introduction

Increasing climate variability is placing growing pressure on agricultural systems, particularly cereal production, which must adapt to increasingly unstable environmental conditions. Spring barley (*Hordeum vulgare* subsp. *distichon* (L.) Körn.) is an important cereal crop with diverse applications in animal feed, human food, and industrial processing, particularly malting and brewing [1]. Compared with other major cereals, barley has a broad capacity to adapt to diverse growing conditions, including relatively poor soils and other environmental constraints, which contributes to its widespread cultivation [1]. Spring barley is cultivated under both intensive and marginal conditions, reflecting considerable environmental plasticity and genetic diversity across contrasting growing environments [1,2]. In the context of sustainable agriculture, optimizing cultivation practices and making effective use of available genetic resources are therefore important strategies for maintaining spring barley productivity under variable growing conditions [2,3].

Among the environmental factors affecting barley performance, temperature and water availability are particularly important because their variability during the growing season can influence phenological development, biomass accumulation, and grain yield [4]. The magnitude and timing of these effects may vary among genotypes and growing environments, leading to differences in phenological development and yield performance across locations and seasons [5,6]. Consequently, the identification of genotypes capable of maintaining stable performance across contrasting growing environments is an important objective of barley breeding programs [7].

Despite these challenges, barley exhibits a strong capacity to adapt to adverse environmental conditions, including low temperatures, water scarcity, and degraded soils, and is generally considered more drought tolerant than wheat [8]. Water availability is a major factor determining barley productivity, with drought stress potentially causing substantial reductions in grain yield [9,10]. Nevertheless, barley can maintain relatively stable performance across diverse environments, partly due to its well-developed root system and suitability for cultivation in different cropping systems [11]. This adaptive capacity, together with the high genetic diversity present in barley, provides opportunities to improve agronomic performance and resource-use efficiency through breeding [1].

Among cultivated barley forms, two-row spring barley is predominantly used in the malting and brewing industries because of its relatively uniform kernel size and favorable quality characteristics. It is also utilized in food processing and, to a lesser extent, as animal feed, depending on grain-quality attributes. In malting barley, grain quality is closely associated with the balance between protein and starch contents, which represents an important criterion for breeding programs targeting specific industrial end uses [12].

Modern breeding programs for spring barley focus on improving grain yield, end-use quality, and tolerance to abiotic stresses, particularly drought [13,14]. Grain yield is a complex quantitative trait determined by several components, including grain number per spike, spike characteristics, grain weight, and thousand-kernel weight [15,16]. Although

these traits are genetically controlled, their expression is influenced by diverse abiotic stresses and environmental conditions, particularly temperature and precipitation during critical stages of crop development [17,18]. Consequently, the simultaneous improvement in yield potential, grain quality, and tolerance to environmental stresses remains a major challenge in spring barley breeding.

This environmental sensitivity, together with the genetic complexity of yield formation, highlights the importance of genotype × environment (G × E) interactions in determining yield performance and stability across contrasting agro-ecological conditions [7]. Disentangling the effects of genotype, environment, and their interaction is therefore essential for identifying genotypes that combine high yield potential with stable performance across environments. Multi-environment trials and statistical approaches such as analysis of variance (ANOVA), correlation analysis, and stability analyses are widely used to characterize G × E interactions and support the identification of stable and well-adapted genotypes [7].

The increasing availability of genomic and phenotypic information provides new opportunities to improve the efficiency of barley breeding and to accelerate genetic gain. The integration of genomic data with phenotypic evaluations can support the identification and selection of superior genotypes, particularly for complex traits influenced by environmental variation [19]. In addition, the extensive genetic diversity present in barley and advances in genomic and analytical tools provide valuable resources for understanding adaptation and developing climate-resilient cultivars suited to contrasting environments [20]. These approaches can complement conventional phenotypic selection and contribute to the development of genotypes combining high productivity, stability, and adaptation to changing environmental conditions.

Despite significant advances in barley breeding and the increasing availability of genomic and phenotypic tools, relatively few studies have jointly evaluated grain yield stability and grain-quality traits across contrasting environments under Romanian growing conditions. Recent multi-environment studies have highlighted the importance of genotype × environment interactions in determining spring barley performance and adaptation across European environments [21]. Therefore, multi-environment evaluations are needed to identify genotypes with favorable yield performance, stability, and grain-quality traits under contrasting agro-ecological conditions in Romania.

The objective of this study was to evaluate the phenotypic variability, grain yield, yield stability, and grain-quality traits of 25 spring barley genotypes, including modern cultivars and breeding lines, assessed over two growing seasons (2024 and 2025) under contrasting agro-ecological conditions in Turda and Fundulea, Romania. Specifically, the study investigated the influence of environmental variation on key agronomic, phenological, and quality traits, including plant height, heading and flowering time, thousand-kernel weight, grain yield, and grain protein and starch content. In addition, the study aimed to identify genotypes with favorable performance and yield stability across environments, supporting the selection of genetic resources for breeding programs targeting productive and adaptable spring barley under variable growing conditions.

2. Materials and Methods

2.1. Experimental Site

The study was conducted in 2024 and 2025 at two experimental sites in Romania with contrasting pedoclimatic conditions: Turda (Cluj County) and Fundulea (Călărași County) (Figure 1) [22].



Figure 1. Geographic location of the experimental sites: the red marker indicates the Agricultural Research and Development Station Turda (ARDS Turda), whereas the yellow marker indicates the National Agricultural Research and Development Institute Fundulea (NARDI Fundulea), Romania.

2.2. Biological Materials

The biological material consisted of 25 spring barley genotypes, including five cultivars developed at ARDS Turda (Turdeana, Daciana, Romanița, Dumbrăvița, and Ioana), one foreign cultivar, RGT Planet [23–25], and nineteen advanced spring barley breeding lines from ARDS Turda (Table 1). The genotypes were evaluated across four location–year environments, corresponding to all combinations of two experimental locations (Turda and Fundulea) and two growing seasons (2024 and 2025): Turda 2024, Turda 2025, Fundulea 2024, and Fundulea 2025.

Table 1. Spring barley genotypes used in the experiment, including advanced breeding lines developed at ARDS Turda and the foreign cultivar RGT Planet.

No.	Genotype	Type	Origin	Status
1	Daciana	two-row	ARDS Turda	Cultivar
2	Turdeana	two-row	ARDS Turda	Cultivar
3	Romanița	two-row	ARDS Turda	Cultivar
4	RGT Planet	two-row	RAGT France	Cultivar
5	Ioana	two-row	ARDS Turda	Cultivar
6	To 2055-21	two-row	ARDS Turda	Breeding line
7	To 2198-13	two-row	ARDS Turda	Breeding line
8	To 2096-10	two-row	ARDS Turda	Breeding line
9	To 2122-17	two-row	ARDS Turda	Breeding line
10	To 2187-18	two-row	ARDS Turda	Breeding line
11	To 2238-18	two-row	ARDS Turda	Breeding line
12	To 2108-18	two-row	ARDS Turda	Breeding line
13	To 2222-18	two-row	ARDS Turda	Breeding line
14	To 2335-15	two-row	ARDS Turda	Breeding line
15	To 2183-17	two-row	ARDS Turda	Breeding line
16	To 2017-93	two-row	ARDS Turda	Breeding line
17	To 2014-99	two-row	ARDS Turda	Breeding line
18	To 2112-21	two-row	ARDS Turda	Breeding line
19	To 2501-16	two-row	ARDS Turda	Breeding line
20	To 2051-10	two-row	ARDS Turda	Breeding line
21	To 2123-01	two-row	ARDS Turda	Breeding line
22	To 2027-10	two-row	ARDS Turda	Breeding line
23	To 2170-01	two-row	ARDS Turda	Breeding line
24	To 2169-12	two-row	ARDS Turda	Breeding line
25	Dumbrăvița	two-row	ARDS Turda	Cultivar

The experiments were established using a randomized complete block design (RCBD) with three replications. Each experimental plot had a harvestable area of 10 m^2 (Figure 2).

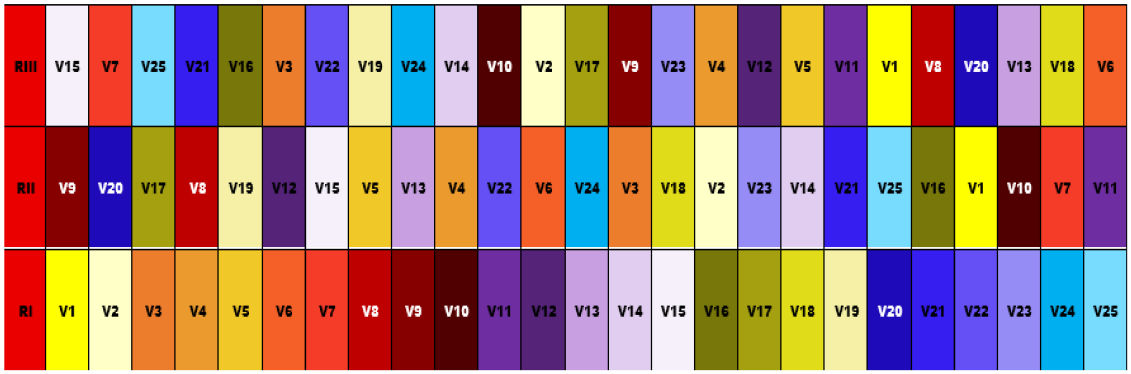


Figure 2. Field layout and randomization of 25 spring barley genotypes in a randomized complete block design (RCBD) with three replications. V, variant (genotype or breeding line); R, replicate (randomization block), I–III. The 25 different colors represent the 25 experimental variants. The same colors are used across all three replicates (RI, RII, and RIII), with different positions reflecting the randomized block design.

2.3. Research Methods

2.3.1. Experimental Sites and Soil Characteristics

The experiments were conducted at two contrasting sites in Romania, Turda and Fundulea, following the long-term management practices of the respective spring barley breeding fields. The soil at the Turda experimental site was classified as a clay illuvial chernozem, similar to a cambic para-rendzina chernozem. The soil had a neutral to slightly alkaline reaction, favorable for plant growth, and a high humus content. The Ap and Bv horizons were characterized by fine textures, slightly alkaline pH values (8.24–8.25), and high humus contents (4.44–4.08%), indicating good topsoil fertility [26].

In contrast, the experimental soil at Fundulea was classified as a typical cambic chernozem, with a groundwater table at a depth of 10–12 m and good nutrient availability. The soil contained 175 mg kg^{-1} soluble K and 70 mg kg^{-1} mobile P, while the humus content in the arable layer was 2.2%. The soil reaction ranged from slightly acidic to neutral, with a pH of 6.7 [27].

2.3.2. Experimental Design and Crop Management

Soil tillage at the Turda experimental site was performed using a moldboard plow (Kuhn Farm Machinery Master 125 T, Hamburg, Germany) [28] to a depth of 28 cm, followed by seedbed preparation with a rotary harrow (Kuhn Farm Machinery HRB 403 D, Hamburg, Germany) [29]. At Fundulea, soil preparation followed the local long-term management protocol and included heavy disking in October, followed by two pre-sowing cultivator passes, with the second performed perpendicular to the sowing direction. Pea was the preceding crop at both experimental sites in both years, and no irrigation was applied during the growing season.

Spring barley was sown within the locally optimal early-sowing period at each location. In 2024, sowing was performed on 22 March at Turda and 27 February at Fundulea; the earlier sowing at Fundulea was possible because of favorable temperatures in late February. In 2025, sowing was performed on 11 March at both locations. At both experimental sites, plots were established using a Wintersteiger experimental plot seeder (Wintersteiger Holding AG, Strasse 1, Ried im Innkreis, Austria) [30] at a uniform seeding rate of 450 viable seeds m^{-2}. The initial plot area was 14 m^2, of which 10 m^2 was retained as the harvestable area after the experimental alleys were established.

2.3.3. Fertilization and Crop Protection

The Fundulea experimental site is primarily used for breeding and evaluating winter barley. Through an established research collaboration, the site was also used to evaluate spring barley genotypes in the present study. Consequently, the experiments were conducted within the existing long-term agronomic management framework of the Fundulea breeding field, including its established soil-preparation, fertilization, and crop-protection practices. This approach allowed the spring barley genotypes to be evaluated under the site-specific agro-ecological and management conditions at Fundulea.

Fertilization regimes differed between the two experimental sites and followed the long-term agronomic management practices routinely used in the respective breeding fields. At Turda, ammonium nitrate (NH_4NO_3, 33.5% N) (Azomureș, Târgu Mureș, Romania) [31] was applied at a rate of 120 kg ha^{-1} in spring, immediately after sowing. At Fundulea, a compound fertilizer containing 18% N and 46% P_2O_5 (Azomureș, Târgu Mureș, Romania) [32] was applied at a rate of 120 kg ha^{-1} one day before the final soil preparation pass, supplying 21.6 kg N ha^{-1} and 55.2 kg P_2O_5 ha^{-1}. An additional 100 kg ha^{-1} of urea was applied in spring, supplying 46 kg N ha^{-1} [33].

The use of phosphorus-containing fertilizer at Fundulea was part of the established long-term fertilization protocol for the breeding field.

At Turda, no fungicides were applied in the spring barley breeding field to allow the evaluation of genotype tolerance to major diseases. Crop maintenance consisted of herbicide and insecticide applications. For weed control, Sekator Progress OD (0.12 L ha^{-1}), containing 100 g L^{-1} amidosulfuron, 25 g L^{-1} iodosulfuron-methyl-Na, and 250 g L^{-1} mefenpyrdiethyl (Bayer, Bucharest, Romania) [34], was applied at the BBCH 20–31 growth stage. In addition, DMA-6, containing 660 g L^{-1} 2,4-D acid equivalent in the form of dimethylamine salt, corresponding to 825 g L^{-1} 2,4-D dimethylamine salt (Corteva, Bucharest, Romania) [35], was also applied for weed control. Pest control was carried out using Leptostar (0.2 L ha^{-1}), containing 200 g L^{-1} acetamiprid (Qemetica, Bucharest, Romania) [36]. All treatments were applied with a MET 1500 sprayer.

At Fundulea, crop protection followed the established local management practices of the breeding field. Weed control was carried out using Axial One (45 g L^{-1} pinoxaden + 5 g L^{-1} florasulam + 11.25 g L^{-1} cloquintocet-mexyl; Syngenta, Bucharest, Romania) [37] at a rate of 1 L ha^{-1}, together with Silwet Gold adjuvant (UPL, Bucharest, Romania) [38]. The first fungicide treatment consisted of Verben (50 g L^{-1} proquinazid + 200 g L^{-1} prothioconazole; Corteva, Bucharest, Romania) [39] at a rate of 1 L ha^{-1} for the control of powdery mildew, rust, and net blotch, combined with the plant growth regulator Moddus (250 g L^{-1} trinexapac-ethyl; Syngenta, Bucharest, Romania) [40] at a rate of 0.6 L ha^{-1} and Silwet Gold adjuvant at 100 mL per 100 L of water. A second fungicide treatment was applied using Mizona (200 g L^{-1} pyraclostrobin + 30 g L^{-1} fluxapyroxad; BASF, Bucharest, Romania) at a rate of 0.7 L ha^{-1} [41].

Because the two experimental sites were managed according to their respective long-term breeding-field practices, differences between locations reflected not only pedoclimatic conditions but also site-specific fertilization and crop-protection regimes. Therefore, the location effect estimated in the statistical analyses should be interpreted as a combined site effect and cannot be attributed exclusively to differences in soil and climate.

The experimental plots at both locations were harvested using a Wintersteiger plot combine harvester (Wintersteiger AG, Ried im Innkreis, Austria) [42].

2.3.4. Trait Evaluation and Grain-Quality Assessment

The quantitative traits evaluated in the experiment included heading date, flowering date, plant height, thousand-kernel weight (TKW), grain yield (GY), grain protein content

(PC), and grain starch content (SC). Heading and flowering were recorded at BBCH growth stages 55 and 61, respectively, and were expressed as the number of days from sowing to each phenological stage. Plant height was measured at full maturity (BBCH 92), from the soil surface to the tip of the awns. These traits are widely used for the phenotypic and agronomic characterization of barley genotypes and breeding materials [27,43,44].

Grain yield was determined by weighing the grain harvested from each experimental plot and expressing the result in kg ha^{-1} after conversion from plot yield. Grain moisture was adjusted to the standard 14% moisture content used for small-grain cereals. Thousand-kernel weight was determined from three representative samples, each consisting of 1000 kernels. Grain protein and starch contents were determined using 500 g grain samples and an Infratec 1241 analyzer (FOSS, Hillerød, Denmark) [45]. Protein and starch contents were expressed as percentages of dry matter (% DM).

2.4. Weather Conditions

Weather conditions during the two growing seasons at Turda (longitude 23°47′ E, latitude 46°35′ N, altitude 427 m) and Fundulea (longitude 26°31′ E, latitude 44°28′ N, altitude 68 m) are presented in Figure 3. Meteorological data were obtained from weather stations located at the Agricultural Research and Development Station Turda and the National Agricultural Research and Development Institute Fundulea [46,47]. Long-term reference values were based on 65-year climatological averages for each location. Both growing seasons were generally characterized by above-average air temperatures and irregular precipitation patterns, although Fundulea experienced larger temperature anomalies and precipitation deficits than Turda. At Turda, mean monthly temperatures exceeded the long-term average during most of the January–July period in both years (Figure 3). Positive temperature deviations reached +4.2 °C in July 2024 and remained high in January (+3.6 °C), March (+3.9 °C) and June (+3.8 °C) 2025, whereas May 2025 was the only month with a slightly negative deviation (−2.0 °C). Rainfall was generally below the long-term average, particularly in June of both years (−48.4 mm in 2024 and −62.2 mm in 2025). Although May 2025 recorded a precipitation surplus (+29.2 mm), rainfall distribution remained highly uneven throughout the growing season. Fundulea experienced warmer conditions than Turda, with larger positive temperature anomalies, especially during January–February 2024 and the summer months. July temperatures exceeded the long-term average by +5.0 °C in 2024 and +3.8 °C in 2025. Precipitation deficits were also greater, particularly in June (−59.3 mm in 2024 and −67.5 mm in 2025), indicating reduced water availability during the growing season. A temporary precipitation surplus occurred in May 2025 (+26.5 mm), but it did not compensate for the overall seasonal moisture deficit. Overall, both locations experienced warmer-than-average growing seasons accompanied by uneven rainfall distribution. Compared with Turda, Fundulea showed higher temperature anomalies and larger precipitation deficits during several months of the growing season.

2.5. Statistical Analysis

All statistical analyses were performed in R version 4.6.1. Descriptive statistics were calculated to characterize the overall variation in the dataset. The effects of year (Y), location (L), genotype (G), and their interactions were evaluated using plot-level observations and factorial analysis of variance (ANOVA) with Type III sums of squares. The model included the main effects of Y, L, and G, together with the Y × L, Y × G, L × G, and Y × L × G interactions; replicate (block) nested within each Y × L combination was included as a blocking term. Type III tests were evaluated using sum-to-zero contrasts. Estimated marginal means were calculated for the year × location combinations and pairwise comparisons were performed using Tukey's adjustment at a significance level of $p < 0.05$. Pearson correlation coefficients among

the seven traits were calculated using the overall means of the 25 genotypes across years and locations (n = 25). The corresponding *p*-values were calculated from these 25 genotype means. Principal component analysis (PCA) was performed on standardized values of the seven evaluated traits using genotype means calculated across the three replications within each year × location environment. The PCA therefore included 100 genotype × environment observations (25 genotypes × 4 environments). Each observation represented one genotype within one environment, and the four environments were identified by their respective year × location combinations. PCA was used as a descriptive multivariate approach to visualize relationships among traits and patterns of environmental differentiation; it was not used for statistical significance testing. Genotype × environment patterns were further investigated separately for each trait using GGE biplot analysis based on genotype means within each environment. The polygon view was used to identify vertex genotypes and their relationships with individual environments, while the Average Environment Coordination (AEC) approach was used to assess genotype performance and stability across environments. In the AEC analysis, mean genotype performance was assessed by projection onto the AEC abscissa, whereas stability was quantified as the absolute perpendicular distance from the AEC abscissa in the PC1–PC2 biplot coordinate system. Smaller distances indicate greater stability across environments. Model assumptions were assessed by visual inspection of residual-versus-fitted and normal Q–Q plots. For grain yield, the robustness of the ANOVA results was additionally evaluated using heteroscedasticity-consistent HC3 standard errors. Data processing, statistical analyses and graphical representations were performed primarily using the R packages dplyr (v. 1.2.1), ggplot2 (v. 4.0.3), ggrepel (v. 0.9.8), emmeans (v. 2.0.4), car (v. 3.1-5), and metan (v. 1.19.0).

(a) ARDS Turda

	Years / Months	January	February	March	April	May	June	July	Average temperature (°C) during the growing season (Mar.–Jul.)
Temperature (°C)	65 year average	−3.3	−0.6	4.4	10	15	18	19.8	13.4
	Deviation (±) °C								
	2024	3.50	7.60	4.40	3.30	0.80	3.70	4.20	16.7
	2025	3.6	0.2	0.39	1.6	−2.0	3.8	1.8	15.3
Rainfall (mm)									Total precipitation (mm) during the growing season (Mar.–Jul.)
	65 year average	21.7	19.2	24.3	45.6	69.4	84.6	78	301.9
	Deviation (±) mm								
	2024	−16.9	−10.0	13.4	−6.8	−8.7	−48.4	−29.0	222.7
	2025	−16.6	−15.4	20.5	−14.3	29.2	−62.2	72.3	347.4

(b) NARDI Fundulea

	Years / Months	January	February	March	April	May	June	July	Average temperature (°C) during the growing season (Mar.–Jul.)
Temperature (°C)	65 year average	−2.4	−0.4	4.9	11.3	17.0	20.8	22.7	15.3
	Deviation (±) °C								
	2024	3.4	8.0	3.6	3.7	−0.6	5.3	5.0	18.7
	2025	6.6	−1.5	4.5	0.4	−1.0	3.5	3.8	17.6
Rainfall (mm)									Total precipitation (mm) during the growing season (Mar.–Jul.)
	65 year average	35.1	32	37.4	45.1	62.5	74.9	71.1	291
	Deviation (±) mm								
	2024	−17.5	−30.6	1.2	17.3	−28.3	−59.3	−25.7	196.2
	2025	−24.3	−9.2	−11.8	−15.5	26.5	−67.5	−28.5	194.2

Figure 3. Meteorological conditions (temperature and precipitation) during January–July 2024 and 2025 at (**a**) ARDS Turda and (**b**) NARDI Fundulea. Source of primary data: Turda Meteorological

Station—longitude: 23°47′ E; latitude: 46°35′ N; altitude 427 m; Fundulea Meteorological Station—longitude 26°31′ E, latitude 44°28′ N, altitude 68 m. The color scales indicate temperature and precipitation values. For temperature, blue represents the lowest values and red the highest values. For precipitation, red represents the lowest values (including deficits), while blue represents positive values. Color intensity varies according to the magnitude of the values.

3. Results

3.1. Descriptive Variation in Grain Yield, Agronomic and Quality Traits

Considerable variation was observed in grain yield and the evaluated agronomic and quality traits (Table 2). Across the 300 plot-level observations, grain yield ranged from 1631 to 7730 kg ha^{-1}, with a mean of 5283 kg ha^{-1} and a descriptive CV of 21.99%. The CV reported here describes the overall variability among plot-level observations and does not represent experimental error. Protein content (PC) also showed considerable variation, with a mean of 14.99% and a CV of 21.13%, whereas starch content (SC) varied much less, with a mean of 60.31% and a CV of 4.29%. Thousand-kernel weight (TKW) ranged from 26.26 to 57.52 g, with an overall mean of 40.72 g (CV = 19.11%). The phenological traits showed lower variability. Days to heading (DTH) and days to flowering (DTF) had mean values of 74.12 and 78.38 days, respectively, while mean plant height (PH) was 96.12 cm. Variability in these traits was relatively low, with CV values ranging from 9.39% for PH to 10.97% for DTH. Skewness values were low for all traits (−0.58 to 0.32), indicating that none of the traits had a strongly asymmetric distribution.

Table 2. Descriptive statistics for grain yield, agronomic, and grain-quality traits across 300 observations.

Trait	Mean	SD	SE	CV (%)	Min	Max	Skewness	Kurtosis
GY	5283	1161.74	67.07	21.99	1631	7730	−0.15	−0.33
PC	14.99	3.17	0.18	21.13	9.50	21.40	0.32	−1.25
SC	60.31	2.59	0.15	4.29	54.60	64.50	−0.58	−0.84
TKW	40.72	7.78	0.45	19.11	26.26	57.52	−0.43	−0.82
DTH	74.12	8.13	0.47	10.97	62.00	89.00	0.15	−1.67
DTF	78.38	8.06	0.47	10.28	66.00	93.00	0.10	−1.68
PH	96.12	9.03	0.52	9.39	72.00	120.00	0.07	−0.47

GY = grain yield (kg ha^{-1}); PC = protein content (%); SC = starch content (%); TKW = thousand-kernel weight (g); DTH = days to heading (days from sowing); DTF = days to flowering (days from sowing); PH = plant height (cm); SD = standard deviation; SE = standard error; CV = coefficient of variation. Mean, SD, SE, Min, and Max are expressed in the units indicated for each trait.

3.2. Effects of Year, Location, Genotype, and Their Interactions

Type III ANOVA, accounting for replicate blocks within each year × location combination, showed significant effects of genotype and year for all seven traits, whereas the location effect was significant for all traits except PH (Table S1). The significant genotype effect ($p < 0.001$ for all traits) indicated substantial variation among the 25 barley genotypes. For GY, year, location, genotype and all interaction terms were highly significant ($p < 0.001$). The significant year × location × genotype interaction (F = 58.82, $p < 0.001$) indicated that the relative performance of the genotypes changed across the four year × location combinations. A similar response was found for PC, SC, TKW, DTH, and DTF, with significant main effects and interactions ($p < 0.001$). For PH, the main effect of location was not significant, although year, genotype and their interaction terms were significant.

The estimated marginal means (EMMs) show these differences among environments more clearly (Table 3). GY was highest at Turda in 2025 (6364 kg ha^{-1}), followed by Turda in 2024 (5626 kg ha^{-1}), Fundulea in 2025 (4992 kg ha^{-1}) and Fundulea in 2024 (4149 kg ha^{-1}),

with all four environments differing significantly from each other. Turda 2025 also had the highest TKW (48.07 g) and PH (104.91 cm), whereas Fundulea 2024 was characterized by the highest PC (19.32%) and the lowest SC (56.64%) and TKW (28.92 g). Heading and flowering occurred earlier at Turda 2024 and Fundulea 2025 and considerably later at Fundulea 2024 and Turda 2025.

Table 3. Estimated marginal means (EMMs ± SE) of grain yield, agronomic, and grain-quality traits across experimental years and locations.

Trait	2024		2025		*p* (Y × L)
	Fundulea	Turda	Fundulea	Turda	
GY	4149 ± 13.30 d	5626 ± 13.30 b	4992 ± 13.30 c	6364 ± 13.30 a	<0.001
PC	19.32 ± 0.04 a	11.78 ± 0.04 d	16.28 ± 0.04 b	12.57 ± 0.04 c	<0.001
SC	56.64 ± 0.05 d	62.96 ± 0.05 a	59.79 ± 0.05 c	61.83 ± 0.05 b	<0.001
TKW	28.92 ± 0.07 d	44.82 ± 0.07 b	41.07 ± 0.07 c	48.07 ± 0.07 a	<0.001
DTH	82.95 ± 0.14 a	66.24 ± 0.14 c	66.68 ± 0.14 c	80.60 ± 0.14 b	<0.001
DTF	86.96 ± 0.13 a	71.00 ± 0.13 c	70.56 ± 0.13 c	85.00 ± 0.13 b	<0.001
PH	93.47 ± 0.35 c	87.86 ± 0.35 d	98.24 ± 0.35 b	104.91 ± 0.35 a	<0.001

Note: Values are estimated marginal means (EMMs) ± standard error (SE). Within each trait, means followed by different letters differ significantly according to Tukey-adjusted pairwise comparisons ($p < 0.05$). The last column shows the *p*-value for the Year × Location interaction from the Type III ANOVA (Table S1). GY = grain yield (kg ha^{-1}); PC = protein content (%); SC = starch content (%); TKW = thousand-kernel weight (g); DTH = days to heading (days from sowing); DTF = days to flowering (days from sowing); PH = plant height (cm).

The distribution of the observed values across the four tested environments is shown in Figure S1.

3.3. Relationships Among Grain Yield, Agronomic and Grain-Quality Traits

Pearson correlation analysis, based on the overall means of the 25 genotypes across years and locations (n = 25), identified several significant relationships among the evaluated traits. The reported *p*-values were calculated using these 25 genotype means (Figure 4). GY was strongly and negatively correlated with PC (r = −0.832, $p < 0.001$), while significant positive correlations were observed with SC (r = 0.609, $p < 0.01$) and TKW (r = 0.767, $p < 0.001$). GY was also negatively correlated with DTH (r = −0.676, $p < 0.001$) and DTF (r = −0.675, $p < 0.001$). The correlation between GY and PH was weaker and not significant (r = −0.328, $p > 0.05$). PC was strongly and negatively correlated with SC (r = −0.812, $p < 0.001$) and TKW (r = −0.667, $p < 0.001$), but was positively correlated with DTH (r = 0.664, $p < 0.001$) and DTF (r = 0.650, $p < 0.001$). The strongest correlation was observed between DTH and DTF (r = 0.987, $p < 0.001$), indicating a close association between these two phenological stages.

The PCA revealed descriptive multivariate patterns that were consistent with the correlation analysis (Figure 5; Table S2). The first two principal components together explained 82.41% of the total variation, with PC1 accounting for 57.47% and PC2 for 24.94%. PC1 was mainly associated with SC (loading = 0.465), PC (−0.461), TKW (0.451), and GY (0.376). GY, TKW and SC were positioned in the same direction, whereas PC was oriented in the opposite direction, consistent with the correlations among these traits. PC2 was more strongly associated with the phenological traits DTH (0.509) and DTF (0.518), as well as with PH (0.513). The four tested environments formed generally distinct groups in the PCA space, with some overlap between Turda 2024 and Fundulea 2025 (Figure 5). Turda 2025 was positioned in the upper part of the biplot, in the direction of DTH, DTF, and

PH, whereas Fundulea 2024 was located on the negative side of PC1, toward PC. Turda 2024 and Fundulea 2025 were located in the lower part of the biplot and were more closely associated with the positive direction of PC1. Overall, the PCA provided a descriptive separation of the four environments and highlighted contrasting multivariate patterns involving grain yield, grain-quality traits, and phenological traits.

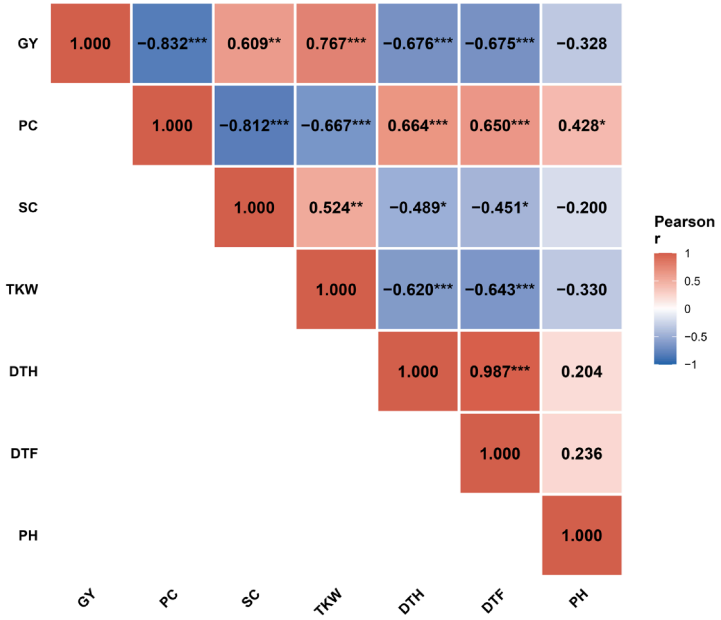


Figure 4. Pearson correlation matrix among grain yield, agronomic and grain-quality traits based on overall means of 25 barley genotypes across years and locations. Pearson correlation coefficients (r) are shown within cells. *, **, *** indicate significance at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

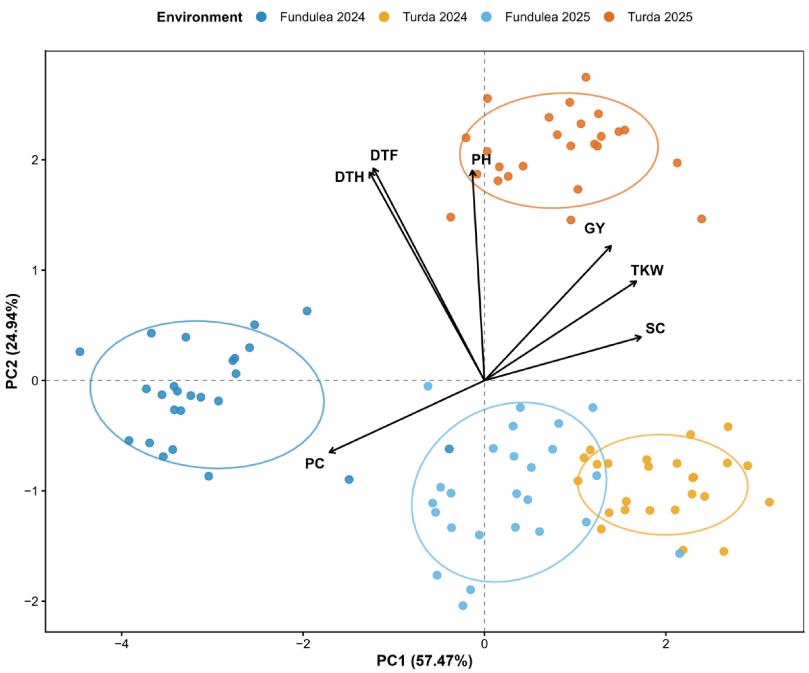
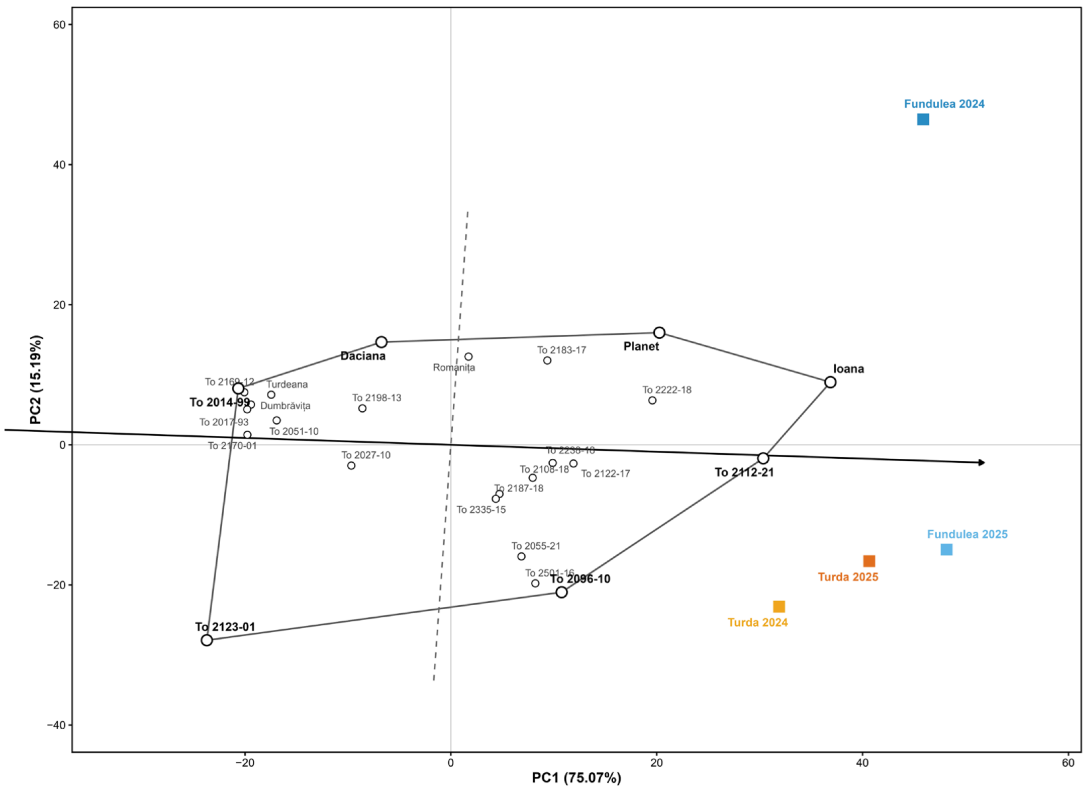


Figure 5. Principal component analysis biplot across four environments. PCA was performed on genotype means calculated across the three replications for each year × location combination. The analysis included 100 genotype × environment observations (25 genotypes × 4 environments). Each point represents the mean of one genotype within an environment; colors indicate the four experimental environments. Ellipses show the dispersion of genotype scores within each environment.

3.4. Genotype Performance and Stability Across Environments

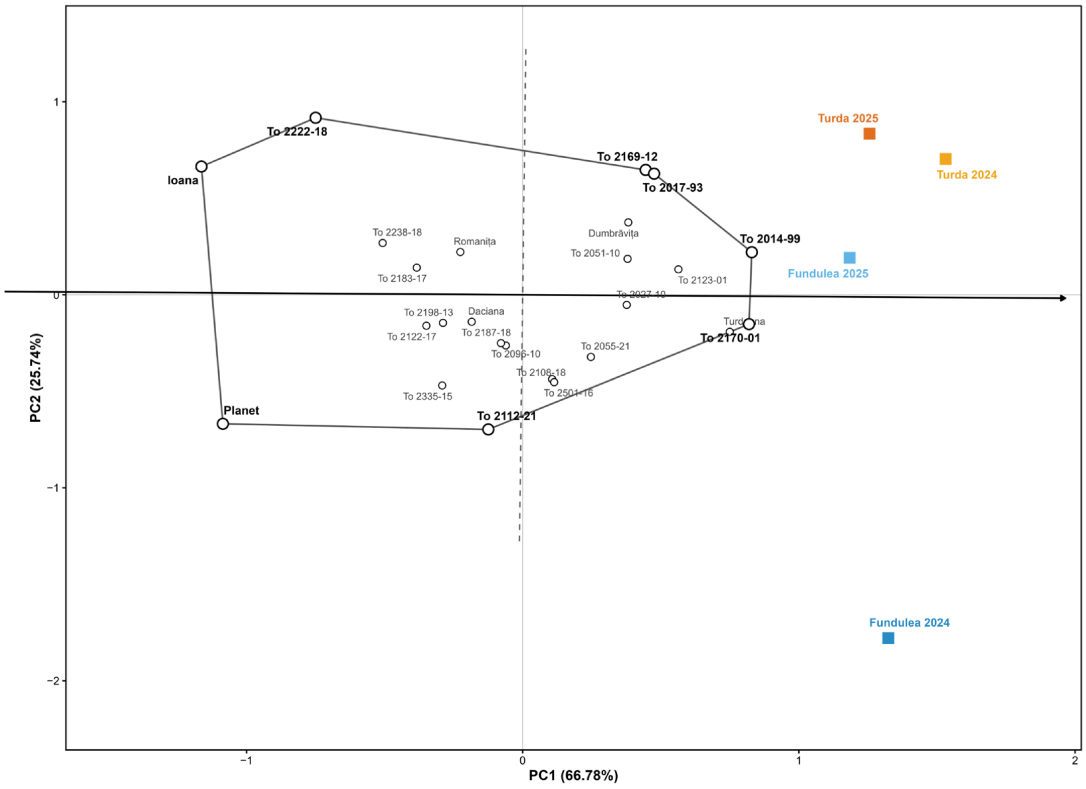
The GGE biplot analysis revealed clear differences in genotype responses across the four tested environments (Figure 6). The first two principal components captured a large proportion of the G + GE variation for all traits, ranging from 78.49% for PH to 92.52% for PC. The polygon views identified different vertex genotypes depending on the trait, reflecting differences in genotype performance across environments. For GY, PC1 and PC2 explained 75.07% and 15.19% of the variation, respectively, accounting for 90.26% in total. The polygon included Ioana, Planet, Daciana, To 2014-99, To 2123-01, To 2096-10, and To 2112-21 as vertex genotypes. The distribution of the four environments in the biplot indicated differences in genotype responses across year × location combinations. Across environments, mean GY ranged from 4149 kg ha^{-1} at Fundulea 2024 to 6364 kg ha^{-1} at Turda 2025. The AEC analysis clearly distinguished mean yield performance from stability. Ioana had the highest mean performance score but had an AEC stability distance of 10.75. In contrast, To 2112-21 had the smallest stability distance (0.43) while maintaining high mean yield performance, indicating the greatest yield stability among the tested genotypes.

PC showed the highest proportion of G + GE variation captured by the first two components (92.52%; PC1 = 66.78%, PC2 = 25.74%). No single genotype had the highest PC across all environments. At Fundulea 2024, the highest values were recorded for To 2170-01 and Turdeana. PC was generally higher at Fundulea than at Turda, with the largest environmental mean recorded at Fundulea 2024. For SC, the first two components accounted for 91.02% of the G + GE variation (PC1 = 62.70%, PC2 = 28.33%). Daciana had the highest observed SC at Fundulea 2024, while Planet ranked first at both Fundulea 2025 and Turda 2024. Environmental means ranged from 56.64% at Fundulea 2024 to 62.96% at Turda 2024, following a pattern opposite to that of PC.

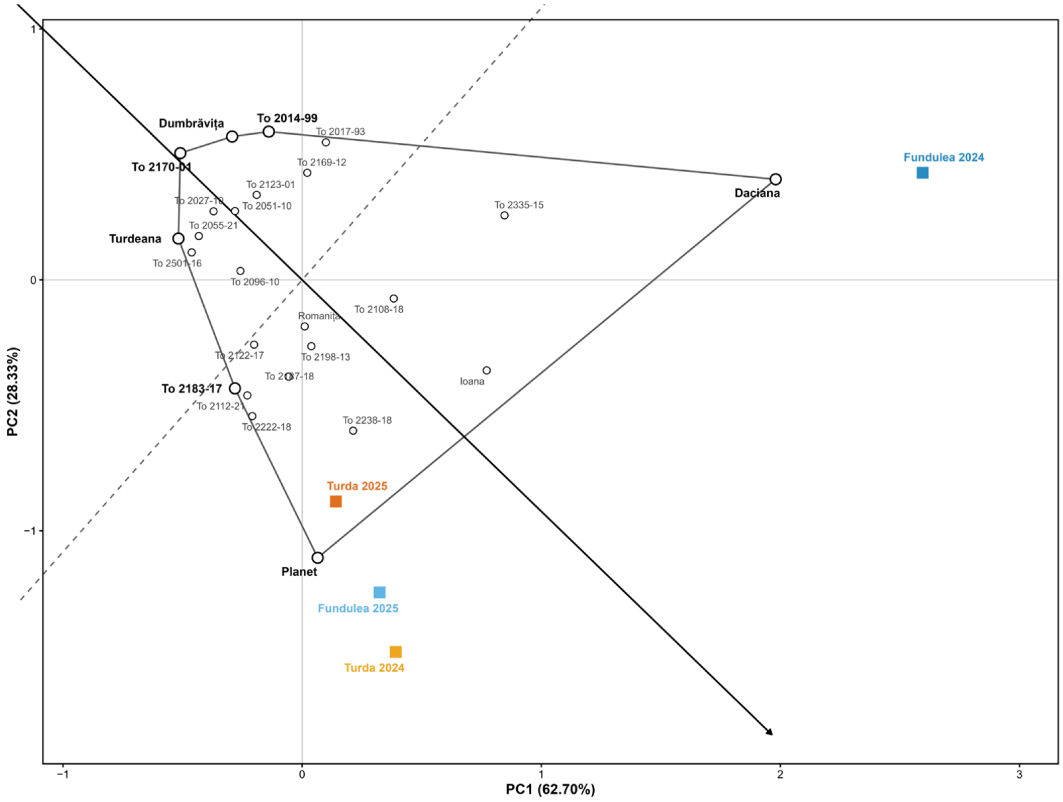


(**a**)

Figure 6. *Cont.*

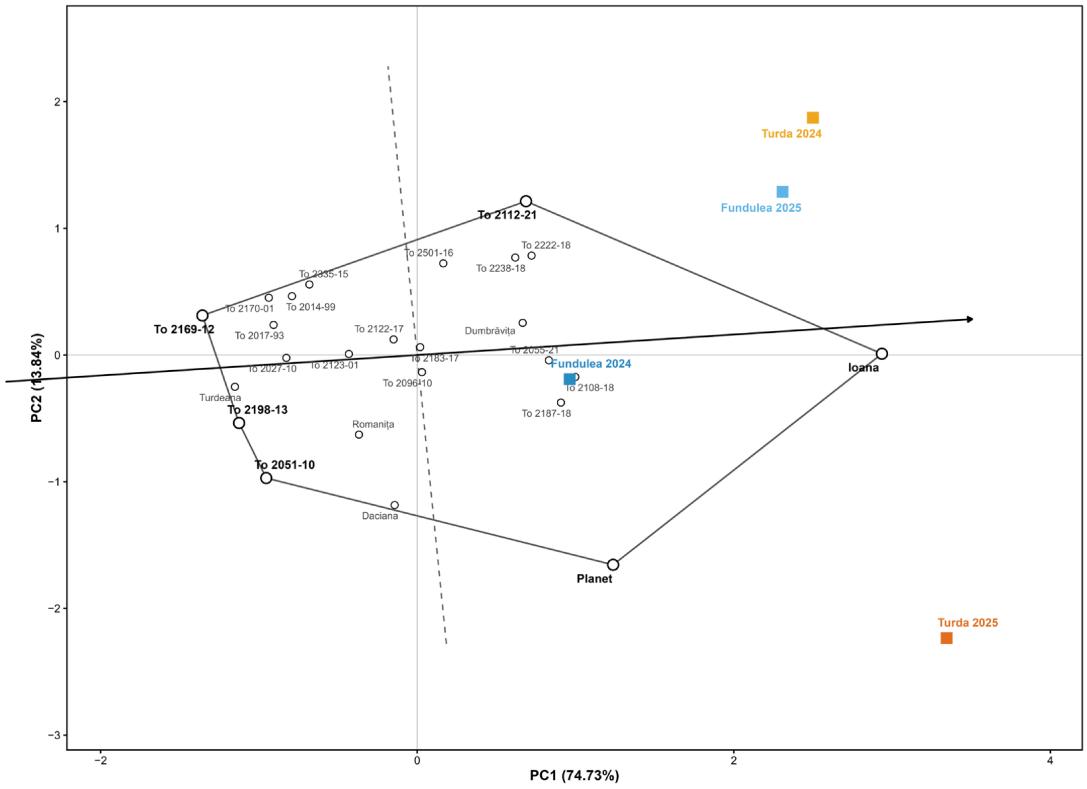


(**b**)

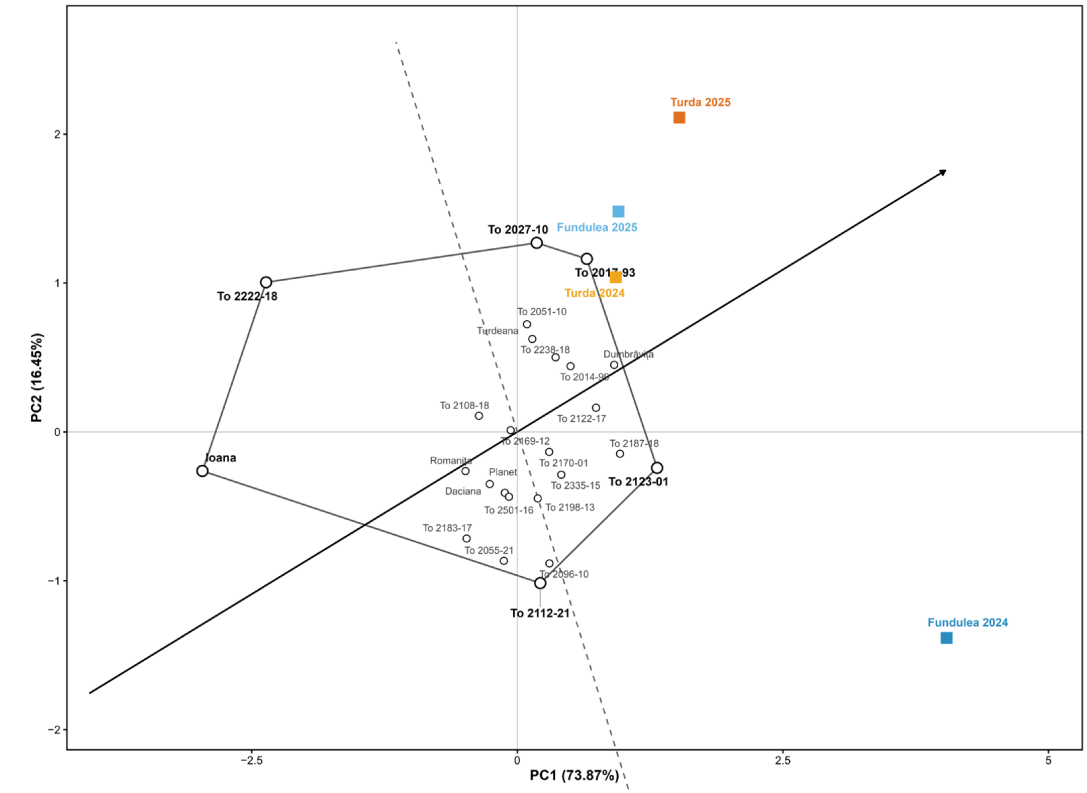


(**c**)

Figure 6. *Cont.*

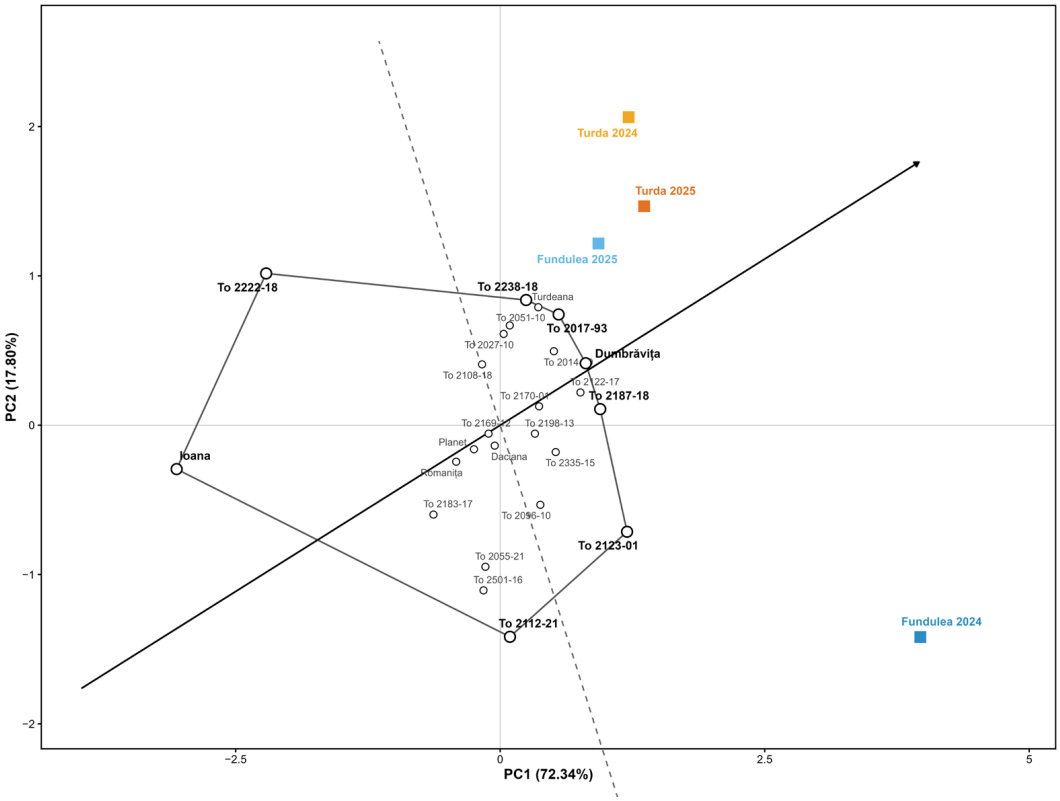


(**d**)

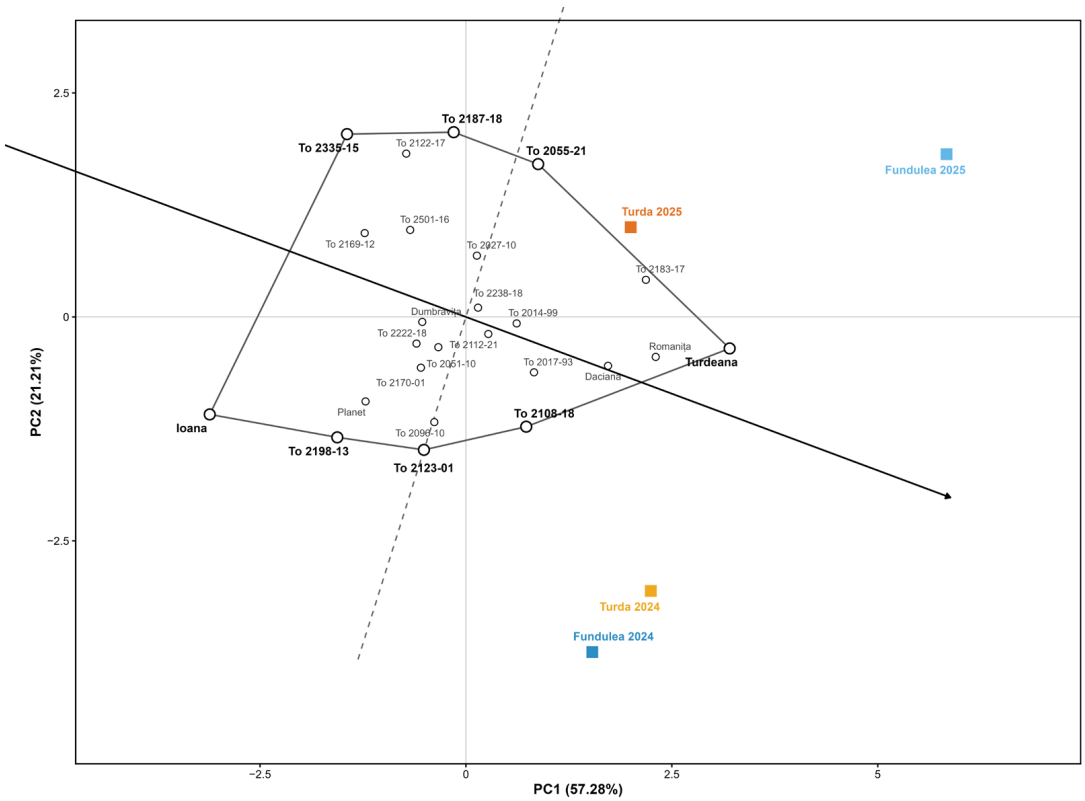


(**e**)

Figure 6. *Cont.*



(**f**)



(**g**)

Figure 6. GGE biplots for GY (**a**), PC (**b**), SC (**c**), TKW (**d**), DTH (**e**), DTF (**f**), and PH (**g**) for 25 barley genotypes evaluated across four tested environments. The polygon connects the vertex genotypes,

representing the most extreme genotypes in the GGE biplot. The Average Environment Coordination (AEC) abscissa (solid line with an arrow) indicates the direction of increasing mean genotype performance, whereas the AEC ordinate (perpendicular dashed line) represents deviations from the AEC abscissa, with shorter distances indicating greater stability.

A more consistent genotype response was found for TKW, for which PC1 and PC2 explained 74.73% and 13.84% of the variation, respectively (88.56% in total). Ioana had the highest observed TKW in all four environments and therefore ranked first consistently for this trait. Mean TKW varied considerably among environments, from 28.92 g at Fundulea 2024 to 48.07 g at Turda 2025.

Clear differences among genotypes were also observed for phenology. For DTH, the first two components explained 90.33% of the G + GE variation. Ioana was the earliest genotype at Fundulea 2024 (71.33 days), Fundulea 2025 (63.67 days) and Turda 2025 (75.33 days), while To 2501-16 was earliest at Turda 2024 (62.67 days). To 2123-01 showed the latest heading at Fundulea 2024 (88.67 days), whereas To 2017-93 and To 2027-10 had the highest DTH at Turda 2025 (84.67 days). DTF showed a very similar pattern, with the first two components explaining 90.14% of the variation. Ioana was again the earliest genotype at Fundulea 2024 (75.33 days), Fundulea 2025 (67.67 days) and Turda 2025 (79.33 days), while To 2501-16 was earliest at Turda 2024 (66.67 days). The latest flowering was observed for To 2123-01 at Fundulea 2024 (92.67 days), while To 2017-93, To 2027-10 and To 2238-18 had the latest flowering at Turda 2025 (87.67 days). The close relationship between DTH and DTF was consistent with their strong positive correlation (r = 0.987, $p < 0.001$).

PH showed more complex response, with the first two components explaining 78.49% of the G + GE variation (PC1 = 57.28%, PC2 = 21.21%). Unlike TKW or phenology, no single genotype consistently occupied the same extreme across environments. The tallest genotypes were To 2096-10 at Fundulea 2024 (101.33 cm), To 2017-93 at Turda 2024 (96.67 cm), Turdeana at Fundulea 2025 (116.33 cm) and To 2108-18 at Turda 2025 (112.67 cm). The shortest genotypes also differed among environments, further illustrating the environment-dependent response of this trait.

Across traits, Ioana showed a favorable combination of high grain yield, high thousand-kernel weight, and early phenology across the tested environments. To 2112-21 showed the greatest yield stability according to the AEC analysis, while other genotypes showed favorable responses for specific traits or environments, including To 2096-10 for grain yield at Turda 2025, To 2170-01, To 2014-99, and To 2169-12 for protein content, and Planet and Daciana for starch content. Table 4 summarizes the genotypes associated with high yield, yield stability, grain-quality traits, early phenology, and environment-specific responses.

Table 4. Summary of spring barley genotypes with potentially useful traits or environment-specific responses identified in the present study.

Breeding Objective/Trait	Genotypes Highlighted	Main Observation in the Present Study
High grain yield	Ioana; To 2112-21	Ioana had the highest overall mean grain yield and ranked first in three of the four environments; To 2112-21 had the second-highest overall mean yield and the smallest AEC stability distance
High thousand-kernel weight	Ioana	Highest TKW in all four environments
Early phenology	Ioana; To 2501-16	Ioana was earliest for heading and flowering in three environments; To 2501-16 was earliest at Turda 2024

Table 4. *Cont.*

Breeding Objective/Trait	Genotypes Highlighted	Main Observation in the Present Study
Protein content (environment-specific high values) Lower protein content	To 2170-01; Turdeana; To 2014-99; To 2169-12 Planet; Ioana	To 2170-01 and Turdeana had the highest PC at Fundulea 2024; To 2170-01 at Fundulea 2025; To 2014-99 at Turda 2024; and To 2169-12 at Turda 2025 Planet showed the lowest environment-specific mean PC (9.70% at Turda 2024), while Ioana had the lowest overall mean PC across the four environments.
Starch content (environment-specific high values)	Daciana; Planet; To 2112-21	Daciana had the highest SC at Fundulea 2024, Planet at Fundulea 2025 and Turda 2024, and To 2112-21 at Turda 2025
Environment-specific high yield	To 2096-10	Highest grain yield at Turda 2025
Yield stability	To 2112-21	Smallest distance from the AEC stability axis among the tested genotypes

Note: The breeding relevance indicated in this table is limited to the four environments evaluated in the present study and requires confirmation in additional multi-environment trials. Because grain protein concentration is an important quality trait for malting barley, genotypes with lower protein content were also highlighted.

4. Discussion

The significant effects of year, location, genotype, and their interactions observed in the present study show that both genetic and environmental factors contributed substantially to variation in grain yield and associated agronomic and quality traits. In particular, the significant year × location × genotype interaction for grain yield indicated that the relative performance of the genotypes changed across the four environments, highlighting the importance of genotype × environment (G × E) interactions in barley. Similar results have been reported in spring barley, where environmental conditions accounted for a large proportion of the variation in grain yield, while significant G × E interactions influenced genotype ranking and performance across environments [48–50]. These findings highlight the importance of evaluating barley genotypes across contrasting environments rather than relying on performance under a single set of growing conditions.

The differences observed among the year–location combinations also show that growing conditions influenced grain yield and associated traits. Under rainfed conditions, barley production is particularly sensitive to variation in precipitation and temperature during the growing season, while drought stress can substantially reduce crop productivity [51,52]. Climatic conditions during the growing season can affect phenology, biomass accumulation, and grain yield, with temperature and water availability being important determinants of barley development and productivity [5,53]. Under abiotic stress, changes in developmental timing may be accompanied by accelerated senescence, reduced photosynthetic activity, and a shorter reproductive and grain-filling period [4,54]. The contrasting temperature and precipitation patterns may have contributed to the differences observed among environments. However, weather variables were analyzed descriptively and were not included as explanatory variables in the statistical models. Therefore, the observed climatic patterns should be considered possible explanations for the variation among environments rather than evidence of direct causal effects. Importantly, the two locations differed not only in their pedoclimatic conditions but also in their long-term fertilization and crop-protection practices. Therefore, the observed location effects reflect the combined influence of agro-ecological and site-specific management conditions and cannot be attributed exclusively to climate or soil. This limitation should be considered when interpreting genotype × environment interactions and genotype performance across locations.

The significant genotype effects detected for all evaluated traits indicate considerable variation among the studied genotypes. Such variation is valuable from a breeding perspective because it provides opportunities to identify genotypes with favorable combinations of agronomic performance, phenology, and grain quality. Understanding genotypic variation and its relationships with grain yield and yield components is essential for the development of effective barley breeding programs [55].

The considerable variation observed in the present study is consistent with previous studies of spring barley, in which grain yield, grain quality, and yield components were strongly influenced by genotype and growing year [56]. Similarly, significant genotype and environmental effects have been reported for important barley grain-quality traits, including protein, starch, and kernel weight [57,58]. The relatively high variability observed for protein content in the present study further supports the importance of considering environmental effects when evaluating grain composition. Consequently, barley genotype selection should consider both grain productivity and quality traits across variable growing conditions.

The relationships identified among grain yield and grain-quality traits provide additional information on the performance of the studied genotypes. Grain yield was strongly and negatively associated with protein content, whereas positive associations were observed with starch content and thousand-kernel weight. The negative association between yield and protein concentration is consistent with previous observations in barley and may partly reflect a dilution effect associated with increased carbohydrate accumulation in high-yielding grains [56]. However, this interpretation should be regarded as a possible explanation rather than a demonstrated causal mechanism. The positive association between grain yield and thousand-kernel weight also shows that kernel weight was closely associated with yield variation among the studied genotypes, in agreement with previous findings in barley [55,56]. The positive relationship between grain yield and starch content, together with the strong negative association between protein and starch, suggests contrasting patterns in the accumulation of these major grain constituents. Similar relationships have been reported for barley grain-quality traits [57,58]. Considering yield, kernel weight, protein content, and starch content together may therefore be useful when selecting genotypes for further breeding and quality evaluation. However, protein and starch concentrations alone are not sufficient to provide a comprehensive assessment of malting quality. Therefore, the present results should not be interpreted as direct evidence of malting suitability.

Although Ioana showed favorable performance for grain yield and thousand-kernel weight, its protein content varied considerably among environments. Mean protein content was 10.60% and 11.57% at Turda in 2024 and 2025, respectively, compared with 16.60% and 14.97% at Fundulea. These differences indicate substantial environmental variation in grain protein concentration. Because protein and starch contents alone do not provide a comprehensive assessment of malting quality, these results should not be interpreted as direct evidence of the malting suitability of Ioana. Phenological traits also showed important associations with grain yield. The negative correlations of grain yield with heading and flowering time suggest that genotypes with earlier heading and flowering tended to perform better under the conditions evaluated in this study. This pattern may be associated with the completion of critical developmental stages before unfavorable conditions occur later in the growing season. Temperature and water availability can substantially influence the duration of developmental stages in cereals, while drought or high temperatures may accelerate reproductive development and shorten the period available for grain filling [5,53]. Thus, earlier development may be advantageous in environments where terminal drought or high temperatures occur during reproductive and grain-filling stages. However, earliness

should not be considered advantageous independently of yield potential, since excessively short developmental periods may restrict biomass accumulation and grain filling. The very strong association between heading and flowering time observed in the present study also reflects the close relationship between these developmental stages and suggests that heading time may provide a useful indication of subsequent flowering time.

The GGE biplot analysis further illustrated the environment-dependent performance of the evaluated genotypes and the difficulty of identifying broadly adapted material. The occurrence of different vertex genotypes for individual traits indicates that genotype superiority was dependent on both trait and environment rather than being universal. Similar patterns have been reported in barley multi-environment trials using GGE biplot and AMMI approaches, where different genotypes showed superiority in particular environments or greater stability across environments [49,59,60]. Among the studied material, Ioana showed a particularly favorable combination of high grain yield and thousand-kernel weight, together with relatively early heading and flowering. It ranked first for grain yield in three of the four environments, had the highest TKW in all four environments, and showed the earliest heading and flowering in three environments. However, these results should be interpreted as evidence of favorable performance within the tested environments, not universal adaptation. The four environments evaluated in this study represent a relatively limited environmental sample and therefore restrict broader conclusions about genotype adaptation and resilience. Further multi-year and multi-location trials across a wider range of agro-ecological conditions are needed to confirm genotype stability and adaptability. Other genotypes showed more specific responses for particular traits or environments and may therefore be useful for breeding objectives focused on specific adaptation or grain-quality characteristics. These results confirm the value of multi-environment testing and GGE biplot analysis for distinguishing broadly favorable genotypes from those showing more specific environmental responses.

From a sustainability perspective, identifying genotypes that maintain favorable agronomic performance across contrasting environments may support more resilient and adaptable barley production systems. However, sustainability was not directly assessed in the present study. Therefore, the observed genotype performance should be regarded as a potential contribution to resilient crop production rather than as direct evidence of improved sustainability. Further studies incorporating agronomic, environmental, and economic indicators are needed for a more comprehensive evaluation of these sustainability implications.

5. Conclusions

The present study revealed substantial variation among genotypes and environments in grain yield, grain quality, phenological traits, and plant height across the 25 barley genotypes evaluated in the tested environments. The significant effects of genotype, year, location, and their interactions, particularly for grain yield, highlighted the importance of genotype × environment interactions in shaping barley performance under contrasting growing conditions.

Grain yield was positively associated with thousand-kernel weight and starch content and negatively associated with protein content. These associations emphasize the importance of considering productivity and grain composition together during genotype selection. However, protein and starch contents represent only selected aspects of grain quality and do not provide a comprehensive assessment of malting quality or other specific end-use characteristics. The strong positive correlation between heading and flowering dates, together with the PCA results, also reflected the close relationship between the evaluated phenological traits.

The GGE biplot analysis revealed substantial differences in genotype performance across environments and helped identify genotypes with favorable responses across the tested environments. Ioana showed a favorable overall combination of traits, with the highest grain yield in three of the four environments, the highest thousand-kernel weight across all environments and early heading and flowering in three environments. This combination of productivity, grain size, and phenological earliness supports the further evaluation of Ioana in barley breeding programs. Other genotypes, including Planet, Daciana, To 2096-10, and To 2170-01, showed favorable responses for specific traits or environments and may therefore provide useful genetic material for targeted selection. To 2112-21 showed the greatest yield stability according to the AEC analysis.

Identifying genotypes with favorable and relatively consistent performance across contrasting environments may support more resilient and adaptable barley production systems. However, because sustainability was not directly assessed in this study, these findings should be interpreted as a potential contribution to sustainable crop production rather than as direct evidence of improved sustainability. Further studies incorporating explicit sustainability indicators are needed to evaluate these broader implications.

The conclusions regarding genotype performance and stability should be interpreted within the scope of the four environments evaluated in this study. The limited number of locations and growing seasons precludes broader conclusions about universal adaptation. Further multi-environment testing across additional years and locations is needed to confirm the observed genotype responses, stability, and adaptability.

Overall, the combined analyses identified genotypes with different strengths across the tested environments. Ioana showed high grain-yield performance and consistently high thousand-kernel weight, while the AEC analysis identified To 2112-21 as the genotype with the greatest yield stability. Grain yield was positively associated with thousand-kernel weight and starch content and negatively associated with protein content, emphasizing the need to consider both productivity and grain composition in genotype selection. However, these results are based on only four location–year environments. Additional evaluation across years and locations is therefore needed to confirm the stability, adaptability, and grain-quality performance of these genotypes.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/su182010547/s1. Figure S1: Distribution of grain yield (GY; (a)), protein content (PC; (b)), starch content (SC; (c)), thousand-kernel weight (TKW; (d)), days to heading (DTH; (e)), days to flowering (DTF; (f)), and plant height (PH; (g)) across years and locations; Table S1: Type III ANOVA for the effects of year, location, genotype, and their interactions on the studied traits; Table S2: Loadings of the evaluated traits on the first two principal components (PC1 and PC2).

Author Contributions: Conceptualization, I.C., L.V. and A.D.O.; methodology, I.C., L.V., E.I.P. and A.D.O.; validation, I.C., F.R., R.R., L.V., E.I.P., A.-M.V., L.Ș. and A.D.O.; formal analysis, I.C. and A.D.O.; investigation, I.C., L.V. and E.I.P.; resources, I.C., L.V. and E.I.P.; data curation, I.C., L.V., E.I.P. and A.D.O.; writing—original draft preparation, I.C.; writing—review and editing, I.C., F.R., R.R., L.V., A.-M.V., L.Ș. and A.D.O.; visualization, A.D.O., R.R.; supervision, I.C., L.V. and A.D.O. All authors have read and agreed to the published version of the manuscript.

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