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Stability Analysis of Quantitative and Qualitative Traits in Lentil Genotypes Across Environments

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Abstract

Lentil (*Lens culinaris* Medik.) is an important grain legume for human nutrition, and the identification of genotypes with stable agronomic and seed quality traits is relevant for breeding and cultivar development. The present study evaluated 14 lentil genotypes, including the cultivars Samos and Dimitra and twelve elite breeding lines (GVLen1–GVLen12), across four environments representing two locations and two growing seasons. Seed yield, thousand-seed weight, pods per plant, seeds per plant, plant height, protein, fat, ash, total starch, dietary fiber, and moisture contents were evaluated. Combined analysis of variance showed significant effects of genotype, environment, and genotype × environment interaction for all traits. Broad-sense heritability was high for all traits, ranging from 92.93% for seed yield to 99.82% for fat content. The Stability Index indicated differences among genotypes in the stability of individual traits, with GVLen11 showing the highest value for seed yield, while particularly high stability values were observed for total starch content. AMMI and GGE analyses further showed differences in genotype responses across environments, with GVLen11 and GVLen12 positioned close to the ideal genotype for seed yield in the GGE analysis. Seed yield was positively correlated with thousand-seed weight ($r = 0.41$, $p < 0.05$) and negatively correlated with fat ($r = -0.44$, $p < 0.05$) and total starch ($r = -0.18$, $p < 0.01$). Multivariate analyses classified the genotypes according to their combinations of agronomic and seed quality traits, while multi-response PLS identified fat, genotype, starch, seeds per plant, pods per plant, and moisture as variables with VIP values above 0.8 in relation to seed yield and thousand-seed weight. These results identified genotypes with stable expression of specific agronomic and seed quality traits and provide information to support the selection of lentil genotypes with desirable trait combinations for further breeding evaluation.

Keywords: *Lens culinaris*; genotype × environment interaction; stability index; heritability; AMMI; GGE biplot; principal component analysis; partial least squares

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1. Introduction

Lentil (*Lens culinaris* Medik.) is one of the oldest domesticated crops and one of the most important grain legumes worldwide [1]. Globally, according to FAOSTAT [2], lentils are cultivated on approximately 5.5 million hectares, with annual production reaching around 7.9 million tonnes. Lentils are produced in diverse agroecological regions and are considered a nutritionally valuable food, being rich in protein, complex carbohydrates, dietary fiber, and folic acid [3]. Plant breeding aims to develop lentil cultivars with high and stable seed yield and resistance to abiotic and biotic stresses [4]. The selection of desirable agronomic and qualitative traits is also important for adaptation and variety development [5]. Lentil production is affected by several constraints, including poor soil fertility, moisture stress, diseases and insect pests, weed competition, and inadequate crop management [6].

Environmental conditions can substantially affect lentil yield, particularly under drought stress, which can negatively affect plant growth and reduce yield [7]. Significant genotype × environment (G × E) interactions have also been reported for lentil grain yield, indicating that genotypes may respond differently to environmental conditions [8,9]. Therefore, the identification of genotypes with high and stable performance across environments is an important objective in lentil breeding [10].

Multi-environment testing is used in lentil breeding programs to evaluate genotype performance across environments and to assess genotype × environment (G × E) interactions for variety recommendation [11]. Genotype and genotype × environment (G × E) interaction effects are important for evaluating the performance and stability of lentil genotypes across different environments [9]. G × E interaction can result in differential responses of genotypes across environments and is therefore important for identifying adaptation targets and test locations [12].

The Stability Index (SI), proposed by Fasoula [13,14], provides a criterion for evaluating the stability of a trait across environments by considering its mean value and variability. This approach has been applied to assess the stability of agronomic and quality traits across different crops and environments [15–17]. The Additive Main Effects and Multiplicative Interaction (AMMI) model combines analysis of variance for the additive effects of genotype and environment with principal component analysis of the non-additive genotype × environment (G × E) interaction [18,19]. This approach can be used to identify mega-environments and target genotypes adapted to specific environments [20]. In lentil, AMMI has been used to investigate genotype × environment interaction and yield stability across multiple environments [6,9,12]. The GGE biplot, introduced by Yan et al. [21], focuses on the genotype and genotype × environment components of multi-environment trial data and provides a graphical approach for evaluating genotypes and identifying mega-environments. The method facilitates the assessment of genotype performance and stability across environments, as well as the evaluation of environments in terms of discriminating ability and representativeness [22,23]. In lentil, GGE biplot analysis has been used to investigate genotype × environment interaction, identify mega-environments, and evaluate yield performance and stability across multiple environments [24].

The present study evaluated 14 lentil genotypes, including two commercial cultivars (Samos and Dimitra) and twelve elite breeding lines (GVLen1–GVLen12), across four environments. The inclusion of commercial cultivars and advanced breeding lines enabled the evaluation of breeding material with different selection histories under the same environmental conditions. The study aimed to provide an integrated assessment of agronomic, morphological, and seed quality traits, considering both genotype performance and trait stability across environments. Specifically, the objectives were to assess trait stability using the Stability Index (SI), AMMI, and GGE biplot analyses; identify genotypes combining de-

sirable trait values with stable performance; and investigate relationships among genotypes and traits using correlation analysis, principal component analysis (PCA), cluster analysis, and multi-response partial least squares (PLS) analysis. It was hypothesized that the evaluated genotypes would differ in their mean performance and stability across environments and that the combined analysis of multiple traits would help identify genotypes with desirable agronomic and seed quality characteristics. The combined application of these complementary approaches provides an integrated assessment of genotype performance, stability, and seed quality, generating information useful for the selection of promising material for further breeding evaluation.

2. Materials and Methods

2.1. Establishment of Crops and Experimental Techniques

Field trials were conducted over two consecutive growing seasons (2022/2023 and 2023/2024) at two locations in Greece: Trikala (120 m above sea level) and Kalambaka (190 m above sea level). Each combination of location and growing season was considered a separate environment (E). Accordingly, the four environments were defined as follows: Environment 1 (E1), Trikala in 2022/2023; Environment 2 (E2), Trikala in 2023/2024; Environment 3 (E3), Kalambaka in 2022/2023; and Environment 4 (E4), Kalambaka in 2023/2024. Soil properties at each experimental site, determined from samples collected at a depth of 0–30 cm, are presented in Table 1. The meteorological conditions recorded during the experimental periods, including average air temperature (°C) and total monthly precipitation (mm), are presented in Figure 1. The meteorological data presented in Figure 1 were obtained from the Hellenic National Meteorological Service (HNMS) and recorded by the measuring stations located in the Trikala and Kalambaka cities, covering the experimental period from January 2022 to December 2024.

Table 1. Soil chemical properties at a depth of 0–30 cm.

	Altitude	Soil Type	pH	$CaCO_3$	NO_3-N	P (Olsen)	K	Organic Matter Content	Soil Electrical Conductivity
Trikala 2022/2023	120 m	Loam (L)	8.00	9.00%	5.0 mg kg^{-1}	16.00 mg kg^{-1}	199.0 mg kg^{-1}	1.70%	347 µS/cm
Trikala 2023/2024	120 m	Loam (L)	8.00	8.48%	6.8 mg kg^{-1}	14.40 mg kg^{-1}	175.0 mg kg^{-1}	1.90%	328 µS/cm
Kalambaka 2022/2023	190 m	Silty Clay (SiC)	8.10	4.89%	11.4 mg kg^{-1}	11.14 mg kg^{-1}	112.4 mg kg^{-1}	2.15%	512 µS/cm
Kalambaka 2023/2024	190 m	Silty Clay (SiC)	8.10	4.52%	10.6 mg kg^{-1}	10.21 mg kg^{-1}	124.2 mg kg^{-1}	2.08%	524 µS/cm

In all four environments, the genotypes were evaluated using a randomized complete block design (RCBD) with four replications. Each plot consisted of five rows, each 10 m long, with a row spacing of 0.25 m and a sowing depth of 5 cm, resulting in a total plot area of 12.5 m^2. Sowing was carried out in mid-November in both growing seasons (2022/2023 and 2023/2024). The seeding rate ranged from 80 to 110 kg ha^{-1}, depending on seed size (small-, medium-, or large-seeded lentil), following the methodology described by Papakosta-Tasopoulou [25], with the aim of maintaining a relatively uniform plant density among genotypes.

At sowing, a basal fertilizer application of 20 kg ha^{-1} nitrogen (N) and 60 kg ha^{-1} phosphorus (P_2O_5) was applied, following Papakosta-Tasopoulou [25]. Weed control was carried out manually after crop establishment, while standard phytosanitary measures for disease and pest management were applied according to local agricultural recommendations. Harvesting took place in mid-June 2023 and 2024.

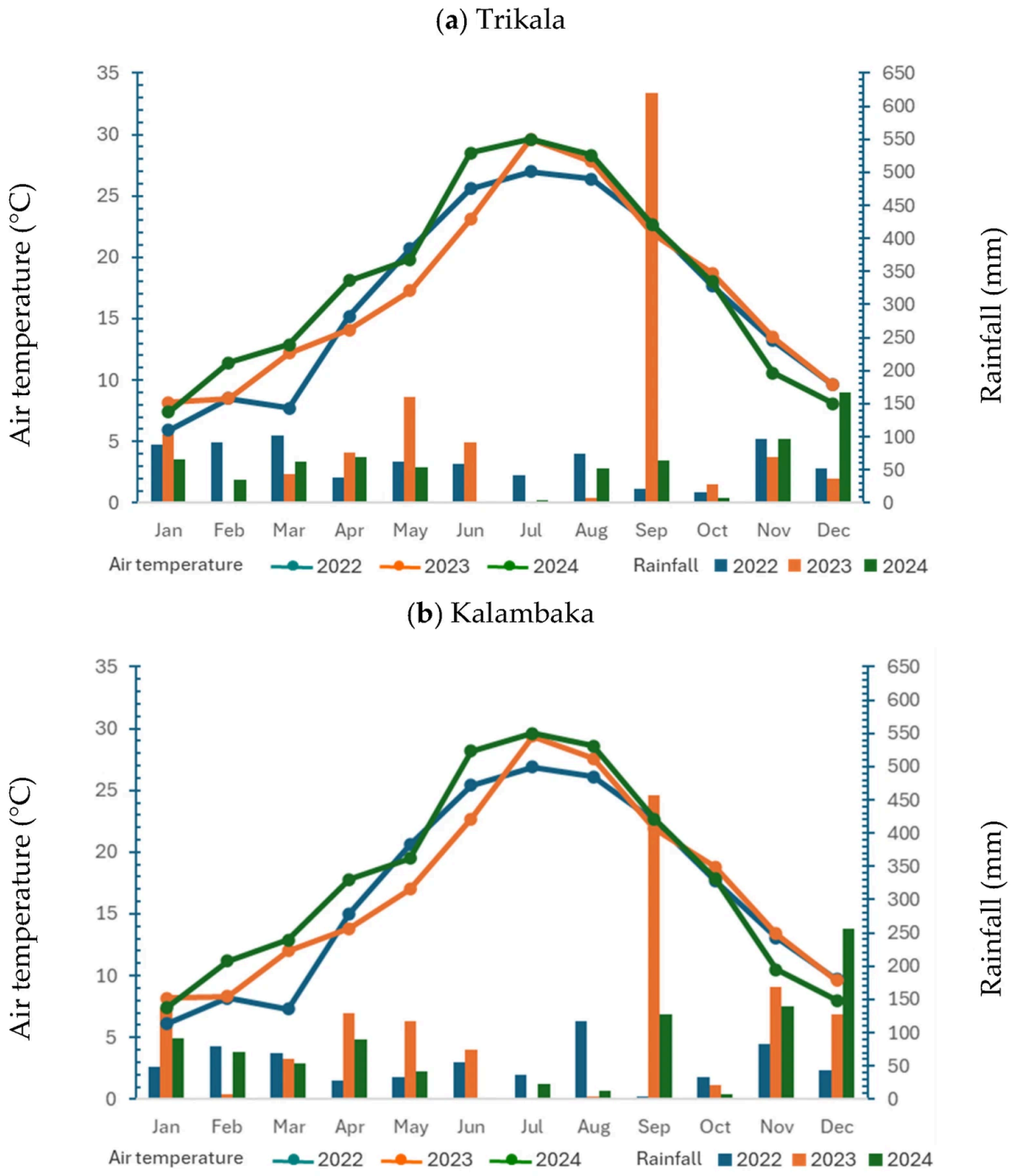


Figure 1. Meteorological data for (**a**) Trikala and (**b**) Kalambaka: average air temperature (°C) and total monthly precipitation (mm).

2.2. Genetic Material

The plant material consisted of two commercial lentil cultivars, Samos and Dimitra, developed by the Institute of Industrial and Forage Crops of ELGO-DIMITRA, along with 12 elite breeding lines (GVLen1–GVLen12). The breeding lines were developed from lentil seed material that had been cultivated by farmers over many years. Four cycles of modified mass selection were conducted, selecting individual plants exhibiting desirable agronomic characteristics. Subsequently, the selected material underwent pure-line selection using the head-to-line evaluation method, as described by Poehlman and Sleper [26].

2.3. Experimental Measurements

The following traits were evaluated: seed yield (kg ha^{-1}), thousand-seed weight (g), number of pods per plant, number of seeds per plant, and plant height (cm).

Seed yield was calculated by weighing the harvested seeds from each plot and expressing the value in kilograms per hectare.

Thousand-seed weight was determined by weighing a randomly selected sample of 1000 seeds.

The number of pods per plant was determined as the average number of mature, seed-bearing pods recorded on ten randomly selected and tagged plants.

The number of seeds per plant was determined by counting the seeds produced by ten randomly selected plants per genotype and calculating the average.

Plant height was recorded in centimeters from the soil surface to the apex of the plant using ten randomly selected and tagged plants.

In addition, seed quality traits—including crude protein content (%), crude fat content (%), ash content (%), total starch content (%), total dietary fiber (TDF) content (%), and moisture content (%)—were analyzed at the Laboratory of Animal Technology, University of Thessaly. Prior to analysis, all seed samples were ground into a fine powder.

The analyses were conducted following standard protocols established by the AOAC:

Crude protein content was estimated using AOAC Official Method 2001.11 [27] for total nitrogen determination. The nitrogen content was then multiplied by 6.25 to calculate the crude protein percentage.

Crude fat content was measured using the Soxhlet extraction method with petroleum ether in accordance with AOAC 920.39 [27].

Moisture content was determined via the AOAC Official Method 925.10 [27], which involves drying the sample to a constant weight. Moisture content was calculated from the loss in mass during drying and expressed as a percentage of the initial sample weight.

Ash content was calculated using AOAC 923.03 [27], a high-temperature incineration technique. Samples were incinerated in a furnace at 550 °C until a constant mass was obtained, then cooled in a desiccator and weighed at room temperature.

Total starch content was assessed using the enzymatic Megazyme Amyloglucosidase/α-Amylase method AOAC Official Method 996.11 [27], an enzymatic procedure using the Megazyme Amyloglucosidase/α-Amylase method (Megazyme International Ireland Ltd., Bray, Ireland). The procedure involves enzymatic hydrolysis of starch using thermostable α-amylase followed by amyloglucosidase, converting α-linked glucose polymers to glucose. The released glucose was quantified using a glucose oxidase–peroxidase (GOPOD) assay.

Total dietary fiber (TDF) content was determined according to AOAC 991.43 [27].

Seed samples from each plot were collected and analyzed separately. For each plot sample, three laboratory determinations were performed, and the mean of the three determinations was used for statistical analysis. Crude protein, crude fat, ash, total starch, and total dietary fiber contents were expressed on a dry matter basis, whereas moisture content was expressed as a percentage of the original sample weight.

2.4. Statistical Analysis

2.4.1. Analysis of Variance

A combined analysis of variance (ANOVA) across environments was conducted to assess differences among genotypes and environments and the genotype × environment (G × E) interaction for all evaluated traits. Statistical analyses were performed using IBM SPSS Statistics version 31 (IBM Corp., Armonk, NY, USA), with statistical significance considered at $p < 0.05$.

2.4.2. Genetic Parameters

Variance components, including those for genotype, genotype × environment interaction, error (e), and replications (r), were estimated from the corresponding mean squares following the procedure described by McIntosh [28]. These components were used to calculate the genetic parameters of the traits studied. Broad-sense heritability (H^2) was calculated using the formulae provided by Johnson et al. [29] and Hanson et al. [30]:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{g\times e}^2}{e} + \frac{\sigma_{re}^2}{r\times e}}$$

The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated for each trait following the method described by Singh and Chaudhary [31]:

$$GCV(\%) = \frac{\sqrt{\sigma_g^2}}{\overline{x}} \times 100$$

$$PCV(\%) = \frac{\sqrt{\sigma_p^2}}{\overline{x}} \times 100$$

where the genotypic variance, phenotypic variance, genotype × environment interaction variance, residual (error) variance, number of replications, number of environments, and overall mean for each trait are represented by σ_g^2, σ_p^2, $\sigma_{g\times e}^2$, σ_{re}^2, r, e, and $\overline{x}$, respectively.

Broad-sense heritability (H^2) was estimated on an entry-mean basis across the four environments.

2.4.3. Stability Index

The Stability Index (SI) was calculated using the following equation [13,14]:

$$SI = (\overline{x}/s)^2$$

where, $\overline{x}$ is the mean value of each trait measured for each genotype included in the study, and s is the corresponding standard deviation. Higher SI values indicate a combination of a higher trait mean and lower variability across environments. For each genotype, the mean and standard deviation used in the SI calculation were determined from its mean trait values across the four evaluated environments.

2.4.4. AMMI and GGE Biplot Analyses

Genotype × environment (G × E) interaction was investigated using the AMMI (Additive Main Effects and Multiplicative Interaction) and GGE (Genotype plus Genotype × Environment) biplot approaches [32]. The AMMI analysis combines the additive effects of genotype and environment obtained from analysis of variance with principal component analysis of the G × E interaction [33]. Singular value decomposition (SVD) was used to decompose the interaction component and characterize the patterns of differential genotype responses across environments [34]. Genotypes with IPCA scores close to zero were considered to have lower interaction with the environments and, consequently, greater stability.

The GGE biplot analysis was based on the genotype main effect and the G × E interaction after removing the environmental main effect [21,35]. The resulting biplots were used to visualize the relationships among genotypes and environments and to examine genotype performance and stability across the evaluated environments. The biplot approach provides a graphical representation of genotype and environment relationships in multi-environment data [36].

All AMMI and GGE biplot analyses were performed using PBTools version 1.4 (International Rice Research Institute, IRRI, Laguna, Philippines).

2.4.5. Correlation and Multivariate Analyses

Pearson's correlation coefficients are calculated to assess relationships among the studied traits ($p < 0.05$). To ensure maximum statistical power and capture the complete phenotypic variance, the correlation analysis was performed on the full raw dataset consisting of 224 observational rows (14 genotypes × 4 environments × 4 independent field replicates). To address the non-independence of data points sharing identical genetic or environmental backgrounds, the true sample size (n = 224) was explicitly declared, and statistical significance thresholds were strictly adjusted using the Benjamini–Hochberg False Discovery Rate (FDR) multiplicity correction.

Principal Component Analysis (PCA), Variable Clustering, Hierarchical Cluster Analysis (HCA), and constellation plots were used to explore multivariate patterns among genotypes and traits. PCA, Variable Clustering, and HCA were performed using the 56 unique genotype × environment combination mean values (14 genotypes × 4 environments). Input values are the arithmetic means of four field replicates per genotype-environment combination, standardized (mean = 0, SD = 1) before analysis. HCA was performed using Ward's minimum variance method and Euclidean distance, mapping the distinct environment-specific performance vectors for each genotype. Multi-response Partial Least Squares (PLS) regression using the SIMPLS algorithm was also performed to evaluate relationships between the studied traits and seed yield and thousand-seed weight. To prevent overfitting and ensure reproducible evaluation boundaries, a 7-fold cross-validation routine was applied internally within the platform. The optimal number of factors was selected by analyzing up to an initial number of 7 factors, using the van der Voet T^2 significance test. All correlation and multivariate analyses were performed using JMP Pro 18 (SAS Institute, Cary, NC, USA).

3. Results

The results of the present study are presented with emphasis on genotype performance, trait variation, and stability across the four evaluated environments. The results of the combined analysis of variance, genetic parameters, stability indices, and multivariate analyses characterize the evaluated lentil genotypes and their responses across environments.

3.1. Analysis of Variance and Genetic Parameters

Table 2 presents the mean squares from the combined analysis of variance across environments for all traits evaluated. Environment, genotype, and genotype × environment interaction effects were highly significant ($p \leq 0.01$) for all traits. Replications within the environment were significant for seed yield, number of pods per plant, number of seeds per plant, plant height, total starch content, and total dietary fiber content, whereas no significant differences were detected for thousand-seed weight, protein, fat, ash, or moisture content.

Table 3 presents the genetic parameter estimates for the evaluated traits across environments. Genotypic variance (σ_g^2) ranged from 0.034 for fat content to 50,899.696 for seed yield, whereas phenotypic variance (σ_p^2) ranged from 0.034 to 54,772.107, respectively. For all traits, phenotypic variance was equal to or slightly higher than genotypic variance, although the differences between the two components were relatively small. The GCV values ranged from 1.584% for total starch content to 22.441% for thousand-seed weight, while the corresponding PCV values ranged from 1.601% to 22.872%. Broad-sense heritability estimates were high for all traits, ranging from 92.93% for seed yield to 99.82% for fat content.

Table 2. Mean squares (m.s.) from the combined analysis of variance across environments for seed yield (kg ha^{-1}), thousand-seed weight (g), number of pods per plant, number of seeds per plant, plant height (cm), crude protein content (%), crude fat content (%), ash content (%), total starch content (%), total dietary fiber content (%), and moisture content (%).

Source of Variation	Seed Yield m.s.	1000-Seed Weight m.s.	Pods per Plant m.s.	Seeds per Plant m.s.	Plant Height m.s.	Protein m.s.	Fat m.s.	Ash m.s.	Starch m.s.	Dietary Fiber m.s.	Moisture m.s.
Environment (E)	62,719.564 **	170.990 **	56.258 **	31.049 **	52.571 **	29.215 **	0.047 **	0.477 **	37.157 **	0.525 **	3.636 **
REPS/Environments	34,204.112 **	3.496 ns	16.969 **	31.456 **	2.868 *	0.005 ns	0.00002 ns	0.00042 ns	0.186 **	0.001 *	0.001 ns
Genotype (G)	876,353.735 **	1510.096 **	224.072 **	429.009 **	170.977 **	25.858 **	0.545 **	1.921 **	12.237 **	8.813 **	4.381 **
Environment × Genotype (E × G)	61,954.224 **	56.387 **	9.250 **	28.679 **	2.221 **	0.862 **	0.001 **	0.039 **	0.249 **	0.090 **	0.207 **
Error	413.589	2.384	1.021	0.993	1.373	0.004	0.00001	0.00023	0.012	0.00036	0.001

Probability values: * $p \leq 0.05$; ** $p \leq 0.01$; ns—not significant.

Table 3. Genetic parameter estimates for the tested traits: seed yield (kg ha^{-1}), thousand-seed weight (g), number of pods per plant, number of seeds per plant, plant height (cm), crude protein content (%), crude fat content (%), ash content (%), total starch content (%), total dietary fiber content (%), and moisture content (%).

Traits	Min.	Max.	Mean	sd	σ_g^2	σ_p^2	GCV (%)	PCV (%)	H² (%)
Seed Yield	1338.00	2437.00	1810.13	254.75	50,899.696	54,772.107	12.464	12.929	92.93
1000-Seed Weight	23.49	64.54	42.48	10.10	90.857	94.381	22.441	22.872	96.27
Pods per Plant	25.74	46.10	35.74	4.13	13.426	14.005	10.251	10.470	95.87
Seeds per Plant	38.76	64.61	52.62	5.73	25.021	26.813	9.506	9.841	93.32
Plant Height	26.12	43.22	35.40	3.49	10.547	10.686	9.174	9.234	98.70
Protein	22.20	28.82	25.66	1.43	1.562	1.616	4.871	4.954	96.67
Fat	0.67	1.36	0.98	0.18	0.034	0.034	18.773	18.790	99.82
Ash	2.97	4.48	3.86	0.35	0.118	0.120	8.874	8.966	97.97
Starch	51.68	56.69	54.64	1.13	0.749	0.765	1.584	1.601	97.96
Dietary Fiber	3.42	6.56	5.13	0.73	0.545	0.551	14.386	14.460	98.98
Moisture	7.78	10.63	9.68	0.58	0.261	0.274	5.276	5.405	95.28

sd—standard deviation, σ_g^2—genotypic variance, σ_p^2—phenotypic variance, GCV—genotypic coefficient of variation, PCV—phenotypic coefficient of variation, H²—broad-sense heritability (%).

3.2. Stability Index

Table 4 presents the Stability Index (SI) values calculated for each genotype and trait across the four evaluated environments. For seed yield, SI values ranged from 127 for G3 (GVLen3) to 471 for G11 (GVLen11). Among the yield-related and morphological traits, the highest SI values were observed for seeds per plant in G6 (GVLen6; 647), plant height in G8 (GVLen8; 685), and pods per plant in G10 (GVLen10; 461). For thousand-seed weight, SI values ranged from 44 for G7 (GVLen7) to 288 for G2 (GVLen2). Regarding seed quality traits, the highest SI values were recorded for protein content in G6 (GVLen6; 2004), fat content in G10 (GVLen10; 1233), ash content in G2 (GVLen2; 1290), total dietary fiber content in G2 (GVLen2; 1528), and moisture content in G10 (GVLen10; 1374).

3.3. AMMI and GGE Biplot Analyses

3.3.1. Seed Yield

For seed yield (Figure 2), PC1 of the AMMI analysis accounted for 41.6% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 92.0% of the G + GE variation (PC1 = 85.1% and PC2 = 6.9%). G12 (GVLen12), G11 (GVLen11), and G4 (GVLen4) showed the highest seed yield, whereas G9 (GVLen9), G6 (GVLen6), and G1 (GVLen1) showed lower performance; G5 (GVLen5), G3 (GVLen3), and G7 (GVLen7) had relatively small IPCA scores, while G12 (GVLen12) and G11 (GVLen11) showed larger absolute IPCA scores, indicating greater contributions to the G × E

interaction. E1 (Trikala 2022/2023) and E4 (Kalambaka 2023/2024) were positioned farther from the average and ideal environment, whereas E2 (Trikala 2023/2024) and E3 (Kalambaka 2022/2023) were located closer to these positions. In the GGE biplot, G11 (GVLen11) was closest to the ideal genotype, followed by G12 (GVLen12), while the which-won-where biplot associated G11 (GVLen11), G3 (GVLen3), G4 (GVLen4), and G5 (GVLen5) with E1 (Trikala 2022/2023) and E3 (Kalambaka 2022/2023), and G12 (GVLen12) with E2 (Trikala 2023/2024) and E4 (Kalambaka 2023/2024).

Table 4. Stability index across environments for the tested traits: seed yield (kg ha^{-1}), thousand-seed weight (g), number of pods per plant, number of seeds per plant, plant height (cm), crude protein content (%), crude fat content (%), ash content (%), total starch content (%), total dietary fiber content (%), and moisture content (%).

Genotypes	Seed Yield	1000-Seed Weight	Pods per Plant	Seeds per Plant	Plant Height	Protein	Fat	Ash	Starch	Dietary Fiber	Moisture
GVLen1	179	124	206	376	646	1023	1035	1085	5063	1027	1045
GVLen2	207	288	256	169	564	1466	1094	1290	5113	1528	900
GVLen3	127	167	411	483	498	1015	1005	1003	5941	1004	1062
GVLen4	246	233	315	552	603	1007	1223	1003	4948	1116	1020
GVLen5	418	85	249	609	643	1002	1029	1011	4582	1018	1100
GVLen6	228	60	350	647	493	2004	1074	1017	4863	1026	1021
GVLen7	364	44	421	265	608	1254	1034	1031	4044	1238	1045
GVLen8	201	111	350	240	685	1005	1192	1005	5228	1020	1010
GVLen9	308	83	440	611	479	1101	1064	1037	5101	1056	1018
GVLen10	246	87	461	332	422	1058	1233	1032	5415	1331	1374
GVLen11	471	186	436	266	522	1109	1018	1001	5257	1010	1117
GVLen12	242	168	270	613	429	1202	1077	1269	4968	1037	1007
Samos	132	189	196	199	537	1132	1028	1126	4795	1079	925
Dimitra	191	77	174	146	501	1011	1014	1005	5205	1036	916

3.3.2. Thousand-Seed Weight

For thousand-seed weight (Figure S1), PC1 of the AMMI analysis accounted for 62.8% of the G × E interaction variation, while the first two principal components of the GGE biplot explained 96.3% of the G + GE variation (PC1 = 90.1% and PC2 = 6.2%). G4 (GVLen4), G2 (GVLen2), G3 (GVLen3), and G1 (GVLen1) recorded the highest thousand-seed weight, whereas G7 (GVLen7), G6 (GVLen6), and G5 (GVLen5) showed lower values; G5 (GVLen5), G3 (GVLen3), and G7 (GVLen7) had relatively small IPCA scores. All environments were positioned within the concentric area associated with the average and ideal environment. In the GGE biplot, G4 (GVLen4), G2 (GVLen2), and G3 (GVLen3) were positioned close to the average and ideal genotype, while the which-won-where biplot showed the relative positions of G1 (GVLen1), G2 (GVLen2), G3 (GVLen3), and G4 (GVLen4) in relation to the four evaluated environments.

3.3.3. Number of Pods per Plant

For the number of pods per plant (Figure S2), PC1 of the AMMI analysis accounted for 44.0% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 93.9% of the G + GE variation (PC1 = 89.1% and PC2 = 4.8%). G9 (GVLen9) and G6 (GVLen6) recorded the highest number of pods per plant, whereas G14 (Dimitra) and G2 (GVLen2) showed lower values; G13 (Samos), G10 (GVLen10), and G5 (GVLen5) showed relatively small IPCA scores. E1 (Trikala 2022/2023), E2 (Trikala 2023/2024), and E3 (Kalambaka 2022/2023) were positioned within the concentric area associated with the average and ideal environment, whereas E4 (Kalambaka 2023/2024) was located farther from these positions. In the GGE biplot, G9 (GVLen9) and G6 (GVLen6) were positioned close to the average and ideal genotype, while the which-won-where biplot

showed the relative positions of G9 (GVLen9), G12 (GVLen12), and G6 (GVLen6) in relation to the four evaluated environments.

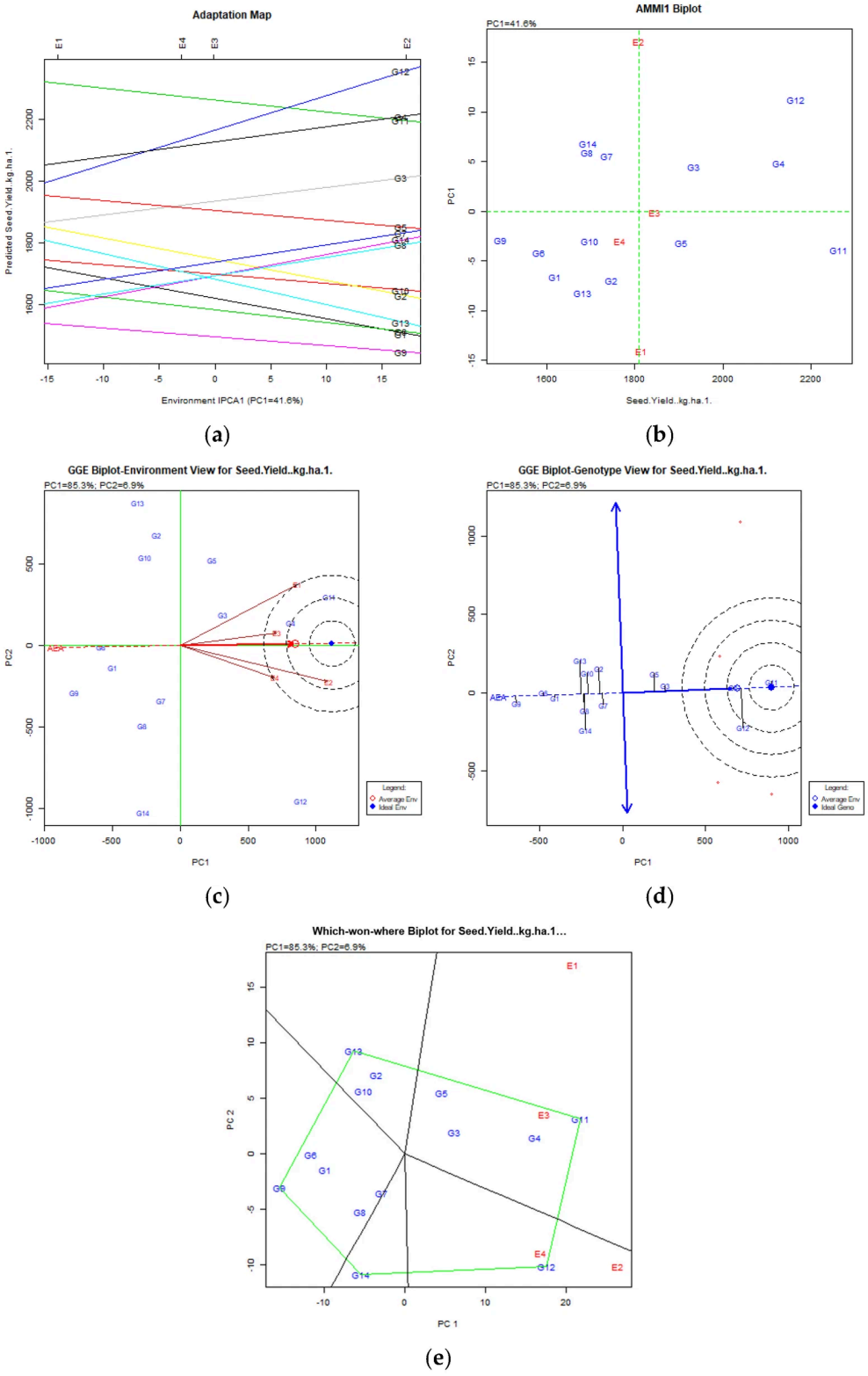


Figure 2. Stability analysis of seed yield (kg ha^{-1}) in lentil genotypes using (**a**) the AMMI adaptation map, (**b**) the AMMI1 biplot, (**c**) the GGE biplot for evaluation of environmental stability, (**d**) the GGE biplot for assessment of genotypic stability, and (**e**) the "which-won-where" GGE biplot illustrating differential genotype responses across environments. In Figure 2d, the red symbols represent the test environments, while the blue symbols represent the lentil genotypes.

3.3.4. Number of Seeds per Plant

For the number of seeds per plant (Figure S3), PC1 of the AMMI analysis accounted for 51.4% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 92.0% of the G + GE variation (PC1 = 85.1% and PC2 = 6.9%). G6 (GVLen6), G5 (GVLen5), G9 (GVLen9), and G12 (GVLen12) recorded the highest number of seeds per plant, whereas G14 (Dimitra) and G2 (GVLen2) showed lower values; G7 (GVLen7), G3 (GVLen3), G4 (GVLen4), and G12 (GVLen12) showed relatively small variation along the IPCA axis. E4 (Kalambaka 2023/2024), E2 (Trikala 2023/2024), and E3 (Kalambaka 2022/2023) were positioned within the concentric area associated with the average and ideal environment, whereas E1 (Trikala 2022/2023) was located farther from these positions. In the GGE biplot, G5 (GVLen5), G6 (GVLen6), G12 (GVLen12), and G9 (GVLen9) were positioned close to the average and ideal genotype, while the which-won-where biplot showed the associations of G5 (GVLen5), G12 (GVLen12), G9 (GVLen9), and G10 (GVLen10) with E2 (Trikala 2023/2024), E3 (Kalambaka 2022/2023), and E4 (Kalambaka 2023/2024), whereas G6 (GVLen6) and G3 (GVLen3) were associated with E1 (Trikala 2022/2023).

3.3.5. Plant Height

For plant height (Figure S4), PC1 of the AMMI analysis accounted for 46.1% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 98.2% of the G + GE variation (PC1 = 96.5% and PC2 = 1.7%). G5 (GVLen5), G2 (GVLen2), G1 (GVLen1), and G12 (GVLen12) recorded the highest plant height, whereas G11 (GVLen11) and G10 (GVLen10) showed lower values; G6 (GVLen6), G7 (GVLen7), and G14 (Dimitra) showed relatively small variation along the IPCA axis. All environments were positioned within the concentric area associated with the average and ideal environment. In the GGE biplot, G5 (GVLen5), G2 (GVLen2), G1 (GVLen1), and G12 (GVLen12) were positioned close to the average and ideal genotype, while the which-won-where biplot showed the relative positions of G1 (GVLen1), G5 (GVLen5), G3 (GVLen3), G13 (Samos), and G12 (GVLen12) in relation to the four evaluated environments.

3.3.6. Crude Protein Content

For crude protein content (Figure 3), PC1 of the AMMI analysis accounted for 66.8% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 97.0% of the G + GE variation (PC1 = 91.0% and PC2 = 6.0%). G8 (GVLen8), G2 (GVLen2), G6 (GVLen6), G12 (GVLen12), and G13 (Samos) recorded the highest crude protein content, whereas G4 (GVLen4) showed lower values; G5 (GVLen5) and G14 (Dimitra) showed relatively small variation along the IPCA axis. E1 (Trikala 2022/2023), E2 (Trikala 2023/2024), and E3 (Kalambaka 2022/2023) were positioned within the concentric area associated with the average and ideal environment, whereas E4 (Kalambaka 2023/2024) was located farther from these positions. In the GGE biplot, G8 (GVLen8), G2 (GVLen2), G6 (GVLen6), G12 (GVLen12), and G13 (Samos) were positioned close to the average and ideal genotype, while the which-won-where biplot showed the relative positions of these genotypes in relation to the four evaluated environments.

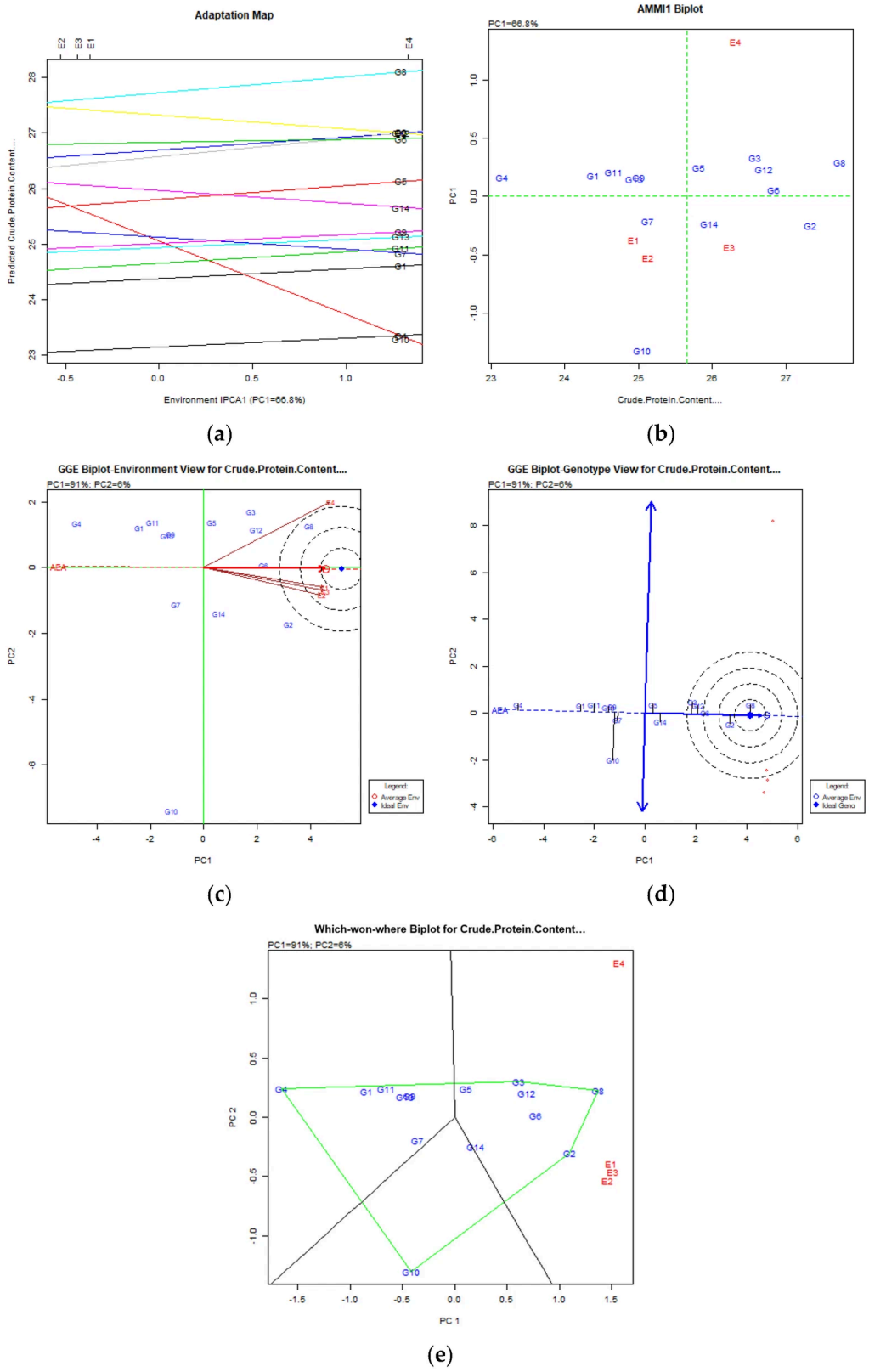


Figure 3. Stability analysis of crude protein content (%) in lentil genotypes using (**a**) the AMMI adaptation map, (**b**) the AMMI1 biplot, (**c**) the GGE biplot for evaluation of environmental stability, (**d**) the GGE biplot for assessment of genotypic stability, and (**e**) the "which-won-where" GGE biplot illustrating differential genotype responses across environments.

3.3.7. Crude Fat Content

For crude fat content (Figure S5), PC1 of the AMMI analysis accounted for 55.9% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 99.8% of the G + GE variation (PC1 = 99.4% and PC2 = 0.4%). G14 (Dimitra), G13 (Samos), G8 (GVLen8), and G6 (GVLen6) recorded the highest crude fat content, whereas G12 (GVLen12), G11 (GVLen11), G1 (GVLen1), and G10 (GVLen10) showed lower values; G4 (GVLen4), G5 (GVLen5), G12 (GVLen12), and G9 (GVLen9) showed relatively small variation along the IPCA axis. All environments were positioned within the concentric area associated with the average and ideal environment. In the GGE biplot, G14 (Dimitra), G13 (Samos), G8 (GVLen8), and G6 (GVLen6) were positioned close to the average and ideal genotype, while the which-won-where biplot showed the relative positions of G7 (GVLen7), G8 (GVLen8), G14 (Dimitra), G13 (Samos), and G6 (GVLen6) in relation to the four evaluated environments.

3.3.8. Ash Content

For ash content (Figure S6), PC1 of the AMMI analysis accounted for 64.1% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 99.8% of the G + GE variation (PC1 = 94.5% and PC2 = 3.5%). G8 (GVLen8), G2 (GVLen2), and G9 (GVLen9) recorded the highest ash content, whereas G3 (GVLen3), G13 (Samos), and G10 (GVLen10) showed lower values; G7 (GVLen7), G11 (GVLen11), and G12 (GVLen12) showed relatively small variation along the IPCA axis. All environments were positioned within the concentric area associated with the average and ideal environment. In the GGE biplot, G8 (GVLen8), G2 (GVLen2), and G9 (GVLen9) were positioned close to the average and ideal genotype, while the which-won-where biplot showed the relative positions of G9 (GVLen9), G8 (GVLen8), G6 (GVLen6), and G5 (GVLen5) in relation to the four evaluated environments.

3.3.9. Total Starch Content

For total starch content (Figure S7), PC1 of the AMMI analysis accounted for 71.0% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 98.5% of the G + GE variation (PC1 = 94.6% and PC2 = 3.9%). G10 (GVLen10), G5 (GVLen5), G2 (GVLen2), and G6 (GVLen6) showed the highest total starch content, whereas G13 (Samos) showed lower values; G1 (GVLen1), G14 (Dimitra), and G7 (GVLen7) showed relatively small variation along the IPCA axis. All environments were positioned within the concentric area associated with the average and ideal environment. In the GGE biplot, G10 (GVLen10), G5 (GVLen5), G2 (GVLen2), and G6 (GVLen6) were positioned close to the average and ideal genotype, while the which-won-where biplot showed the relative positions of G10 (GVLen10), G2 (GVLen2), G5 (GVLen5), G9 (GVLen9), and G7 (GVLen7) in relation to the four evaluated environments.

3.3.10. Total Dietary Fiber Content

For total dietary fiber content (Figure S8), PC1 of the AMMI analysis accounted for 62.2% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 99.1% of the G + GE variation (PC1 = 98.0% and PC2 = 1.1%). G13 (Samos), G12 (GVLen12), G8 (GVLen8), and G10 (GVLen10) recorded the highest total dietary fiber content, whereas G14 (Dimitra) showed lower values; G1 (GVLen1), G8 (GVLen8), G7 (GVLen7), and G3 (GVLen3) showed relatively small variation along the IPCA axis. All environments were positioned within the concentric area associated with the average and ideal environment. In the GGE biplot, G13 (Samos), G12 (GVLen12), G8 (GVLen8), and G10 (GVLen10) were positioned close to the average and ideal genotype,

while the which-won-where biplot showed the relative positions of G13 (Samos), G12 (GVLen12), G8 (GVLen8), G10 (GVLen10), and G4 (GVLen4) in relation to the four evaluated environments.

3.3.11. Moisture Content

For moisture content (Figure S9), PC1 of the AMMI analysis accounted for 61.3% of the G × E interaction variation, whereas the first two principal components of the GGE biplot explained 95.6% of the G + GE variation (PC1 = 88.0% and PC2 = 7.6%). G13 (Samos), G3 (GVLen3), G11 (GVLen11), G5 (GVLen5), and G12 (GVLen12) recorded the highest moisture content, whereas G4 (GVLen4) showed lower values; G14 (Dimitra), G2 (GVLen2), G9 (GVLen9), and G1 (GVLen1) showed relatively small variation along the IPCA axis. E1 (Trikala 2022/2023) and E3 (Kalambaka 2022/2023) were positioned within the concentric area associated with the average and ideal environment, whereas E2 (Trikala 2023/2024) and E4 (Kalambaka 2023/2024) were located outside this area. In the GGE biplot, G13 (Samos), G3 (GVLen3), G11 (GVLen11), G5 (GVLen5), and G12 (GVLen12) were positioned close to the average and ideal genotype, while the which-won-where biplot associated G3 (GVLen3), G10 (GVLen10), G5 (GVLen5), and G11 (GVLen11) with E3 (Kalambaka 2022/2023), E1 (Trikala 2022/2023), and E2 (Trikala 2023/2024), whereas G13 (Samos) was associated with E4 (Kalambaka 2023/2024).

3.4. Correlation and Multivariate Analyses

3.4.1. Correlation Analysis

Table S1 and Figure 4 present the correlations calculated across all $n = 224$ individual experimental observations. Seed yield was positively correlated with thousand-seed weight ($r = 0.41$, $p < 0.05$) and negatively correlated with fat content ($r = -0.44$, $p < 0.05$) and total starch content ($r = -0.18$). Due to the expanded sample size of the raw data matrix ($n = 224$), the weak negative correlation observed between total starch content and seed yield ($r = -0.18$) was determined to be statistically significant ($p < 0.01$) even after conservative FDR multiplicity adjustments.

Total starch content was negatively correlated with thousand-seed weight ($r = -0.23$, $p < 0.01$) and positively correlated with protein content ($r = 0.41$, $p < 0.05$). Fat content was negatively correlated with thousand-seed weight ($r = -0.36$) and positively correlated with plant height and protein content ($r = 0.19$ and 0.23, respectively). Total dietary fiber content was negatively correlated with ash and total starch content ($r = -0.25$ and -0.25, respectively). Moisture content was negatively correlated with thousand-seed weight and ash content ($r = -0.25$ and -0.35, respectively).

3.4.2. Principal Component Analysis (PCA) and Variable Clustering Analysis

Principal component analysis (PCA) showed that the first two components explained 21.04% and 18.14% of the total variation, respectively, accounting for 39.18% of the cumulative variation (Table S2). Although definitive stability conclusions cannot be drawn based on these first two parameters alone, extending the analysis to include additional higher-dimensional principal components allows for more reliable insights about the multi-trait performance profile and environmental adaptation of individual genotypes. By expanding the model to encompass five principal components with eigenvalues > 1, the cumulative explained variance reaches 77.83% (PC3 = 15.0%, PC4 = 12.4%, PC5 = 11.2%). This multi-component matrix space (Figure 5b, Table S2) captures the greater part of the experimental information, providing a robust, scientifically valid baseline to support conclusions about genotype-specific phenotypic stability and macro-environmental adaptation patterns across the evaluated traits.

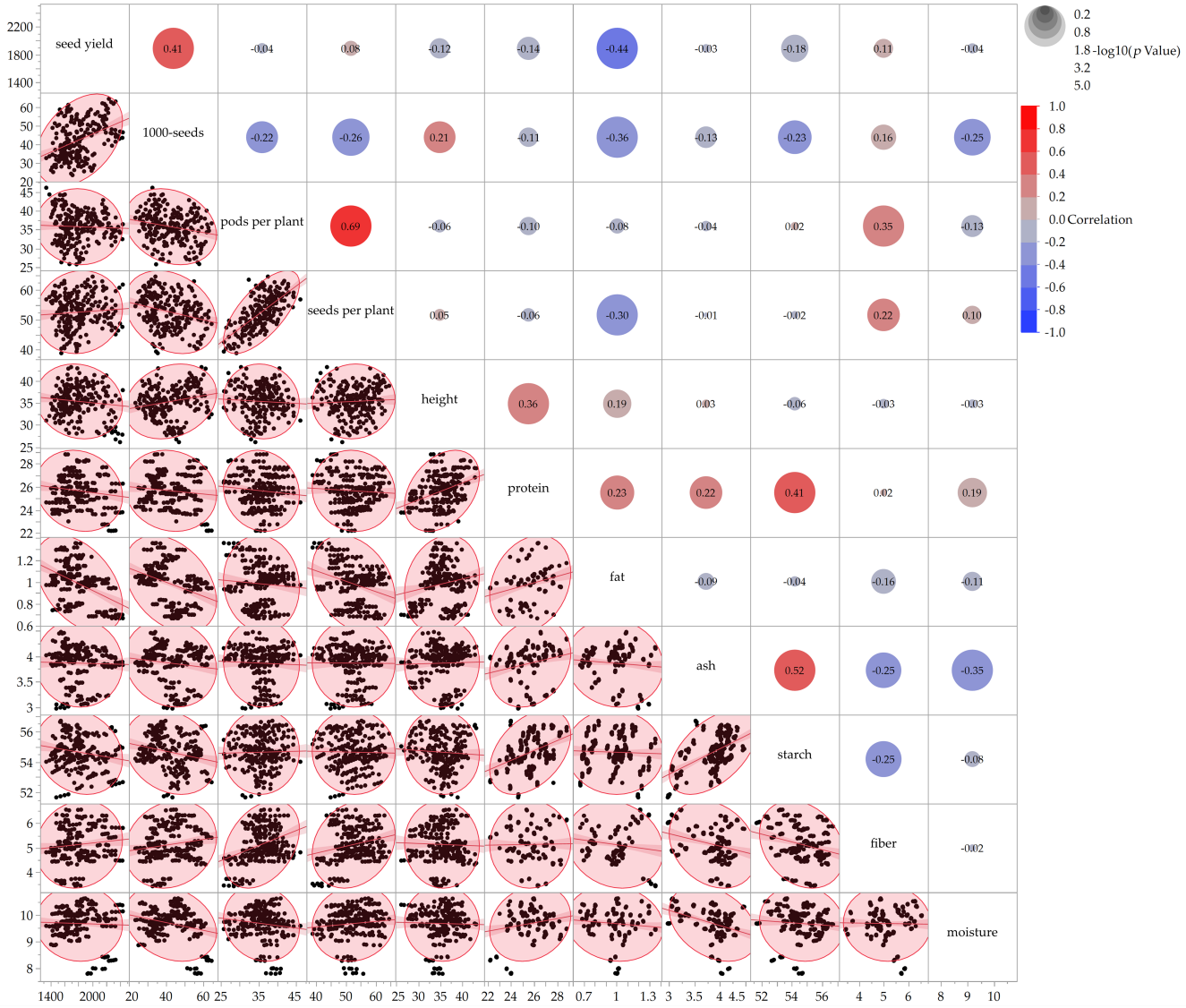


Figure 4. Multivariate correlation matrix among the 11 agronomic and seed quality traits. The lower triangle shows scatterplots with 95% bivariate normal density ellipses, while the upper triangle presents Pearson correlation coefficients (r). Red and blue indicate positive and negative correlations, respectively, with color intensity representing correlation strength. Circle size represents the statistical significance of the correlations.

The genotype score plot showed a broad distribution of observations across the PC1–PC2 space, with differences in the positioning of genotypes, which were further resolved across the higher-dimensional axes (PC3-PC3). Observations of G2 (GVLen2) and G14 (Dimitra) were predominantly located on the positive side of PC1 and negative side of PC2, whereas observations of G6 (GVLen6) and G9 (GVLen9) were mainly positioned in the upper part of the plot. G4 (GVLen4) observations were predominantly located in the lower-left region, while G3 (GVLen3) observations were mainly distributed toward the negative side of PC1. Beyond the first two dimensions, the remaining principal components revealed distinct spatial clusters. For instance, genotypes of group G4 separated prominently along the negative axis of Components 3 and 5. Furthermore, genotypes that overlapped in the first two dimensions showed clear segregation when evaluated across Components 3, 4, and 5.

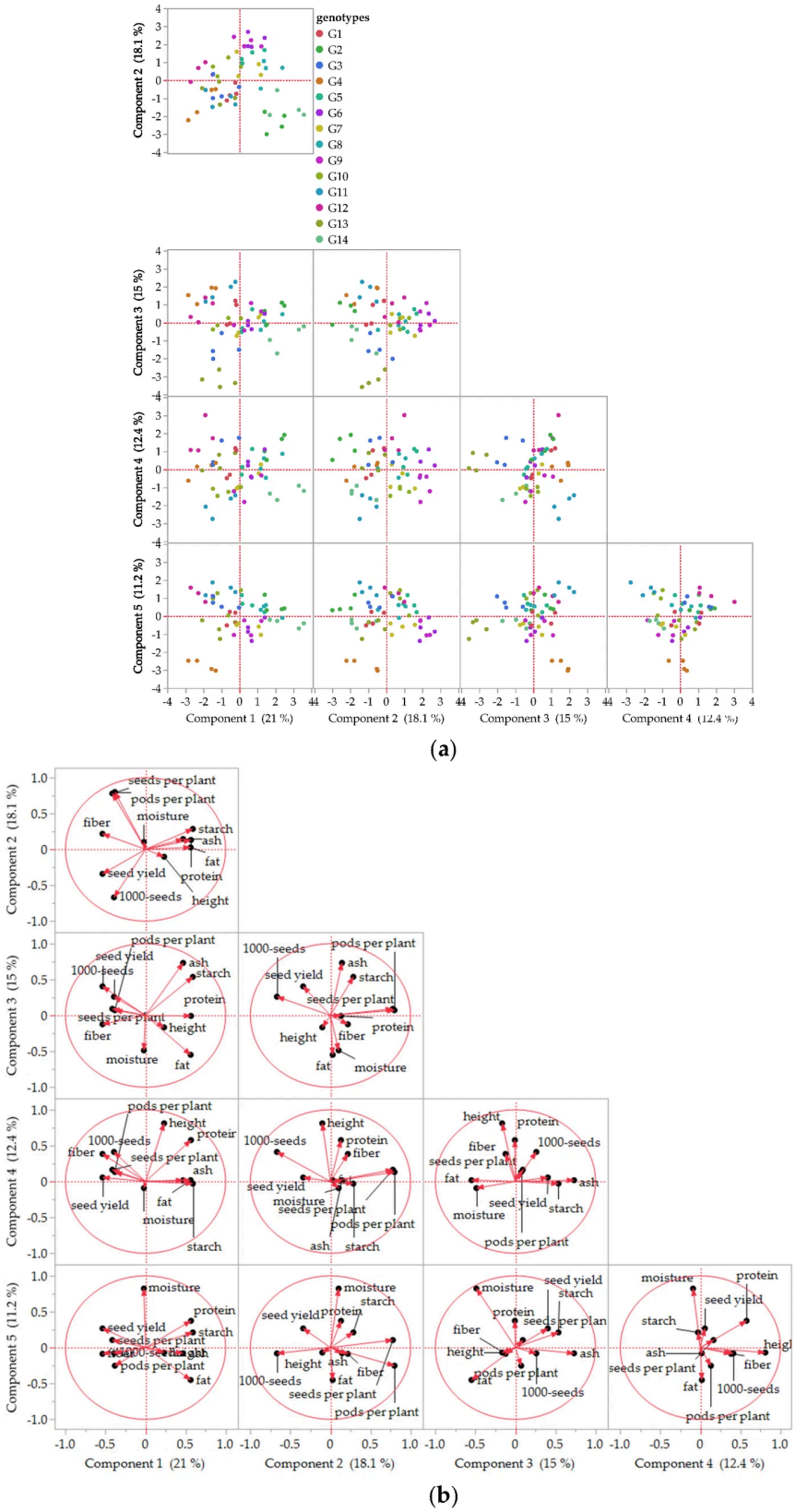


Figure 5. Principal component analysis (PCA) of the 14 genotypes based on 11 agronomic and seed quality traits: (**a**) score plot and (**b**) variable loading plot matrix: the matrix displays the pairwise projections across all five extracted principal components with eigenvalues > 1 (PC1 to PC5). Individual data points represent the 56 distinct Genotype × Environment combination means.

The loading plot showed positive loadings of pods per plant and seeds per plant on PC2, whereas seed yield and thousand-seed weight were oriented toward the negative side of PC2. Fat was positioned toward the positive side of PC1 and slightly below the PC2 = 0 axis, while starch, ash, and protein were associated with the positive side of PC1. Fiber was positioned on the negative side of PC1 and the positive side of PC2, whereas moisture was located close to the origin (Figure 5).

Variable Clustering grouped the 11 agronomic and seed quality traits into five clusters, accounting for 68.4% of the variation explained by the clustering solution (Table S3). Cluster 1 included seed yield, thousand-seed weight, and fat, whereas Cluster 2 comprised pods per plant, seeds per plant, and fiber. Cluster 3 included protein, ash, and starch, while moisture and plant height formed individual clusters (Tables S3 and S4). The clustering structure was consistent with the correlation patterns among the traits. Seed yield and thousand-seed weight were positively correlated ($r = 0.41$), whereas seed yield and fat content showed a negative correlation ($r = -0.44$). Pods per plant and seeds per plant showed a strong positive correlation ($r = 0.69$), while their correlations with seed yield were weak ($r = -0.04$ and $r = 0.08$, respectively). The grouping of traits therefore reflected their observed correlation structure across the evaluated genotypes.

3.4.3. Hierarchical Cluster Analysis

The hierarchical clustering analysis (Figure 6) separated the 14 genotypes into six distinct groups based on their multivariate profiles across the 11 traits. G1 (GVLen1), G2 (GVLen2) and G14 (Dimitra) formed one group, while G5 (GVLen5), G6 (GVLen6), G9 (GVLen9), G7 (GVLen7) and G8 (GVLen8) formed a second group. G3 (GVLen3) and G13 (Samos) were grouped together, whereas G12 (GVLen12) and G4 (GVLen4) formed separate groups, and G10 (GVLen10) and G11 (GVLen11) constituted another group. The heatmap showed similar patterns of trait values among genotypes within the respective clusters. At the trait level, seed yield and thousand-seed weight formed a closely associated pair, while pods per plant and seeds per plant were also closely clustered. The remaining traits were progressively joined to these groups at greater hierarchical distances, indicating different levels of similarity among the traits.

3.4.4. Partial Least Squares Analysis

The multi-response Partial Least Squares (PLS) regression was performed using the SIMPLS algorithm, with seed yield and thousand-seed weight as response variables. Block cross-validation with a K-Fold configuration (7-Fold) identified a 4-factor model as the optimal configuration, corresponding to the minimum Root Mean PRESS of 0.496768 (Figure 7; Table S5). The selected model explained 30.39% of the variance in the X predictors and 82.55% of the variance in the Y responses (Tables S5 and S6). The first factor accounted for 54.65% of the variance in the responses, while factors 2–4 contributed cumulatively to the remaining explained variation (19.78%, 5.94%, and 2.17%, respectively). The VIP analysis identified four of the 11 X predictors with VIP values above the 0.8 reference threshold: fat, starch, number of seeds per plant, and number of pods per plant (Figure 8). Fat had the highest VIP value (approximately 1.75), followed by starch (which also exceeded 1.0), whereas seeds per plant and pods per plant had VIP values below 1.0, but above the 0.8 baseline threshold. Plant height, crude protein, ash, dietary fiber, moisture, environment, and the broader genotype category average fell below the 0.8 reference threshold.

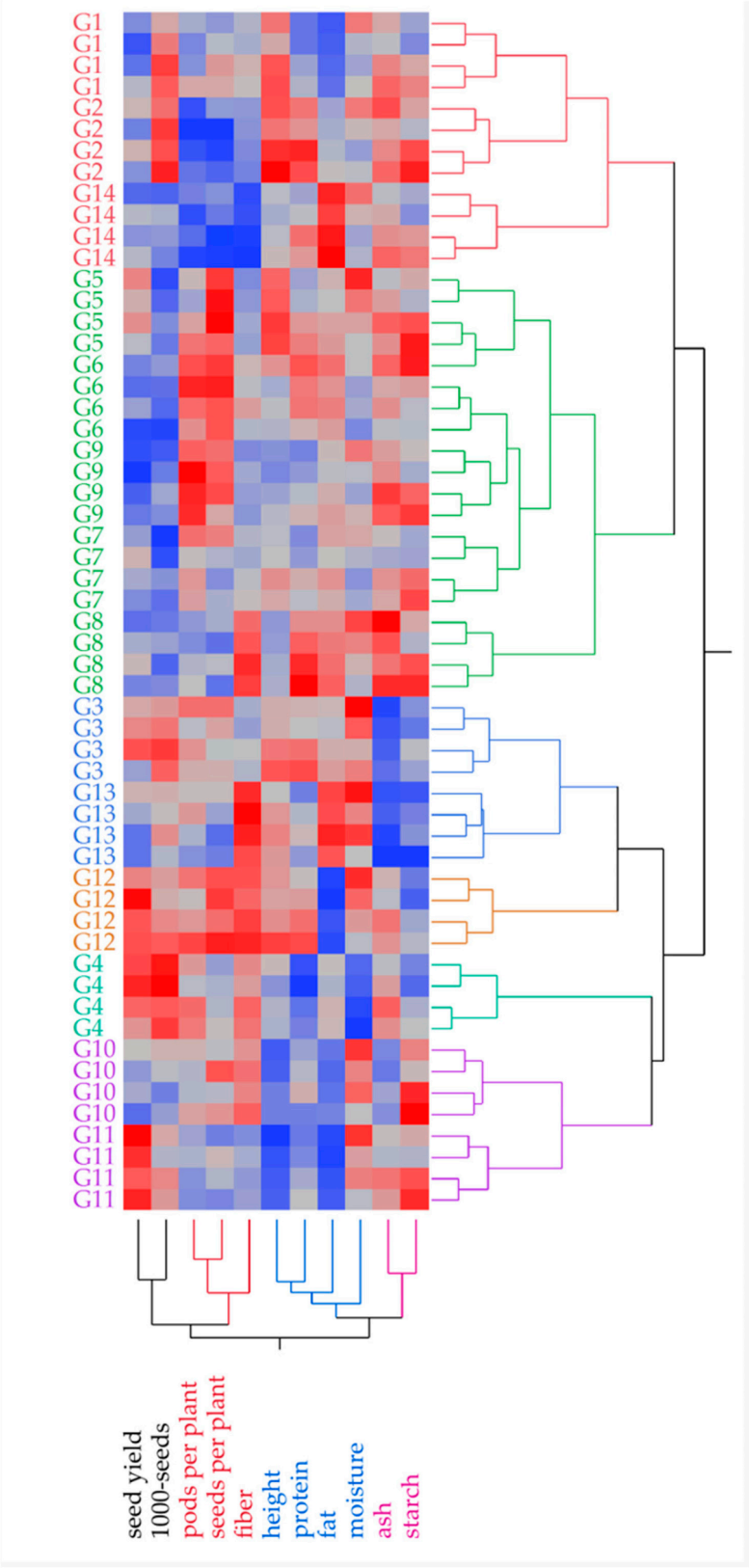


Figure 6. Two-way hierarchical clustering dendrogram and heatmap of the 14 genotypes and 11 agronomic and seed quality traits. Colors represent standardized values (red = higher values, grey= medium values, blue = lower values).

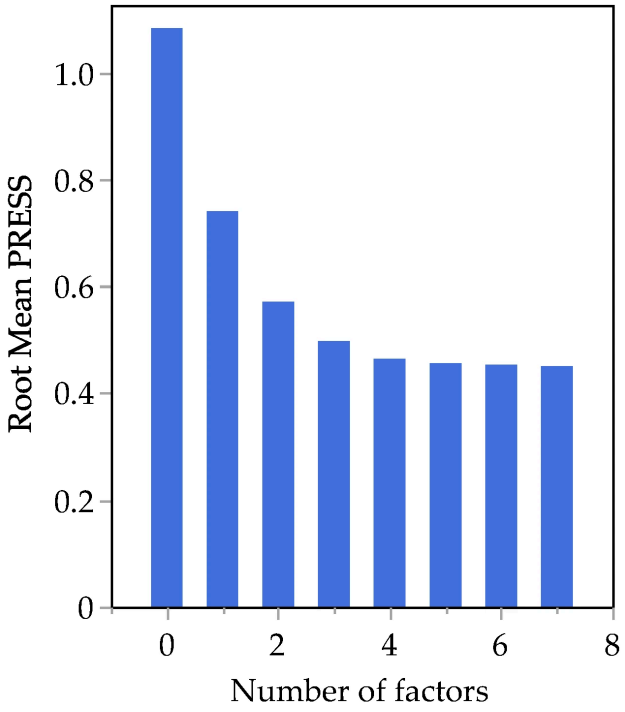


Figure 7. Root Mean PRESS (Predicted Residual Sum of Squares) values across different numbers of extracted PLS factors. Lower values indicate lower cross-validated prediction error.

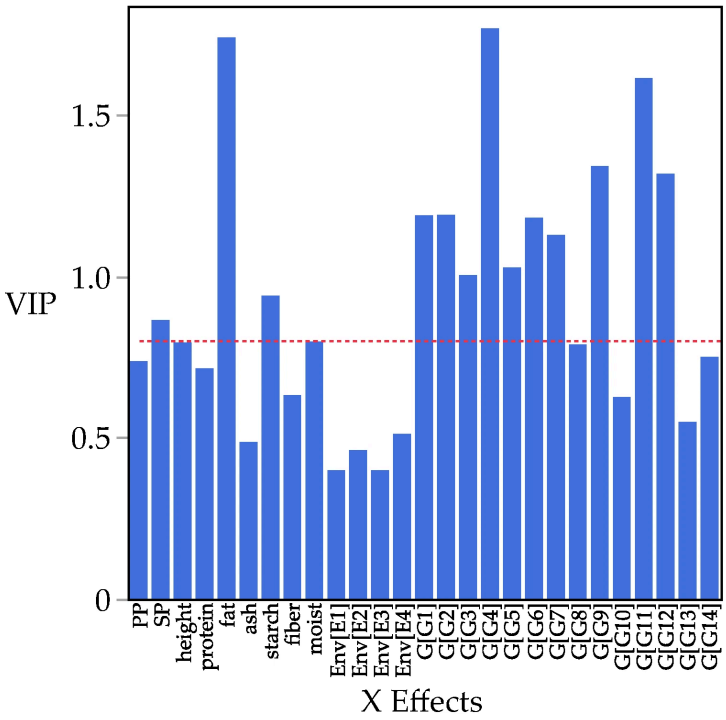


Figure 8. Variable Importance in Projection (VIP) scores for the 11 predictors in the multi-response SIMPLS model. The vertical bar chart shows the relative predictive importance of each predictor for the simultaneous estimation of seed yield and thousand-seed weight. The horizontal dashed line indicates the VIP reference threshold of 0.8 (number of pods per plant (PP), number of seeds per plant (SP), plant height (height), crude protein content (protein), crude fat content (fat), ash content (ash), total starch content (starch), total dietary fiber content (fiber), moisture content (moist), Environment (Env), Genotype (G)).

The VIP scores and standardized regression coefficients from the optimal 4-factor SIMPLS model are presented in Figure 9. Fat showed the highest VIP value for both responses, at approximately 1.75, and had negative standardized coefficients for both seed yield and thousand-seed weight. Starch also showed VIP values above 1.0 for both responses and negative coefficients. Seeds per plant showed contrasting coefficient directions between the two responses, with a positive coefficient for seed yield and a negative coefficient for thousand-seed weight. Pods per plant and moisture showed minor contributions with near-zero standardized coefficients across the response margins. For the categorical predictors, individual elite genotypes (specifically G4 and G11) demonstrated the highest significant VIP peaks, showing highly pronounced positive standardized regression coefficients simultaneously for both seed yield and thousand-seed weight. The remaining predictors had VIP values below the 0.8 reference threshold.

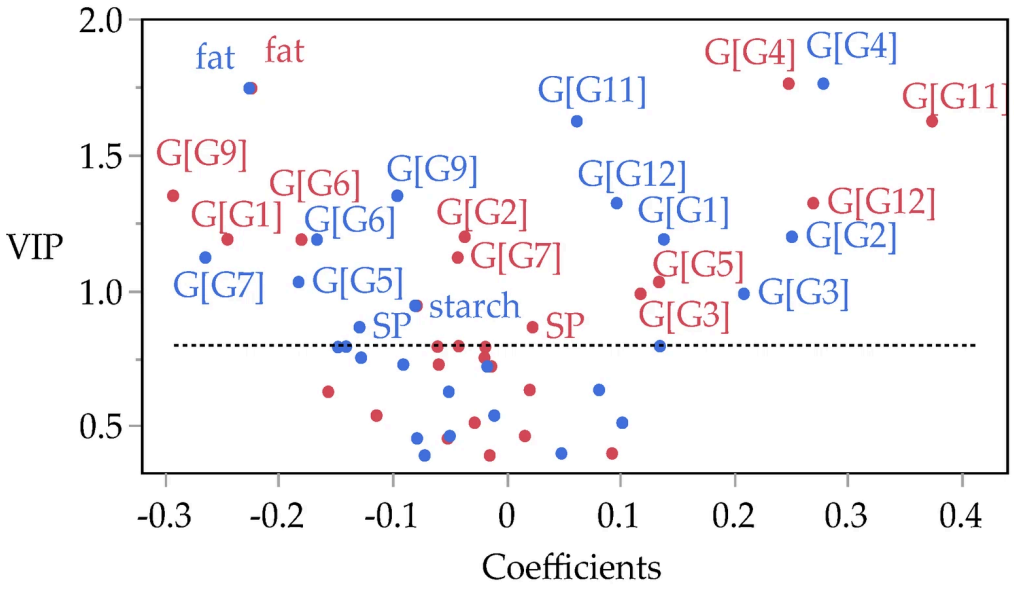


Figure 9. Variable Importance in Projection (VIP) scores versus standardized regression coefficients for the 11 predictors (number of pods per plant (PP), number of seeds per plant (SP), plant height (height), crude protein content (protein), crude fat content (fat), ash content (ash), total starch content (starch), total dietary fiber content (fiber), moisture content (moist), Environment (Env), Genotype (G)) in the optimal 4-factor SIMPLS model. The vertical axis represents VIP values, while the horizontal axis represents standardized regression coefficients. Red and blue symbols indicate the relationships of the predictors with seed yield (SY) and thousand-seed weight (1000S), respectively. The horizontal dashed line indicates the VIP reference threshold of 0.8. Below the threshold line, the labels are not shown to be clearer in the image.

The multi-response prediction profiler (Figure 10) showed distinct predicted response profiles across the range of the evaluated predictors. Fat and starch displayed negative slopes for both seed yield and thousand-seed weight, with the steepest decline observed for fat. Seeds per plant showed opposite response directions for the two target variables, with an increasing predicted seed yield and a decreasing predicted thousand-seed weight. The profiles for environment showed highly stable, flat response lines across all levels (E1–E4), while the genotype factor levels showed substantial variation, characterized by steep peaks for G4 and G11 that simultaneously optimized predicted yield and seed weight margins. The vertical dashed lines indicate the baseline settings for the predictors, while the horizontal reference lines indicate the baseline settings for the responses. The shaded regions represent the 95% confidence intervals around the predicted response profiles.

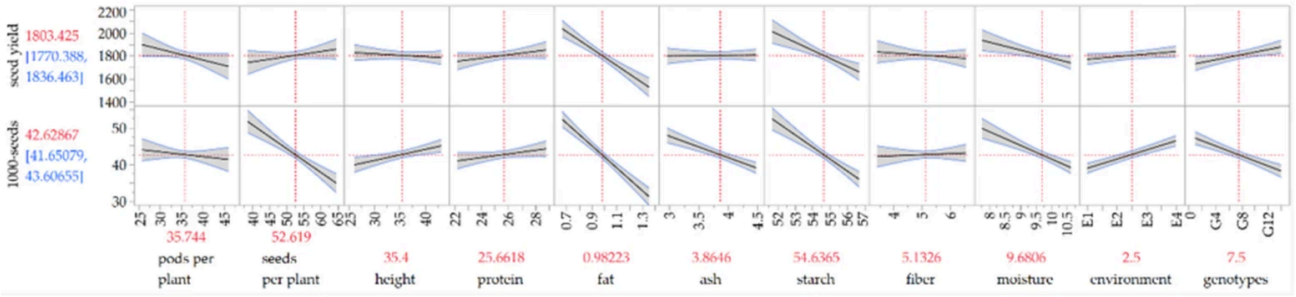


Figure 10. Multi-response prediction profiler for the optimal 4-factor SIMPLS model. The profile matrix shows the predicted responses of seed yield (SY) and thousand-seed weight (1000S) across the levels of the agronomic, structural, compositional and categorical predictors. Solid lines represent the predicted response profiles, with shaded regions indicating the 95% confidence intervals. The vertical dashed lines and horizontal reference lines indicate the baseline settings for the predictors and responses, respectively. (number of pods per plant (PP), number of seeds per plant (SP), plant height (height), crude protein content (protein), crude fat content (fat), ash content (ash), total starch content (starch), total dietary fiber content (fiber), moisture content (moist), Environment (Env), Genotype (G)).

The constellation plot (Figure 11) showed a clear hierarchical separation of the 14 genotypes into two major groups. The first group comprised G3 (GVLen3), G13 (Samos), G12 (GVLen12), G4 (GVLen4), G10 (GVLen10), and G11 (GVLen11), whereas the second included G1 (GVLen1), G2 (GVLen2), G14 (Dimitra), G5 (GVLen5), G6 (GVLen6), G7 (GVLen7), G8 (GVLen8), and G9 (GVLen9). Within these two major groups, several smaller clusters were distinguished based on the branching structure and distances among genotypes.

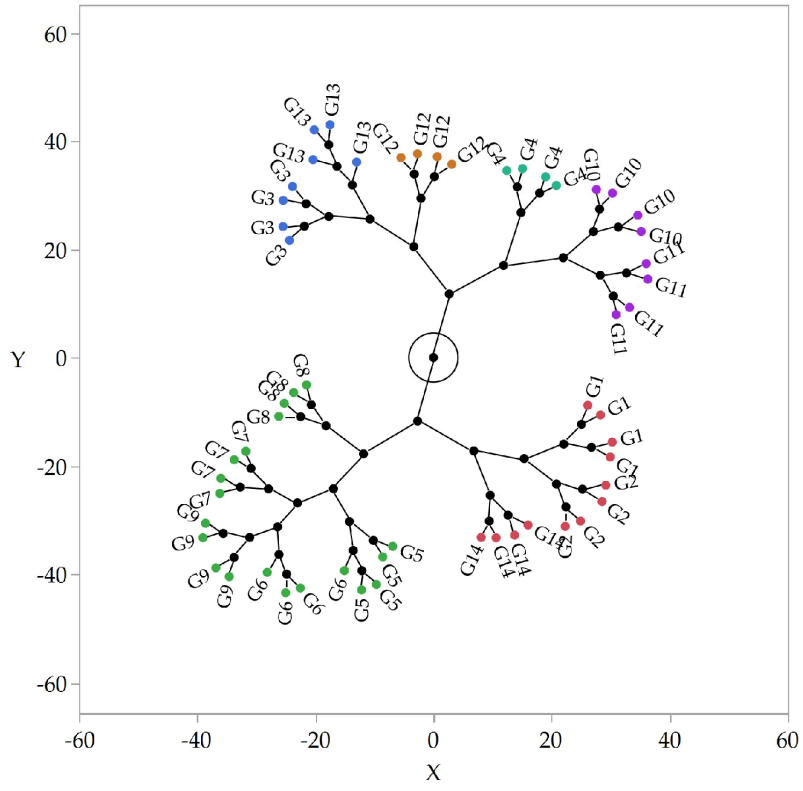


Figure 11. Constellation plot illustrating the hierarchical clustering relationships and global distances among the 14 genotypes.

4. Discussion

The evaluation of the 14 lentil genotypes across four environments showed variation in agronomic, morphological, and seed quality traits, as well as significant genotype × environment interaction for all evaluated traits. The combined use of stability and multivariate analyses allowed differences among genotypes and their responses across environments to be examined from complementary perspectives. The main findings are discussed below in relation to trait variation, stability, genotype responses, and relationships among traits.

4.1. Genotype × Environment Interaction and Genetic Variation

The combined analysis of variance showed significant effects of environment, genotype, and genotype × environment interaction for all evaluated traits. The significant genotype effects indicate genetic differences among the evaluated lentil genotypes, while the significant environmental effects reflect differences in trait expression among the four environments. The significant G × E interaction shows that the genotypes differed in their responses across environments and that their relative performance varied among environments [8,37].

The genetic parameter estimates showed small differences between genotypic and phenotypic variance estimates, which was also reflected in the close GCV and PCV estimates. Genetic variation differed among traits, with the highest GCV observed for thousand-seed weight (22.441%) and the lowest for total starch content (1.584%). GCV and PCV have been widely used to assess genetic variability among lentil genotypes, with PCV generally exceeding GCV in several studies [38–42]. Broad-sense heritability was high for all traits, ranging from 92.93% for seed yield to 99.82% for fat content. The relatively small differences between genotypic and phenotypic variance estimates suggest a limited contribution of environmental variance relative to genetic variance under the conditions evaluated. High broad-sense heritability has also been reported for several agronomic traits in lentil, although estimates varied among traits and experimental populations [38–45]. The high heritability estimates indicate that the observed differences among genotypes were largely associated with genetic variation within the four environments studied. These results indicate greater relative genetic variation for thousand-seed weight and lower relative genetic variation for total starch content among the evaluated genotypes. The significant G × E interaction also shows that these genetic differences were expressed differently across environments. Similar genotype, environment, and G × E effects have been reported in lentil multi-environment trials, where genotype responses and stability differed across environments [8,37,46].

4.2. Stability of Agronomic and Seed Quality Traits

The Stability Index (SI) showed differences among genotypes in the stability of the evaluated traits across the four environments. Similar differences in genotype stability across environments have been reported in lentil, where significant genotype, environment, and genotype × environment effects were observed for several agronomic and yield-related traits, with different genotypes identified as stable depending on the stability criterion and trait considered [46]. According to the criterion used in the present study, higher SI values indicate greater stability of the respective trait. For seed yield, GVLen11 showed the highest SI (471), whereas GVLen3 showed the lowest value (127). For the other agronomic and morphological traits, the highest SI values were observed for seeds per plant in GVLen6 (647), plant height in GVLen8 (685), pods per plant in GVLen10 (461), and thousand-seed weight in GVLen2 (288).

Differences in stability were also observed among the seed quality traits. The highest SI values were recorded for protein content in GVLen6 (2004), fat content in GVLen10 (1233), ash content and dietary fiber in GVLen2 (1290 and 1528, respectively), and moisture content in GVLen10 (1374). Similar trait-dependent differences in stability have been reported for

seed quality traits evaluated across multiple environments, with stability patterns differing among traits and genotypes [17,47]. Total starch content showed particularly high SI values, ranging from 4044 in GVLen7 to 5941 in GVLen3, reflecting the relatively small variation in starch content in relation to its mean value across the evaluated environments. These SI values should be interpreted only among genotypes for the same trait, since the magnitude of the index is influenced by the scale and mean value of each trait. The differences in SI among genotypes within each trait indicate that stability was trait-specific, with different genotypes showing higher stability for different agronomic and seed quality traits. From a breeding perspective, differences in stability among traits may provide useful information for identifying traits with more consistent expression across environments. Previous studies have associated differences in stability with differences in trait expression and inheritance patterns [48–52]. Accordingly, the SI results provide a basis for comparing the relative stability of genotypes within each agronomic or seed quality trait, rather than ranking different traits according to their SI values.

4.3. AMMI and GGE Analyses

The AMMI and GGE analyses provided complementary information on genotype responses across the four environments. For seed yield, the first AMMI principal component accounted for 41.6% of the G × E interaction variation, while the first two GGE components explained 92.0% of the G + GE variation. G12 (GVLen12), G11 (GVLen11), and G4 (GVLen4) recorded the highest seed yield, whereas G5 (GVLen5), G3 (GVLen3), and G7 (GVLen7) showed relatively small IPCA scores. In the GGE biplot, G11 (GVLen11) was closest to the ideal genotype, followed by G12 (GVLen12), while the which-won-where biplot showed different genotype associations with the evaluated environments. Similar genotype-specific responses and differences in stability across environments have been reported in lentil using AMMI and GGE approaches [6,12,24]. These results indicate that genotype responses for seed yield differed among environments. The differences between genotype positions in the GGE biplot and their IPCA scores highlight that high mean performance and stability are distinct aspects of genotype evaluation. In particular, the results should be interpreted in relation to the four environments examined, without assuming broader adaptation beyond the conditions of this study.

A similar pattern was observed for thousand-seed weight. G4 (GVLen4), G2 (GVLen2), G3 (GVLen3), and G1 (GVLen1) recorded the highest values, while G5 (GVLen5), G3 (GVLen3), and G7 (GVLen7) showed relatively small IPCA scores. The GGE biplot placed G4 (GVLen4), G2 (GVLen2), and G3 (GVLen3) close to the ideal genotype, while the which-won-where biplot indicated similar genotype responses across the four environments. GGE biplot analyses in lentil have similarly been used to identify high-performing and stable genotypes and to examine genotype associations with different environments [6,24]. These findings further demonstrate that genotype rankings and stability patterns may differ depending on the trait considered. For crude protein content, G8 (GVLen8), G2 (GVLen2), G6 (GVLen6), G12 (GVLen12), and Samos showed high values and were positioned close to the ideal genotype. AMMI analyses of lentil have also shown that genotype responses to environments can differ among traits, with significant G × E interaction and different patterns of IPCA scores and stability among genotypes [6,12]. The different genotype patterns observed among traits indicate that genotype responses to the environments were trait-dependent.

4.4. Correlation and Multivariate Analyses

4.4.1. Relationships Among Agronomic and Seed Quality Traits

The correlation analysis indicated that the relationships among yield components and seed quality traits were trait-specific. The positive association between seed yield and thousand-seed weight indicates that higher values of one trait were associated with higher values of the other one, whereas the negative associations of seed yield with fat and total starch indicate that higher values of these traits were associated with lower seed yield across the evaluated data, even though there is no linear relationship. These negative associations indicate that genotypes with higher fat and starch contents tended to have lower seed yield in the evaluated dataset, although the correlations were relatively weak and do not necessarily imply a direct biological trade-off. Similar positive associations between seed yield and seed weight have been reported in lentil genotypes [41,45,53]. The strong relationship between pods per plant and seeds per plant reflects the close association between these two yield components, consistent with previous findings in lentil [40]. The associations among protein, starch, fat, fiber, and ash also indicate that changes in one seed quality trait may be accompanied by changes in other components of seed composition. In lentil, protein and starch have been reported to be negatively correlated [54]. The observed relationships among agronomic and seed quality traits highlight the importance of considering several characteristics simultaneously when evaluating genotypes for breeding purposes. These relationships provide a basis for interpreting the multivariate analyses and for considering multiple traits in the evaluation of the tested genotypes.

4.4.2. Multivariate Relationships Among Traits

The PCA revealed that genotypic variation is driven by differences in growth and seed quality traits. Yield-related traits, particularly seed yield and thousand-seed weight, were positioned separately from the group formed by pods per plant and seeds per plant along the second principal component, while protein, ash, and starch were associated with another part of the multivariate structure. Fat and fiber also drove the separation of genotypes, whereas moisture had minimal impact, remaining close to the origin in the first two principal components. The first two principal components explained 39.18% of the total variation, indicating that the two-dimensional projection captured only part of the multivariate structure. Consequently, the observed separation of traits and genotypes in this projection should be interpreted with caution. While yield-related and seed quality traits differentiated the genotypes, a standard two-dimensional projection hid some of these relationships. By expanding the analysis to a five-component score plot matrix, distinct spatial clustering profiles are revealed that would otherwise remain hidden (Figure 5b). Similar applications of higher-dimensional PCA for characterizing variation and relationships among yield and agronomic traits have been reported in lentil germplasm [55,56].

4.4.3. Variable Clustering Analysis

The Variable Clustering analysis further separated the traits according to their patterns of variation. Seed yield, thousand-seed weight, and fat were grouped together, while pods per plant, seeds per plant, and fiber formed another group. Protein, ash, and starch constituted a separate group, whereas plant height and moisture remained as individual clusters. This structure corroborated the main relationships identified among the traits, confirming that genotypes differed by combinations of traits rather than a single trait. The grouping of yield-related traits separately from the protein–ash–starch group also indicates that agronomic and seed quality traits contributed differently to the overall differentiation of the evaluated genotypes. The resulting clusters describe associations among traits based on their variation across the evaluated dataset and do not necessarily indicate direct

biological or causal relationships between them. Multivariate analyses combining PCA and clustering have similarly been used to characterize genetic diversity and group lentil genotypes according to their trait profiles [57,58].

4.4.4. Genotype Differentiation Based on Trait Profiles

The hierarchical clustering and constellation analyses further differentiated the genotypes according to their overall trait profiles. The groups identified by these analyses reflected differences in the combinations of agronomic and seed quality traits among the genotypes and provided additional information on their multivariate relationships. The resulting genotype groupings reflect overall phenotypic similarity across the evaluated traits and environments, rather than stability or superiority for any individual trait. Similar clustering approaches have been used in lentil to differentiate genotypes according to their trait profiles and genetic diversity [55,57].

4.4.5. Multi-Response PLS Analysis

The multi-response PLS analysis identified fat, starch, number of seeds per plant, and number of pods per plant as variables with VIP values above the 0.8 reference threshold. Among the categorical factors, the broader environment factor and the average genotype variance fell below the 0.8 baseline, albeit individual elite lines (specifically G4 and G11) emerged as highly significant, top-ranking predictors in the multivariate profile. VIP values reflect the relative importance of variables in the PLS model and should not be interpreted as measures of statistical significance. Fat and starch showed negative associations with both seed yield and thousand-seed weight, as indicated by their negative standardized regression coefficients and steep downward trends in the multi-response prediction profiler, whereas the seeds per plant showed opposite associations with the two responses, highlighting a distinct source-sink trade-off. These associations describe the relationships captured by the predictive model and do not, by themselves, establish direct causal or physiological mechanisms. The PLS results therefore identified differences in the multivariate profiles associated with seed yield and thousand-seed weight among the evaluated genotypes and environments. This shows that geographical variations across E1–E4 maintained remarkably stable baseline profiles, whereas genetic variations across G1–G14 drove the primary shifts in agronomic and quality trait performance. The relative contribution of the environment and genotype terms should nevertheless be interpreted within the scope of the fitted PLS model and the four environments evaluated. Partial least squares regression has also been applied to lentils to identify phenotypic variables associated with seed yield and to identify the most informative predictors for yield prediction [59].

4.5. Breeding Implications and Practical Relevance

The results of the present study provide useful information for the selection of lentil genotypes based on the stability of agronomic and seed quality traits. The differences observed among genotypes indicate that no single genotype showed the highest stability for all evaluated traits. GVLen11 showed the highest SI for seed yield, while GVLen6 showed the highest SI for the number of seeds per plant and protein content. GVLen10 showed the highest SI for fat and moisture content, whereas GVLen2 showed the highest SI for thousand-seed weight, ash, and dietary fiber. These genotype-specific responses indicate that the choice of breeding material depends on the target trait and the objectives of the breeding program.

The high protein content observed in Samos, together with the performance of GVLen8, GVLen2, GVLen6, and GVLen12, also highlights the potential value of the evaluated material for improving seed quality. The genotypes identified for individual traits may serve as

candidates for further evaluation and, where supported by their overall agronomic and quality profiles, for use in crossing programs. The results also emphasize the importance of combining trait performance with stability when selecting parental material, rather than relying exclusively on mean values.

4.6. Study Limitations and Future Perspectives

The present study was conducted using 14 lentil genotypes across four environments representing two locations and two growing seasons. Although this experimental design allowed the evaluation of genotype responses under different environmental conditions, the results are limited to the locations and growing seasons included in the study. Further evaluation across a wider range of locations, years, and environmental conditions would be necessary to establish the consistency of the observed genotype responses under broader production conditions.

Another consideration is that the different stability and multivariate approaches used in this study assess complementary aspects of genotype performance and trait relationships. Their results should therefore be interpreted together, taking into account the specific characteristics and objectives of each analysis. Future studies could further investigate the stability of seed quality traits and the relationships between agronomic performance and seed composition, particularly in breeding materials selected for their potential use in food and feed applications.

5. Conclusions

The evaluation of 14 lentil genotypes across four environments showed significant effects of genotype, environment, and genotype × environment interaction for all evaluated traits. GVLen11 showed the highest Stability Index for seed yield, while GVLen11 and GVLen12 were positioned close to the ideal genotype in the GGE analysis. These results indicate that GVLen11 combined favorable seed yield stability with a favorable position in the GGE analysis under the evaluated conditions. Specific genotypes showed high stability for specific traits, including GVLen6 for seeds per plant and protein content, GVLen8 for plant height, GVLen10 for pods per plant, fat and moisture content, GVLen2 for ash and dietary fiber, and GVLen3 for total starch content. The multivariate analyses further showed that the genotypes differed in their combinations of agronomic and seed quality traits. The combined consideration of Stability Index values, heritability estimates, and trait correlations may provide additional information for identifying traits with consistent expression and desirable associations for selection. These findings provide information to support the selection of lentil genotypes with specific stable and desirable trait combinations and their further evaluation in multi-environment breeding trials. The identification of genotypes showing trait-specific stability provides a basis for their further evaluation as potential sources of desirable characteristics in lentil breeding programs. Their stability and performance require confirmation across additional locations and growing seasons.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture16222550/s1. Table S1: Pairwise correlation coefficients (r), confidence intervals, and significance levels among agronomic performance and seed quality characteristics (n = 224)*; Table S2: Eigenvalues and proportion of total variance explained by the principal components for 11 agronomic and seed quality traits; Table S3: Summary of variable clustering for the 11 agronomic and seed quality traits; Table S4: Standardized component loadings for the 11 agronomic and seed quality traits across the extracted principal components; Table S5: Partial least squares (PLS) model selection diagnostics and variance summary; Table S6: Proportion of variance and cumulative variation explained by individual latent factors in the multi-response SIMPLS model; Figure S1: Stability analysis of thousand-seed weight; Figure S2: Stability analysis of

number of pods per plant in lentil genotypes; Figure S3: Stability analysis of number of seeds per plant in lentil genotypes; Figure S4: Stability analysis of plant height (cm) in lentil genotypes; Figure S5: Stability analysis of crude fat content; Figure S6: Stability analysis of ash content (%) in lentil genotypes; Figure S7: Stability analysis of total starch content; Figure S8: Stability analysis of total dietary fiber content; Figure S9: Stability analysis of moisture content (%).

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