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Assessment of Seed Quality and Kernel Morphological Trait Stability in Two Maize Hybrids Across Four Growing Seasons

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Abstract

Maize seed quality and kernel morphological traits are important determinants of grain utilization and are influenced by both genetic factors and growing-season conditions. This study evaluated the stability of seed quality and kernel morphological traits in two commercial maize hybrids (Costanza and LG 3535) across four growing seasons, three row spacing systems, and two plant density levels. Seed quality traits (protein, fat, ash, starch, crude fiber, and moisture content) and kernel morphological traits (length, width, and thickness) were evaluated using univariate and multivariate statistical analyses. Significant effects of hybrid, growing season, row spacing, and their interactions were detected for most evaluated traits. Growing-season variability influenced seed composition and kernel morphology, while row spacing and plant density further contributed to trait expression. Costanza exhibited greater stability for most traits, particularly starch content and kernel morphology, whereas LG 3535 showed more variable responses across growing seasons and row spacing combinations. Correlation and multivariate analyses revealed strong associations among starch content, kernel width, and kernel thickness, whereas protein, ash, and crude fiber were less closely associated with kernel size traits. These findings demonstrate the importance of hybrid × growing-season interactions in shaping maize kernel characteristics and highlight the value of multi-environment evaluation for identifying hybrids with stable kernel quality traits under Mediterranean production conditions.

Keywords: grain composition; maturity class; row spacing; plant density; multivariate analysis; Mediterranean conditions

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1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops worldwide, serving as a major source of food, feed, and industrial raw material [1]. Beyond grain yield, seed quality and kernel morphological traits play a crucial role in determining end-use value,

processing efficiency, and overall crop utilization [2]. Traits such as protein, starch, fat, and fiber content, as well as kernel dimensions (length, width, and thickness), are increasingly considered essential components of maize improvement programs, particularly under intensifying agricultural systems and changing climatic conditions [2,3]. Kernel size-related traits are important determinants of grain development, yield formation, and grain quality and are increasingly considered in modern maize breeding programs [4,5].

The expression of these traits is strongly influenced by genetic factors, environmental conditions, and their interactions [6]. In multi-environment agricultural systems, genotype × environment (G × E) interaction represents a key determinant of genotype performance, often resulting in differential responses across locations, years, and management practices and thereby complicating the identification of stable and superior genotypes [7,8]. Stability across environments is therefore a fundamental requirement for commercial cultivars, as stable genotypes are characterized by low interaction with environmental conditions while maintaining favorable performance under optimal environments [9–11]. In this context, Fasoulas [12] proposed the ratio of mean to standard deviation as a simple stability metric, later refined by Fasoula [13,14] in its squared form, providing a more robust criterion for plant breeding applications.

G × E interactions arise from differences in genotype sensitivity to environmental conditions, including biotic and abiotic stresses, which affect genotypes unequally and often result in inconsistent performance across environments [15–17]. Consequently, multi-environment trials have consistently demonstrated significant genotype, environment, and interaction effects, emphasizing the need for reliable stability assessment tools to support breeding decisions and cultivar selection across contrasting environments [18]. Recent studies have further shown that year-to-year climatic variability may substantially affect maize grain quality, kernel characteristics, and trait stability, highlighting the importance of evaluating hybrid responses across multiple growing seasons [19,20].

Various statistical approaches have been developed to evaluate stability and genotype × environment (G × E) interaction patterns. Analysis of variance (ANOVA) remains one of the most widely used approaches for partitioning genotype, environment, and interaction effects in multi-environment trials [21,22], while principal component analysis (PCA) allows the exploration of multivariate structure among traits [23]. Furthermore, AMMI (Additive Main Effects and Multiplicative Interaction) and GGE (Genotype plus Genotype × Environment) biplot methodologies have been widely adopted to interpret G × E interactions and identify genotypes with stable performance across environments [8,24–26]. Recent investigations have confirmed the usefulness of AMMI and GGE approaches for evaluating both genotype × environment and genotype × year interactions in maize and for identifying stable hybrid performance under contrasting production conditions [27,28].

Most studies investigating genotype × environment interactions in maize have focused primarily on grain yield and agronomic performance, whereas comparatively less attention has been given to the combined evaluation of seed quality attributes and kernel morphological characteristics. Understanding the stability of these traits is important because grain composition and kernel morphology directly influence grain utilization, processing efficiency, and end-use quality [20]. Furthermore, information regarding the consistency of these traits across growing seasons and management conditions remains limited, particularly for commercially important hybrids differing in maturity class (FAO group). Therefore, integrating stability assessment with multivariate analyses may provide a more comprehensive understanding of trait expression and hybrid performance under variable production conditions.

The present study focused on two commercially important maize hybrids belonging to different maturity classes (FAO groups), thereby representing contrasting developmental

patterns and adaptation strategies. Although the limited number of genotypes limits broad generalization, the experimental design allows a detailed assessment of hybrid responses for seed quality and kernel morphological traits across growing seasons and management conditions.

Therefore, the present study aimed to evaluate the stability of seed quality and kernel morphological traits in two maize hybrids across four growing seasons, row spacing systems, and plant density levels. Specifically, the objectives were to (i) quantify trait variability and stability using a hierarchical Stability Index approach, (ii) explore multivariate relationships among traits using principal component analysis (PCA), (iii) characterize hybrid × growing-season interactions using AMMI and GGE biplot methodologies, and (iv) identify hybrids exhibiting consistent performance across growing seasons and management conditions. It was hypothesized that seed quality and kernel morphological traits differ in their stability across growing seasons and management practices and that hybrids belonging to different FAO maturity groups exhibit distinct stability responses.

2. Materials and Methods

2.1. Experimental Conditions

Field experiments were conducted over four consecutive growing seasons (2009–2012) at the experimental farm of the Technological Education Institute of Western Macedonia, Florina, Greece (40°46′ N, 21°22′ E; 705 m a.s.l.).

The experimental field was characterized by a sandy loam soil texture, consisting of 61.2% sand, 27.6% silt, and 11.2% clay, with a pH of 6.25 and an organic matter content of 1.29%.

Climatic conditions, including mean monthly air temperature (°C) and precipitation (mm), were recorded throughout the experimental period (Figure 1). Seasonal variation in temperature and precipitation was observed among growing seasons, providing contrasting climatic conditions for evaluating hybrid responses and trait stability.

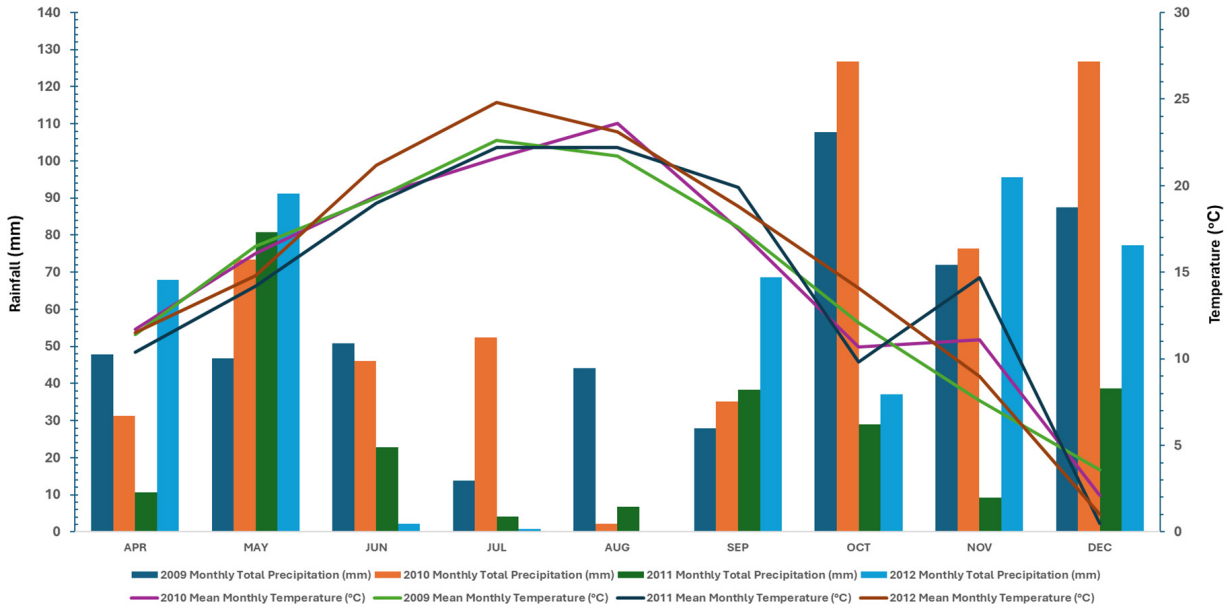

Figure 1. Monthly total precipitation (mm) and mean temperature (°C) in Florina during the 2009–2012 experimental period.

2.2. Experimental Design

The experiment was arranged in a split-split plot design with four replications per year. Row spacing, maize hybrid, and plant density were assigned to main plots, sub-plots,

and sub-subplots, respectively, allowing the simultaneous evaluation of management and genetic effects on seed quality and kernel morphological traits. This design was chosen to facilitate the practical implementation of row spacing and plant density treatments under field conditions.

Three planting systems were evaluated: single rows with 75 cm spacing, single rows with 50 cm spacing, and a twin-row system consisting of paired rows 25 cm apart and 50 cm between pairs.

Two commercial F1 maize hybrids differing in maturity class (FAO group) were used, namely Costanza (FAO 660; Pioneer Hi-Bred International, Princeton, IL, USA) and LG 3535 (FAO 500; Limagrain Europe SAS, Saint-Beauzire, France), along with two plant density levels (75,000 and 112,500 plants ha^{-1}). The selection of hybrids with contrasting maturity classes was intended to facilitate the evaluation of potential differences in trait stability and adaptation across growing seasons and management conditions.

Each experimental plot measured 15 m^2 (3 m $\times$ 5 m). The number of rows varied according to row spacing treatment (four rows for 75 cm spacing, six rows for 50 cm spacing, and twin-row configurations), while plot area remained constant across all treatments.

2.3. Crop Management

Sowing was carried out in early May each year (14 May 2009, 7 May 2010, 6 May 2011, and 11 May 2012) using a self-propelled Wintersteiger precision plot seeder. The experimental field was left fallow during the growing season preceding the establishment of each experiment. Seeds were sown at higher density and subsequently thinned at the two-leaf stage by manually removing excess seedlings to achieve the target plant populations for each density treatment.

A uniform fertilization regime was applied across all treatments, consisting of 150 kg N ha^{-1} and 75 kg P ha^{-1} at sowing, followed by an additional 135 kg N ha^{-1} at the six-leaf stage. Weed control was achieved through post-emergence application of dicamba (48% SL) at a rate of 600 mL ha^{-1}. Irrigation was supplied through a sprinkler irrigation system at approximately 40–50 mm per application, with irrigation intervals adjusted according to rainfall and crop requirements to prevent water stress during crop development.

Harvesting was conducted at physiological maturity in each year (25 November 2009, 17 December 2010, 15 December 2011, and 30 October 2012) using a self-propelled Wintersteiger plot combine harvester.

2.4. Seed Trait Measurements

After harvest, seed samples were collected separately from each experimental plot and hybrid. Each experimental unit was treated independently, and from each plot-specific sample, representative subsamples (approximately 100 g) were taken for laboratory analyses of chemical composition and quality traits. All laboratory determinations were performed at the Laboratory of Animal Nutrition, University of Thessaly, under standardized analytical conditions.

Prior to analysis, seed samples were air-dried to approximately 13% moisture content, manually cleaned, and ground using a laboratory mill to obtain a homogeneous powder. The ground material was stored at 4 °C until further analysis.

Approved methods of the American Association of Cereal Chemists (AACC) were used for the determination of fat (%), ash (%), crude protein (CP, %), and moisture (%), including Soxhlet extraction with petroleum ether (AACC Method 30-25.01), ash determination (AACC Method 08-01), the Kjeldahl method for total nitrogen (modified AACC Method 46-12, with protein expressed as CP = total N $\times$ 6.25), and moisture analysis (AACC Method 44-15A) [29]. In more detail, 3 g of ground homogenized seed sample, previously

dried for fat analysis, was subjected to Soxhlet extraction using petroleum ether for 4 h. The solvent was then evaporated using a rotary evaporator, and the remaining residue was dried at 100 °C to constant weight. This residue was reported as crude fat (ether extract) percentage [29].

For ash determination, 3 g of ground homogenized seed samples were weighed into previously ignited porcelain crucibles, cooled in a desiccator, and weighed at room temperature. The samples were then incinerated in a furnace at 550 °C until constant weight was reached, cooled in a desiccator, and reweighed [29].

Official methods of the Association of Official Analytical Chemists (AOAC) were applied for the determination of crude fiber (%) (AOAC 991.43) and total starch (%) (AOAC Method 996.11) [30].

Kernel morphological traits, including kernel length, kernel width, and kernel thickness (mm), were measured on a random subsample of 20 kernels per plot using a digital Vernier caliper with 0.01 mm precision. Twenty kernels per experimental unit were considered sufficient to obtain representative estimates of average kernel dimensions while maintaining analytical efficiency. Final values for each trait were expressed as the mean of the 20 measurements.

2.5. Statistical Analysis

Data were analyzed using a mixed-model analysis of variance (ANOVA) appropriate for the split-split plot experimental design, following Steel and Torrie [21]. Analyses were conducted across twelve experimental conditions defined by the combination of four years (2009–2012) and three row spacing treatments, according to Stratilakis and Goulas [31]. For the AMMI and GGE analyses, each year × row spacing combination was considered a distinct environment.

Hybrid and plant density were considered fixed effects, whereas replications were treated as random effects. The statistical model included the main effects of environment, hybrid, and plant density, as well as their two- and three-way interactions. Appropriate error terms for each source of variation were used to test the significance of effects based on the split-split plot structure. The ANOVA was performed using IBM SPSS Statistics v31 (IBM Corp., Armonk, NY, USA).

Pearson's correlation coefficients were calculated to assess relationships among seed quality and kernel morphological traits, and statistical significance was considered at $p < 0.05$. Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and constellation plots were used to explore multivariate patterns among traits across environments. PCA was used to identify major patterns of variation. HCA was performed using Ward's minimum variance method. Prior to analysis, all variables were standardized to eliminate the effect of different measurement scales, and Euclidean distance was used as the dissimilarity metric. The resulting clusters were visualized using heat maps combined with dendrograms. Constellation plots were generated to visualize relationships among samples and variables in a network-based representation. All correlation and multivariate analyses were performed using JMP 18 (Statistical Discovery LLC, Cary, NC, USA).

2.6. Stability Index (SI) Analysis

The Stability Index (SI) for seed quality and kernel morphological traits was calculated following Fasoula [13,14] as the squared ratio of the trait mean ($\overline{x}$) to its standard deviation (s):

$$\mathrm{SI} = \left(\frac{\overline{x}}{s}\right)^2$$

A multi-level application of the SI was used to assess stability at different levels of variation. First, overall SI values were calculated across all environments for each maize hybrid, representing global genotype stability (Table 2). Second, SI was computed for each of the twelve environments defined by year (2009–2012) and row spacing treatments (R1: 75 cm, R2: 50 cm, R3: twin-row system) (Table 3). Third, SI values were estimated separately for each row spacing treatment to evaluate stability associated with agronomic practice within each hybrid (Table 4). Finally, year-specific SI values were calculated to assess temporal stability across growing seasons (Table 5). Higher SI values indicate greater trait stability across the evaluated conditions, whereas lower SI values indicate greater variability and lower stability.

2.7. Multi-Environment Analysis Using AMMI and GGE Biplots

Genotype × environment (G × E) interactions were analyzed using the AMMI (Additive Main Effects and Multiplicative Interaction) and GGE (Genotype plus Genotype × Environment) biplot methodologies [32]. The AMMI approach combines analysis of variance for the additive effects of genotypes and environments with principal component analysis applied to the interaction component [33]. In this context, the genotype × environment interaction matrix is decomposed using singular value decomposition (SVD), allowing separation of main effects from interaction patterns represented by multiplicative interaction terms [34]. Genotypes with interaction principal component axis (IPCA1) scores close to zero were considered more stable across environments. The GGE biplot methodology focuses on genotype main effects together with genotype × environment interactions, enabling visualization of genotype performance and environmental relationships [25,35]. This graphical approach, originally introduced by Gabriel [36], presents both genotypes and environments in the same plot, facilitating the identification of superior and stable genotypes as well as representative environments. All AMMI and GGE analyses, including biplot construction, were performed using PBTools version 1.4 (International Rice Research Institute, IRRI, Laguna, Philippines). Given that only two hybrids were evaluated, AMMI and GGE analyses were used primarily as complementary exploratory tools to visualize genotype × environment interaction patterns rather than to support broad genotype ranking or breeding recommendations.

2.8. Integration of SI with AMMI and GGE Analyses

Stability Index (SI)-based metrics were integrated with AMMI and GGE biplot analyses to provide a comprehensive assessment of genotype performance and stability. While SI quantifies trait stability across environments, AMMI and GGE describe the patterns of genotype × environment interactions. Therefore, these approaches are complementary rather than alternative, offering a more comprehensive understanding of the structure and stability of genotype responses across the defined environments. The combined use of these approaches was intended to provide complementary information on trait consistency, multivariate relationships, and genotype × environment interaction patterns.

3. Results

Variation in seed quality and kernel morphological traits was assessed using analysis of variance. Stability across environments, agronomic treatments, and years was evaluated using the Stability Index (SI). Genotype × environment interactions and adaptation patterns were examined using AMMI, GGE biplot, and principal component analysis (PCA). Relationships among traits were investigated using correlation analysis, while hierarchical clustering analysis (HCA), heat map visualization, and constellation plots were used to explore grouping patterns among traits and genotypes across environments.

3.1. Analysis of Variance for Seed Quality and Kernel Morphological Traits

The analysis of variance revealed significant effects of environments, hybrids, and plant density on all seed quality and kernel morphological traits (Table 1). Environment had a significant effect ($p \leq 0.001$) on all evaluated traits, including protein, fat, ash, starch, crude fiber, moisture, and kernel morphological characteristics.

Table 1. Analysis of variance (mean squares) for seed quality and kernel morphological traits of maize.

Source of Variation	df	Protein m.s.	Fat m.s.	Ash m.s.	Starch m.s.	Crude Fiber m.s.	Moisture m.s.	Kernel Length m.s.	Kernel Thickness m.s.	Kernel Width m.s.
Environment	11	2.569 ***	0.514 ***	0.287 ***	28.954 ***	0.655 ***	1.687 ***	2.013 ***	1.401 ***	0.752 ***
Replications within Environment	36	0.00009 ^{ns}	0.0001 ^{ns}	0.0001 ^{ns}	0.0003 *	0.0002 ^{ns}	0.001 ^{ns}	0.0001 ^{ns}	0.0001 ^{ns}	0.0001 ^{ns}
Hybrid	1	381.411 ***	0.033 ***	0.029 ***	22.327 ***	0.401 ***	27.598 ***	232.091 ***	0.088 ***	3.498 ***
Hybrid × Environment	11	0.892 ***	0.219 ***	0.110 ***	2.868 ***	0.455 ***	0.795 ***	0.399 ***	0.488 ***	0.113 ***
Plant density	1	18.331 ***	0.370 ***	0.063 ***	116.084 ***	1.774 ***	3.331 ***	10.252 ***	12.184 ***	5.263 ***
Plant density × Environment	11	0.324 ***	0.070 ***	0.151 ***	2.224 ***	0.132 ***	0.126 ***	0.272 ***	0.166 ***	0.100 ***
Hybrid × Plant density	1	0.00008 ^{ns}	0.526 ***	0.220 ***	0.128 ***	0.143 ***	1.213 ***	0.055 ***	0.069 ***	0.068 ***
Hybrid × Plant density × Environment	11	0.212 ***	0.145 ***	0.058 ***	2.730 ***	0.020 ***	0.082 ***	0.098 ***	0.206 ***	0.072 ***
Error	108	0.0001	0.0001	0.0001	0.0002	0.0002	0.001	0.0001	0.0001	0.0001

Note: m.s. = mean squares. Significance levels: *** $p \leq 0.001$; * $p \leq 0.05$; ^{ns} = not significant. Protein, fat, ash, starch, crude fiber, and moisture are expressed in %; kernel length, kernel thickness, and kernel width are expressed in mm.

Hybrid effects were significant ($p \leq 0.001$) for all traits, indicating genetic differences between the two maize hybrids. Plant density also significantly affected all measured traits, with noticeable effects on starch content and kernel morphological characteristics, including kernel length, kernel thickness, and kernel width.

All two-way interactions (Hybrid × Environment, Plant density × Environment, and Hybrid × Plant density) were significant for the majority of traits and trait combinations, indicating differential responses across environments. The three-way interaction (Hybrid × Plant density × Environment) was also significant for all traits, confirming the complexity of trait expression under combined environmental and agronomic variation. Replication effects within environments were generally not significant, except for starch content. This indicates the importance of combined analysis between factors.

The significant environmental effects reflected the substantial variation among year × row spacing combinations, indicating that seasonal climatic variability and crop spatial arrangement both contributed to trait expression.

3.2. Stability Index of Maize Hybrids Across All Environments

Substantial differences in Stability Index (SI) values were observed between Costanza and LG 3535 across all environments (Table 2). For seed quality traits, starch content showed SI values of 2045 in Costanza and 1116 in LG 3535. Protein content showed SI values of 437 and 153, respectively, while fat content showed SI values of 338 and 154. Ash content SI values were 79 and 73 for Costanza and LG 3535, respectively, whereas crude fiber SI values were 114 and 72, respectively. Moisture content showed higher SI values in LG 3535 (896) compared with Costanza (751).

Table 2. Stability Index (SI) values of maize hybrids for seed quality and kernel morphological traits across all environments.

Hybrid	Protein	Fat	Ash	Starch	Crude Fiber	Moisture	Kernel Length	Kernel Thickness	Kernel Width
Costanza	437	338	79	2045	114	751	1056	399	258
LG 3535	153	154	73	1116	72	896	380	359	241

Note: Higher SI values indicate greater trait stability across environments.

Kernel morphological traits also differed between hybrids. Kernel length SI values were 1056 in Costanza and 380 in LG 3535, while kernel thickness SI values were 399 and 359, and kernel width SI values were 258 and 241, respectively.

3.3. Stability Index of Maize Hybrids Across Twelve Environments

The Stability Index (SI) values for seed quality and kernel morphological traits across twelve environments defined by year (2009–2012) and row spacing treatments (R1: 75 cm, R2: 50 cm, R3: twin-row system) are presented in Table 3.

Table 3. Stability Index (SI) values for seed quality and kernel morphological traits across twelve environments defined by year (2009–2012) and row spacing treatments (R1: 75 cm, R2: 50 cm, R3: twin-row system).

Environment	Description	Protein	Fat	Ash	Starch	Crude Fiber	Moisture	Kernel Length	Kernel Thickness	Kernel Width
E1	2009 × R1	33	233	203	3086	280	1372	81	1326	376
E2	2009 × R2	37	699	563	3858	90	983	106	600	494
E3	2009 × R3	28	214	38	3473	84	591	108	436	301
E4	2010 × R1	53	320	592	3171	512	1696	79	1250	439
E5	2010 × R2	31	381	88	3683	129	1103	58	517	351
E6	2010 × R3	110	283	152	1500	215	509	108	252	264
E7	2011 × R1	30	558	272	3205	225	877	79	1677	409
E8	2011 × R2	33	345	158	4440	162	408	81	1586	400
E9	2011 × R3	47	485	132	2331	704	150	145	406	143
E10	2012 × R1	28	441	124	4876	40	2129	136	517	993
E11	2012 × R2	29	1394	113	7088	328	2917	81	1659	237
E12	2012 × R3	44	758	103	2817	775	624	138	526	418

Note: Higher SI values indicate greater stability across environments.

Considerable variation in SI values was observed across environments for all evaluated traits. Protein content SI values ranged from 28 (E3, 2009 × R3) to 110 (E6, 2010 × R3). Fat content SI values ranged from 214 (E3) to 1394 (E11). Ash content SI values ranged from 38 (E3) to 592 (E4). Starch content SI values ranged from 1500 (E6) to 7088 (E11). Crude fiber SI values ranged from 40 (E10) to 775 (E12), while moisture content SI values ranged from 150 (E9) to 2917 (E11).

Kernel morphological traits also showed variation across environments. Kernel length SI values ranged from 58 (E5) to 145 (E9), kernel thickness SI values ranged from 252 (E6) to 1677 (E7), and kernel width SI values ranged from 143 (E9) to 993 (E10).

3.4. Stability Index of Maize Hybrids Across Row Spacing Treatments

Table 4 presents the Stability Index (SI) values for seed quality and kernel morphological traits of LG 3535 across three row spacing treatments (R1: 75 cm, R2: 50 cm, R3: twin-row system).

Table 4. Stability Index (SI) of seed quality and kernel morphological traits for each maize hybrid across row spacing treatments (R1: 75 cm, R2: 50 cm, R3: twin-row system).

Row Spacing	Hybrid	Protein	Fat	Ash	Starch	Crude Fiber	Moisture	Kernel Length	Kernel Thickness	Kernel Width
R1	Costanza	350	580	137	2534	222	1090	802	675	360
R2	Costanza	831	384	129	2706	99	1027	2330	671	617
R3	Costanza	426	219	110	1866	141	679	863	246	204
R1	LG 3535	143	89	125	898	64	1513	326	307	197
R2	LG 3535	475	268	70	1473	80	1378	534	371	403
R3	LG 3535	103	366	58	1314	164	666	524	670	208

Note: Higher SI values indicate greater stability across row spacing treatments.

Protein content SI values ranged from 103 (LG 3535, R3) to 831 (Costanza, R2). Fat content SI values ranged from 89 (LG 3535, R1) to 580 (Costanza, R1). Starch content SI values ranged from 898 (LG 3535, R1) to 2706 (Costanza, R2). Ash content SI values ranged from 58 (LG 3535, R3) to 137 (Costanza, R1), while crude fiber SI values ranged from 64

(LG 3535, R1) to 222 (Costanza, R1). Moisture content SI values ranged from 666 (LG 3535, R3) to 1513 (LG 3535, R1).

Kernel morphological traits also varied across hybrids and treatments. Kernel length SI values ranged from 326 (LG 3535, R1) to 2330 (Costanza, R2), kernel thickness SI values ranged from 246 (Costanza, R3) to 675 (Costanza, R1), and kernel width SI values ranged from 197 (LG 3535, R1) to 617 (Costanza, R2).

3.5. Year-Specific Stability Index of Maize Hybrids

Table 5 presents the year-specific Stability Index (SI) values for seed quality and kernel morphological traits of Costanza and LG 3535 across four growing seasons (2009–2012). The SI values describe trait stability within each year across genotypes.

Table 5. Year-specific Stability Index (SI) of seed quality and kernel morphological traits for each maize hybrid.

Year	Hybrid	Protein	Fat	Ash	Starch	Crude Fiber	Moisture	Kernel Length	Kernel Thickness	Kernel Width
2009	Costanza	693	653	96	3697	227	2910	1981	574	362
2010	Costanza	735	465	127	2174	211	2402	1924	516	353
2011	Costanza	476	845	187	3192	178	1373	1931	479	362
2012	Costanza	896	1584	109	5596	92	3730	2140	727	409
2009	LG 3535	305	140	56	2167	135	2834	712	461	384
2010	LG 3535	203	233	138	4551	148	2405	543	274	384
2011	LG 3535	355	382	176	2027	182	1056	648	1006	370
2012	LG 3535	304	379	118	3561	158	1346	772	628	677

Note: Higher SI values indicate greater stability within each year.

In Costanza, protein SI values ranged from 476 (2011) to 896 (2012), while in LG 3535 they ranged from 203 (2010) to 355 (2011). Fat content SI values ranged from 465 (2010) to 1584 (2012) in Costanza and from 140 (2009) to 382 (2011) in LG 3535. Starch content SI values ranged from 2174 (2010) to 5596 (2012) in Costanza and from 2027 (2011) to 4551 (2010) in LG 3535.

Ash content SI values ranged from 96 (2009) to 187 (2011) in Costanza and from 56 (2009) to 176 (2011) in LG 3535, while crude fiber SI values ranged from 92 (2012) to 227 (2009) in Costanza and from 135 (2009) to 182 (2011) in LG 3535. Moisture content SI values ranged from 1373 (2011) to 3730 (2012) in Costanza and from 1056 (2011) to 2834 (2009) in LG 3535.

Kernel morphological traits also showed variation across years. Kernel length SI values ranged from 1924 (2010) to 2140 (2012) in Costanza and from 543 (2010) to 772 (2012) in LG 3535. Kernel thickness SI values ranged from 479 (2011) to 727 (2012) in Costanza and from 274 (2010) to 1006 (2011) in LG 3535, while kernel width SI values ranged from 353 (2010) to 409 (2012) in Costanza and from 370 (2011) to 677 (2012) in LG 3535.

3.6. Correlation Matrix of Seed Quality and Kernel Morphological Traits

The correlation coefficients among seed quality and kernel morphological traits of maize are presented in Figure 2. Protein content was positively correlated with ash ($r = 0.26$), crude fiber ($r = 0.34$), moisture ($r = 0.60$), and kernel length ($r = 0.76$), while showing a weak negative association with fat content ($r = -0.11$). Fat content exhibited positive correlations with starch ($r = 0.59$) and kernel morphological traits, including kernel length ($r = 0.30$), kernel thickness ($r = 0.53$), and kernel width ($r = 0.48$), and a negative association with crude fiber ($r = -0.58$). Ash content was positively associated with protein ($r = 0.26$) and crude fiber ($r = 0.27$), whereas negative correlations were observed with starch ($r = -0.47$) and kernel size-related traits, particularly kernel thickness ($r = -0.36$) and kernel width ($r = -0.38$).

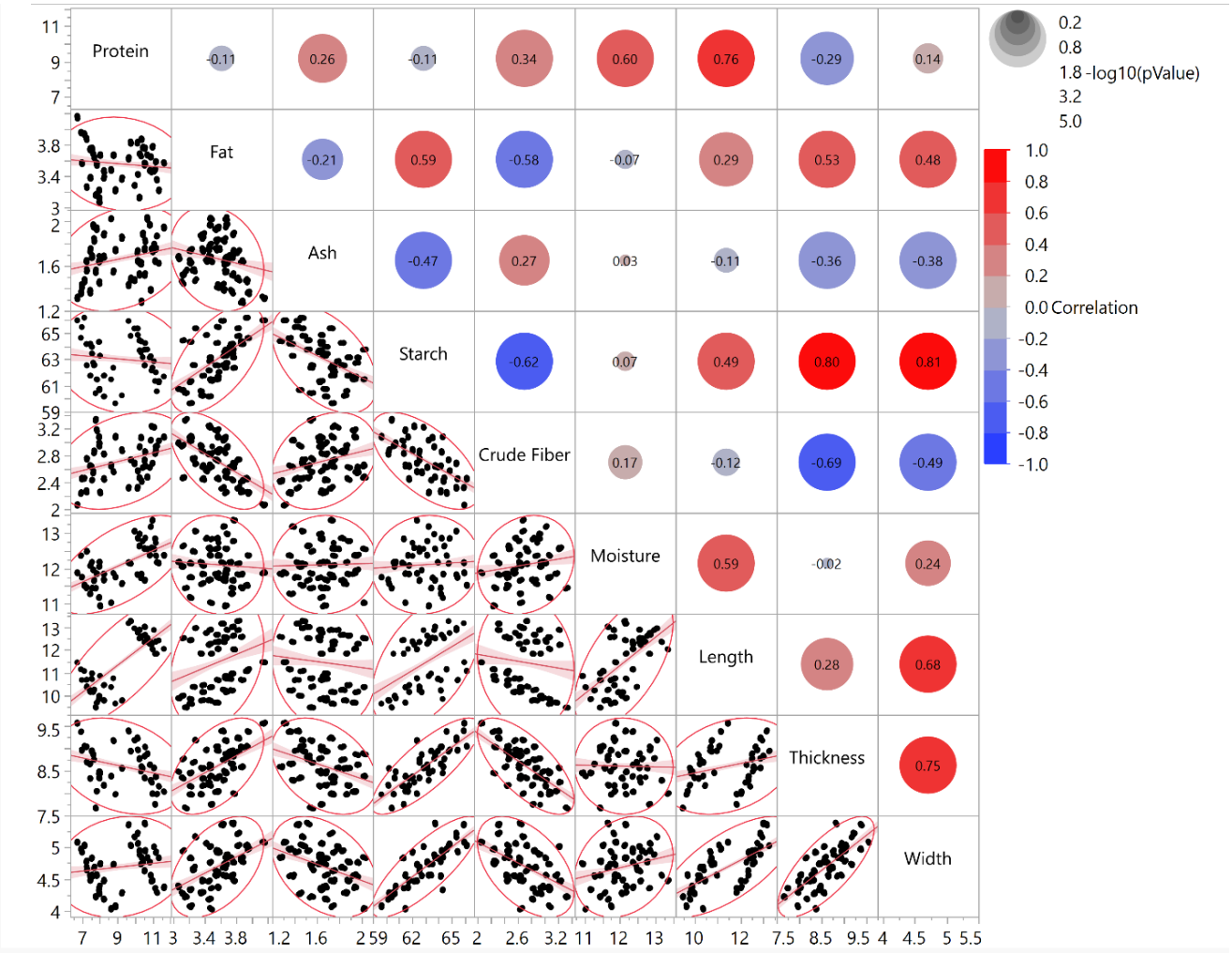

Figure 2. Correlation matrix showing Pearson correlation coefficients among seed quality traits (protein, fat, ash, starch, crude fiber, and moisture content) and kernel morphological traits (kernel length, kernel width, and kernel thickness) of maize. Positive correlations are indicated by warmer colors and negative correlations by cooler colors, while color intensity reflects the strength of the association.

Starch content showed a strong positive correlation with kernel morphological traits, including kernel length (r = 0.49), kernel thickness (r = 0.81), and kernel width (r = 0.81), as well as with fat content, while being negatively associated with ash (r = −0.47) and crude fiber (r = −0.62). Crude fiber content exhibited positive correlations with protein (r = 0.34), ash (r = 0.27), and moisture (r = 0.17), and strong negative associations with fat (r = −0.58), starch (r = −0.62), kernel thickness (r = −0.69), and kernel width (r = −0.49).

Moisture content was positively correlated with protein (r = 0.60), showed a weak positive association with crude fiber (r = 0.17), and was positively associated with kernel length (r = 0.59) and kernel width (r = 0.24), while associations with fat and starch were weak or non-significant. Kernel morphological traits were interrelated, showing positive correlations ranging from moderate to strong (r = 0.28–0.81), particularly with starch accumulation and, to a lesser extent, fat content.

3.7. Multivariate Analysis of Seed Quality and Kernel Morphological Traits Across Maize Environments

Multivariate statistical analyses were performed to evaluate relationships among maize combinations and to identify patterns of variation in seed quality and kernel morphological traits across years, row spacing systems, hybrids, and plant density levels. Heat map and hierarchical clustering analysis, principal component analysis (PCA), and constellation plot analysis were used to examine similarities among samples and associations among the evaluated traits. For the multivariate analyses, row spacing treatments were coded as R1 (single rows at 75 cm spacing), R2 (single rows at 50 cm spacing), and R3 (twin-row system). Hybrids were coded as H1 (Costanza) and H2 (LG 3535), whereas plant density levels were coded as D1 (75,000 plants ha^{-1}) and D2 (112,500 plants ha^{-1}). Environments were defined as combinations of year and row spacing treatment: E1 = 2009 × R1, E2 = 2009 × R2, E3 = 2009 × R3, E4 = 2010 × R1, E5 = 2010 × R2, E6 = 2010 × R3, E7 = 2011 × R1, E8 = 2011 × R2, E9 = 2011 × R3, E10 = 2012 × R1, E11 = 2012 × R2, and E12 = 2012 × R3.

3.7.1. Heat Map and Hierarchical Clustering of Seed Traits

The heat map and hierarchical clustering analysis revealed clear differentiation among the evaluated maize combinations for seed quality and kernel morphological traits (Figure 3).

The sample dendrogram separated the evaluated combinations into five major clusters. The upper cluster consisted predominantly of H1-D1 combinations from environments E1-E9, including E1-H1-D1, E3-H1-D1, E5-H1-D1, E6-H1-D1, E7-H1-D1, E8-H1-D1, and E9-H1-D1. These combinations were characterized by relatively higher values for protein, kernel length, and moisture content, whereas starch, kernel width, and kernel thickness generally exhibited lower relative values.

The second cluster consisted mainly of H1 combinations associated with D2 density from environments E1-E9 together with several H1-D1 combinations. Compared with the lower sections of the dendrogram, these combinations also exhibited relatively higher values for protein and kernel length, while width and thickness remained comparatively lower.

A large middle cluster was composed predominantly of H2 combinations from environments E1-E9, particularly under R1 and R2 spacing systems. These combinations generally exhibited lower relative values for protein, starch, width, and thickness, whereas several samples showed relatively higher values for ash and crude fiber.

Another cluster located below the central branch included mainly H2 combinations from both earlier environments and the 2012 environments (E10-E12). Several combinations within this cluster, particularly E11-H2-D1 and E12-H2-D1, exhibited increased relative values for starch, width, and thickness compared with the middle H2 cluster.

The lower cluster included combinations E3-H2-D1, E10-H2-D1, E10-H1-D1, E11-H1-D2, E12-H1-D2, E11-H1-D1, and E12-H1-D1. These samples exhibited the highest relative values for starch, kernel width, and kernel thickness across the entire heat map.

The variable dendrogram grouped the evaluated traits into three major clusters. Protein, kernel length, and moisture content formed one cluster, ash and crude fiber formed a second cluster, whereas fat, starch, kernel width, and kernel thickness formed a third closely associated cluster. Among these variables, starch, width, and thickness exhibited the strongest similarity in variation patterns across the evaluated maize combinations.

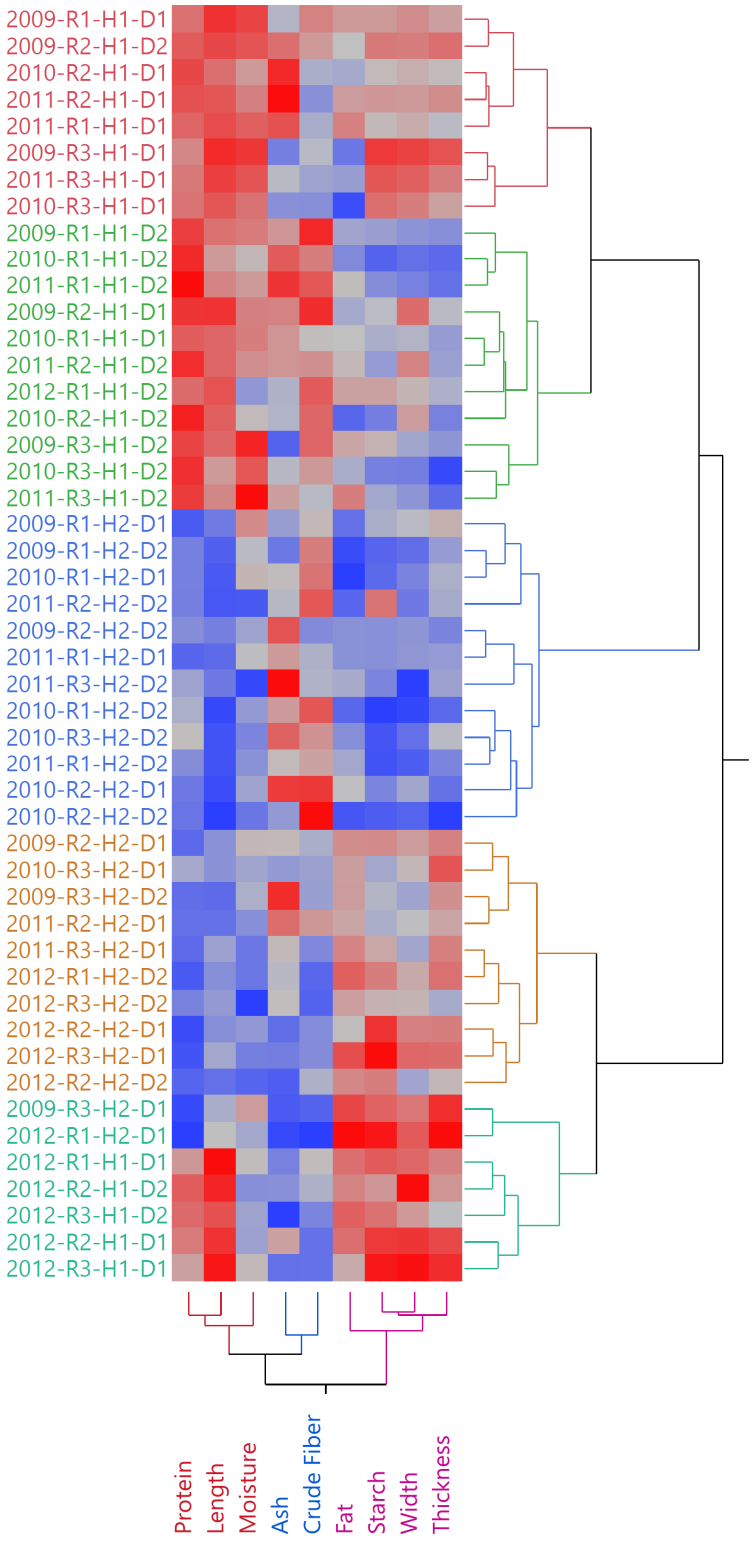

Figure 3. Heat map and hierarchical clustering analysis of seed quality and kernel morphological traits across all maize treatment combinations (year × row spacing × hybrid × plant density). The color scale ranges from low standardized values (blue) to high standardized values (red). The dendrogram illustrates similarity patterns among treatments and traits based on their multivariate profiles and reveals groups of combinations with similar trait expression.

3.7.2. Principal Component Analysis of Seed Quality and Kernel Morphological Traits in Maize Across Environments

Principal component analysis (PCA) explained a substantial proportion of the total variability among the evaluated samples, with the first principal component (PC1) accounting for 44.8% of the total variation and the second principal component (PC2) accounting for 27.4%, resulting in a cumulative explanation of 72.2% (Figure 4).

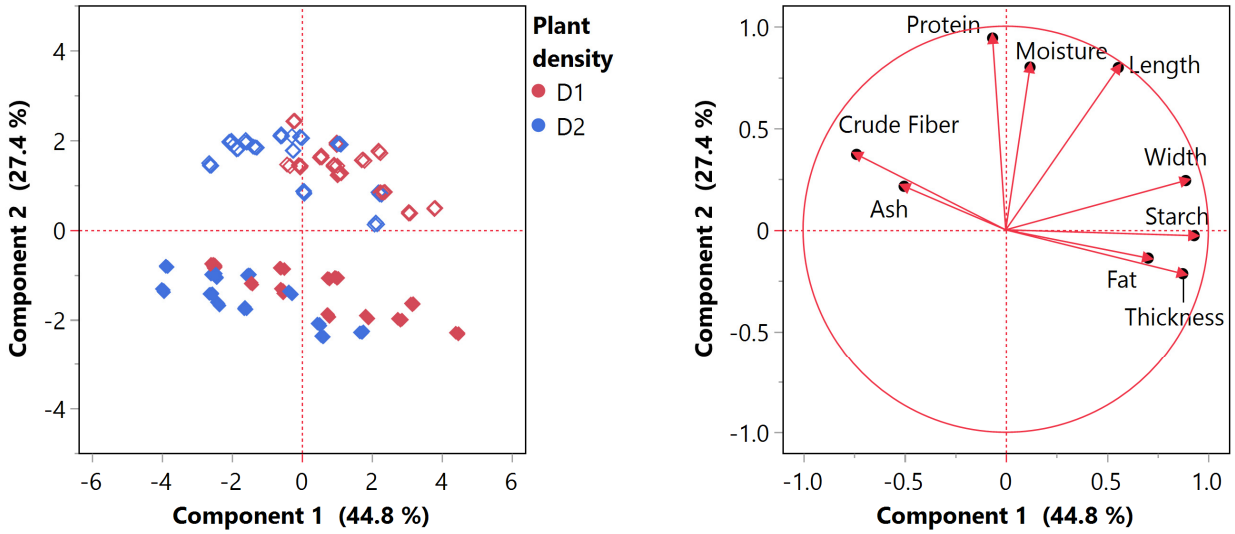

Figure 4. Principal component analysis (PCA) of seed quality and kernel morphological traits across maize combinations. The score plot presents the distribution of samples according to the first two principal components (PC1 and PC2), explaining 44.8% and 27.4% of the total variation, respectively. Open symbols represent H1 (Costanza), whereas filled symbols represent H2 (LG 3535). Symbol colors indicate plant density treatments (D1 and D2). The loading plot illustrates the contribution and association of seed quality and kernel morphological traits to the principal components.

The loading plot showed that starch content, kernel width, kernel thickness, and fat content were strongly associated with positive PC1 values, as their vectors were oriented toward the positive side of the horizontal axis and clustered closely together. This pattern indicates positive relationships among these traits across the evaluated treatments. In contrast, crude fiber and ash were positioned on the negative side of PC1, demonstrating an opposite pattern of variation relative to starch- and kernel size-related traits. Protein and moisture content were more strongly associated with PC2 because their vectors were directed toward the upper part of the loading plot, indicating that these variables contributed mainly to vertical differentiation among samples.

The score plot revealed a clear separation primarily associated with maize hybrids rather than plant density treatments. Open symbols, corresponding to H1, were predominantly distributed in the upper half of the PCA space and were characterized by positive PC2 values. Based on the loading plot, these samples were more closely associated with protein and moisture content. In contrast, filled symbols, corresponding to H2, were mainly located in the lower half of the diagram and were more strongly associated with starch content, kernel width, kernel thickness, and fat content, as indicated by their positioning toward the positive side of PC1 and negative PC2 values.

Plant density effects were comparatively less noticeable. Samples corresponding to D1 and D2 showed substantial overlap within both hybrids, indicating that plant density contributed less to the overall multivariate differentiation than hybrid grouping. Nevertheless, some dispersion along PC1 was observed within individual hybrid groups, suggesting that environmental and row spacing combinations also contributed to trait variation patterns.

The PCA results revealed stronger separation patterns associated with hybrid identity than with plant density treatments.

3.7.3. Constellation Plot Analysis of Seed Quality and Kernel Morphological Traits

The constellation plot revealed clear grouping patterns among the evaluated maize combinations according to hybrid, plant density, year, and row spacing treatment (Figure 5).

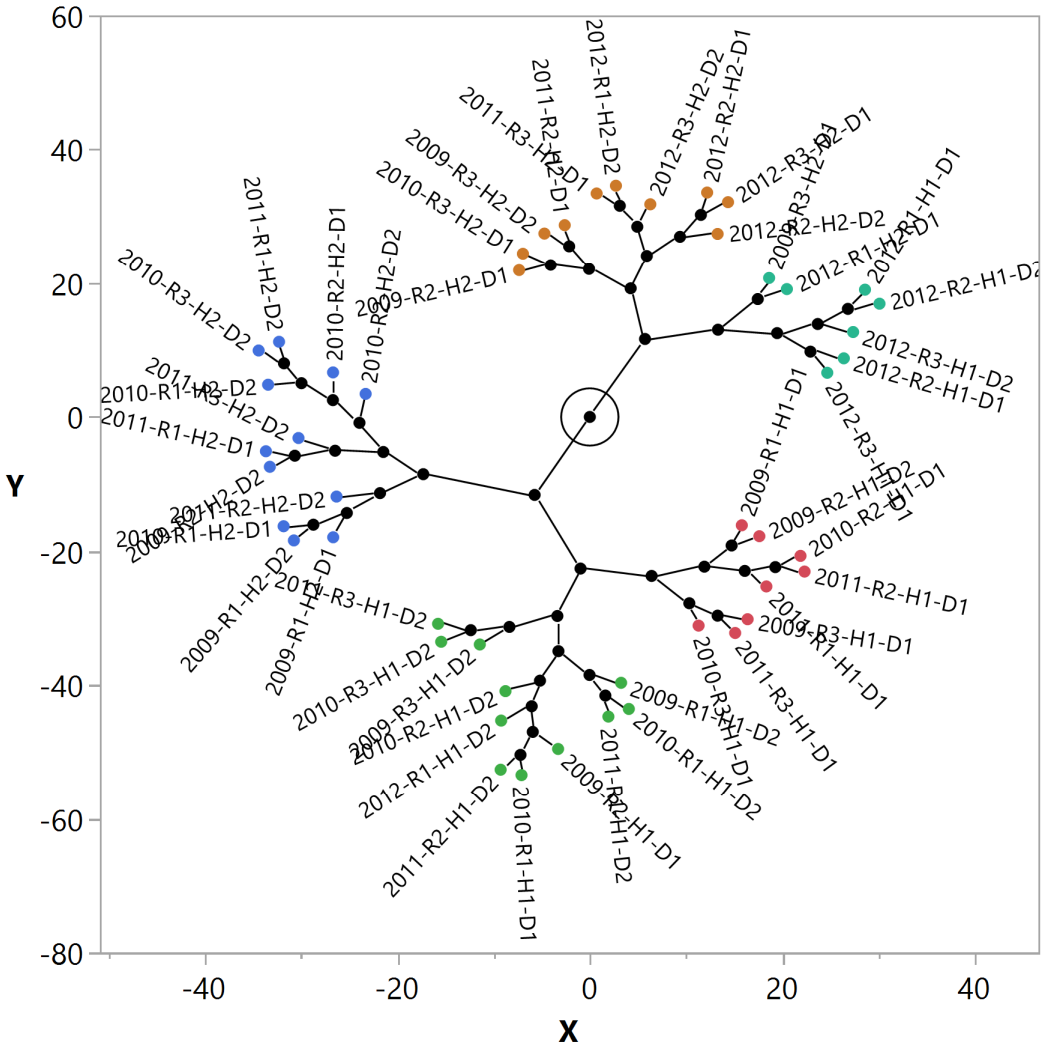

Figure 5. Constellation plot illustrating multivariate relationships among maize treatment combinations (year × row spacing × hybrid × plant density) based on seed quality and kernel morphological traits. The spatial proximity of points indicates greater similarity in multivariate trait expression, whereas greater distances indicate increased differentiation among treatment combinations. Clustering patterns facilitate the visualization of relationships among years, hybrids, row spacing treatments, and plant densities.

A distinct branch on the left side of the plot consisted predominantly of H2-D2 combinations, including E4-H2-D2, E7-H2-D2, E5-H2-D2, E8-H2-D2, E1-H2-D2, and E2-H2-D2. These combinations formed a relatively compact group, indicating similar multivariate trait profiles.

The upper branch of the constellation plot was composed mainly of H2 combinations from R2 and R3 environments, particularly from E3, E5, E6, E11, and E12. This branch included combinations such as E3-H2-D1, E6-H2-D1, E5-H2-D1, E11-H2-D1, E12-H2-D1, and E12-H2-D2. These combinations occupied closely connected positions within the same branch structure, suggesting relatively similar patterns among the evaluated seed traits.

On the right side of the plot, a separate branch included mainly H1 combinations from the 2012 environments, particularly E10-H1-D1, E11-H1-D1, E11-H1-D2, E12-H1-D1, and E12-H1-D2. These combinations formed a compact subgroup distinct from most earlier-year H1 combinations.

The lower-right branch consisted mainly of H1-D1 combinations from E1–E9, whereas the lower-central branch contained predominantly H1-D2 combinations, including E1-H1-D2, E2-H1-D2, E4-H1-D2, E5-H1-D2, E7-H1-D2, and E10-H1-D2.

The constellation plot revealed clear multivariate grouping patterns associated with hybrid, plant density, year, and row spacing treatment combinations across the evaluated maize samples.

3.8. AMMI and GGE Analyses

The additive main effects and multiplicative interaction (AMMI) model and genotype plus genotype × environment (GGE) biplot analysis were used to evaluate genotype × environment (G × E) interactions for seed quality and kernel morphological traits across the evaluated maize environments. AMMI1 biplots, adaptation maps, and "which-won-where" GGE biplots were used to assess genotype performance, stability, and specific adaptation patterns across environments.

For these analyses, genotypes were coded as G1 (Costanza) and G2 (LG 3535). Environments were coded as E1–E12 according to year × row spacing combinations: E1 = 2009 × R1, E2 = 2009 × R2, E3 = 2009 × R3, E4 = 2010 × R1, E5 = 2010 × R2, E6 = 2010 × R3, E7 = 2011 × R1, E8 = 2011 × R2, E9 = 2011 × R3, E10 = 2012 × R1, E11 = 2012 × R2, and E12 = 2012 × R3.

3.8.1. Protein Content

The AMMI analysis for protein content (Figure 6) revealed substantial genotype × environment interaction. Genotype G1 exhibited higher mean protein content than G2. Environments were unevenly distributed around the origin, indicating differential responses across environments. E4 and E9 were close to the origin, suggesting greater stability, whereas E8 and E12 showed stronger interaction effects. The "which-won-where" biplot indicated that G1 was associated mainly with E2, E5, and E7, while G2 was associated with E12. Most environments were closer to G1 along PC1, indicating a tendency for wider adaptation across the evaluated conditions and higher protein performance of G1 across environments.

3.8.2. Fat Content

For fat content, the AMMI model (Figure S1) revealed substantial genotype × environment interaction. The AMMI1 biplot showed small differences in mean fat content between genotypes, with G1 exhibiting slightly higher values than G2. Environments were more dispersed around the origin compared with protein content, indicating stronger crossover interaction and greater environmental influence. E2, E5, E9, and E12 were close to the origin, suggesting greater stability, whereas E1, E3, E4, and E10 showed stronger interaction effects. The "which-won-where" biplot indicated that E1, E4, E7, and E11 were associated with G1, while E3, E6, and E10 were associated with G2, indicating no consistently superior genotype across environments.

3.8.3. Ash Content

The AMMI analysis for ash content (Figure S2) revealed substantial genotype × environment interaction. The AMMI1 biplot showed similar mean ash content values between genotypes, indicating limited differences in performance. Environments E2, E4, E5, and E6 were close to the origin, suggesting greater stability, whereas E1, E3, E11, and

E12 showed stronger interaction effects and contributed more to genotype differentiation. The "which-won-where" biplot showed a distribution of environments across the PC1 axis, indicating specific adaptation patterns of the genotypes under different environmental conditions. Ash content was influenced more by genotype × environment interaction than by differences in genotype mean performance.

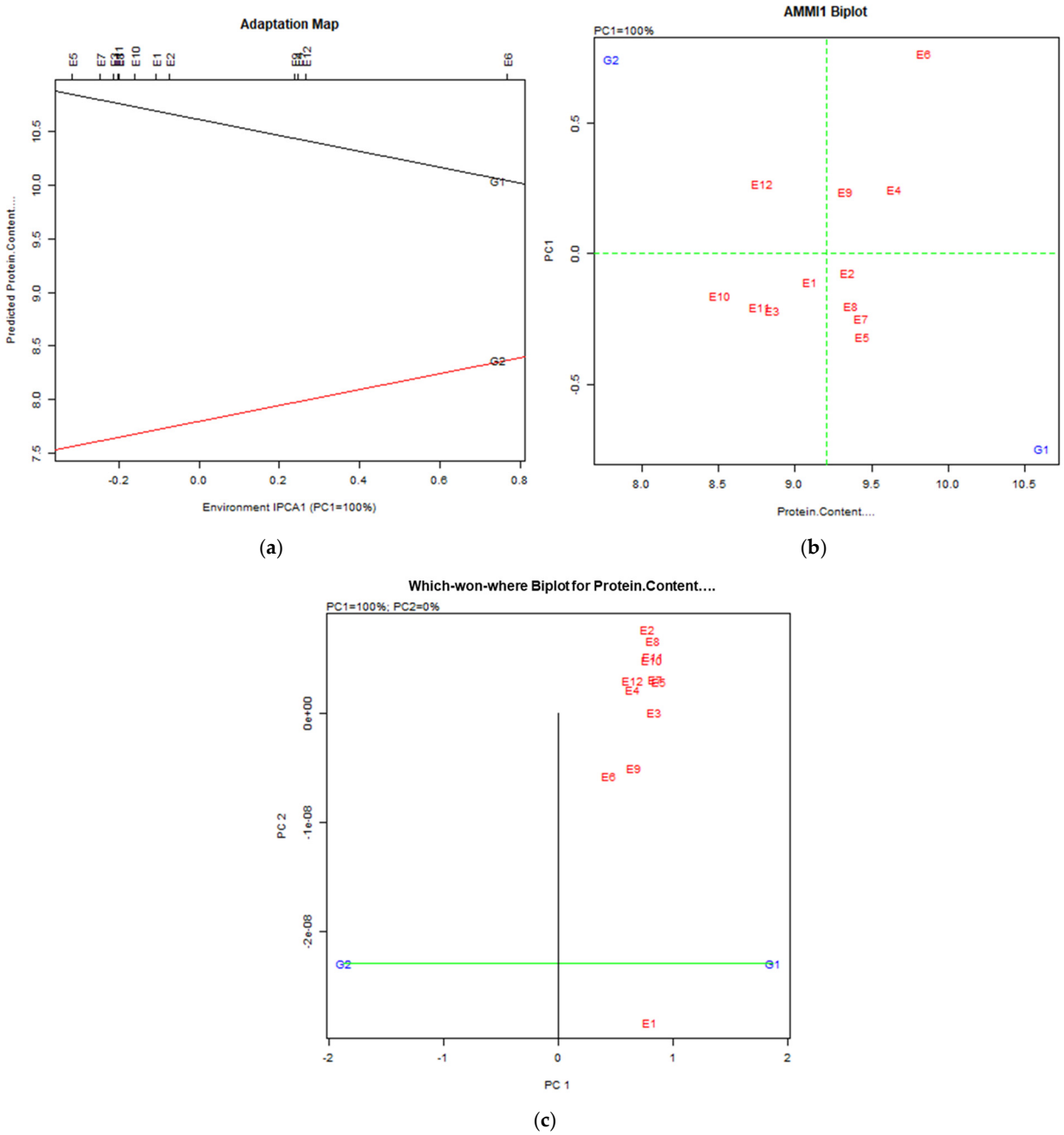

Figure 6. Stability analysis of crude protein content in maize hybrid seeds. (**a**) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids; (**b**) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability; (**c**) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments.

3.8.4. Starch Content

The AMMI analysis for starch content (Figure S3) revealed substantial genotype × environment interaction. The AMMI1 biplot indicated slightly higher mean starch content in G1 than in G2. Environments were distributed on both sides of the PC1 axis, indicating differential responses across environments. E2, E3, E9, and E12 were close to the origin, suggesting greater stability, whereas E10 and E11 showed stronger interaction effects and contributed more to genotype differentiation. The "which-won-where" biplot showed that G1 was associated with E1, E3, E4, E5, E6, E7, E9, and E12, while G2 was associated with E8, E10, and E11. Most environments were closer to G1 along PC1, indicating a tendency for wider adaptation across the evaluated conditions of G1, whereas G2 was associated with fewer environments.

3.8.5. Crude Fiber Content

For crude fiber content, the AMMI analysis (Figure S4) revealed substantial genotype × environment interaction. The AMMI1 biplot showed small differences in mean crude fiber content between genotypes, with G1 exhibiting slightly higher values. E5, E7, and E12 were close to the origin, indicating greater stability and weaker interaction effects, whereas E3, E8, and E10 were farther from the origin and contributed more to genotype differentiation. The "which-won-where" biplot showed that G1 was associated with E1, E2, E3, E7, E9, and E10, while G2 was associated with E4, E5, E6, E8, and E11, indicating strong genotype × environment interaction and environment-specific performance differences.

3.8.6. Moisture Content

The AMMI analysis for moisture content (Figure S5) demonstrated clear genotype × environment interaction. The AMMI1 biplot indicated slightly higher mean moisture content in G1 than in G2. E2, E4, E7, and E12 were close to the origin, suggesting greater stability, whereas E8, E9, E10, and E11 showed stronger interaction effects and contributed more to genotype differentiation. The "which-won-where" biplot showed that G1 was associated with E3, E6, E8, and E9, while G2 was associated with E1, E4, E5, E10, and E11. Most environments were concentrated on the positive side of PC1, indicating closer association with G1 and indicating a tendency for wider adaptation across the evaluated conditions for this genotype.

3.8.7. Kernel Length

The AMMI analysis for kernel length (Figure S6) revealed substantial genotype × environment interaction. The AMMI1 biplot indicated higher mean kernel length in G1 than in G2. E1, E2, and E7 were close to the origin, suggesting greater stability, whereas E5 and E10 showed stronger interaction effects and contributed more to genotype differentiation. The "which-won-where" biplot showed that G1 was associated with E1, E4, E5, E7, E8, and E11, while G2 was associated with E3, E6, E9, E10, and E12. Most environments were positioned on the positive side of PC1 and were more closely associated with G1, indicating a tendency for wider adaptation across the evaluated conditions and superior kernel length performance of G1 across environments. G2 was associated with lower mean values and fewer environments.

3.8.8. Kernel Width

The AMMI analysis for kernel width (Figure S7) revealed substantial genotype × environment interaction. The AMMI1 biplot showed small differences in mean kernel width between genotypes, with G1 exhibiting slightly higher values than G2. E1, E5, and E8 were close to the origin, indicating greater stability and weaker interaction effects,

whereas E10 and E11 showed stronger interaction effects and contributed more to genotype differentiation. The "which-won-where" biplot showed that G1 was associated with E2, E5, E8, E9, and E11, while G2 was associated with E1, E3, E6, and E10. Environmental distribution across the positive side of PC1 indicated differential responses of the genotypes across environments. Kernel width was strongly influenced by environmental conditions and genotype × environment interaction.

3.8.9. Kernel Thickness

The AMMI analysis for kernel thickness (Figure S8) revealed substantial genotype × environment interaction. The AMMI1 biplot showed slightly higher mean kernel thickness in G1 than in G2, although differences between genotypes were small. E1, E4, and E8 were close to the origin, indicating greater stability, whereas E5 and E10 showed stronger interaction effects and contributed more to genotype differentiation. The "which-won-where" biplot showed that G1 was associated with E2, E5, E7, and E11, while G2 was associated with E3, E6, and E10. Environmental separation along the PC1 axis indicated specific adaptation patterns for the two genotypes. Kernel thickness was influenced by both genotype mean performance and genotype × environment interaction.

4. Discussion

The variation in seed quality and kernel morphological traits across environments reflects the considerable influence of genotype, environment, and their interaction on maize performance [16,37,38]. In the present study, environments were defined as combinations of growing season and row spacing treatment, allowing the assessment of both seasonal and management-related sources of variation. The significant genotype × environment (G × E) interaction confirms the environment-dependent expression of traits, as widely reported in maize breeding studies [7,39]. Breeding studies have shown that simultaneous selection for both performance and stability across environments can enhance genetic gain and adaptation, highlighting the importance of integrated breeding strategies [40]. Similar patterns of variation in biochemical and kernel morphological traits have also been reported in diverse maize germplasm under contrasting environmental conditions [41]. Multivariate approaches, including PCA, AMMI, and GGE biplot, further reveal the complex structure of genotype performance and trait expression across environments [7,8,24,42].

4.1. Effects of Environment, Hybrid, and Plant Density on Seed Traits

The significant effects of year × row spacing combinations, hybrid, and plant density indicate that maize kernel quality and morphology are controlled by the combined influence of genetic, environmental, and management factors. This finding confirms that both genetic background and environmental conditions substantially influenced the expression of nutritional and morphological kernel characteristics. Similar findings have been widely reported in maize multi-environment studies, where genotype × environment interactions are considered a major source of phenotypic variation and stability differences among hybrids [24,39]. Recent studies have confirmed the importance of environmental variation and genotype × environment interactions in determining maize performance and grain quality. Wang et al. [19] demonstrated that meteorological conditions before and after anthesis significantly affected grain nutritional composition and kernel characteristics, whereas Bongianino et al. [20] reported that environmental effects explained a substantial proportion of variation in grain quality traits across diverse maize genotypes. Similarly, Ma et al. [27] and Engida et al. [43] highlighted the major contribution of genotype × environment interactions to hybrid adaptation and stability across contrasting production conditions.

Environmental effects significantly influenced traits related to seed composition, including protein, starch, fat, and moisture content, suggesting strong environmental regulation of grain filling and assimilate accumulation processes. In the present study, environmental variation was primarily associated with differences among growing seasons and row spacing treatments. Annual fluctuations in temperature and precipitation likely influenced grain filling dynamics and assimilate partitioning, thereby contributing to the observed variation in seed composition and kernel morphology. Hybrid effects further confirmed the importance of genetic variability in determining seed quality and morphology, particularly for nutritionally important traits such as protein and starch content [44]. The observed differences between Costanza (FAO 660) and LG 3535 (FAO 500) further suggest that maturity class may contribute to differential responses of seed quality and kernel morphological traits under varying growing conditions, although this hypothesis was not directly tested in the present study. Plant density and row spacing also significantly affected most traits, supporting previous findings that agronomic practices influence maize kernel development and grain characteristics through modifications in resource availability and plant competition [45,46]. Moreover, the significant interaction effects observed in the present study indicate that hybrid performance and trait stability were strongly dependent on environmental and agronomic conditions, consistent with observations reported by Greveniotis et al. [47].

4.2. Stability Index Responses Across Environments, Row Spacing Treatments, and Growing Seasons

The Stability Index (SI) revealed genotype × environment × agronomic treatment interactions affecting seed quality and kernel morphological traits across environments, row spacing treatments, and growing seasons. Costanza generally showed higher SI values for protein, starch, fat, crude fiber, and most kernel morphological traits, whereas LG 3535 exhibited relatively greater stability for moisture content, indicating genotype-specific regulation of kernel composition under environmental variability. SI variation across environments, particularly for starch, moisture, and kernel size traits, indicates their high sensitivity to environmental fluctuations. Similar genotype-dependent stability patterns in maize grain quality have been reported, where environmental variability and genotype × environment interactions strongly influenced kernel composition and morphology [48,49]. These observations are also consistent with Bongianino et al. [20], who reported that environmental conditions accounted for a substantial proportion of variation in grain quality traits, while the relative contribution of genotype and environment differed among maize germplasm groups, highlighting the complexity of quality trait stability across environments. Comparable findings in wheat further support the strong environmental influence on starch- and moisture-related traits under Mediterranean conditions [50]. Agronomic factors, particularly row spacing and plant density, also contributed to SI variation, indicating that agronomic practices interact with genotype and environment in shaping trait expression. Significant effects of plant density and row configuration on maize morphological and yield-related traits have been previously demonstrated [47], supporting their role as key modulators of stability expression. Year-to-year variation highlighted the influence of climatic variability on trait stability, with Costanza exhibiting more consistent performance across seasons, particularly for starch accumulation and kernel morphology. The importance of seasonal climatic variation is supported by Wang et al. [19], who demonstrated that temperature, rainfall, and accumulated thermal units before and after anthesis significantly affected grain nutritional quality and kernel weight components in maize. This agrees with previous SI-based studies identifying Costanza among the most stable maize hybrids across diverse Greek environments [51], as well as with AMMI- and stability-based studies reporting that certain maize genotypes may

combine high performance with stable expression across environments, depending on genotype × environment structure and trait architecture [52,53]. Similarly, Engida et al. [43] identified maize hybrids combining superior performance and stability across a wide range of stress and non-stress environments, demonstrating the usefulness of AMMI and GGE methodologies for identifying broadly adapted genotypes. According to Fasoulas [9], high SI values may reflect traits under stronger genetic control and higher heritability, as stability was associated with relatively simpler (oligogenic) genetic control and higher heritable components. Recent SI-based evaluations in barley further support this, indicating that highly stable traits are associated with stronger genetic control and improved selection response under Mediterranean conditions [54]. Integrated evidence from wheat, cotton, and barley supports the view that seed quality stability is governed by interacting genetic, environmental, and management factors rather than isolated effects [55,56], highlighting the need for multi-environment evaluation in genotype selection. Costanza expressed broader and more consistent stability across traits and environments, whereas LG 3535 showed more selective stability, particularly for moisture-related traits, suggesting contrasting adaptive strategies under Mediterranean agroecological variability, with implications for breeding programs aiming to improve both compositional stability and stress tolerance. The observed differences in stability between Costanza and LG 3535 may also be partly explained by their maturity classification. Because the two hybrids belong to different FAO maturity groups (FAO 660 and FAO 500, respectively), they differ in growth duration, resource utilization patterns, and exposure to environmental conditions during grain filling. Consequently, maturity class may contribute to differences in the stability of both kernel composition and kernel morphology across environments. Greveniotis et al. [57] reported that maize hybrids belonging to the intermediate to high maturity group (FAO 500–700) generally exhibit comparable or superior performance compared to very early or very late maturity hybrids due to better adaptation to the length of the growing season. In the present study, Costanza (FAO 660) falls within this optimal maturity range for the environmental conditions of northern Greece, whereas LG 3535 (FAO 500) represents a relatively earlier hybrid. This difference in maturity class may help explain the broader stability and more consistent performance of Costanza across environments and may be associated with better adaptation to local growing season conditions and environmental resources. In summary, traits with high stability indices may be more effectively exploited in breeding programs due to their greater phenotypic consistency across environments [49,54,55,58].

4.3. Interpretation of Correlation Matrix Results

The correlation analysis revealed that protein content was positively associated with ash, crude fiber, moisture, and kernel length, while showing a weak negative relationship with fat content. Similar associations among grain quality traits have been reported in maize under multi-environment conditions [48,49], suggesting that kernel composition and morphology are regulated by interconnected assimilate allocation processes during grain filling. Such associations may also reflect differences in the duration and efficiency of grain filling among environments and genotypes. Fat content showed positive correlations with starch and kernel morphological traits, whereas it was negatively associated with crude fiber. Similar associations between kernel physical characteristics and starch-related traits have been reported in maize [59,60], suggesting an association between kernel morphology and storage component deposition during grain development. Starch content exhibited strong positive relationships with kernel morphological traits and fat content, while being negatively associated with ash and crude fiber. This is consistent with the central role of starch as the primary carbon reserve in seeds, where increased carbohydrate accumulation is often accompanied by reduced relative proportions of structural and mineral components

during seed development [61], indicating compositional trade-offs during endosperm filling. The positive association between starch accumulation and kernel dimensions further suggests that larger kernels possess a greater capacity for reserve deposition during seed development. Crude fiber showed consistent negative associations with fat, starch, and kernel size traits, supporting an antagonistic relationship between structural tissue formation and storage compound accumulation. Moisture content was positively associated with protein and kernel size traits, while its relationships with fat and starch were weak, consistent with the complex genetic and environmental regulation of grain moisture dynamics in maize [62]. Finally, kernel morphological traits were strongly intercorrelated and positively associated with starch accumulation, highlighting their role as integrative indicators of kernel growth and assimilate partitioning during grain development, consistent with previous reports describing the relationship between starch deposition, kernel structure, and grain development processes [59,60]. These correlations suggest a balance between storage compounds and structural tissue, which may contribute to differences in kernel development and final seed quality across environments.

4.4. Multivariate Analysis of Seed Quality and Kernel Morphological Traits

The combined multivariate analyses, including heat map with hierarchical clustering, principal component analysis (PCA), and constellation plot, revealed clear differences in seed quality and kernel morphological trait expression among maize hybrids under contrasting environmental and agronomic conditions. The heat map demonstrated that clustering patterns were associated with specific combinations of hybrid, year, row spacing treatment, and plant density. Several H1 (Costanza) combinations were associated with relatively higher protein content, kernel length, and moisture content, whereas specific H2 (LG 3535) combinations, particularly under certain environments and row spacing treatments, were associated with increased starch content, kernel width, and kernel thickness. Hierarchical clustering of traits further highlighted coordinated variation among starch, width, thickness, and fat, while protein, crude fiber, and ash exhibited partially distinct clustering patterns. This clustering pattern suggests that kernel dimensions and reserve accumulation are physiologically interconnected processes, as larger kernels generally possess a greater capacity for starch and lipid deposition during grain filling. Conversely, the distinct grouping of protein, crude fiber, and ash may reflect differences in assimilate partitioning and tissue composition during kernel development. Similar multivariate approaches have been widely used for the exploration of complex trait relationships and genetic diversity patterns in crop studies [7,63].

PCA explained 72.2% of total variability, with PC1 positively associated with starch, kernel width, kernel thickness, and fat content, and negatively associated with crude fiber and ash, whereas PC2 was mainly associated with protein and moisture content. Score plots showed clearer separation between hybrids than plant densities, suggesting a stronger influence of genetic background on kernel composition. This finding indicates that genetic background was the primary determinant of kernel compositional profiles, whereas agronomic practices mainly modulated trait expression within each genotype. Similar PCA-based multivariate approaches have been widely applied for visualization and interpretation of complex biological datasets and trait relationships [10,64].

The constellation plot provided an additional visualization of multivariate relationships, showing structured networks among years, hybrids, row spacing, and plant density treatments. Clustering patterns indicated differentiation according to year and hybrid background, while plant density effects were less noticeable but still contributed to local grouping structures. Collectively, the constellation plot confirmed the presence of substantial multivariate variability among maize combinations and highlighted the combined

influence of year, hybrid, and row spacing treatment on seed quality and kernel morphological trait expression across environments. The tighter clustering of several Costanza combinations suggests a tendency toward greater multivariate consistency across year × row spacing combinations, although this pattern should be interpreted cautiously given the limited number of hybrids evaluated.

4.5. AMMI and GGE Analysis of Seed Quality and Kernel Morphological Traits

The combined AMMI and GGE analyses revealed substantial genotype × environment interactions for most seed quality and kernel morphological traits, indicating differential genotype responses across the evaluated maize environments. The AMMI1 biplots showed that environmental combinations differed considerably in their contribution to interaction effects, with some environments positioned close to the origin and therefore exhibiting greater stability, whereas others contributed more strongly to genotype differentiation. This pattern indicates that seasonal conditions and row spacing combinations did not affect all traits uniformly but rather altered the relative performance of the two hybrids depending on the trait considered. Such differential responses are characteristic of crossover genotype × environment interactions and complicate the identification of universally superior genotypes. Similar AMMI interpretation approaches have been widely used for evaluating genotype performance, interaction patterns, and environmental stability in multi-environment trials [33,65].

The GGE biplots further illustrated patterns of specific adaptation among genotypes and environments through the "which-won-where" visualization approach. Several traits showed broader environmental association for G1 (Costanza), whereas G2 (LG 3535) exhibited adaptation to fewer or more specific environmental combinations depending on the evaluated trait. The dispersion of environments across the PC1 and PC2 axes also indicated the presence of crossover interactions and differential genotype responses under contrasting environmental conditions. Because environments in the present study were defined by combinations of year and row spacing treatment, crossover interactions likely reflected the combined effects of seasonal climatic variability and modifications in plant spatial arrangement. These results emphasize that kernel compositional and morphological traits are highly responsive to both environmental fluctuations and agronomic management practices. Similar applications of GGE biplot analysis for identifying adaptation patterns, mega-environment structure, and genotype stability have been extensively reported in multi-environment crop studies [7,25].

The combined AMMI and GGE analyses demonstrated that both genotype background and year × row spacing combinations contributed substantially to the expression and stability of seed quality and kernel morphological traits. Importantly, these multivariate approaches complemented the Stability Index analysis by identifying not only the magnitude of stability differences but also the specific environmental combinations responsible for genotype discrimination and adaptation patterns. Comparable approaches have also been successfully applied in maize and other crop species for the investigation of seed quality stability and genotype × environment interactions across diverse cultivation conditions [49,50,56].

Maize seed quality and kernel morphological traits were considerably influenced by genotype, year × row spacing combinations, and agronomic practices. The integration of stability analysis, multivariate statistics, and AMMI/GGE approaches provided complementary information regarding trait expression, genotype adaptation, and environmental responsiveness. Costanza generally exhibited a tendency toward broader stability and adaptation across the evaluated year × row spacing combinations, whereas LG 3535 showed more specific responses depending on environmental conditions and trait type.

These findings may provide useful information for breeding and agronomic strategies aimed at improving seed quality stability under Mediterranean agroecological conditions. However, because only two commercial hybrids differing in FAO maturity group were evaluated, the present findings should be considered indicative rather than broadly representative of maize germplasm diversity. Further studies involving a larger number of genotypes are required to confirm the observed adaptation and stability patterns.

5. Conclusions

This study showed that seed quality and kernel morphological traits in maize are strongly influenced by genotype, year-to-year climatic variation, and agronomic management. The combined influence of these factors resulted in considerable variation in trait expression across the evaluated year × row spacing combinations, highlighting the complex nature of kernel quality and morphology under multi-environment conditions.

Environmental variation associated with differences among growing seasons and row spacing treatments was a major driver of variation in seed composition and kernel morphology, particularly for protein and starch content and kernel size-related traits. Agronomic practices, including plant density and row spacing, also contributed to trait variability, emphasizing their importance in modulating kernel development and compositional characteristics.

Among the two evaluated commercial hybrids, Costanza (FAO 660) generally exhibited greater stability and more consistent performance across the evaluated year × row spacing combinations, particularly for starch content and kernel morphological traits. In contrast, LG 3535 (FAO 500) showed more specific responses to environmental variation, especially for moisture-related traits, suggesting differences in adaptive responses associated with maturity class.

The combined use of Stability Index, correlation analysis, PCA, AMMI, and GGE methodologies provided complementary information on trait relationships, genotype adaptation, and the environmental combinations contributing to trait stability and differentiation. These approaches improved the understanding of kernel quality responses under variable production conditions and demonstrated the value of integrating univariate and multivariate methodologies in multi-environment studies.

Because only two commercial hybrids differing in FAO maturity group were evaluated, the present conclusions should be considered indicative rather than broadly representative of maize genetic diversity. Nevertheless, the findings highlight the importance of multi-environment evaluation for identifying hybrids with stable seed quality and kernel morphological characteristics under Mediterranean production conditions and provide a basis for future studies involving a broader range of maize germplasm.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture16131385/s1, Figure S1: Stability analysis of fat content in maize hybrid seeds. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments; Figure S2: Stability analysis of ash content in maize hybrid seeds. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the

distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments; Figure S3: Stability analysis of starch content in maize hybrid seeds. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments; Figure S4: Stability analysis of crude fiber content in maize hybrid seeds. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments; Figure S5: Stability analysis of moisture content in maize hybrid seeds. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments; Figure S6: Stability analysis of seed length in maize hybrids. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments; Figure S7: Stability analysis of seed width in maize hybrids. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments. Figure S8: Stability analysis of seed thickness in maize hybrids. (a) AMMI adaptation map illustrating the structure of hybrid maize genotype × environment interactions and the relative stability of the evaluated hybrids. (b) AMMI1 biplot showing the relationship between mean performance and interaction effects, allowing a general assessment of hybrid performance and stability. (c) "Which-won-where" GGE biplot presenting the distribution of maize hybrids across environments and indicating patterns of adaptation. Hybrids located closer to the "ideal genotype" are considered to combine higher mean performance with greater stability across the evaluated environments.

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