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A Comparative Study on Stability of Seed Characteristics in Vetch and Pea Cultivations

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Abstract: Stability for yield and seed quality across environments are desirable traits for varieties used for the support of livestock, and such specific varieties of common vetch (*Vicia sativa* L.) and peas (*Pisum sativum* L.) are highly demanded from farmers. The objective of this study was to investigate the stability performance of seed quality attributes on six common vetch genotypes and five pea genotypes. The genotypes' stability traits were based on seed quality characteristics of peas and common vetch under low-input vs. conventional cultivation systems. Significantly positive or negative correlations between the main traits in all cultivation schemes were found. Based on these findings, improving certain traits that exhibit qualitative inheritance is expected to be an efficient indirect way to improve seed quality stability, more easily in the case of peas. It was evident from comparisons that even in low-input farming systems, varieties showed stable performance. Analysis of variance (ANOVA), GGE biplot on main traits, and AMMI analysis all resulted in statistically significant variations between genotypes, environments, and farming practices. This analysis resulted in specific pea varieties and vetch cultivars that were stable for various regions and farming systems on seed quality traits.

Keywords: protein; starch content; seed quality; peas; vetch

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1. Introduction

Animal nutrition requires high-quality protein feed and a good balance of other ingredients. The two main crops that support livestock in Greece, providing protein and useful ingredients, are peas and common vetch. Pea (*Pisum sativum* L.) is a desirable crop for feeding farm animals [1–3]. Thus, Greek cultivars were developed almost twenty years ago in order to cover animal feeding demands all over Greece and in many distinct environments. Pea cultivations are found in a variety of agro-ecological zones, making this crop very useful to support livestock [1–5]. Legumes constitute a significant food source in developing countries, according to Elamine et al. [6]. On a worldwide basis, many crops that are thought to be neglected may actually be critical to animal feeding. A few species of grain legumes are among them, such as common vetch (*Vicia sativa* L.) and grass-pea (*Lathyrus sativus* L.) [7–9]. In the Mediterranean and central Asian regions, common vetch is a significant legume that is grown for feed grain and forage [10–12].

Almost all animal species on farms could use field peas as a source of protein. *Vicia sativa* L., known as common vetch, is a popular legume crop due to its versatile uses, mainly as a basic protein source, and also due to its suitability for organic and minimal-input farming systems [13,14]. The seven attributes that define seed quality and are important to animals are water, minerals, significant proteins, carbohydrates (usually starch), fats, fiber, vitamins, and minerals.

Stability is of great importance in selecting genotypes for different growing systems and environmental conditions [15,16]. The capacity for performance under practically any environmental challenge is known as stability [17]. In this way, Fasoulas proposed a simple coefficient to predict the stability performance of genotypes based on the coefficient of variation. Later, Fasoula [18] proposed some modifications to this basic coefficient.

In pea and vetch cultivations, several researchers evaluated stability using different statistical tools (GxE classic statistics, GGE and AMMI biplot analysis, cluster and regression analysis) for yield characteristics [19–22], mainly in multi-location environments, as summarized briefly by Greveniotis et al. [2,3,13,14]. In peas, cluster analysis was shown by Acikgoz et al. [19] to be more effective than traditional stability analysis. Sayar [23] succeeded in revealing consistent genotypes in common vetch (*Vicia sativa* L.). Macák et al. [24] reckoned that pea grain yield may be more stable with high fertilizer levels. Because farmers who utilize peas as livestock feed use specific cultivation techniques, performance under low-input settings must be considered.

Pea breeders' main objective is to boost seed yield in order to maximize plant productivity and enable peas to be used more widely in different agricultural production systems [25].

The selection of varieties must take into account their ability to adapt to a variety of environments, especially low-input ones, and, therefore, both breeders and agronomists must perform significant experimentation.

The objective of this study was to ascertain the seed characteristics' stability of pea and vetch genotypes, for the traits of protein, fat, ash, starch, crude fiber, and moisture using various stability indexes, focusing on genotypic behavior in both high-input and low-input production systems. Based on the observations of Fasoulas [17] and Fasoula [26], Greveniotis et al. [27,28] employed the stability index to estimate the heritability of several variables. The type of heritability of characteristics and the type of stability performance are both identified by our method, which also analyzes stability performance.

2. Materials and Methods

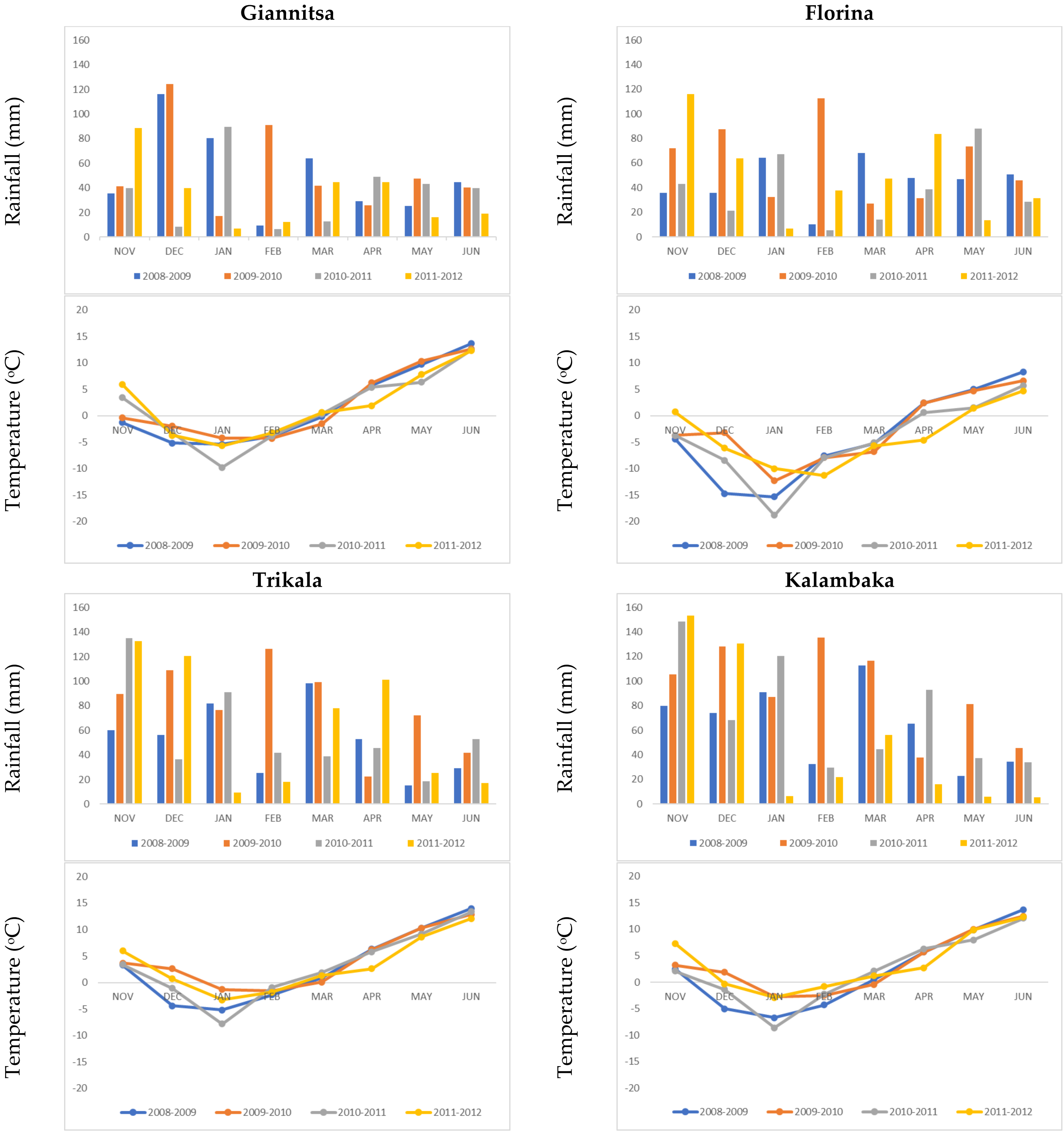
2.1. Establishment of Crops and Experimental Techniques

The field trials were established in four locations (Florina and Giannitsa in Northern Greece; Kalambaka and Trikala in Central Greece), which varied in terms of soil type, elevation (Table 1), and characteristics of the environment.

Table 1. Coordinates, elevation, and type of soil for the experimental settings.

Environments	Longitude	Latitude	Elevation (m)	Soil Texture
Northern Greece 1—Giannitsa	22°39′ E	40°77′ N	10	clay (C)
Northern Greece 2—Florina	21°22′ E	40°46′ N	705	sandy loam (SL)
Central Greece 1—Trikala	21°64′ E	39°55′ N	120	sandy clay loam (SCL)
Central Greece 2—Kalambaka	21°65′ E	39°64′ N	190	silty clay (SiC)

Experimentation lasted four successive seasons of growth (2008–2009, 2009–2010, 2010–2011, and 2011–2012), and mean monthly temperatures and precipitation values according to daily recordings are shown in Figure 1 for each experimental area.


Giannitsa

Florina

Trikala

Kalambaka

Figure 1. Meteorological data (average monthly air temperatures in °C and total monthly rainfall in mm) according to daily records, through four growing seasons (2008–2009, 2009–2010, 2010–2011, and 2011–2012).

Vetch: Six well-established varieties of common vetch were chosen to be cultivated utilizing a strip-plot design: cvs. The varieties were Alexandros, Filippos, Omiros, Pigasos, Tempi, and Zefyros. Alexandros, Pigasos, Tempi, and Zefyros were created at the Institute of Industrial and Forage Crops (Hellenic Agricultural Organization Demeter, Greece). Filippos was developed by Zouliamis Nikolaos (Greece), and Omiros is a creation of P. Agrafiotis & Sons GP (Greece).

Within each plot, the chosen cultivars were planted in random order. Each plot included seven rows that were each five meters long, with 0.25 m separating each row, for a total plot area of 8.75 m^2. Crops were planted early in November 2010 and 2011 and harvested late in June 2011 and 2012, respectively.

Conventional and low-input agricultural techniques were employed. For the conventional farming approach, the plots were treated before sowing, adding 30 and 50 kg ha^{-1} of nitrogen and P_2O_5 to the soil, respectively.

In order to practice low-input farming, no fertilizers or other agro-chemicals were used in any of the four study locations during the experiment. The fields had previously been used to produce bread wheat and legumes in rotation without the use of supplemental nutrients or other agro-chemicals. The area of experimentation underwent manual weed control.

Pea: Five cultivars of peas, common in Greek cultivations, were chosen for cultivation using a strip-plot design: cvs. The cultivars were Olympos, Pisso, Livioletta, Vermio, and Dodoni. Olympos, Vermio, and Dodoni were created at the Institute of Industrial and Forage Crops (Hellenic Agricultural Organization Demeter, Greece). Pisso was developed by Zouliamis Nikolaos (Greece), and Livioletta is of German origin. Characteristics of the cultivars have been provided previously by Greveniotis et al. [2].

Establishing a strip-plot design with a plot size of 8.75 m^2 and the five genotypes randomly distributed within each plot, all varieties were seeded during early in November 2008 and 2009. For each plot, there were four replications. According to the planting rate, each plot had seven rows that were five meters long and twenty-five centimeters apart. A total of 120 seeds were sown at a depth of 4 cm per square meter. Experiments were planted in early November 2008 and 2009, and harvested in late June 2009 and 2010, respectively.

Low-input and conventional agricultural systems were chosen as the two different types of cultivation methodologies. The standard farming approach treated the plots before sowing, adding 40 kg ha^{-1} of nitrogen and 80 kg ha^{-1} of P_2O_5 to the soil. No fertilizers or other agrochemicals were used during the trial because it was low-input agriculture. The fields had been planted in a two-year cycle of bread wheat and legumes without the use of agrochemicals or supplemental nutrients before the experiment was started in 2008. Only hand labor was used to control the weeds.

2.2. Measurements

All attributes were measured in the University of Thessaly's Laboratory of Animal Technology. Traits measured were crude protein content (%), crude fat (%), ash content (%), total starch (%), crude fiber content (%), and moisture (%).

Prior to the analyses, samples were grounded. Crude protein content (%) was determined by applying the American Association of Cereal Chemists (AACC) method 44-15.02 [29] for total nitrogen, using the Kjeldahl procedure, followed by multiplication by factor 6.25. The procedure for total nitrogen determination is as follows: after the organic matter of the sample is destroyed by sulfuric acid in the presence of a catalyst, the reaction products are alkalized, the released ammonia is distilled, and it is then collected in a boric acid solution before being titrated with a volumetric standard hydrochloric acid solution.

Crude fat (%) was determined by employing extraction with petroleum ether using the Soxhlet extraction apparatus (AACC method 30-25.01) [29]. In order to determine moisture (%), an air-oven method was applied (AACC method 44-15.02) [29]. For measuring ash content (%) the sample was heated to 550 °C in a furnace until it reached a consistent weight, then it was cooled in a desiccator and weighed once it had cooled to room temperature, according to AACC method 08-01.01 [29]. An enzyme-based assay (AACC method 76-13.01) [29] was used to determine the amount of total starch (%). Specifically, the amount of total starch was determined by enzymatically converting the α-linked-glucose carbohydrate to glucose and then detecting the released glucose using the Megazyme Amyloglucosidase/alpha-Amylase protocol. Lastly, method AACC 32-10.01 [29] was employed to calculate crude fiber (%), which includes a series of sulfuric acid and sodium hydroxide digestions, followed by drying, weighing, and ignition of the insoluble residue, and, finally, calculation of crude fiber from the ignition loss.

2.3. Data Analysis

Taking into account the stability index $(\overline{x}/s)^2$, stability estimates were generated, where $\overline{x}$ and s represent the entry mean yield and standard deviation, respectively [18,26].

According to Steel et al. [30], the Pearson coefficient was used to analyze trait correlations, and SPSS version 25 was used to determine the significance of every statistic at $p < 0.05$. For each characteristic, a statistical analysis was carried out using the free edition of PB Tools employing AMMI1 and (GGE) Biplot analysis as the statistical tools.

Following the suggestions made by McIntosh [31], the variance components were estimated using the mean squared values of the genotypes, genotype environment, error, and replicates. This enabled us to determine the genetic parameters for the examined characteristics in the following ways.

The heritability in a broad sense (H^2) was estimated based on Johnson et al. [32] and Hanson et al. [33], as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{gxe}^2}{e} + \frac{\sigma_{re}^2}{rxe}}$$

The genotypic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV) were determined for every examined attribute in accordance with Singh and Chaudhary [34]:

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

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

where the genotypic variance, phenotypic variance, genotype × environment variance, residual variance (error), and overall mean for every examined attribute are, in turn, denoted by σ_g^2, σ_p^2, σ_{gxe}^2, σ_{re}^2, and $\overline{x}$, respectively.

2.4. The Multi-Environment Evaluation AMMI Tool

The AMMI analysis is a software tool utilized in the experimental multi-environment analysis in order to explore the GEI complex. The AMMI software arranges the data in a two-way table for GEI. From these tables, the least squares are estimated and used to produce a two-way ANOVA for an additive model for the main effects and a value to express the residuals' interaction [35].

This AMMI software tool generates figures of the adaptation map and AMMI1 biplot with the two axes depicting the factor (X axis) and the PC1 value (Y axis). Based on the data, if the PC1 value is low, then the distance from the X-axis is short, which means that the analyzed factor is stable for all environments. Based on the AMMI1 biplot, the stable genotypes, which are desirable, are those having higher values on the trait performance X-axis (right position) and are closer to the Y-axis of the PC1 values.

GGE analysis is for genotype main effect (G) combined with genotype by environment interaction (GE), which makes it the main component of variance that is applied in the assessment of genotypes. In mathematical terms, GGE consists of the genotype by environment (GxE) data matrix from which the environment means are subtracted. In two-way data, a GGE biplot depicts the GGE of a genotype by environment. The methodology of GGE biplot originates from the multi-environmental analysis of genotype trials (MET) data using graphical tools and is easily adapted to different kinds of two-way data.

Using the GGE biplot over environments, the most stable and desirable environment is that placed near the average and ideal environment. With regard to the genotypes and the GGE biplot, the ideal and desirable genotypes (productivity combined with stability) were those that were placed in the zone of the average genotype dot and close to the ideal genotype.

The GGE and AMMI1 biplot analysis tools create biplots showing how each genotype performed in all environments. In this manner, each genotype can easily be characterized for performance and stability in a simple way. The software used was the PB tools v1.4 free version (International Rice Research Institute, Laguna, Philippines).

3. Results

3.1. Vetch Seed Analysis

ANOVA results are given in Table 2, and all traits' main effects showed significant differences. GXE interaction was highly significant for all attributes, revealing the relationship between phenotypic expression and environmental conditions under which the genotypes were cultivated. These results led to further analysis of our data for stability estimations, GGE biplots, and AMMI1 analysis.

Table 2. Mean squares (m.s.) from an ANOVA for the evaluated attributes across environments and cultivation techniques: crude protein percent of dry matter (%DM), fat (%DM), ash content (%DM), starch (%DM), crude fiber (%DM), and moisture (%) for common vetch.

Source of Variation	**Crude Protein (%DM) m.s.**	**Fat (%DM) m.s.**	**Ash (%DM) m.s.**	**Starch (%DM) m.s.**	**Crude Fiber (%DM) m.s.**	**Moisture (%) m.s.**
Environments (E)	35.116 **	0.635 **	0.801 **	13.218 **	2.574 **	5.291 **
REPS/Environments	0.087 **	0.003 **	0.001 ns	0.157 *	0.003 ns	0.014 ns
Varieties (G)	141.09 **	1.756 **	7.969 **	83.712 **	23.736 **	45.457 **
Environments × Varieties (G × E)	0.073 **	0.003 **	0.008 **	1.665 **	0.015 **	0.911 **
Error	0.050	0.001	0.004	0.107	0.003	0.014

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

Stability estimations are presented in Tables 3–5. Table 3 includes calculations for all characteristics tested across environments. Starch content showed the highest index values, while crude protein showed values over 200. Low values (less or close to 100) were found for the rest of the characteristics measured. The stability estimates were not significantly affected by the minor differences between the two agricultural systems, but in some instances (in the Kalambaka area for starch content and in Giannitsa and Trikala for crude protein content), the stability indices were greater in low-input experiments.

Table 3. Stability index estimates for seed chemical composition parameters for common vetch in two farming systems across environments.

	Environments	**Crude Protein (%DM)**	**Fat (%DM)**	**Ash (%DM)**	**Starch (%DM)**	**Crude Fiber (%DM)**	**Moisture (%)**
Conventional	Giannitsa	230	54	112	1716	64	139
	Florina	253	51	126	1247	52	104
	Trikala	277	57	122	1108	55	110
	Kalambaka	250	129	101	1764	45	89
Low-Input	Giannitsa	292	65	126	1027	45	64
	Florina	241	66	138	1385	49	116
	Trikala	305	64	132	1127	48	100
	Kalambaka	274	98	114	2520	43	89
Conventional and Low-Input	Giannitsa	243	54	110	1263	42	88
	Florina	229	49	121	1217	41	94
	Trikala	268	53	118	1057	44	106
	Kalambaka	247	102	100	1778	35	86

The differences between the six genotypes are shown in Table 4. Alexandros and Zefyros displayed the highest values for starch content (4147, 4064 vs. 3543, 2355, respectively) and ash content (642, 717 vs. 847, 916, respectively). However, it was revealed that Alexandros and Zefyros present steady performance for protein content, even in low-input cultivating systems where stability is often slightly higher. Comparisons between conventional and low-input farming systems generally indicated minor variations. This is a crucial finding for the adoption of productive cultivars using low-input farming techniques. Alexandros, Omiros, and Pigasos showed high values for crude protein content in conventional farming.

Table 4. Stability index estimates for seed chemical composition parameters for common vetch in two farming systems across genotypes.

	Genotypes	**Crude Protein (%DM)**	**Fat (%DM)**	**Ash (%DM)**	**Starch (%DM)**	**Crude Fiber (%DM)**	**Moisture (%)**
Conventional	Filippos	461	58	633	3276	344	303
	Omiros	515	82	315	2940	1040	199
	Alexandros	508	38	642	4147	925	347
	Tempi	524	90	590	2730	864	96
	Zefyros	506	39	847	3543	732	424
	Pigasos	691	106	442	3582	577	460
Low-Input	Filippos	630	103	711	3247	436	301
	Omiros	736	87	362	2965	365	167
	Alexandros	616	59	711	4064	605	230
	Tempi	517	106	627	2715	592	458
	Zefyros	610	69	916	2355	660	191
	Pigasos	650	125	531	2679	625	624
Conventional and Low-Input	Filippos	473	65	419	2770	132	303
	Omiros	513	76	268	2530	111	146
	Alexandros	490	44	445	3369	201	275
	Tempi	461	85	427	2380	191	122
	Zefyros	458	47	585	2375	148	267
	Pigasos	574	96	339	2832	140	365

For all variables examined across the two cultivation systems, stability indices (Table 5) incorporate genotypic and environmental behavior (conventional and low-input). Extreme stability index values were displayed by Trikala and Kalambaka due to the contribution of certain environments, favoring the two varieties Alexandros and Zefyros. Omiros in Giannitsa and Filippos, and Omiros and Pigasos in Trikala showed some extreme values over 1000 for crude protein content.

Table 5. Combined trait stability index estimates for seed chemical composition parameters for common vetch in two farming systems across genotypes and environments.

	Genotypes	**Crude Protein (%DM)**	**Fat (%DM)**	**Ash (%DM)**	**Starch (%DM)**	**Crude Fiber (%DM)**	**Moisture (%)**
			Giannitsa				
Conventional	Filippos	650	737	960	5194	1098	409
	Omiros	463	902	1071	5065	1560	682
	Alexandros	585	1168	1079	4678	1557	678
	Tempi	702	1005	1310	5743	1578	511
	Zefyros	612	883	1229	4967	2411	847
	Pigasos	625	1190	823	5796	1044	527

Table 5. *Cont.*

	Genotypes	Crude Protein (%DM)	Fat (%DM)	Ash (%DM)	Starch (%DM)	Crude Fiber (%DM)	Moisture (%)
Giannitsa							
Low-Input	Filippos	864	704	925	5006	1688	957
	Omiros	1407	1142	1192	4900	2670	1018
	Alexandros	957	717	1182	4534	1727	940
	Tempi	780	924	1296	5493	1748	570
	Zefyros	907	855	1443	6790	2133	1095
	Pigasos	897	1215	921	4444	2288	530
Conventional and Low-Input	Filippos	718	326	488	4114	97	530
	Omiros	613	444	587	3981	92	446
	Alexandros	635	242	605	3788	213	787
	Tempi	628	417	656	4404	180	232
	Zefyros	583	230	758	5771	252	401
	Pigasos	653	412	472	5351	162	339
Florina							
Conventional	Filippos	725	764	906	5579	1929	980
	Omiros	942	889	727	4059	1296	952
	Alexandros	689	782	985	4928	1468	901
	Tempi	502	1144	1113	4707	1340	1029
	Zefyros	691	789	1376	4597	2025	911
	Pigasos	923	1112	838	4824	1736	976
Low-Input	Filippos	630	911	1070	5366	465	2860
	Omiros	854	654	794	4202	729	2661
	Alexandros	774	1065	1047	4765	435	2200
	Tempi	475	843	1299	4477	472	2424
	Zefyros	761	761	1438	4035	453	2834
	Pigasos	822	996	1014	4742	525	1884
Conventional and Low-Input	Filippos	519	223	535	4289	662	232
	Omiros	661	327	487	3385	261	160
	Alexandros	633	139	562	3956	628	236
	Tempi	472	218	668	3759	95	234
	Zefyros	562	129	770	2898	421	126
	Pigasos	742	250	522	3869	182	118
Trikala							
Conventional	Filippos	560	666	929	4543	1144	443
	Omiros	908	755	733	4513	1319	8201
	Alexandros	613	750	1057	6062	1039	575
	Tempi	766	1156	917	5417	2247	827
	Zefyros	741	1082	1780	4533	1949	727
	Pigasos	1580	1240	1007	4442	1461	1159
Low-Input	Filippos	1085	736	1066	4403	2605	488
	Omiros	1400	1285	914	4298	3442	513
	Alexandros	908	578	1176	5749	2647	685
	Tempi	948	1018	850	5209	2183	539
	Zefyros	1011	777	1994	4379	1603	434
	Pigasos	729	1264	1198	4301	1845	1010
Conventional and Low-Input	Filippos	623	113	551	3671	204	456
	Omiros	826	383	530	3620	183	948
	Alexandros	635	339	627	4600	303	644
	Tempi	697	367	561	4250	288	517
	Zefyros	658	312	911	3597	194	554
	Pigasos	792	358	590	3565	212	1146

Table 5. *Cont.*

	Genotypes	Crude Protein (%DM)	Fat (%DM)	Ash (%DM)	Starch (%DM)	Crude Fiber (%DM)	Moisture (%)
		Kalambaka					
Conventional	Filippos	483	645	957	4553	1322	958
	Omiros	794	667	932	5540	2032	816
	Alexandros	742	1168	1173	5680	1692	1095
	Tempi	772	1183	1296	5567	1717	1021
	Zefyros	833	1106	1000	4545	1426	499
	Pigasos	971	728	689	5077	1652	1024
Low-Input	Filippos	733	757	1034	4415	1316	445
	Omiros	938	640	1024	5302	1135	440
	Alexandros	782	835	1263	5476	2338	454
	Tempi	792	681	1496	5462	2155	439
	Zefyros	825	1133	1141	7557	1614	533
	Pigasos	1093	673	885	4887	1356	607
Conventional and Low-Input	Filippos	556	487	509	3692	212	248
	Omiros	738	369	509	4248	85	182
	Alexandros	669	696	607	4415	173	499
	Tempi	675	437	683	4297	190	365
	Zefyros	675	885	666	2010	125	263
	Pigasos	841	381	441	3986	157	656

Genetic parameters are presented in Table 6. Differences were found between minimum and maximum values for almost all traits. Genotypic expression in the phenotype was great, and thus, heritability indices were found to be very high (from 97.99 to 99.95).

Table 6. Genetic parameter estimates for seed chemical composition parameters for common vetch.

Traits	Min.	Max.	Mean	sd	σ_g^2	σ_p^2	GCV (%)	PCV (%)	H^2 (%)
Crude protein content (%)	22.13	30.87	26.74	1.809	2.2034	2.2045	5.551	5.553	99.95
Fat content (%)	0.96	1.95	1.34	0.222	0.0274	0.0275	12.351	12.362	99.83
Ash content (%)	3.04	4.76	3.81	0.374	0.1244	0.1245	9.257	9.262	99.90
Starch content (%)	46.75	53.65	50.23	1.422	1.2820	1.3080	2.254	2.277	98.01
Crude fiber content (%)	2.6	5.35	4.03	0.645	0.3706	0.3709	15.107	15.112	99.94
Moisture content (%)	7.23	12.21	9.21	0.995	0.6960	0.7103	9.058	9.151	97.99

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

Correlations between traits are presented in Table 7. Almost all correlations between traits studied were significant, especially the positive correlations between crude protein content and fat content (0.476) and starch content (0.201). Strong negative correlations were found between crude protein content and fiber content (−0.588), crude protein and moisture content (−0.576), and crude protein and ash content (−0.423). The stability of crude protein and starch content is fully compatible due to the positive correlation found.

Table 7. Correlations between seed chemical composition parameters for common vetch.

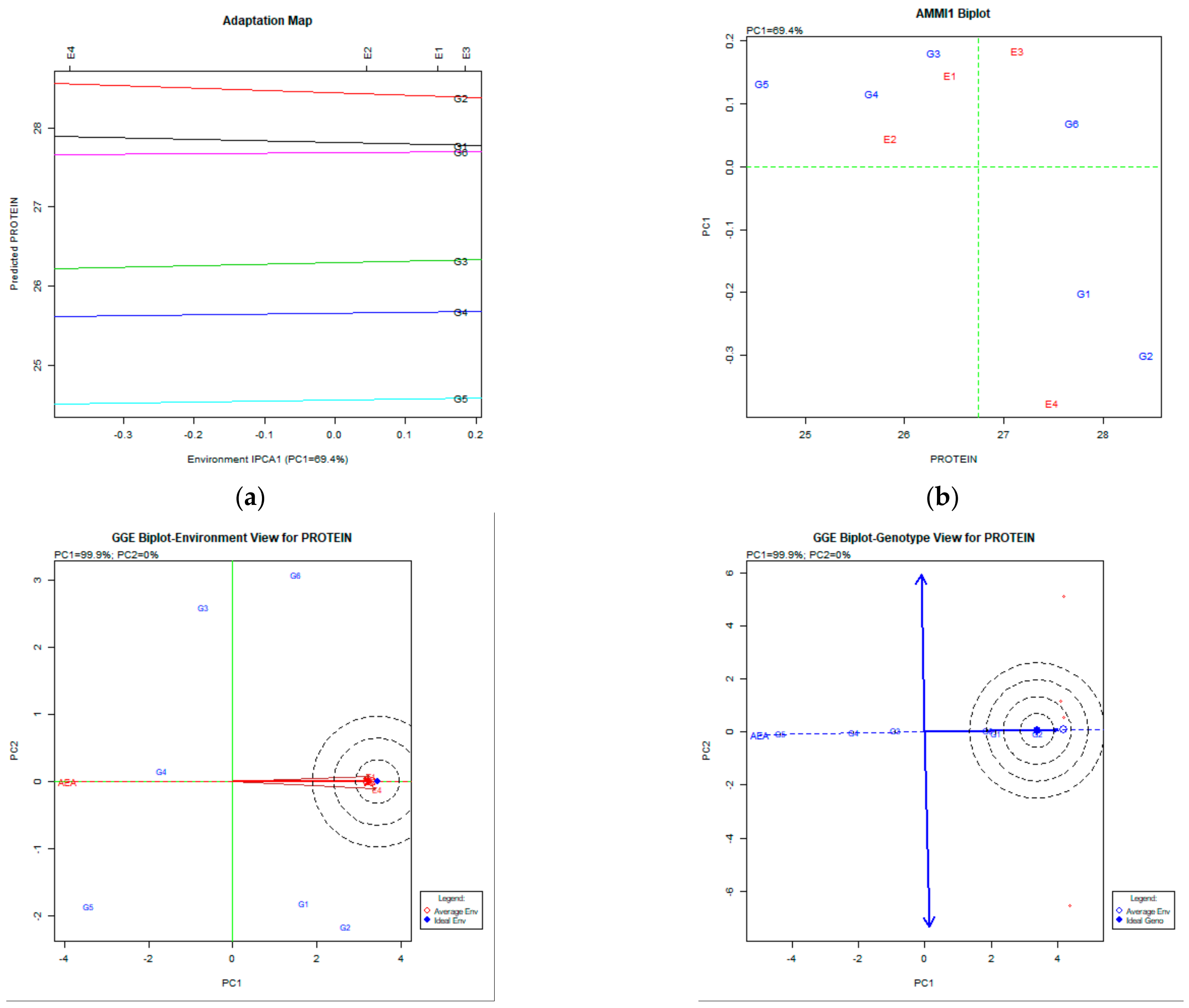
	Crude Protein (%DM)	Fat (%DM)	Ash (%DM)	Starch (%DM)	Crude Fiber (%DM)
Fat content (%)	0.476 **				
Ash content (%)	−0.423 **	−0.315 **			
Starch content (%)	0.200 **	0.126 *	−0.151 **		
Crude fiber content (%)	−0.588 **	−0.305 **	0.369 **	−0.038	
Moisture content (%)	−0.576 **	−0.312 **	0.365 **	−0.712 **	0.114 *

Significant correlations: * at the 0.05 level (2-tailed), ** at the 0.01 level (2-tailed).

The AMMI Tool for Multi-Environment Evaluations in Common Vetch

The performance of each genotype in various environments is simply depicted using biplots created by the GGE and AMMI1 biplot analysis. By utilizing a simple tool designed for the purpose, the produced biplots may clearly and quickly characterize each genotype for stability and performance.

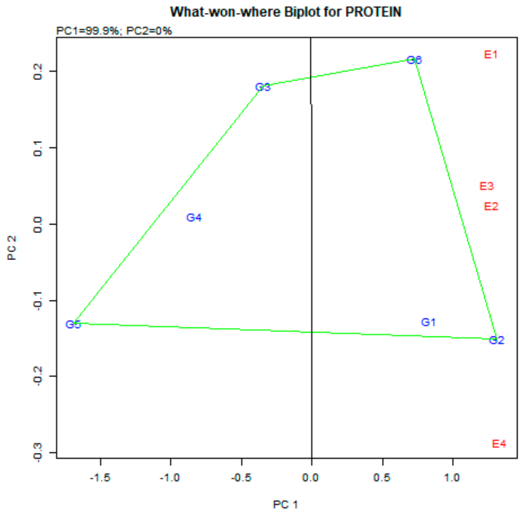
Figure 2a–e shows the stability analysis utilizing both AMMI and GGE biplots for protein content (%DM).



(**a**) (**b**)

(**c**) (**d**)

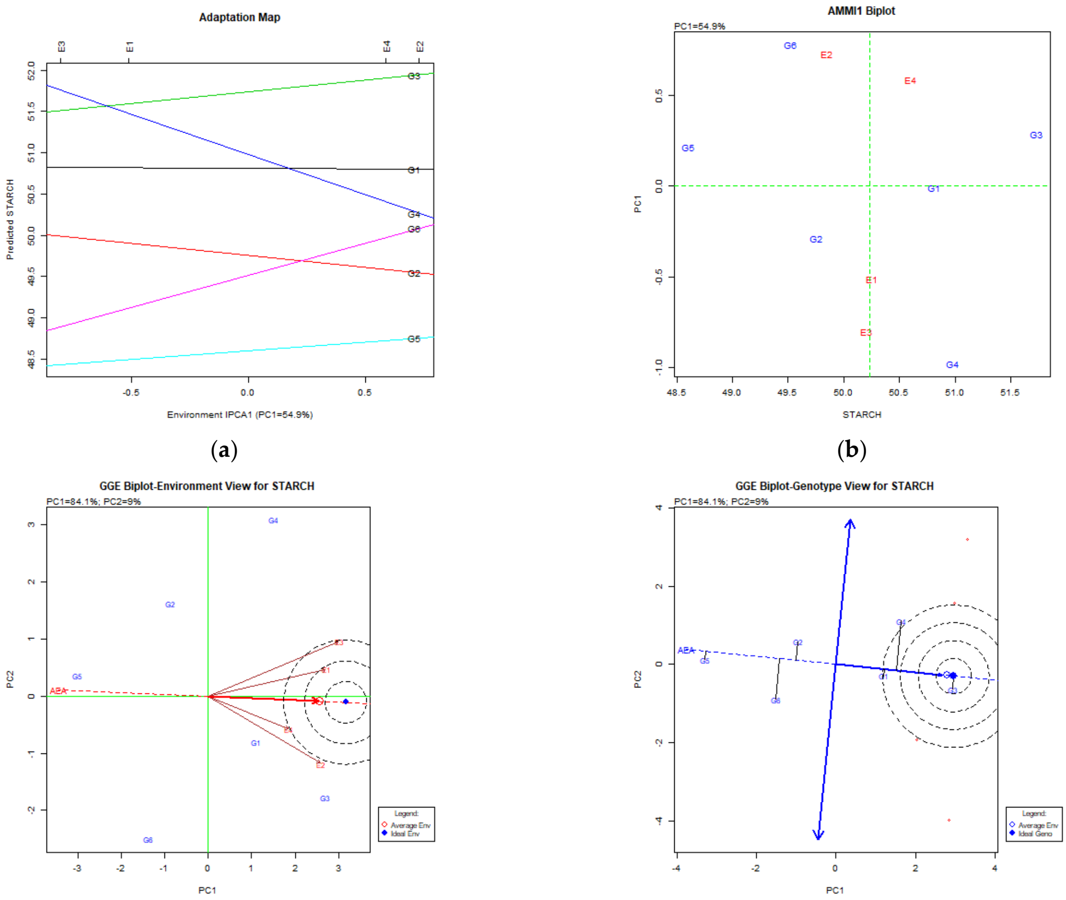
Figure 2. *Cont.*



(**e**)

Figure 2. Stability analysis for the trait of protein content (%) based on (**a**) adaptation; (**b**) AMMI1 biplot; (**c**) GGE biplot depicting the environmental stability; (**d**) GGE biplot showing the genotypic stability. The genotypes closer to the ideal genotype are recommendable. (**e**) GGE which-won-where biplot depicting specific adaptability of genotypes over environments.

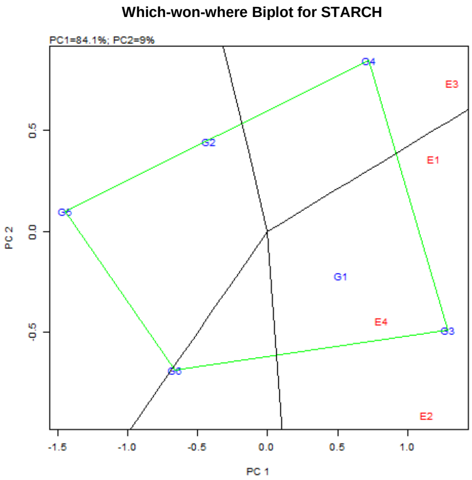
Figure 3a–e illustrates the stability analysis for starch content (%DM) using both AMMI and GGE biplots.



(**a**) (**b**)

(**c**) (**d**)

Figure 3. *Cont.*



(**e**)

Figure 3. Stability analysis for the trait of starch content (%) based on (**a**) adaptation map; (**b**) AMMI1; (**c**) GGE biplot depicting the environmental stability; (**d**) GGE biplot for genotypes depicting the genotypic stability. The genotypes closer to the ideal genotype are recommendable. (**e**) GGE which-won-where biplot depicting specific adaptability of genotypes over environments.

For AMMI analysis and based on the figure of adaptation map, the genotypes which expressed high values on the axis of trait performance along with nearly parallel lines to the PC1 were the desirable ones, as this behavior indicates environmental stability.

The desirable genotypes for the AMMI1 biplot were those having high values on the axis of trait performance (right position, x-axis,) and were closer to the axis of the PC1.

With regard to the GGE biplot over environments, the stable and preferable environment is placed closer to the ideal and/or average environment.

For the GGE biplot regarding the genotypes, the more advantageous ones (productive and stable) are depicted closer to the ideal genotype and in the zone of the ideal genotype dot.

3.2. Pea Seed Analysis

ANOVA results are given in Table 8. The main effects for all pea characteristics also showed significant differences. For all variables, the GXE interaction was highly significant, revealing the relation between phenotypic expression and environmental conditions under which the genotypes were cultivated. These results led to further analysis of our data for stability estimations, GGE biplots, and AMMI1 analysis.

Table 8. ANOVA mean squares (m.s.) over environments and farming methods for examined parameters: crude protein (CP) percent of dry matter (%DM), fat (%DM), ash (%DM), starch (%DM), crude fiber (%DM), and moisture (%) for peas.

Source of Variation	**Crude Protein (%DM) m.s.**	**Fat (%DM) m.s.**	**Ash (%DM) m.s.**	**Starch (%DM) m.s.**	**Crude Fiber (%DM) m.s.**	**Moisture (%) m.s.**
Environments (E)	1.433 **	0.146 **	0.990 **	3.144 **	0.146 **	2.271 **
REPS/Environments	0.082 ns	0.001 ns	0.02 ns	0.069 ns	0.03 ns	0.031 ns
Varieties (G)	79.791 **	0.933 **	1.959 **	77.020 **	1.982 **	29.241 **
Environments × Varieties (G × E)	1.079 **	0.211 **	0.070 **	2.840 **	0.397 **	1.903 **
Error	0.063	0.001	0.001	0.078	0.003	0.025

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

Stability estimations are presented in Tables 9–11. Table 9 includes calculations for all attributes tested across environments. Starch content showed the highest index values, while crude protein and ash content showed values over 200. Low values (lower or close to 100) were found for the rest of the characteristics measured. The stability estimates were not significantly affected by the minor differences between the two farming systems, but in some instances (in the Florina area), stability indices were greater in low-input experiments. Crude protein content showed a higher value in Giannitsa.

Table 9. Stability index estimates for seed chemical composition parameters for peas in two farming systems across environments.

	Environments	**Crude Protein (%DM)**	**Fat (%DM)**	**Ash (%DM)**	**Starch (%DM)**	**Crude Fiber (%DM)**	**Moisture (%)**
Conventional	Giannitsa	1094	26	344	1547	287	94
	Florina	231	77	159	1598	294	327
	Trikala	380	57	277	1589	236	245
	Kalambaka	599	78	135	881	163	87
Low-Input	Giannitsa	381	31	800	1470	345	144
	Florina	327	96	240	3001	294	303
	Trikala	623	61	573	2135	710	74
	Kalambaka	347	30	489	1250	724	76
Conventional and Low-Input	Giannitsa	572	26	476	1507	316	115
	Florina	262	84	194	2066	291	289
	Trikala	476	59	378	1802	319	111
	Kalambaka	432	32	215	1046	255	82

Table 10 depicts the differences between varieties. Olympos showed the highest values for starch content, but other varieties were better in other traits, such as Pisso and Vermio, for crude protein content. Olympos demonstrated consistent results even in low-input cropping systems, where stability is often slightly greater. Comparisons between conventional and low-input farming systems generally indicated little variation. This is a crucial discovery for the adoption of high-yielding cultivars in low-input cropping practices.

Table 10. Stability index estimates for seed chemical composition parameters for peas in two farming systems across genotypes.

	Genotypes	**Crude Protein (%DM)**	**Fat (%DM)**	**Ash (%DM)**	**Starch (%DM)**	**Crude Fiber (%DM)**	**Moisture (%)**
Conventional	Olympos	2448	59	4043	4526	280	328
	Pisso	2487	70	2357	2727	534	1274
	Livioletta	1556	64	526	5288	324	531
	Vermio	2027	33	193	4037	206	347
	Dodoni	794	35	3369	5531	186	83
Low-Input	Olympos	1384	40	1318	5721	568	62
	Pisso	2160	133	683	2536	915	1429
	Livioletta	1932	284	553	2609	1856	944
	Vermio	4109	26	682	3486	1395	407
	Dodoni	1763	126	1489	2170	403	151
Conventional and Low-Input	Olympos	1774	44	1243	4957	377	101
	Pisso	1973	79	600	2586	664	1368
	Livioletta	1224	106	548	3128	385	688
	Vermio	2592	28	304	3790	349	313
	Dodoni	1109	55	1172	2927	251	106

The stability indices for the two farming methods (conventional and low-input) that included genotypic and environmental behavior are shown in Table 11. The behavior of several varieties was influenced differentially by various settings and growing methods. In some environments and for some varieties, the starch concentration displayed extreme index values that were close to or over 10,000. Olympos had the highest index results for crude protein content in Florina and Giannitsa, while other varieties, such as Dodoni and Vermio, may be more dependable and productive in the same regions under low-input farming systems.

Table 11. Combined trait stability index for seed chemical composition parameters for peas in two farming systems across genotypes and environments.

	Genotypes	**Crude Protein (%DM)**	**Fat (%DM)**	**Ash (%DM)**	**Starch (%DM)**	**Crude Fiber (%DM)**	**Moisture (%)**
Giannitsa							
Conventional	Olympos	8913	1680	4026	8194	2970	1092
	Pisso	6910	1651	4041	8715	2440	1830
	Livioletta	5924	1294	175	9084	3105	1140
	Vermio	9798	1853	3545	8532	3406	1464
	Dodoni	8170	1532	4431	9545	3475	1784
Low-Input	Olympos	7345	1537	3181	9267	2966	1308
	Pisso	8548	1496	3652	9780	3701	1674
	Livioletta	7339	1279	3099	9583	3877	1902
	Vermio	8174	1374	4262	9313	2944	1465
	Dodoni	8487	1143	3497	9823	3467	1426
Conventional and Low-Input	Olympos	2019	1719	3440	8151	540	138
	Pisso	7686	28	613	6045	2205	1663
	Livioletta	6216	115	260	9799	747	1296
	Vermio	2805	1035	2425	5221	220	1061
	Dodoni	5466	638	1827	2210	1831	241
Florina							
Conventional	Olympos	9404	1730	4594	10663	3087	1287
	Pisso	8576	1365	3392	9042	2604	1209
	Livioletta	8644	1672	4445	9290	2849	1285
	Vermio	8277	1198	3773	9118	2831	1640
	Dodoni	6057	1527	4301	8599	3113	1731
Low-Input	Olympos	6212	1574	4049	8424	3799	1112
	Pisso	5950	1292	3792	9431	3963	1957
	Livioletta	6190	1162	4069	8594	2832	1226
	Vermio	9595	1174	4184	8700	3165	1334
	Dodoni	8561	1420	4813	9163	2891	1465
Conventional and Low-Input	Olympos	7767	164	839	9219	2411	85
	Pisso	1146	522	877	6427	345	1514
	Livioletta	1213	153	3263	4882	333	1078
	Vermio	7584	633	4196	4924	1092	1116
	Dodoni	1584	185	790	5548	2731	1653
Trikala							
Conventional	Olympos	5230	1505	4523	9582	2978	1800
	Pisso	6855	1671	3520	9339	3038	1849
	Livioletta	7769	1534	4014	8412	2928	1438
	Vermio	8877	1362	3987	8922	3424	1378
	Dodoni	6791	1198	4064	8892	2865	1340

Table 11. *Cont.*

	Genotypes	Crude Protein (%DM)	Fat (%DM)	Ash (%DM)	Starch (%DM)	Crude Fiber (%DM)	Moisture (%)
Trikala							
Low-Input	Olympos	6293	1607	3991	9703	3039	1448
	Pisso	7670	1321	3497	9087	3857	1684
	Livioletta	6668	1508	4066	9224	3334	1890
	Vermio	9118	1779	3759	8987	3315	1576
	Dodoni	8218	1184	3466	8610	2851	1545
Conventional and Low-Input	Olympos	5670	306	1617	10287	2621	365
	Pisso	5222	311	432	9502	1692	1881
	Livioletta	1718	636	1849	4738	2282	1410
	Vermio	8794	207	1805	9339	1071	518
	Dodoni	7721	429	3501	6251	241	527
Kalambaka							
Conventional	Olympos	7291	1308	3240	10337	4048	1321
	Pisso	8298	1323	4687	9149	2556	1149
	Livioletta	8904	1791	3692	9251	2661	1564
	Vermio	8800	1343	3613	9060	3281	1351
	Dodoni	8476	1449	4122	9923	3896	1454
Low-Input	Olympos	6106	1506	3580	9752	2956	1335
	Pisso	9598	1351	3366	8140	3069	1105
	Livioletta	6695	1697	3975	2355	2695	1668
	Vermio	8238	1194	3542	7049	2772	1532
	Dodoni	7157	1461	3841	8397	3872	1421
Conventional and Low-Input	Olympos	6903	673	2105	4010	1072	534
	Pisso	3140	113	4117	9151	1810	1056
	Livioletta	1731	311	2016	3783	593	1719
	Vermio	6479	27	578	8353	386	240
	Dodoni	6016	676	1365	9742	1979	697

Genetic parameters are presented in Table 12. Large variances were also seen in the examined features of peas. Phenotypic expression was generally high, but not for all traits, and varied from 77.38 (fat content) to 98.65 (crude protein content).

Table 12. Estimations of genetic parameters for seed chemical composition parameters for peas.

Traits	Min.	Max.	Mean	sd	σ_g^2	σ_p^2	GCV (%)	PCV (%)	H^2 (%)
Crude protein content (%)	20.13	25.08	22.85	1.15	1.2299	1.2467	4.8530	4.8861	98.65
Fat content (%)	1.11	2.10	1.56	0.24	0.0113	0.0146	6.8011	7.7313	77.38
Ash content (%)	2.94	3.99	3.29	0.21	0.0295	0.0306	5.2142	5.3099	96.43
Starch content (%)	47.02	52.83	49.68	1.31	1.1591	1.2034	2.1671	2.2082	96.31
Crude fiber content (%)	5.02	6.53	5.61	0.33	0.0248	0.0310	2.8041	3.1357	79.97
Moisture content (%)	7.74	12.25	9.51	0.92	0.4272	0.4569	6.8741	7.1094	93.49

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

Correlations between traits are presented in Table 13. Nearly all of the studied trait correlations were significant. Particularly favorable correlations exist between crude protein with ash (0.653), starch (0.449), fiber (0.343), and fat (0.271) contents. Crude protein and moisture content were found to be significantly inversely related (−0.540).

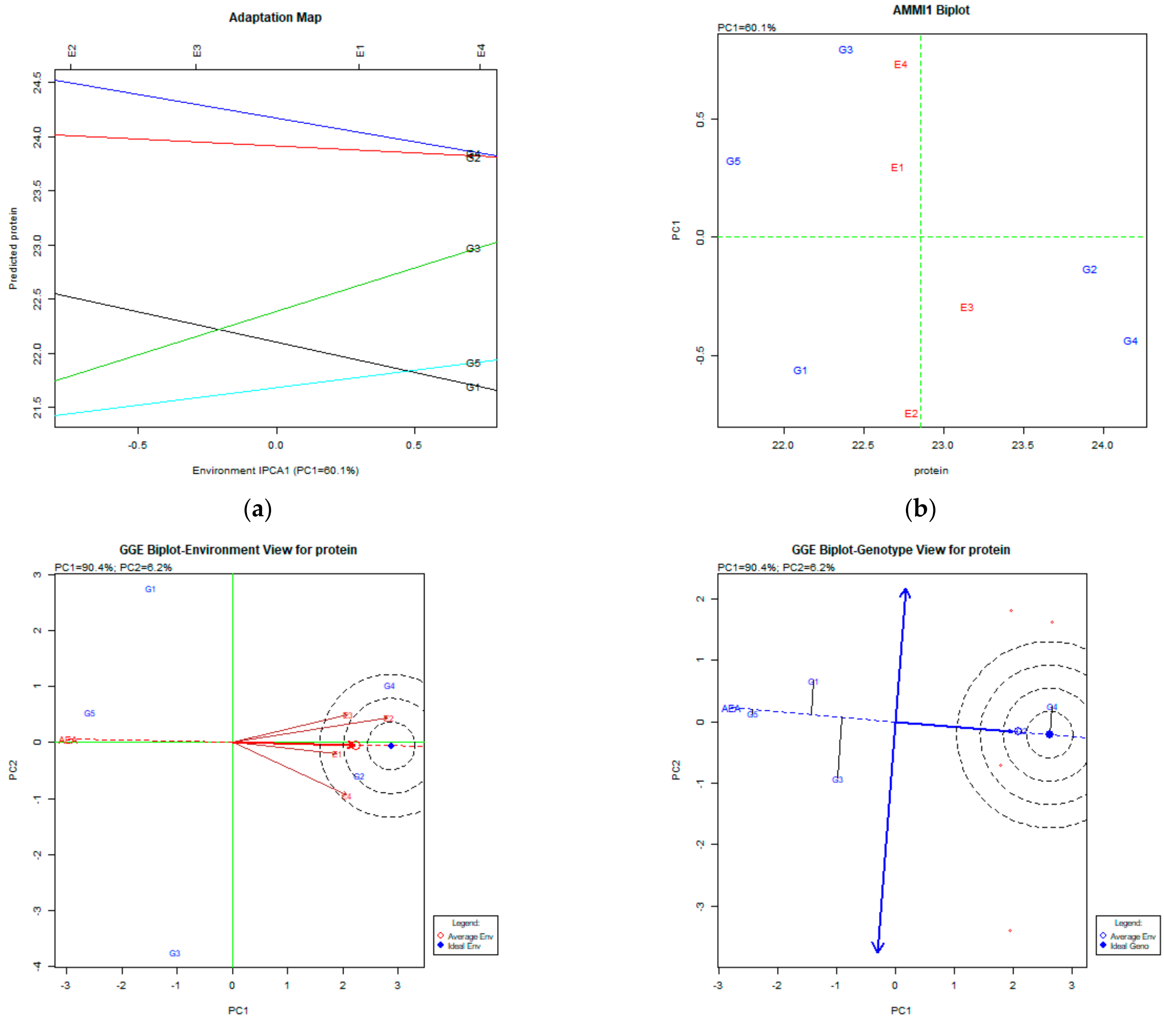
Table 13. Correlations between seed chemical composition parameters for peas.

	Crude Protein (%DM)	Fat (%DM)	Ash (%DM)	Starch (%DM)	Crude Fiber (%DM)
Fat (%DM)	0.271 **				
Ash (%DM)	0.653 **	0.174 **			
Starch (%DM)	0.449 **	0.173 **	0.527 **		
Crude fiber (%DM)	0.343 **	0.069	0.357 **	0.373 **	
Moisture (%)	−0.540 **	−0.377 **	−0.396 **	−0.619 **	−0.390 **

** Correlations significant at the 0.01 level (2-tailed).

The AMMI Tool for Multi-Environment Evaluations in Peas

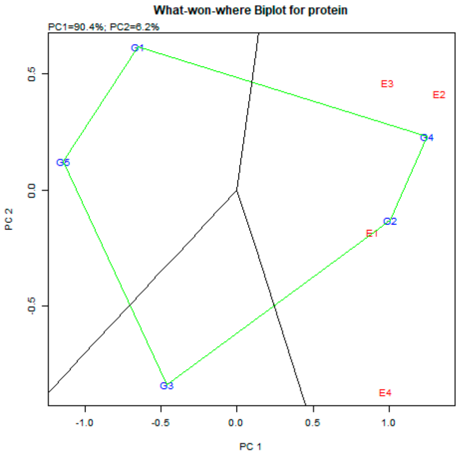
The analysis for stability combining AMMI and GGE biplots of protein content (%) is shown in Figure 4a–e.



(**a**) (**b**)

(**c**) (**d**)

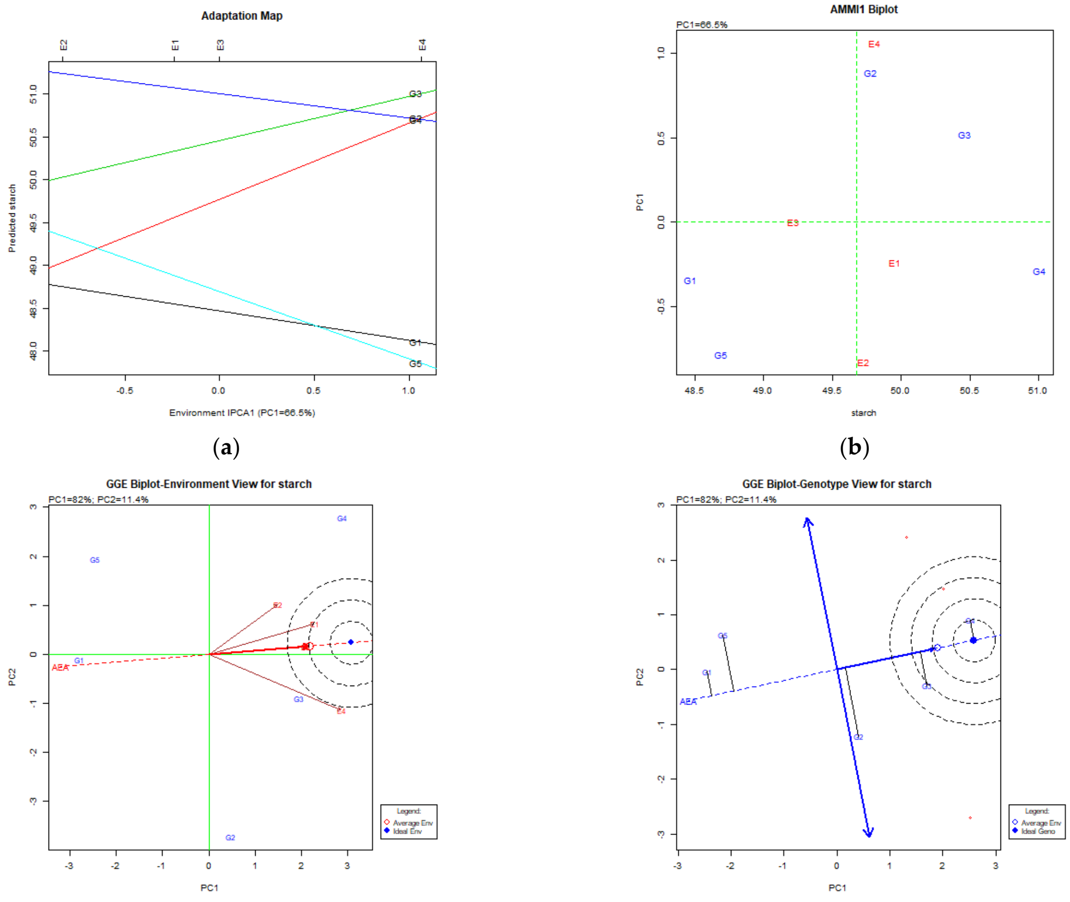
Figure 4. *Cont.*



(**e**)

Figure 4. Stability analysis for the trait of protein content (%) based on (**a**) adaptation map; (**b**) AMMI1 biplot; (**c**) GGE biplot depicting the environmental stability; (**d**) GGE biplot for genotypes representing the genotypic stability. The genotypes closer to the ideal genotype are recommendable. (**e**) GGE which-won-where biplot depicting specific adaptability of genotypes over environments.

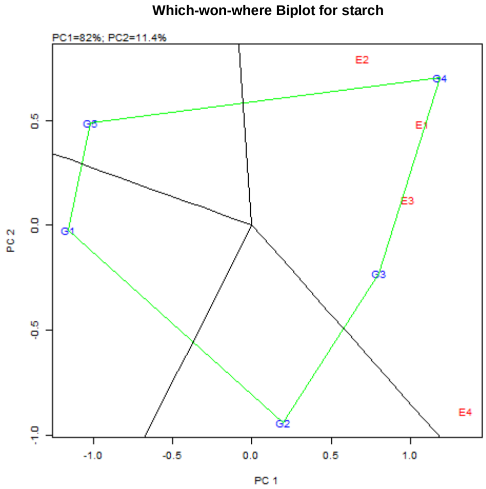
The analysis for stability combining AMMI and GGE biplots for starch content is shown in Figure 5a–e.



(**a**) (**b**)

(**c**) (**d**)

Figure 5. *Cont.*



(**e**)

Figure 5. Stability analysis for the trait of starch content (%) based on (**a**) adaptation; (**b**) AMMI1 biplot; (**c**) GGE biplot representing the environmental stability; (**d**) GGE biplot for genotypes depicting the genotypic stability. The genotypes closer to the ideal genotype are recommendable. (**e**) GGE which-won-where biplot depicting specific adaptability of genotypes over environments.

According to the adaptation map figure and the AMMI analysis, the genotypes with high values on the trait performance axis that expressed a line almost parallel to the PC1 axis were the most preferred, as this demonstrates stability throughout the many experimental environments.

The desirable genotypes based on the AMMI1 biplot were the ones expressing elevated values on the axis of performance of the trait (right position, x-axis,) and closer to the axis of the PC1.

As far as the GGE biplot over environments, the stable and desirable environment was one that was situated close to the optimal and average environment.

For the GGE biplot regarding the genotypes, the preferable ones (considering productivity and stability) were those depicted in close proximity to the optimal genotype and within its zone.

4. Stability Analysis, Comparative Results, and Discussion

High protein and starch content are considered the main traits to define seed quality as an animal feed, among the seven categories of seed characteristics [36–39]. An extended analysis of nutritional value for common vetch is presented by Huang et al. [40] and for peas by Castell et al. [41] and Bestianelli et al. [42]. Although conventional and low-input cultivation methods performed differently in our research regarding seed quality performance, overall, the two cultivation methods had no impact on the stability of the examined variables. Combining the two farming systems with the GGE biplot analysis showed that the low-input cropping method was the most reliable for seed quality varieties in every setting, as well as in some particular areas/environments.

The use of AMMI and GGE biplots can divide genotypes into groups based on the environmental traits' similarities. It is very useful in selecting genotypes characterized by environmental stability. The quality trait stability is influenced by GxE, so AMMI and GGE are suitable environmental analysis methods for the stability selection of desirable genotypes.

4.1. Crude Protein (%DM) in Peas

AMMI analysis produced the adaptation map (Figure 2a) and AMMI1 biplot figures (Figure 2b) for the trait of crude protein (%DM) in peas. Both biplot figures explained a percentage of total variability (69.4%), which makes it possible to draw conclusions. The adaptation map and AMMI1 figures show that the most reliable and desirable genotypes were G2 (Omiros) and G1 (Filippos), followed by G6 (Pigasos). All the previously mentioned genotypes had stable performance on protein content, which is the main desirable trait for seed quality. The characteristic's broad distribution allows for the discrimination of the most desirable genotypes for that trait. Nearly all of the variability was explained by the GGE analysis (99.9%) (PC1:99.9%, PC2: 0%); therefore, only the first PC1 explains the whole variability. The GGE biplot for the environments (Figure 2c) shows that the environments were similar, so they were placed very near the average environment. The genotype view of the GGE biplot (Figure 2d) revealed that the stable one that was identical to the optimal genotype was the G2 (Omiros), followed by the G1 (Filippos) and G6 (Pigasos), which were very productive and stable since they were positioned around the optimal genotype in a circular pattern. The which-won-where biplot (Figure 2e) showed that all genotypes had good adaptation in the environments E3 (Trikala) and E2 (Florina), but the genotypes G2 (Omiros) and G1 (Filippos) had better adaptability in the E4 (Kalambaka) environment and the G6 (Pigasos) genotype in the E1 (Giannitsa) environment.

4.2. Starch Content (%DM) in Peas

The analysis by AMMI, as depicted by the adaptation map (Figure 3a) and AMMI1 biplot (Figure 3b), expressed a PC1:54.9% of total variability for the trait of starch (%DM), which is quite high for further interpretation. In both figures, it was obvious that the most productive genotypes with a high percentage of starch and the most stable genotypes were G3 (Alexandros), G1 (Filippos), and G4 (Tempi). GGE biplot analysis explained 93.1% (PC1:84.1%, PC2: 9.0%) of the total variability. According to the environment view of the GGE biplot (Figure 3c) and regarding the trait of starch content, all environments were diverse but quite stable since they were placed on the perimeter of the far concentric area of the ideal environment. The genotype view of the GGE biplot (Figure 2d) shows that the most stable genotype was the G3 (Alexandros), while the G1 (Filippos) and the G4 (Tempi) came next. Regarding the which-won-where biplot (Figure 3e), the stable genotype over all environments was the G3 (Alexandros), whereas the G3 (Alexandros) showed relative stability in E4 (Kalambaka) and E2 (Florina) environments and the G4 (Tempi) in E3 (Trikala) and E1 (Giannitsa) environments.

4.3. Crude Protein (%DM) in Common Vetch

Regarding the trait of protein content (%) in vetch, AMMI analysis created the adaptation map figure (Figure 4a) and the AMMI1 biplot (Figure 4b). Both types of analysis depicted in the figures explained a percentage of the total variability (60.1%), which is sufficient to draw conclusions. The AMMI1 figure and adaptation map show that the stable and desirable genotypes with the highest protein content were the G2 (Pisso) and G4 (Vermio). The trait of high protein content combined with stability over environments is the main desirable characteristic for seed quality. The range of the trait was high, which discriminates the most desirable genotypes for that trait. In total, 96.8% of the overall variability was explained by the GGE analysis (PC1:90.4%, PC2: 6.2%), which is extremely high for variety discrimination and stability over environments. The environment view of the GGE biplot (Figure 4c) shows that the experimentation environments were somewhat similar and were positioned close to the average environment dot. The GGE biplot for genotype view (Figure 4d) reveals that G4 (Vermio) was the most stable genotype and, by the figure shown identical to the ideal one, is followed by G2 (Pisso), both of which are of extremely high quality and stability because they are located in the zone of the ideal genotype. According to Figure 4e, the genotype G4 (Vermio) has demonstrated good adaptation to the environments E2 (Florina) and E3 (Trikala), and G2 (Pisso) to the environments E1 (Giannitsa) and E4 (Kalambaka). Both pea and common vetch cultivations generally exhibited the same behavior for seed quality stability for protein content.

4.4. Starch Content (%DM) in Common Vetch

The adaption map of the AMMI analysis (Figure 5a) and the biplot of the AMMI1 (Figure 5b) expressed a PC1:66.1% of the total variability for starch content, which was demonstrated to be rather high for further interpretation. In both figures, it was obvious that the most productive genotypes with a high percentage of starch and relatively stable genotypes were G3 (Livioletta), G2 (Pisso), and G4 (Vermio). The analysis of the GGE biplot explained 93.4% (PC1:82.0%, PC2: 11.4%) of the total variability. The environment view of the GGE biplot (Figure 5c) shows that all experimentation environments regarding the specific trait were diverse but quite stable since they were placed out and near the perimeter of the far-center area of the dot of the optimal environment. The G4 (Vermio) genotype was the most stable, followed by the G3 (Livioletta) genotype, according to the GGE biplot for genotypes (Figure 5d). According to the results given in Figure 5e, the G3 (Livioletta) genotype was the most reliable across every environment, whereas the G4 (Vermio) showed relative stability in the E1 (Giannitsa) and E2 (Florina) environments and the G3 (Livioletta) in the E3 (Trikala) and E4 (Kalambaka) environments.

Extended GXE interactions for animal feed plants were also reported by Hood-Niefer et al. [43], who recommended certain varieties for special environments. Yihunie and Gesesse [44] and Sayar and Han [45] used the GGE biplot, while according to Bocianowski et al. [21], AMMI analysis was able to define specific cultivars for specific settings in pea studies.

Comparisons between peas and vetch showed that crude protein and starch content stability are highly heritable for vetch and for peas, but stability indices were higher for peas in many environments and for certain varieties.

Data from the stability index may also be used to estimate the degree of heredity for a variety of quantitative or qualitative variables.

Greveniotis et al. [2,3,13,14] stated that high indices indicate possibly high heritability for certain characteristics. This is quite obvious in the case of starch content, which seems to be more qualitative than other characteristics measured, and thus can be improved more easily by breeders [17].

In our research, peas showed more positive and strong correlations between traits than did vetch. Greveniotis et al. [2,3,13,14] showed positive relationships for other variables in common vetch and peas. Significant associations were discovered for a number of field pea traits by Georgieva et al. [46]. Greveniotis et al. [13,14] identified common vetch associations in the same way for additional traits. Sayar [47] and Tiryaki et al. [48] depicted significant correlation coefficients, which are useful for breeders.

Due to the high correlations found in our work, indirect selection on seed traits' stability may be performed for almost all traits for peas. If breeders can manage to improve certain traits, then stability is expected to improve for the rest of the main traits. The linearity found was acceptable for indirect breeding. Indirect selection on seed traits' stability in common vetch may concern protein content by improving the most stable characteristic, which is starch content, since the stability of crude protein and starch content are fully compatible due to the positive correlation found. Greveniotis et al. [2,3,13,14], for vetch, peas, etc., showed that it is possible to improve the most heritable and stable characteristics and indirectly improve all the rest of the main characteristics that are correlated with each other.

5. Conclusions

According to assessments of numerous characteristics, a number of traits in vetch and peas were significantly positively associated. Due to strong correlations, almost all pea trait traits are amenable to indirect selection on seed characteristics' stability. Since the stabilities of crude protein and starch content are fully compatible due to the positive correlation discovered, indirect selection on the seed traits' stability in common vetch may concern protein content by improving the most stable characteristic, which is starch content.

Comparisons between conventional and low-input farming systems typically identified genotypes that displayed extremely consistent performance, even under low-input cropping systems. It is also possible to determine the quantitative or qualitative heritability of a number of traits using information from the stability index.

The results of the AMMI analysis when combined with the GGE biplot and the ANOVA data show that environments and genotypes considerably interact, and the farming system (low-input or traditional) also plays a role. We must therefore suggest particular genotypes of field pea for particular geographies and agricultural systems in order to obtain the most reliable performance.

The primary features of seed quality are thought to be more stable in pea varieties. We recommend Olympos for stable pea varieties on seed quality because it showed the highest levels of crude protein content in Florina and Giannitsa, whereas Dodoni and Vermio may be more productive and stable in the same regions under low-input farming systems, and Livioletta may show widespread adaptation. We recommend Alexandros and Zefyros in Trikala and Kalambaka for stable vetch varieties. Extreme values for the crude protein content were shown by Omiros in Giannitsa and Filippos, Omiros, and Pigasos in Trikala.

Due to climate change, which is the greatest obstacle to field tests, cultivar adaptation research must be ongoing.

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