



Article Stability, the Last Frontier: Forage Yield Dynamics of Peas under Two Cultivation Systems

Vasileios Greveniotis ^{1,2,*}, Elisavet Bouloumpasi ³, Stylianos Zotis ^{2,†}, Athanasios Korkovelos ⁴ and Constantinos G. Ipsilandis ⁵

- ¹ Hellenic Agricultural Organization Demeter, Institute of Industrial and Forage Crops, 41335 Larissa, Greece
- ² Department of Agricultural Technology, Technological Educational Institute of Western Macedonia, 53100 Florina, Greece
- ³ Department of Agricultural Biotechnology and Oenology, International Hellenic University, 66100 Drama, Greece; elisboul@abo.ihu.gr
- ⁴ Directorate of Water Management of Thessaly, Decentralized Administration of Thessaly—Central Greece, 41335 Larissa, Greece; athanasios.korkovelos@apdthest.gov.gr
- ⁵ Regional Administration of Central Macedonia, Department of Agriculture, 54622 Thessaloniki, Greece; ipsigene@gmail.com
- * Correspondence: vgreveni@mail.com; Tel.: +30-241-067-1285
- † Deceased.

Abstract: The stability of performance may be proved to be the last frontier for adopting certain genotypes in various cultivation systems and environments. The main objective of the present study was to analyze the forage yield stability performance of seven pea (Pisum sativum L.) genotypes based on various stability indices. The genotype behavior was studied based on the yield of peas under both conventional and low-input cultivation systems. Five cultivars of peas (broadly distributed) and two lines were used in a strip-plot design. Significant positive correlations were detected between forage yield and some other traits. This way, forage yield stability may be indirectly improved by improving certain traits showing qualitative inheritance. Comparisons revealed that genotypes exhibited stable performance, even in low-input farming systems. AMMI analysis, GGE biplot, and analysis of variance (ANOVA) combination showed statistically significant differences between genotypes and environments and the farming system. Our analysis depicted specific cultivars of peas for different areas and farming systems to attain highly stable performance. Vermio was confirmed to be a stable genotype for forage yield performance in low-input farming in Trikala and Kalambaka areas, while Pisso was indicated as the best in Florina and Giannitsa areas in low-input farming. The two pea lines exhibited stable performance in Giannitsa and Florina areas, especially in low-input conditions. The stable behavior of some genotypes in these conditions may be useful for farmers that raise livestock in mountainous areas. The genetic parameters show that the selection for fresh forage yield and dry matter yield in breeding programs is expected to be effective.

Keywords: low-input; AMMI; GGE biplot; trait stability index; pea (Pisum sativum L.)

1. Introduction

The pea (*Pisum sativum* L.) is a very useful crop for supporting livestock [1,2]. Pea cultivations can be found in a wide range of agro-climatic zones; the potential of this crop and its high nutritional value are referred to in many related publications [3,4]. Field pea is often used as a main protein source, as it has a rich and unique protein profile, different from other natural protein sources [5]. On the basis of the aforementioned points, our study focused on estimating the genetic potential needed to support the growth and yield performance of pea cultivations in various environmental conditions and, subsequently, to propose the best cultivars.

The stability of performance could be crucial for adopting certain genotypes in various cultivation systems and environments [6]. Stability may be dependent on cultivar tolerance



Citation: Greveniotis, V.; Bouloumpasi, E.; Zotis, S.; Korkovelos, A.; Ipsilandis, C.G. Stability, the Last Frontier: Forage Yield Dynamics of Peas under Two Cultivation Systems. *Plants* **2022**, *11*, 892. https://doi.org/10.3390/ plants11070892

Academic Editors: Fouad S. Maalouf and Dil Thavarajah

Received: 19 February 2022 Accepted: 25 March 2022 Published: 27 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). to various biotic and abiotic factors in certain environments [7]. It is the ability to perform satisfactorily under almost any difficulty and cope with it during the whole growing season. Fasoulas [7], and later Fasoula [8], who used the squared form of reversed Coefficient of Variation as a stability criterion (stability index), tried to describe the plant's behavior under different circumstances.

Total yield performance is a multi-factor function: genotypes (G), environment (E), and the genotype by environment (GE) [9]. Plant breeders take into account the various implications of GEI in their breeding programs. GEI reduces the correlation between phenotypic and genotypic variability, decreasing heritability [10]. Without GEI analysis, the selection is insufficient for secure gains across selection cycles [11], making stability a high priority in every breeding program. Stability is necessary for the development of new, successful genotypes [12]. Breeders select for positive genotypic stability or try to minimize environmental variability that results in GEI [13].

In peas, many researchers assessed stability using varying approaches and methods, such as $G \times E$ classic statistics, cluster, and regression analysis, especially for yield [14–17], some of them in multi-location environments. Genotype by year interaction was found to be important for morpho-productive traits and biogas production in soybean [18] and in quantitative traits of field pea [19]. All these researchers tried to define the best genotypes suitable for various environments. Genotypes displaying high means of yield components, along with a low degree of fluctuations in different locations or seasons, are considered more adaptive or stable [7,20]. In our novel approach, high adaptability and stability of performance are realized when the stability criterion used shows high values; thus, all methods proper for this analysis must be taken into account. Acikgoz et al. [14] showed that cluster analysis was more efficient than classic stability analysis. The most recent research involves GEI analysis, and that concept was part of our study, too, involving ANOVA, GGE (genotype main effect (G) plus genotype by environment interaction (GE)) AMMI Biplot analysis and correlations, using additional data to support primary field research in order to improve the efficiency of estimations [21]. The predictive accuracy of such research trials is described in previous work based on AMMI analyses [22]. It is essential to depict that all analysis methods must be properly selected for each kind of data, including raw data and stability indices [23].

The interaction of genotypes with the environment defined many different approaches to cope with stability problems [24] since a wide range of parameters affect cultivar behavior [25]. Al-Aysh et al. [26], assessing genotype by environment interaction, found a few stable pea lines, while Sayar [27] succeeded in revealing stable genotypes in common vetch (*Vicia sativa* L.).

AMMI analysis, which is the acronym of additive main effects and multiplicative interaction, is useful and commonly used in the estimation and evaluation of GEI. AMMI is a hybrid model that combines ANOVA and principal component analysis (PCA) and creates easily understandable figures regarding the GEI [10,28,29].

Gabriel [30] first reported the GGE (i.e., G + GE) biplot analysis, and it has since been applied in diverse topics such as economics, business, medicine, genetics, and ecology [31]. This method has been applied by many agricultural scientists for many crops [32–36]. Such an analysis can be conducted on data for traits and genotypes and depict their relationship, not only genotypes and environments [37].

Macák et al. [37] investigated the performance of field peas and conventional and low-input tillage. These researchers concluded that pea grain yield could be bolstered by incorporating the chloromass of the previous crop along with the application of fertilizer (to preserve performance in high levels). Hanáčková and Candráková [38] reported high yields in pea cultivations under low- or no-tillage conditions; however, they found that conventional treatments showed a higher protein content. Performance under low-input conditions must be taken into consideration because of the cultivation practices followed by farmers that use peas as feed for their livestock. Peas are an interesting cultivar for animal feeding; thus, the highly productive varieties combined with a high quality of forage are preferable. The exploitation of the genetic parameters of the traits for breeding purposes is desirable. This knowledge is fundamental for effective breeding programs. Therefore, in order to initiate any breeding program, the exploitation of suitable parameters, such as the genotypic coefficient of variation GCV, heritability in a broad sense (H^2) is necessary.

In the present study, the main scope was to determine the forage yield stability of pea genotypes along with various correlated traits based on the innovative approach of estimating the stability index, with the specific aim of studying pea genotypes' behavior under both conventional and low-input cultivation systems. Greveniotis et al. [23,39] used a stability index, based on Fasoulas [7] and Fasoula [8] remarks, as an estimation of the heritability of various traits, leading to clear discrimination between qualitative and quantitative traits. Our approach includes stability performance analysis and reveals the stability performance and the kind of heritability of traits.

2. Results

2.1. ANOVA and Descriptive Statistics on the Stability Index

Regarding the ANOVA table (Table 1), the main effects for all traits expressed significant differences. The $G \times E$ interaction showed significant differences for all traits. Multiple interactions involving genotypes, the environment, and the cultivation system were found to be very significant, especially for forage yield, and these data must be analyzed in combination with the genotype performance within each environment and cultivation system in order to define the best genotype for specific conditions. Days to flowering showed no multiple interactions. Main stem length showed no cultivation \times environment interaction. Days to flowering showed no multiple interactions.

Table 1. Mean squares (m.s.) from analysis of variance over environments and cultivation methods for tested traits: days to 50% flowering, main stem length (cm), main stem thickness (mm), fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%), and ash content % of dry matter.

Source of Variation	Days to 50% Flowering	Main Stem Length (cm)	Main Stem Thickness (mm)	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content (%)	Ash Content % of Dry Matter
	m.s.	m.s.	m.s.	m.s.	m.s.	m.s.	m.s.
Environments (E) REPS/Environments Genotypes (G)	163.29 ** 1.16 ns 499.53 **	68.52 ** 17.02 ns 155.90 **	0.09 ** 0.01 ns 0.02 *	28,290,714 ** 6,632,777 ** 168,425,535 **	1,938,835 ** 316,336 ** 8,653,394 **	3.95 ** 8.41 ** 17.54 **	3.71 ** 10.31 ** 5.92 **
Genotypes \times Cultivation	2.01 *	48.89 *	0.04 **	102,070,746 **	5,364,623 **	1.18 **	0.44 **
Genotypes \times Environ- ments (G \times E)	5.37 **	32.28 *	0.03 **	17,196,116 **	828,043 **	2.77 **	1.44 **
Cultivations	334.99 **	207.37 **	0.04 *	36,668,636 **	1,557,247 **	98.63 **	42.84 **
Cultivation × Environ- ments	2.31 *	32.49 ns	0.08 **	31,475,811 **	1,614,424 **	0.36 **	20.09 **
Cultivation × Geno-	1.03 ns	42.24 **	0.04 **	26,630,013 **	1,270,305 **	0.29 **	0.18 **
Error	0.95	2.128	0.01	1,609,523	69,682	0.020	0.03

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

To better analyze the performance of the genotypes in different environments and estimate the stability of each genotype for all traits, we used AMMI and GGE analysis as the most appropriate tools. For all traits, the GE was much more significant than G effects, and additional AMMI analysis was needed.

Stability estimations based on the calculation of the stability index of each trait are presented in Tables 2–4. The tabulated stability index data across environments for the seven characteristics under study are listed in Table 2. Days to 50% flowering and main stem length showed generally high indices (over 1000) in many cases. Fresh forage yield

showed low indices. The low-input farming system seems to improve stability indices in many cases for forage yield.

In Table 3, the behavior of genotypes in all farming systems is shown. CultivarsPisso, Livioletta, and Vermio showed stability performance for forage yield and a few other traits, such as the main stem length and days to flowering—especially for Livioletta, which was generally more stable regarding forage yield.

Table 4 combines data for genotypes across environments and farming systems. This table is useful to depict the most stable genotype for a certain area (environment) and the selected farming system. In the Florina area and for forage yield, cv. Pisso displayed stable performance—the Giannitsa area stability index was over 600. Vermio was the best in the Trikala area (over 500), but only for low-input systems, not conventional.

Table 2. Trait stability index across environments for two farming systems: days to 50% flowering, main stem length (cm), main stem thickness (mm), fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%), and ash content % of dry matter.

	Environments	Days to 50% Flowering	Main Stem Length (cm)	Main Stem Thickness (mm)	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content %	Ash Content % of Dry Matter
	Giannitsa	1735	544	301	41	44	379	117
Conventional	Florina	2127	675	533	59	57	284	108
Conventional	Trikala	3391	927	1012	38	44	402	115
	Kalambaka	3173	2726	3009	57	57	357	83
	Giannitsa	2091	1816	484	60	61	449	119
Low input	Florina	2142	1344	664	94	126	438	123
Low-Input	Trikala	2987	2575	422	68	79	446	130
	Kalambaka	2461	1531	649	120	148	435	109
	Giannitsa	1824	720	367	49	51	343	104
Conventional and Low-input	Florina	2074	905	593	73	79	307	107
	Trikala	2881	1348	593	46	53	340	113
<u> </u>	Kalambaka	2458	1860	1065	73	80	315	85

Table 3. Trait stability index across genotypes for the two farming systems: days to 50% flowering, main stem length (cm), main stem thickness (mm), fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%), and ash content % of dry matter.

	Genotypes	Days to 50% Flowering	Main Stem Length (cm)	Main Stem Thickness (mm)	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content %	Ash Content % of Dry Matter
Conventional	Olympos	4766	888	1124	97	121	354	133
	Pisso	7190	2155	1735	140	148	475	115
	Livioletta	7544	1661	388	135	135	459	133
	Vermio	5127	1650	738	88	99	533	111
	Dodoni	4208	1539	881	58	64	453	81
	Zt1	3881	579	476	68	66	540	123
	Zt2	5786	847	364	84	86	526	118
Low-input	Olympos	5693	1885	690	87	108	472	146
	Pisso	7613	1751	1016	51	52	486	129
	Livioletta	8642	1286	408	139	204	471	118
	Vermio	7799	1542	362	125	193	438	116
	Dodoni	5006	2130	476	59	55	623	122
	Zt1	8035	993	343	151	202	541	153
	Zt2	10,911	3094	638	64	71	544	107
Conventional and Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	4487 6254 6589 5305 4090 4080 6836	1194 1731 1413 1462 1648 630 1347	846 1292 395 473 586 386 468	93 46 114 83 59 85 71	115 48 130 98 60 86 75	325 460 347 390 407 461 337	126 119 118 104 84 120 95

Table 4. Combined trait stability index across genotypes and environments for the two farming systems: days to 50% flowering, main stem length (cm), main stem thickness (mm), fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%), ash content % of dry matter.

	Genotypes	Days to 50% Flowering	Main Stem Length (cm)	Main Stem Thickness (mm)	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content %	Ash Content % of Dry Matter
				Giai	nnitsa			
Conventional	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	3420 4193 6005 5724 3471 2905 4254	5714 9510 800 933 2586 8231 2239	591 1778 239 834 412 293 298	294 569 147 71 30 44 98	507 437 247 73 32 43 97	436 600 574 663 510 864 786	134 130 186 75 71 147 143
Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	4118 6867 7437 6470 5693 5386 7046	2902 6588 13,802 9720 26,290 8439 6798	760 411 327 684 1184 430 1474	130 633 439 90 24 399 82	140 720 470 132 23 528 59	402 893 598 461 921 704 598	146 143 100 81 124 133 128
Conventional and Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	3600 4795 5997 5870 4228 3668 4391	2443 4764 1621 467 2573 268 2417	684 708 289 406 577 186 355	190 538 88 64 29 56 81	233 509 107 74 28 55 65	315 619 424 475 464 692 491	133 124 123 79 81 130 101
				Flo	orina			
Conventional	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	4556 4709 7338 8348 5397 4315 9480	$1110 \\ 1626 \\ 11,783 \\ 3337 \\ 3092 \\ 4055 \\ 4485$	1957 3689 350 358 729 399 725	178 791 146 269 174 215 98	196 513 139 442 170 240 124	590 746 603 511 706 643 386	148 96 104 186 87 145 90
Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	7142 5487 8024 11,714 7228 4205 9946	1362 1700 2574 3077 3662 9218 4165	2903 2236 941 404 801 326 499	31 307 149 113 55 291 284	39 444 511 299 78 395 329	734 375 470 549 967 812 823	162 109 140 151 127 166 116
Conventional and Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	5354 5101 7078 8853 5516 4151 8990	849 1705 4333 2523 3340 3239 2881	2451 1225 506 381 746 384 592	57 95 97 114 78 248 149	70 93 122 168 104 311 192	486 531 309 469 596 632 397	144 104 107 155 102 153 101
	Olympos	89/5	3180	2478	kala 32	30	485	183
Conventional	Pisso Livioletta Vermio Dodoni Zt1 Zt2	11,898 20,733 11,755 11,729 16,981 10,124	2917 3844 4665 1154 1810 17,502	$ \begin{array}{r} 24701 \\ 496 \\ 1098 \\ 2544 \\ 1666 \\ 2158 \\ \end{array} $	79 268 114 86 114 75	75 178 172 178 112 102	327 387 549 558 527 460	178 169 107 129 92 150
Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	5348 9580 12,243 13,050 12,591 24,270 22,794	12,101 908 7686 12,343 3145 3716 8975	854 2804 1106 228 201 901 351	375 256 64 569 479 196 109	486 267 109 796 391 339 82	772 450 236 936 796 476 396	144 128 155 97 121 132 139
Conventional and Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	5520 8503 10,698 8453 9319 9155 11,099	4435 1122 2202 7203 1216 2563 12,133	640 2079 680 393 374 1007 647	63 18 110 81 142 155 72	78 20 143 102 225 176 76	506 380 255 417 515 465 243	145 159 169 101 111 103 103

	Genotypes	Days to 50% Flowering	Main Stem Length (cm)	Main Stem Thickness (mm)	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content %	Ash Content % of Dry Matter		
		Kalambaka								
Conventional	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	11,945 38,704 27,429 8138 16,768 13,542 3435	1617 2185 2785 13,133 9926 5841 12,901	1364 8494 2088 4768 3175 3894 3572	222 100 190 103 121 163 58	275 112 241 110 163 188 51	885 902 463 531 856 463 507	90 112 176 121 162 169 87		
Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	9304 8098 14,546 11,685 7719 21,627 12,166	1443 4612 442 6158 2190 4766 3613	678 1367 189 777 958 1075 1116	125 297 361 317 195 177 47	$164 \\ 545 \\ 419 \\ 470 \\ 463 \\ 264 \\ 54$	897 781 844 518 873 804 952	146 156 98 160 121 152 72		
Conventional and Low-input	Olympos Pisso Livioletta Vermio Dodoni Zt1 Zt2	6814 8481 10,069 6461 7493 4138 5731	1591 2977 815 7037 3521 3336 3069	966 2505 372 1340 1517 1756 1694	153 62 227 166 151 80 53	203 71 231 180 188 104 55	516 681 496 452 564 431 334	110 132 128 114 86 117 77		

 Table 4. Cont.

Days to 50% flowering showed some extreme values, and for certain areas, it was 38,704. Generally, it was the most stable trait, with values over 10,000 in many cases. The main stem length showed a very stable behavior, with values over 1000.

Days to 50% flowering showed some extreme values for certain areas; for example, it was found to be 38,704 for cv. Pisso in Kalambaka (cv. Livioleta was 27,429). Generally, it was the most stable trait, with values over 10,000 in many cases. The main stem length showed a very stable behavior, with values over 1000. For some cultivars or lines, there were a few extreme values over 10,000 (line Zt2, cv. Olympos and cv. Vermio)—but also line Zt2, cv. Vermio in Kalambaka, cv. Olympos in Trikala, and cv. Livioletta in Giannitsa and in Florina, respectively, depending on the environment or cultivation method. Evidently, interactions led specific genotypes to exhibit varying behaviors for stability according to the environment or cultivation method.

2.2. The AMMI Tool for Multi-Environment Evaluations

The AMMI model is a widely used statistical tool in the analysis of multi-environment experiments. The purpose of the tool is to understand the complex GEI. In the AMMI model, the data are represented by a two-way table of GEI means. In complete tables, least-squares estimation is equivalent to fitting an additive two-way ANOVA model for the main effects and applying a single value decomposition to the interaction residuals [40].

Using this statistical tool, AMMI software can generate the adaptation map and AMMI1 biplot, where one axis is the axis of the factor, and the other is the PC1 value. When the PC1 value and its distance from the X-axis are close, the factor analyzed is stable. Regarding the AMMI1 biplot, the desirable genotypes were those with a high value on the axis of trait performance (*x*-axis, right position) and close to the center of the PC1 axis (near zero).

GGE stands for the genotype's main effect (G) plus the genotype by environment interaction (GE), which is the only source of variation that is relevant to genotype evaluation. Mathematically, GGE is the genotype by environment data matrix after the environmental means are subtracted. A GGE biplot is a biplot that displays the GGE of a genotype through two-way environmental data. The GGE biplot methodology originates from the graphical analysis of multi-environment genotype trials (MET) data but is equally applicable to all other types of two-way data.

Regarding the GGE biplot for environments, the most stable environment was that placed close to the dot of the ideal and average environment and in the concentric area of the ideal environment dot. In terms of the GGE biplot for genotypes, the desirable genotypes (stable and productive) were those placed near the ideal genotype and in the concentric area of the ideal genotype dot.

The AMMI1 and $G \times E$ biplot analysis created biplots depicting the performance of the genotypes in different environments. The biplots created serve as a simple tool that can easily characterize each genotype for performance and stability.

The stability analysis using both AMMI and GGE biplot for days to 50% flowering is depicted in Figure 1a–d.

AMMI1 Biplot



(d)

8 **E**4 99 Predicted days.to.50..flowering 158 00 PC1 8 90 154 2 0.0 -0.5 0.5 1.0 154 156 158 160 ent IPCA1 (PC1=71.5%) days.to.50..flo (a) (b) GGE Biplot-Environment View for days.to.50..flow GGE Biplot-Genotype View for days.to.50..flov rina 96%; PC2=2.8% PC2=2.89 ₽ 50 0 S ę Average Er
 Ideal Env 10 -5 -5 0 PC1 PC1

2

2

Adaptation Man

(c)

4 m



The stability analysis using both AMMI and GGE biplot for main stem length is depicted in Figure 2a–d.

Figure 2. Stability analysis for the trait of main stem length (cm) based on (**a**) adaptation map, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**b**) AMMI1 biplot, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**c**) GGE biplot depicting the environmental stability over time for the desirable genotypes placed near the ideal environment; (**d**) GGE biplot for genotypes depicting the genotypic stability in different environments. The desirable genotypes are those placed near the concentric region of the ideal genotype.



Data from the main stem thickness used in AMMI and GGE biplot analysis (Figure 3a–d) show that this trait was very environmentally dependent.

Figure 3. Stability analysis for the trait of main stem thickness (mm) based on: (**a**) adaptation map, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**b**) AMMI1 biplot, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**c**) GGE biplot depicting the environmental stability over time for the desirable genotypes placed near the ideal environment; (**d**) GGE biplot for genotypes depicting the genotypic stability in different environments. The desirable genotypes are those placed near the concentric region of the ideal genotype.



The stability analysis using both AMMI and GGE biplot for fresh forage yield is shown in Figure 4a–d.

Figure 4. Stability analysis for the trait of fresh forage yield (kg ha⁻¹) based on: (**a**) adaptation map, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**b**) AMMI1 biplot, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**c**) GGE biplot depicting the environmental stability over time for the desirable genotypes placed near to the ideal environment; (**d**) GGE biplot for genotypes depicting the genotypes stability in different environments. The desirable genotypes are those placed near the concentric region of the ideal genotype.



The stability analysis of dry matter yield using AMMI and GGE biplot is depicted in Figure 5a–d.

Figure 5. Stability analysis for the trait of dry matter yield (kg ha⁻¹) based on: (**a**) adaptation map, where the environmental stability of the genotypes is visualized by the X-axis (PC1) and the performance of the trait for the genotypes is tested by the Y-axis; (**b**) AMMI1 biplot, where the environmental stability of the genotypes is visualized by the X-axis (PC1) and the performance of the trait for the genotypes is visualized by the X-axis (PC1) and the performance of the trait for the genotypes is tested by the Y-axis; (**c**) GGE biplot depicting the environmental stability over time for the desirable genotypes placed near to ideal environment; (**d**) GGE biplot for genotypes depicting the genotypic stability in different environments. The desirable genotypes are those placed near the concentric region of the ideal genotype.



The stability analysis using AMMI and GGE biplot for forage dry matter crude protein content is presented in Figure 6a–d.

Figure 6. Stability analysis for the trait of forage dry matter crude protein content % based on: (a) adaptation map, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is visualized by the *Y*-axis; (b) AMMI1 biplot, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (c) GGE biplot depicting the environmental stability over time for the desirable genotypes placed near the ideal environment; (d) GGE biplot for genotypes depicting the genotypic stability in different environments. The desirable genotypes are those placed near the concentric region of the ideal genotype.



The stability analysis for the ash content % of dry matter trait, using the AMMI and GGE biplot, is presented in Figure 7a–d.

Figure 7. Stability analysis for the trait of ash content % of dry matter based on: (**a**) adaptation map, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**b**) AMMI1 biplot, where the environmental stability of the genotypes is visualized by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *X*-axis (PC1) and the performance of the trait for the genotypes is tested by the *Y*-axis; (**c**) GGE biplot depicting the environmental stability over time for the desirable genotypes placed near the ideal environment; (**d**) GGE biplot for genotypes depicting the genotypes are those placed near the concentric region of the ideal genotype.

For AMMI analysis, as visualized by the adaptation map figure, the most desirable genotypes were those placed high on the axis of trait performance, showing a nearly parallel line to the PC1 axis, which was an indication of stability in different environments.

For the AMMI1 biplot, the desirable genotypes were those placed high on the axis of trait performance (*x*-axis, right position) and close to the center of the PC1 axis (near the zero point).

Regarding the GGE biplot for environments, the most stable environment was that placed close to the dot of the ideal and average environment and in the concentric area of the ideal environment dot.

Concerning the GGE biplot for genotypes, the desirable genotypes (stable and productive) were those placed close to the ideal genotype and in the concentric area of the ideal genotype dot.

2.3. Exploratory Data Analysis of Peas

In order to estimate the phenotypic distances among genotypes, the clustering method of Ward was performed and formed clusters based on the traits tested. The clusters were formed based on the fresh forage yield and dry matter yield and the relations among them.

2.4. Genotypic and Phenotypic Coefficients of Variation and Heritability

In Table 5, estimations of genetic parameters for the traits are tabulated. The genetic parameters, along with the heritability in a broad sense, were estimated for all traits except the trait of main stem thickness. The parameters show that there is enough phenotypic variability for all traits. Furthermore, a large portion of phenotypic variability was genotypic, and this is desirable for geneticists in order to select superior genotypes for all traits. The heritability for all traits ranged from 99.4% to 83.8%. These estimates of heritability combined with the high percentage of genetic variability to the phenotype and the high diversity for all traits indicates that the selection of new varieties would be effective.

Table 5. Estimations of genetic parameters for tested traits: days to 50% flowering, main stem length (cm), main stem thickness (mm), fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%), and ash content % of dry matter.

Traits	Min	Max	Mean	sd	σ_g^2	σ_p^2	GCV (%)	PCV (%)	H ² (%)
Days to 50% flowering	148.1	165.7	156.3	3.38	7.76	7.81	1.78	1.79	99.4
Main stem length (cm)	85.2	99.8	92.3	2.98	2.04	2.31	1.55	1.65	88.4
Main stem thickness (mm)	2.78	3.76	3.21	0.14	-	-	-	-	-
Fresh forage yield (kg ha $^{-1}$)	17,257	34,932	23,947	3201	2,205,760	2,631,649	6.20	6.77	83.8
Dry matter yield (kg ha^{-1})	4080	7930	5531	714.2	114,321	135,209	6.11	6.48	84.6
Forage dry matter crude protein content (%)	17.8	23.3	20.3	1.14	0.251	0.274	2.47	2.58	91.4
Ash content % of dry matter	7.29	12.45	9.9	0.98	0.081	0.093	2.87	3.08	87.0

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

2.5. Correlations between All Characteristics

In Table 6, correlations between all traits are tabulated. Many correlations were statistically significant, especially between forage yield and traits such as the main stem length (r = 0.203), dry matter yield (r = 0.974), and forage dry matter crude protein content (r = 0.100).

	Days to 50% Flowering	Main Stem Length (cm)	Main Stem Thickness (mm)	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content %
Main stem length (cm)	-0.061					
Main stem thickness (mm)	-0.004	-0.231 **				
Fresh forage yield (kg ha^{-1})	0.032	0.203 **	0.016			
Dry matter yield (kg ha $^{-1}$)	0.028	0.210 **	0.009	0.974 **		
Forage dry matter crude protein content (%)	0.289 **	0.006	0.004	0.100 *	0.084	
Ash content % of dry matter	0.100 *	-0.050	0.048	-0.084	-0.091	0.676 **
	* 0 1		1 1 (2 1 1 1) 44			1 (2) 11 11

Table 6. Correlations between all traits measured: days to 50% flowering, main stem length (cm), main stem thickness (mm), fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%), and ash content % of dry matter.

* Correlations significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

3. Discussion

Farmers and breeders need both high and stable performance regarding forage yield. In our work, the two cultivation systems (conventional and low-input) displayed differences in genotype-yielding performance, but overall estimations on various pea characteristics seemed to be unaffected. In combination with GGE biplot analysis, the two farming systems revealed the most stable genotypes across all environments, as well as those more stable in specific environments and farming systems. Additionally, some genotypes exhibited stability in low-input conditions. Generally, very significant GGE interactions were recorded. Sayar and Han [41], based on ANOVA findings, state that $G \times E$ interaction is the most important concept to deal with. In our work, $G \times E$ interaction was revealed due to multiple interactions recorded for many traits. Sayar's work [27] was based on AMMI analysis in order to define cultivar interactions with the environment. We described the interactions of each trait of pea cultivars and lines across different environments as follows:

3.1. Days to 50% Flowering

Regarding days to 50% flowering, AMMI analysis produced the figures adaptation map (Figure 1a) and AMMI1 biplot (Figure 1b). Both figures explained a portion of the total variability (71.5%), which is high enough for the genotype \times environment (Gx) variation. Both the adaptation map and AMMI1 figures show that the most stable genotypes for environments E1 (Gianitsa) and E2 (Florina) were G6 (Zt1) and G5 (Dodoni), the late genotypes, whereas, for E3 (Trikala) and E4 (Kalambaka), the most stable genotypes were the early ones, G1 (Olympos), G2 (Pisso) and G7 (Zt1). The GGE analysis explained a total variability of 98.8% (PC1:96%, PC2: 2.8%), which was very high. The GGE biplot of the environment (Figure 1c) shows that all environments were quite similar and in the concentric circles of the ideal environment. The GGE biplot for the genotype view (Figure 1d) shows that all genotypes were very stable in all environments; the early genotypes were G1 (Olympos) and G2 (Pisso), and the late genotypes were G6 (Zt1) and G5 (Dodoni). The ideal for cultivation genotypes depends on what is desirable among early and late ones.

3.2. Main Stem Length (cm)

Regarding the main stem length, AMMI analysis produced the figures adaptation map (Figure 2a) and AMMI1 biplot (Figure 2b). Both figures explained a portion of the total variability (62.5%), which is high enough for conclusions. Both the adaptation map and AMMI1 figures show that the most stable genotypes were G2 (Pisso) and G1 (Olympos), where G2 (Pisso) had the highest performance for the main stem length trait. The GGE analysis explained a total variability of 90% (PC1:66.2%, PC2: 24.6%), which is very high. The GGE biplot for the environment view (Figure 2c) shows that all environments were very diverse, where the E1 (Giannitsa) environment was very close to the average environment.

The GGE biplot for the genotype view (Figure 2d) shows that the most stable genotype and identical to the ideal genotype was G2 (Pisso), followed by the G1 (Olympos) genotype, which was very stable but with lower performance for this trait.

3.3. Main Stem Thickness (mm)

The AMMI analysis via the adaptation map (Figure 3a) and AMMI1 biplot expressed the PC1: 48.4% of the total variability. In both figures, there no clear pattern was found for stability, but the genotypes G3 (Livioletta), G4 (Vermio), and G2 (Pisso) were relatively stable. The GGE biplot analysis explained 70.0% (PC1:38.5%, PC2:31.5%) of the total variability. The GGE biplot for the environment view (Figure 3c) shows that all environments were very diverse, and no environment was placed near the average environment. The GGE biplot for the environment view (Figure 3d) shows that relative stable genotypes were G3 (Livioletta), G5 (Dodoni), and G7 (Zt2), but all were placed out of the concentric circles of the ideal genotype.

3.4. Fresh Forage Yield (kg ha^{-1})

The AMMI analysis explained a portion (57.1%) of PC1's total variability. Both the adaptation map (Figure 4a) and AMMI1 biplot (Figure 4b) show that the relatively stable genotypes were G2 (Pisso), G6 (Zt1), and G1 (Olympos), while G2 (Pisso) had the highest fresh forage yield. The GGE biplot analysis expressed 93.3% (PC1:79.3%, PC2:14.0%) of the total variability. The GGE biplot for the environment view shows that E1 (Giannitsa) and E4 (Kalambaka) were close to the average environment, and all environments were very diverse. The GGE biplot for the genotype view shows that the most desirable genotype was G2 (Pisso), followed by G6 (Zt1), which was less stable, and G1 (Olympos), which was less productive than the other two but very stable. AMMI analysis assisted Sayar [27] in recommending the best cultivars for fresh forage yield in certain cultivation areas.

3.5. Dry Matter Yield (kg ha⁻¹)

The AMMI analysis as presented from the adaptation map and AMMI1 biplot figures explained the PC1: 62.7% of the total variability. Both the adaptation map (Figure 5a) and AMMI1 biplot (Figure 5b) show that the most stable genotypes were G2 (Pisso), G6 (Zt1), and G1 (Olympos). The most productive genotype was G2 (Pisso), followed by G6 (Zt1) and G1 (Olympos). The GGE biplot analysis explained 95.5% (PC1:77.9%, PC2:15.6%) of the total variability. The GGE biplot for environment view shows that E1 (Giannitsa) and E4 (Kalambaka) were close to the average environment, and all environments were very diverse. The GGE biplot for the genotype view shows that the most desirable genotype was G2 (Pisso), followed by G6 (Zt1) which was less stable, and G1 (Olympos), which was less productive than the other two but very stable. Acikgoz et al. [14] investigated the dry matter, yield relations, and $G \times E$ interactions and concluded after a comparison of cluster and stability analyses that the stability analysis failed to recommend cultivars for different regions.

3.6. Forage Dry Matter Crude Protein Content %

The AMMI analysis explained the PC1: 78.2% of the total variability, which is quite high. Both the adaptation map (Figure 6a) and AMMI1 biplot (Figure 6b) figures show that the most productive genotypes were G2 (Pisso), G6 (Zt1), and G5 (Dodoni), which showed relatively low stability. The GGE biplot analysis explained 97.2% (PC1:79.9%, PC2:17.3%) of the total variability. The GGE biplot for the environment view shows that E2 (Florina), E1 (Giannitsa), and E4 (Kalambaka) were close to the average environment. The GGE biplot for the genotype view shows that the most desirable genotype was G2 (Pisso), G6 (Zt1), and G5 (Dodoni), which showed relatively low stability. Only the G6 (Zt1) genotype was placed in the concentric region of the ideal genotype, which indicates relatively acceptable stability and performance for this trait.

3.7. Ash Content % of Dry Matter

The AMMI analysis explained the PC1: 68.8% of the total variability, which is quite high. Both the adaptation map (Figure 7a) and AMMI1 biplot (Figure 7b) figures show that the most productive genotypes were G3 (Livioletta), followed by G6 (Zt1) and G2 (Pisso). The most stable one was the G3 (Livioletta) genotype. The GGE biplot analysis explained 93.6% (PC1:66.9%, PC2:26.7%) of the total variability. The GGE biplot for the environment view shows that E4 (Kalambaka) and E3 (Trikala) were close to the average environment. The GGE biplot for the genotype view shows that the most desirable genotypes were G3 (Livioletta) and G6 (Zt1), which showed relatively low stability. The G3 (Livioletta) genotype was placed nearly identical to the ideal genotype, which means that it has acceptable stability and performance for this trait.

3.8. Genotypic and Phenotypic Coefficients of Variation and Heritability

The traits of days to 50% flowering, the main stem length, main stem thickness, dry matter crude protein, and ash are components of fresh and dry forage yield. The traits of fresh forage yield and dry forage yield seem to have high variability, as described by the min and max of Table 5. The heritability estimate was 83.8% and 84.6%, respectively. These values are high [42]. The genetic variability and the GCV of these two traits are the highest among all other traits. This combination of high heritability (H^2) and high GCV is an indication that the variation among genotypes was largely due to the additive genetic part [43]. Abebe et al. [44] suggested that high heritability in these values, in a broad sense, indicate that the characters under study are less influenced by the environment in their expression. This means that the direct selection of the traits of fresh forage yield and dry forage yield could be effective. As far as the other traits, the heritability (H^2) was high, and the GCV was high to moderate, so the selection of these traits could be effective as well. The findings of this genetic analysis for the traits tested suggest that the selection of productive genotypes in order to create new varieties that are stable in all environments for conventional and organic cultivation is possible. The estimates of genetic parameters of forage dry matter crude protein content characterized by high heritability and high genetic variability, and GCV indicates that selecting for better quality, as described by the protein content, is possible.

3.9. Correlations between Traits

In our study, many correlations between traits displayed statistically significant results. Statistically significant correlations are useful for indirect breeding and selection of traits that show low stability through more stable traits that promote adaptation [7]. Positive correlations were also reported for other traits in common vetch and peas by Greveniotis et al. [2,45,46]. Georgieva et al. [47] reported significant correlations for many traits in field peas. We found positive relationships between the fresh forage yield and dry matter yield, which were expected, but also the main stem length and crude protein content.

Correlation studies are very important in the genetic improvement of cultivars [48,49]. Singh et al. [50] reported significant correlations between seed yield per plant and harvest index, as well as the biological yield per plant, plant height, number of seeds per pod, number of primary branches per plant, number of pods per plant, and 100-seed weight. Days to maturity and 100-seed weight and number of pods per plant showed a weak negative correlation with the seed yield per plant. In our results, the most interesting correlation was between the fresh forage yield and the stable characteristic, 'stem length', for indirect breeding purposes [46]. Linearity was not satisfactory in many cases due to low correlation coefficients. Cacan et al. [1] reported interesting yield performances for forage pea lines. They also reported statistically significant correlations between many traits studied. Kosev and Mikić [51] also reported high and significant correlations between many traits in peas and, most of all, with significant linearity.

Sayar and Han [41] used GGE biplot analysis in two growing seasons. Their results showed that two lines and cultivar Kirazli were superior for fresh forage yield, dry matter

yield, plant height, and days to 50% flowering. PC2 scores of these genotypes were found near zero, making them stable genotypes. Bocianowski et al. [16] reported that AMMI analysis managed to depict certain cultivars for certain environments regarding seed yield. This was an encouraging result for practical farming.

Sayar and Han [41], as well as Yihunie and Gesesse [52], reported that the GGE biplot could be used as a tool for the discrimination of pea genotypes according to their productivity and stability and the selection of the most suitable genotype for cultivation.

Uzun et al. [53] assessed the dry matter performance for peas used for their forage yield. He reported that semi-leafless lines had significantly better standing ability than leafed peas. The leaf type had no effect on lodging scores at the seed-harvesting stage. Yihunie and Gesesse [52] used a GGE-biplot of field peas genotypes and defined the ideal genotype. Among the twelve environments used, three environments were the best for discrimination, while one genotype was found to be the most stable, the highest yielding, and it was recommended for wider cultivation in Northwestern Ethiopia and similar areas. Georgieva et al. [25] also reported the specific adaptation of certain genotypes in field peas. In our study, Vermio proved to be a stable genotype for forage yield performance in low-input farming in the Trikala and Kalambaka area, while Pisso was the best in Florina and Giannitsa areas and low-input farming systems. The two pea lines exhibited stable performance in Giannitsa and Florina areas, especially in low-input conditions. Livioletta was also a stable genotype.

3.10. Exploratory Data Analysis of Peas

To provide a certain classification for the studied pea genotypes and cultivation systems, a heat map (Figure 8) was carried out. Cluster analysis was previously used for classification purposes for various genetic materials (e.g., maize, sweet cherry), sometimes in combination with principal components analysis [54–56]. The available data were divided into groups of increasing dissimilarity. Based on these results, the peas were divided into two distinct clusters (C1 and C2), each one having two subclusters (SC1, SC2, SC3, and SC4, respectively). Grouping for each subcluster revealed differences among pea cultivations. More specifically, SC1 consisted of low-input cultivated genotypes, which were characterized by a lower forage yield and dry matter yield, as well as lower ash content. SC2 included various other subgroups, mainly containing conventionally cultivated genotypes, which exhibited mostly low forage and dry matter yields. SC3 contained genotypes with higher yields, with two distinct subgroups, one cultivated conventionally and the other cultivated with low input. Lastly, SC4 included genotypes cultivated conventionally during the first growing season of experimentation and exhibiting higher main stems. There were no identified specific clusters based on locality.



Figure 8. Dendrogram of two-way clustering based on the variables measured in peas using Ward's method on the standardized data to define distances between clusters. Blue areas in the map dendrogram indicate low values, whereas the red areas indicate high values.

4. Materials and Methods

4.1. Crop Establishment and Experimental Procedures

Four different locations (Table 7) were employed for the field experiments, two of them in Northern Greece and another two in Central Greece, divergent regarding soil type, altitude, and environmental conditions.

Table 7. Coordinates, altitude, soil type, and cultivation information for the environments of the experiment.

Environments	Longitude	Latitude	Elevation (m)	Soil Texture	Planting Date	Harvesting Date
Giannitsa	22°39′ E	40°77′ N	10	Clay (C)	Early November 2008 and 2009	Late May 2009 and 2010
Florina	21°22′ E	40°46′ N	705	Sandy loam (SL)	Early November 2008 and 2009	Late May 2009 and 2010
Trikala	21°64′ E	39°55′ N	120	Sandy clay loam (SCL)	Early November 2008 and 2009	Late May 2009 and 2010
Kalambaka	21°65′ E	39°64′ N	190	Silty clay (SiC)	Early November 2008 and 2009	Late May 2009 and 2010

Five cultivars (common in Greek cultivations) of peas, namely, cv. Olympos, cv. Pisso, cv. Livioletta, cv. Vermio, and cv. Dodoni, and two lines (Zt1, Zt2) were used.

Two types of cultivation approaches were selected: low-input and conventional farming systems. The plots cultivated under the conventional farming system were fertilized before sowing so that 40 kg ha⁻¹ Nitrogen and 80 kg ha⁻¹ P₂O₅ were added into the soil. For low-input cultivation, no fertilizers or other agrochemicals were applied during the experiment, while prior to the establishment of the experiment in 2008, the fields had been in a two-year rotation consisting of bread wheat/legumes without nutritional supplementation or other agrochemical inputs. Weeds were fully controlled by hand.

All genotypes were sown in early November 2008 and 2009 according to a strip-plot design, with the seven genotypes randomized within each plot and a plot size of 8.75 m². Replications were four for each plot. Each plot consisted of seven (7) rows 5 m long, spaced at 25 cm, and the number of plants per plot was around 1000 according to the sowing rate. The number of seeds was 120 per m², and the depth of sowing was 4 cm.

4.2. Climatic Conditions

Experimentation lasted two growing seasons (2008–2009 and 2009–2010), and the mean monthly air temperatures (maximum, minimum, mean) and rainfall data during the study period are provided in Table 8 for each experimental area based on daily records.

Year and Environments	Mean Monthly Maximum Temperature (°C)	Mean Monthly Minimum Temperature (°C)	Mean Temperature (°C)	Rainfall (mm)
Giannitsa 2008–2009	22.9	-0.1	10.5	51.5
Giannitsa 2009–2010	23.3	0.6	11.0	55.5
Florina 2008–2009	21.0	-5.7	7.1	44.1
Florina 2009–2010	21.3	-3.8	8.0	62.3
Trikala 2008–2009	23.4	1.3	11.0	55.8
Trikala 2009–2010	24.4	2.9	12.0	85.1
Kalambaka 2008–2009	21.1	0.4	10.8	68.4
Kalambaka 2009–2010	23.5	2.2	11.7	98.8

Table 8. Climatic conditions for the examined environments during the cultivation period (November–May).

4.3. Measurements

For each plot, the number of days from the sowing date to 50% of the flowering time was recorded. Ten random plants of each plot were selected at the flowering time and measured from the ground level to the top point with a ruler (1 mm sensitivity) after extending the plants upward. The arithmetic mean of the measurements (in cm) was accepted as the 'main stem length' for each plot. The main stem thickness (mm) was calculated by measuring the stem diameter at the top, middle, and bottom of each stem selected. These traits also served as correlation variables.

The chloromass (fresh forage) obtained from each plot right after harvesting in full flowering time was weighed, and the value was converted to a hectare basis in order to calculate the 'fresh forage yield (kg ha⁻¹)'. After, fresh forage samples (0.5 kg), harvested from each experimental plot, were placed in a drying oven at 70 °C for 48 h, left to cool, and weighed; the dry matter yield was determined for each plot, followed by the calculation on a hectare basis in order to obtain the 'dry matter yield (kg ha⁻¹)'.

In order to analyze the forage dry matter crude protein content (%) and ash content % of the dry matter, the forage dry matter was ground to pass through a 1 mm sieve and subsequently mixed for the analysis. Ash content was determined according to AOAC Official Method 942.05 [57], while total nitrogen was determined using AOAC Official Method 988.05 [57], followed by total protein content estimation.

4.4. Data Analysis

The experimental design was a combined analysis of seven genotypes in four replications over four locations for two cultivation systems and two years of experimentation. The formal ANOVA should include the interaction of years \times locations or years \times genotypes, etc., which were not the aims of our study and made no practical sense. To overcome such a problem, we created a simpler ANOVA with one degree of interaction less, and it did not affect the precision of the analysis for the genotypes in different environments, so we conducted an ANOVA as follows. In order for the ANOVA to be more informative, the combination of each year and location was assigned as an environment in the general meaning since locations and years contribute to the effect of the environment on the genotypes. In this way, we have fewer interactions in the ANOVA table and do not affect the variance of genotypes and the $G \times E$ (genotype \times environment) interaction, which is crucial for proceeding in the stability analysis. Stability estimations were based on the stability index $(\overline{x}/s)^2$, where \overline{x} and s are the entry mean trait and the standard deviation, respectively [8,58]. Trait correlations were examined using the Pearson coefficient according to Steel et al. [59], and the significance of all the statistics was checked at p < 0.05 using SPSS ver. 25. Stability analysis was performed using the free version of PB Tools v1.4 (International Rice Research Institute, Laguna, Philippines) over locations and years for each characteristic and the statistical tools were the AMMI1 and (GGE) biplot analysis.

The mean squared values of genotypes, genotype \times environment, error, and replicates were used to estimate the variance components following the methods suggested by McIntosh [60], which were used for the estimation of genetic parameters for the tested traits as follows:

Heritability in a broad sense (H²) was calculated according to Johnson et al. [42] and Hanson et al. [61]:

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

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated for all tested traits according to Singh and Chaudhary [62]:

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

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

where σ_g^2 , σ_p^2 , σ_{gxe}^2 , σ_{re}^2 , and \overline{x} are the genotypic variance, phenotypic variance, genotype × environment variance, residual variance (error), and overall mean for each tested trait, respectively.

The mathematical processing of the data was performed by hierarchical cluster analysis (HCA) using Ward's method. HCA analysis was performed using JMP 14 (SAS Institute Inc., Cary, NC, USA). The results from the cluster analysis are presented in a dendrogram.

5. Conclusions

Correlations among various characteristics showed significant positive relationships between the forage yield along with the dry matter yield and forage dry matter crude protein content. Indirect forage yield stability improvement may be performed by improving the main stem length, which generally showed high stability indices.

Comparisons between conventional and low-input farming systems generally revealed genotypes that displayed highly stable performance, even in low-input farming systems. Stability index data could also serve to estimate the kind of heritability of various traits, either quantitative or qualitative.

AMMI analysis, and consequently, a GGE biplot, along with ANOVA data, showed that there is a strong interaction between genotypes and environments, as well as the farming system (conventional or low-input). Therefore, the necessity arises to propose certain genotypes of field peas for specific areas and farming systems so as to obtain the most stable performance. The Vermio cultivar proved to be a stable genotype for forage yield performance in low-input farming in Trikala and Kalambaka areas, while Pisso was the best in Florina and Giannitsa areas and low-input farming. The two pea lines displayed stable performance in Giannitsa and Florina areas, especially in low-input conditions. The stable behavior of some genotypes in low-input farming systems could be valuable for farmers that raise livestock in mountainous areas.

The genetic parameters showed that all traits were of high heritability and moderate to high GCV, and the direct selection for fresh forage yield and dry matter yield was expected to be effective.

Limitations of this study are related to the differences in environmental data through time (across years). Low rainfall may significantly affect the genotype behavior across different environments.

Author Contributions: Conceptualization, V.G. and S.Z.; methodology, V.G. and S.Z.; investigation, V.G. and E.B.; statistical analysis, A.K., E.B., C.G.I. and V.G., writing—original draft preparation, V.G. and C.G.I.; writing—review and editing, E.B. and A.K.; visualization, A.K. and V.G.; supervision, S.Z., project administration, S.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Cacan, E.; Kokten, K.; Bakoglu, A.; Kaplan, M.; Bozkurt, A. Evaluation of some forage pea (*Pisum arvense* L.) lines and cultivars in terms of herbage yield and quality. *Harran Tarım Ve Gıda Bilimleri Derg.* **2019**, *23*, 254–262.
- Greveniotis, V.; Bouloumpasi, E.; Zotis, S.; Korkovelos, A.; Ipsilandis, C.G. Yield components stability assessment of peas in conventional and low-input cultivation systems. *Agriculture* 2021, *11*, 805. [CrossRef]

- 3. Elzebroek, T.; Wind, K. Guide to Cultivated Plants; CAB International: Oxfordshire, UK, 2008; p. 496.
- 4. Tan, M.; Koc, A.; Dumlu, G.Z. Morphological characteristics and seed yield of East Anatolian local forage pea (*Pisum sativum* ssp. *arvense* L.) ecotypes. *Turk. J. Field Crop.* **2012**, *17*, 24–30.
- Arif, U.; Ahmed, M.J.; Rabbani, M.A.; Arif, A.A. Assessment of genetic diversity in pea (*Pisum sativum* L.) landraces based on physic-chemical and nutritive quality using cluster and principal component analysis. *Pak. J. Bot.* 2020, 52, 575–580. [CrossRef]
- 6. Vafias, B.; Goulas, C.; Lolas, G.; Ipsilandis, C.G. A triple stress effect on monogenotypic and multigenotypic maize populations. *Asian J. Plant Sci.* 2007, *6*, 29–35. [CrossRef]
- Fasoulas, A.C. *The Honeycomb Methodology of Plant Breeding*; Department of Genetics and Plant Breeding, Aristotle University of Thessaloniki: Thessaloniki, Greece, 1988; p. 168.
- 8. Fasoula, V.A. Prognostic Breeding: A new paradigm for crop improvement. Plant Breed. Rev. 2013, 37, 297–347.
- Yan, W.; Kang, M.S. *GGE Biplot Analysis: A Graphical Tool for Breeders, Geneticists and Agronomists*; CRC Press: Boca Raton, FL, USA, 2003.
 Karimizadeh, R.; Mohammadi, M.; Shefazadeh, M.K. A review on parametric stability analysis methods: Set up by Matlab program. *Int. J. Agric.* 2012, 2, 433–442.
- 11. Kang, M.S.; Gauch, H.G. Genotype-by-Environment Interaction; CRC Press: Boca Raton, FL, USA, 1996.
- 12. Lin, C.S.; Binns, M.R.; Lefkovitch, L.P. Stability analysis: Where do we stand? Crop Sci. 1986, 26, 894–900. [CrossRef]
- Jinks, J.; Pooni, H. The genetic basis of environmental sensitivity. In Proceedings of the 2nd International Conference on Quantitative Genetics, Raleigh, NC, USA, May–June 1987; Weir, B.S., Eisen, E.J., Goodman, M.M., Namkoong, G., Eds.; Sinauer: Sunderland, MA, USA, 1988; pp. 505–522.
- Acikgoz, E.; Ustun, A.; Gul, İ.; Anlarsal, A.E.; Tekeli, A.S.; Nizam, İ.; Avcioglu, R.; Geren, H.; Cakmakcı, S.; Aydinoglu, B.; et al. Genotype × environment interaction and stability analysis for dry matter and seed yield in field pea (*Pisum sativum* L.). Span. J. Agric. Res. 2009, 7, 96–106. [CrossRef]
- 15. Ceyhan, E.; Kahraman, A.; Ates, M.K.; Karadas, S. Stability analysis on seed yield and its components in peas. *Bulg. J. Agric. Sci.* **2012**, *18*, 905–911.
- 16. Bocianowski, J.; Ksiezak, J.; Nowosad, K. Genotype by environment interaction for seeds yield in pea (*Pisum sativum* L.) using additive main effects and multiplicative interaction model. *Euphytica* **2019**, *215*, 191. [CrossRef]
- Rana, C.; Sharma, A.; Sharma, K.C.; Mittal, P.; Sinha, B.N.; Sharma, V.K.; Chandel, A.; Thakur, H.; Kaila, V.; Sharma, P.; et al. Stability analysis of garden pea (*Pisum sativum* L.) genotypes under North Western Himalayas using joint regression analysis and GGE biplots. *Genet. Resour. Crop. Evol.* 2021, 68, 999–1010. [CrossRef]
- Popović, V.; Vučković, S.; Jovović, Z.; Rakaščan, N.; Kostić, M.; Ljubičić, N.; Mladenović-Glamočlija, M.; Ikanović, J. Genotype by year interaction effects on soybean morphoproductive traits and biogas production. *Genetika* 2020, 52, 1055–1073. [CrossRef]
- Lakić, Ž.; Stanković, S.; Pavlović, S.; Krnjajić, S.; Popović, V. Genetic variability in quantitative traits of field pea (*Pisum sativum* L.) genotypes. *Czech J. Genet. Plant Breed.* 2018, 54, 1–7. [CrossRef]
- 20. Amin, M.; Mohammad, T.; Khan, A.J.; Irfaq, M.; Ali, A.; Tahir, G.R. Yield stability of spring wheat (*Triticum aestivum* L.) in the North West Frontier Province, Pakistan. *Songklanakarin J. Sci. Technol.* **2005**, *27*, 1147–1150.
- 21. Gauch, H.G. A simple protocol for AMMI analysis of yield trials. *Crop Sci.* **2013**, *53*, 1860–1869. [CrossRef]
- 22. Ebdon, J.S.; Gauch, H.G. Direct validation of AMMI predictions in turfgrass trials. Crop Sci. 2011, 51, 862–869. [CrossRef]
- 23. Greveniotis, V.; Bouloumpasi, E.; Zotis, S.; Korkovelos, A.; Ipsilandis, C.G. Estimations on Trait Stability of Maize Genotypes. *Agriculture* **2021**, *11*, 952. [CrossRef]
- 24. Tsenov, N.; Atanasova, D.; Nankova, M.; Ivanova, A.; Tsenova, E.; Chamurliiski, P.; Raykov, G. Approaches for grading breeding evaluation of winter wheat varieties for grain yield. *Sci. Work. Institute Agric.-Karnobat* **2014**, *3*, 21–35.
- Georgieva, N.; Kosev, V. Model of forage pea (*Pisum sativum* L.) cultivar in conditions of organic production. *Bulg. J. Agric. Sci.* 2020, 26, 91–95.
- Al-Aysh, F.; Kotmaa, H.; Al-Shareef, A.; Al-Serhan, M. Genotype-environment interact ion and stability analysis in garden pea (*Pisum sativum L.*) landraces. *Agric. Forest.* 2013, 59, 183–191.
- Sayar, S.M. Additive Main Efects and Multiplicative Interactions (AMMI) analysis for fresh forage yield in common vetch (*Vicia Sativa L.*) genotypes. *Agric. For.* 2017, 63, 119–127.
- Gauch, H.G.; Zobel, R.W. AMMI analysis of yield trials. In *Genotype-by-Environment Interaction*; Kang, M.S., Gauch, H.G., Eds.; CRC Press: Boca Raton, FL, USA, 1996; pp. 85–122.
- 29. Islam, M.R.; Anisuzzaman, M.; Khatun, H.; Sharma, N.; Islam, M.Z.; Akter, A.; Biswas, P.S. AMMI analysis of yield performance and stability of rice genotypes across different haor areas. *Eco-Friendly Agril. J.* 2014, *7*, 20–24.
- 30. Gabriel, K.R. The biplot graphic display of matrices with application to principal component analysis. *Biometrika* **1971**, *58*, 453–467. [CrossRef]
- Yan, W.; Tinker, N.A. Biplot Analysis of Multi-Environment Trial Data: Principles and Applications. *Can. J. Plant Sci.* 2006, 86, 623–645. [CrossRef]
- 32. Yan, W. Singular Value Partitioning for Biplot Analysis of Multi-environment Trial Data. Argon. J. 2002, 94, 990–996.
- 33. Kaya, Y.; Akcura, M.; Taner, S. GGE Biplot Analysis of Multi Environment Yield Trials in Bread Wheat. *Turk. J. Agric. For.* 2006, *30*, 325–337.
- Ilker, E.; AykutTonk, F.; Caylak, O.; Tosun, M.; Ozmen, I. Assessment of Genotype × Environment Interactions for Grain Yield in Maize Hybrids Using AMMI and GGE Biplot Analyses. *Turk. J. Field Crop.* 2009, 14, 123–135.

- 35. Ahmadi, J.; Vaezi, B.; Shaabani, A.; Khademi, K. Multi-environment Yield Trials of Grass Pea (*Lathyrus sativus* L.) in Iran Using AMMI and SREG GGE. *J. AgrIC. Sci. Technol.* **2012**, *14*, 1075–1085.
- Mortazavian, S.M.M.; Nikkhah, H.R.; Hassani, F.A.; Sharif-al-Hosseini, M.; Taheri, M.; Mahlooji, M. GGE Biplot and AMMI Analysis of Yield Performance of Barley Genotypes Across Different Environments in Iran. J. AgrIC. Sci. Technol. 2014, 16, 609–622.
- Macák, M.; Candráková, E.; Đalovic, I.; Prasad, P.V.V.; Farooq, M.; Korczyk-Szabó, J.; Kovácik, P.; Šimanský, V. The Influence of Different Fertilization Strategies on the Grain Yield of Field Peas (*Pisum sativum* L.) under Conventional and Conservation Tillage. *Agronomy* 2020, 10, 1728. [CrossRef]
- Hanáčková, E.; Candráková, E. The influence of soil cultivation and fertilization on the yield and protein content in seeds of common pea (*Pisum sativum* L.). Agriculture 2014, 60, 105–114. [CrossRef]
- 39. Greveniotis, V.; Sioki, E.; Ipsilandis, C.G. Estimations of fibre trait stability and type of inheritance in cotton. *Czech J. Genet. Plant Breed.* **2018**, *54*, 190–192. [CrossRef]
- 40. Koundinya, A.V.V.; Ajeesh, B.R.; Hegde, V.; Sheela, M.N.; Mohan, C.; Asha, K.I. Genetic parameters, stability and selection of cassava genotypes between rainy and water stress conditions using AMMI, WAAS, BLUP and MTSI. *Sci. Hortic.* **2021**, *281*, 109949.
- Sayar, M.S.; Han, Y. Forage Yield Performance of Forage Pea (*Pisum sativum spp. arvense* L.) Genotypes and Assessments Using GGE Biplot Analysis. *J. Agric. Sci. Technol.* 2016, 18, 1621–1634.
- Johnson, H.W.; Robinson, H.E.; Comstock, R.E. Estimate of genetic and environmental variability in soybean. Agron. J. 1955, 47, 314–318. [CrossRef]
- Al-Ashkar, I.; Al-Suhaibani, N.; Abdella, K.; Sallam, M.; Alotaibi, M.; Seleiman, M.F. CombiningGenetic and Multidimensional-Analyses to Identify InterpretiveTraits Related to Water ShortageTolerance as an Indirect Selection Toolfor Detecting Genotypes of DroughtTolerance in Wheat Breeding. *Plants* 2021, 10, 931. [CrossRef]
- 44. Abebe, T.; Alamerew, S.; Tulu, L. Genetic variability, heritability and genetic advance for yield and its related traits in rainfed lowland rice (*Oryza sativa* L.) genotypes at Fogera and Pawe, Ethiopia. *Adv. Crop Sci. Technol.* **2017**, *5*, 272. [CrossRef]
- 45. Greveniotis, V.; Bouloumpasi, E.; Zotis, S.; Korkovelos, A.; Ipsilandis, C.G. Assessment of interactions between yield components of common vetch cultivars in both conventional and low-input cultivation systems. *Agriculture* **2021**, *11*, 369. [CrossRef]
- Greveniotis, V.; Bouloumpasi, E.; Zotis, S.; Korkovelos, A.; Ipsilandis, C.G. A Stability Analysis Using AMMI and GGE Biplot Approach on Forage Yield Assessment of Common Vetch in Both Conventional and Low-Input Cultivation Systems. *Agriculture* 2021, 11, 567. [CrossRef]
- Georgieva, N.; Nikolova, I.; Kosev, V. Association study of yield and its components in pea (*Pisum sativum* L.). *Int. J. Pharmacogn.* 2015, 2, 536–542.
- 48. Sanwal, S.K.; Singh, B.; Singh, V.; Mann, A. Multivariateanalysis and its implication in breeding of desired planttype in garden pea (Pisum sativum). *Indian J. Agric. Sci.* 2015, *85*, 1298–1302.
- 49. Kumar, A.V.R.; Sharma, R.R. Character association studies in garden pea. Indian J. Hortal. 2006, 63, 185–187.
- 50. Singh, S.K.; Singh, V.P.; Srivastava, S.; Singh, A.K.; Chaubey, B.K.; Srivastava, R.K. Estimation of correlation coefficient among yield and attributing traits of field pea (*Pisum sativum* L.). *Legume Res.* **2018**, *41*, 20–26. [CrossRef]
- 51. Kosev, V.; Mikić, A. Assessing relationships between seed yield components in spring-sown field pea (*Pisum sativum* L.) cultivars in Bulgaria by correlation and path analysis. *Span. J. Agric. Res.* **2012**, *10*, 1075–1080. [CrossRef]
- 52. Yihunie, T.A.; Gesesse, C.A. GGE Biplot analysis of genotype by environment interaction in field pea (*Pisum sativum* L.) genotypes in North Western Ethiopia. *J. Crop Sci. Biotechnol.* **2018**, *21*, 67–74. [CrossRef]
- 53. Uzun, A.; Bilgili, U.; Sincik, M.; Acikgoz, E. Yield and quality performances of forage type pea strains contrasting leaf types. *Eur. J. Agric.* **2005**, *22*, 85–94. [CrossRef]
- 54. Greveniotis, V.A.; Giourieva, V.S.; Bouloumpasi, E.C.; Sioki, E.J.; Mitlianga, P.G. Morpho-physiological Characteristics and Molecular Markers of Maize Crosses Under Multi-location Evaluation. *J. Agric. Sci.* **2018**, *10*, 79–90. [CrossRef]
- 55. Greveniotis, V.; Bouloumpasi, E.; Tsakiris, I.; Sioki, E.; Ipsilandis, C. Evaluation of Elite Open-Pollinated Maize Lines in Two Contrasting Environments. J. Agric. Sci. 2018, 10, 85–101. [CrossRef]
- Ganopoulos, I.; Moysiadis, T.; Xanthopoulou, A.; Ganopoulou, M.; Avramidou, E.; Aravanopoulos, F.A.; Kazantzis, K. Diversity of morpho-physiological traits in worldwide sweet cherry cultivars of GeneBank collection using multivariate analysis. *Sci. Hortic.* 2015, 197, 381–391. [CrossRef]
- 57. AOAC. Official Methods of Analysis, 18th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA, 2005.
- 58. Fasoula, V.A. A novel equation paves the way for an everlasting revolution with cultivars characterized by high and stable crop yield and quality. In Proceedings of the 11th National Hellenic Conference in Genetics and Plant Breeding, Orestiada, Greece, 31 October–2 November 2006; Hellenic Scientific Society for Genetics and Plant Breeding: Orestiada, Greece, 2006; pp. 7–14.
- 59. Steel, R.G.D.; Torrie, H.; Dickey, D.A. Principles and Procedures of Statistics. A Biometrical Approach, 3rd ed.; McGraw-Hill: New York, NY, USA, 1997; p. 666.
- 60. McIntosh, M.S. Analysis of Combined Experiments. Agron. J. 1983, 75, 153–155. [CrossRef]
- 61. Hanson, G.; Robinson, H.F.; Comstock, R.E. Biometrical studies on yield in segregating population of Korean Lespedeza. *Agron. J.* **1956**, *48*, 268–274. [CrossRef]
- 62. Singh, R.K.; Chaudhary, B.D. *Biometrical Methods in Quantitative Genetic Analysis*; Kalyani Publishers: New Delhi, India, 1977; p. 304.