



Article A Stability Analysis Using AMMI and GGE Biplot Approach on Forage Yield Assessment of Common Vetch in Both Conventional and Low-Input Cultivation Systems

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Abstract: Common vetch (Vicia sativa L.) is a widespread legume crop in the Mediterranean megaenvironment, due to its versatile uses and its compatibility with organic and low-input farming systems. However, its adaptation to various such environments should be studied and varieties suitable for forage yield must be selected. This study aimed to explore forage yield stability of common vetch varieties based on the stability index, with a specific target to explore common vetch variety behavior in various environments. Six Greek varieties of common vetch were used over four environments for two years. The cultivation was conducted using a strip plot with the varieties randomized within each plot in both conventional and low-input cultivation systems. (Alexandros) and (Tempi) varieties showed stability for days to 50% of flowering (index >4000), while (Pigasos) and (Zefyros) for fresh forage yield (>200) across environments. Combined estimations, also showed stability of (Pigasos) and (Zefyros) for fresh forage yield. Comparisons between the conventional and low-input farming systems generally showed minor differences but revealed varieties that exhibit stable performance even in the low-input farming systems, where stability is generally a little higher. The AMMI and GGE biplot analysis depicted the stability performance of the varieties regarding the traits under experimentation. As far as the fresh forage and dry matter yield, (Zefyros) was the most stable and productive variety over all others. Correlations between traits displayed the positive relation of fresh forage yield with days for 50% flowering and dry forage yield. Positive correlations may be proved useful for indirect breeding through traits with high stability leading to the selection of traits that show low stability.

Keywords: low-input; stability; indirect breeding; biplot analysis

1. Introduction

Common vetch (*Vicia sativa* ssp. *sativa* L.) is one of the most important livestock legumes in the Mediterranean mega-environment due to its multiple uses, its high nutritional value, and its ability to grow in different environmental conditions [1]. Turkey holds the first place regarding common vetch grain production among Mediterranean countries, followed by Spain, Italy, and Greece, while Greece is also fourth with regard to the harvested area in the Mediterranean basin [2]. According to official data, annual



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Copyright: © 2021 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/). production in vetches was approximately 57,060 tons in 2017, while the cultivated area was 31,977 ha [3].

Increased nutritional needs for livestock require the introduction of animal feed legumes in crop rotations [4]. Common vetch is considered among the best options to be part of crop rotations, especially in lower rainfall areas, and a good alternative to cereal monoculture, as it produces higher seed and protein yields, in comparison [5]. Another advantage of vetch cultivations is their compatibility with organic and low-input farming systems. Its usefulness is based on the exploitation of atmospheric nitrogen yielding satisfactory in certain cultivation areas [6]. Vetch-cereal intercrops produce considerably higher protein yields on the soil without any need for N-fertilizers. It is a usual approach to cultivate local varieties or mixtures among them, to maintain yield under low-input farming systems that support mainly livestock [7].

Climate change is a challenge to ensuring food and feed for the increasing population in the 21st century. Various climatic environments require varieties with good and stable performance to ensure production [8]. On the other hand, the adoption of organic farming principles under low-input conditions requires also the use of proper genetic materials to ensure stability [7,9]. Stability is a difficult target for both breeders and farmers that have to cope with the specific adaptability of varieties [10,11]. Ecological processes are combined with biodiversity, low inputs, and adapted cultivation cycles in local conditions. Appropriate varieties for forage yield must be selected based on adaptation in various, and especially low-input, environments and, therefore, extensive experimentation is needed both by breeders and agronomists. In faba beans, biomass yield, seed yield, and yield components were studied together with plant height, earliness, and water use efficiency [12]. Both genotype, environment, and also their interaction affected the studied characteristics. The environment was the major source of variation, while genotype displayed a much smaller effect. GGE-biplot analysis for high yield and stability across different environments revealed three genotypic types: (a) well adapted either for biomass, (b) seed yield, and (c) with high adaptation ability for both traits. Fasahat et al. [13], summarized the concept Genotype x Environment interactions, for breeding purposes in various crops, to facilitate the choice of breeders to select the appropriate method.

A recent work summarized stability parameters, especially for long-term field experiments [14]. The authors suggest that relative surveys and research works must include:

- (i) data quality and methodological approaches in the analysis of yield stability that are as transparent as possible,
- (ii) testing for and deal with outliers,
- (iii) investigation on potentially confounding factors in the statistical model,
- (iv) exploratory pathways on the need for detrending of yield data,
- (v) account for temporal autocorrelation,
- (vi) the choice for the stability measures and consider the correlation between some of the measures,
- (vii) consideration on the dependence of stability measures on the mean yield,
- (viii) temporal trends of stability, and
- (ix) reports on standard errors and statistical inference of stability measures where possible.

AMMI model is a hybrid model that combines analysis of variance (ANOVA) and principal component analysis (PCA) [15,16]. GGE biplot clearly visualizes interactions and cross-impact between genotypes, environments, and total interaction [17].

The main aim of the present work was to explore forage yield stability of common vetch varieties based on stability index, with a specific target to investigate common vetch variety behavior in various environments. Our novel approach is based on the principles described by Fasoulas [18], regarding stability parameters of genotypes and also on the stability index as described and applied by Fasoula [19]. Greveniotis et al., [20], used successfully a trait stability index to describe the type of trait inheritance in cotton and to depict the varieties better adapted in certain environments.

2. Materials and Methods

2.1. Crop Establishment and Experimental Procedures

Field experiments were conducted during two growing seasons (2010–2011 and 2011–2012) in four different locations. Two locations in Northern Greece and two locations in Central Greece were selected, varying in soil type and altitude. Coordinates according to the WGS 1984 geographic coordinate system are provided.

- (A) InGiannitsa, Northern Greece (latitude, 40°77′ N;longitude, 22°39′ E;elevation,10 m a.s.l.). The soil type was clay (C): sand, 9.1%; silt, 37.5%; clay, 53.8%. The chemical properties of the soil were as follows: conventional: N-NO₃, 15.1 mg kg⁻¹; P-Olsen, 17.4 mg kg⁻¹; K, 274 mg kg⁻¹; pH_{H20}, 7.69; organic matter, 3.26%; and CaCO₃, 5.23(%).Low-input system: N-NO₃, 16.1 mg kg⁻¹; P-Olsen, 15.9 mg kg⁻¹; K, 261 mg kg⁻¹; pH_{H20}, 7.65; organic matter, 3.51%, and CaCO₃, 5.37(%).
- (B) In the farm of the Technological Educational Institute of Western Macedonia in Florina, northern Greece (latitude, 40°46' N;longitude, 21°22' E;elevation, 705 m a.s.l.). The soil type was characterized as a sandy loam (SL): sand, 62%; silt, 26.9%; clay, 11.1%. The chemical properties of the soil were as follows: conventional: N-NO₃, 16.1 mg kg⁻¹; P-Olsen, 26.4 mg kg⁻¹; K, 236 mg kg⁻¹; pH_{H20}, 6.32; organic matter, 1.29%; and CaCO₃, 1.7(%). Low-input system: N-NO₃, 17.4 mg kg⁻¹; P-Olsen, 25.1 mg kg⁻¹; K, 224 mg kg⁻¹; pH_{H20}, 6.29; organic matter, 1.32%, and CaCO₃, 1.9(%).
- (C) In Trikala, Central, Greece (latitude, 39°55' N; longitude, 21°64' E; elevation,120 m a.s.l.). The soil type was characterized as sandy clay loam (SCL): sand, 48.6%; silt, 19.2%; clay, 32.2%. The chemical properties of the soil were as follows: conventional: N-NO₃, 12.7 mg kg⁻¹; P-Olsen, 11.8 mg kg⁻¹; K, 168 mg kg⁻¹; pH_{H20}, 8.15; organic matter, 2.21%; and CaCO₃, 7.54(%). Low-input system: N-NO₃, 13.6 mg kg⁻¹; P-Olsen, 11.5 mg kg⁻¹; K, 176 mg kg⁻¹; pH_{H20}, 8.11; organic matter, 2.39%, and CaCO₃, 7.63(%).
- (D) In Kalambaka, Central Greece (latitude, 39°64' N;longitude, 21°65' E;elevation, 190 m a.s.l.). The soil type was silty clay (SiC): sand, 14.6%;silt, 41.2%;clay, 44.2%. The chemical properties of the soil were as follows: conventional: N-NO₃, 11.39 mg kg⁻¹; P-Olsen, 7.62 mg kg⁻¹; K, 96.3 mg kg⁻¹; pH_{H20}, 8.05; organic matter, 2.14%; and CaCO₃, 3.58(%). Low-input system: N-NO₃, 12.01 mg kg⁻¹; P-Olsen, 7.56 mg kg⁻¹; K, 98.7 mg kg⁻¹; pH_{H20}, 8.08; organic matter, 2.21%, and CaCO₃, 3.65(%).

Those locations were selected deliberately because of their varied environmental conditions. Basic weather data (mean monthly temperatures in °C and rainfall in mm) for each experimental site based on daily records, for the two growing seasons of the experimentation, are given in Figure 1.

Common vetch (*Vicia sativa* L.) varieties well established in Greece were selected for cultivation using a strip-plot design with the six varieties (namely cv. (*Alexandros*), cv. (*Filippos*), cv. (*Omiros*), cv. (*Pigasos*), cv. (*Tempi*), and cv. (*Zefyros*). The characteristics of the selected varieties are given next [21].

(*Alexandros*) was developed in Greece. It is very stable and well adapted to Greek environments. This variety is characterized by high hay yield, has early flowering, and is suited for green manure. It develops rich fresh forage, although its development in early stages is slow. It yields hay 4000–6500 kg ha⁻¹ and grain 1500–2200 kg ha⁻¹.

(*Filippos*) was bred in Greece, it is characterized by high hay yield, with high protein content, also could be used for silage. It is sown mainly in autumn.

(*Omiros*) was developed in Greece and is well adapted to Greek environments. It is very productive. The advantage (*Omiros*) has over other varieties is its winter growth and vigor combined with good frost tolerance (can withstand the cold up to -10 °C). It is suitable as green manure. It provides rich hay with a high content of pods and a high yield in seed. The protein content of the seed ranges from 22% to 27% and provides a good source of energy and phosphorus for livestock fodder.

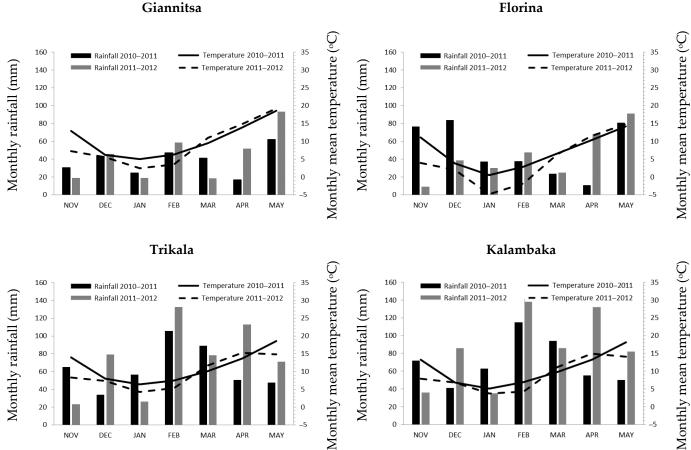


Figure 1. Basic weather data (mean monthly temperature in °C and rainfall in mm) based on daily records, through two growing seasons.

(Pigasos) was developed in Greece for hay and grain production. It is well adapted to all environments, and well resistant to drought. It is characterized by the good early establishment, is categorized in early to mid-early varieties, and develops rich chloromass. It produces hay 4000–6000 kg ha⁻¹ and grain 1500–2000 kg ha⁻¹.

(Tempi) was developed in Greece for hay and grain production. It is well adapted to Greek environments and is adequately drought tolerant. It develops rich chloromass and is proposed for use as green manure, as well as mixed-planted with oats. It yields hay $4000-6000 \text{ kg ha}^{-1}$ and grain $1500-2000 \text{ kg ha}^{-1}$.

(Zefyros) was developed in Greece for hay production. It is well adapted to Greek environments and is adequately drought tolerant. It is categorized into mid-early varieties. It yields hay 4000–6500 (kg ha⁻¹ and grain 1500–2200 kg ha⁻¹.

The selected varieties were installed randomized within each plot. Seven rows five meters long consisted of each plot, the distances between rows were 0.25m, with a total plot size amounting to 8.75 m^2 .

Low-input and conventional farming systems were applied. The plots cultivated under the conventional farming system were fertilized before sowing so that 30 and 50 kg ha⁻¹, Nitrogen and P₂O₅, respectively were added into the soil.

For low-input cultivation, no fertilizers or other agrochemicals were applied during the experiment in all four different locations, while prior to the establishment of the experiment in 2010, the fields had been in a two-year rotation consisting of bread wheat/legume without nutritional supplementation or other agrochemical inputs.

Weeds were controlled by hand in the experimental area. The selected varieties were sown in early November during November 2010 and November 2011 for growing seasons

2010–2011 and 2011–2012, respectively, and were harvested in early May 2011 and May 2012 for growing seasons 2010–2011 and 2011–2012, respectively, upon reaching full flowering.

2.2. Measurements

Traits measured were:

- 1. Days to 50% flowering: This corresponds to the number of days passing from the sowing date to 50% of the flowering time; it was recorded for each plot [22].
- 2. Main stem length: Corresponds to the mean height of ten random plants of each plot, measured from ground level to the top point with a ruler (1 mm sensitivity) after extending the plants upward at flowering time. The arithmetic mean of the measurements was accepted as the main stem length for each plot.
- 3. The number of main stems per plant: This corresponds to the number of first stems accounted for from the bottom part of the plants in flowering time. The number occurred as an arithmetic mean of values obtained from ten plants of each plot, situated in different parts of the plot
- 4. Fresh forage yield (kg ha⁻¹): Corresponds to the weight of chloromass (fresh forage) obtained from each plot right after harvesting in full flowering time and subsequent calculation on a hectare basis.
- 5. Dry matter yield (kg ha⁻¹): Corresponds to the dry matter percent per plot calculated after fresh forage samples (0.5 Kg), harvested from each experimental plot were placed at 70 °C for 48 h in a drying oven, left to cool, and weighed. Then, dry matter yield was determined for each plot, and the value was converted to a hectare basis.
- 6. Forage dry matter crude protein content (%): Forage dry matter was passed through a 1 mm sieve and subsequently mixed for the analysis. Total N was determined using AOAC Official Method 988.05 [23] and then the total protein content was estimated.
- 7. Ash content (%) of dry matter: Ash was analyzed using AOAC Official Method 942.05 [23].

2.3. Data Analysis

Data primarily analyzed via ANOVA over environments and cultivation practice to experience if there are significant differences for all traits investigated in this study. For the ANOVA table to be more informative the combination of each year and location was assigned as the environment. In this way, we have fewer interactions in the ANOVA table and do not affect the variance of Genotypes (varieties) and the $G \times E$ (Genotype × Environment) interaction which is crucial for proceeding in the stability analysis.

Stability index calculation $(\bar{x}/s)^2$, where \bar{x} and s are the entry mean yield (etc.) and standard deviation, respectively, was employed for stability estimations [10,19]. Pearson coefficient according to Steel et al. [24] was applied for trait correlations, and statistical significance of all data was checked at p < 0.05 with SPSS ver. 25 statistical software. As a precondition, we took into account the suggestions of Reckling et al. [14]. For the computation of AMMI and (GGE) biplot analysis for interactions used the free version of PB Tools v1.4 (International Rice Research Institute, Laguna, Philippines).

The AMMI model is a widely used statistical tool in the analysis of multi-environment experiments. The purpose is to understand the complex GEI. In the AMMI model the data are represented by a two-way table of GEI means. In the complete tables, least squares estimation is equivalent to fitting an additive two-way ANOVA model for the main effects and applying a singular value decomposition to the interaction residuals [25].

Using this statistical tool AMMI software generates mainly 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 then the factor analyzed is stable. Regarding the AMMI1 biplot, the desirable varieties were those having 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 genotype main effect (G) plus genotype by environment interaction (GE), which is the only source of variation that is relevant to cultivar evaluation. Mathe-

matically, GGE is the genotype by environment data matrix after the environment means

are subtracted. A GGE biplot is a biplot that displays the GGE of a genotype by environment twoway data. The GGE biplot methodology originates from the graphical analysis of multienvironment variety 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 ideal and average environment and in the concentric area of the ideal environment dot. As far as the GGE biplot for varieties, the desirable varieties (stable and productive) were those which placed near to the ideal variety and in the concentric area of the ideal variety dot.

3. Results

Regarding the ANOVA table, the main effects for all traits expressed significant differences. Furthermore, the G × E (varieties × environment) interaction, showing significant differences for all traits (Table 1). To clarify the performance of the varieties along environments and estimate the stability of each variety for all traits, Fasoula [19] method along with AMMI and GGE analysis were performed. For almost all traits studied, the interaction between cultivation system, variety, and environments was very significant (except for dry matter). This finding means that we have to suggest certain varieties for certain environments and cultivation systems. GxE interaction (Table 1 (m.s. 533.910^{**} for fresh forage yield)) and Cultivation × environment interaction were very intense for all traits. Especially for fresh forage yield Cultivation x variety mean square was found also very significant (Table 1 (m.s.: 115.869^{**})).

Stability estimations based on the stability index are presented in Tables 2–4. In Table 2, calculations across environments are included for all traits measured. Fresh forage yield stability exhibited values over 100 and especially in two environments over 200. Low values (<100) were found for forage dry matter crude protein content (%) and ash content (%) of dry matter and in some cases for a number of stems per plant. Days to 50% of flowering showed extremely high values (>2000). The two farming systems presented slight differences, not significantly affecting stability estimations, but in some cases, stability indices were higher in low-input experiments.

Source of Variation	Days to 50% Flowering	Main Stem Length	Number of Stems per Plant	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)	114.6 **	76.67 **	0.283 **	3353.77 **	306.9 **	7.523 **	0.438 **
REPS/Environments	63.05 **	124.5 **	0.972 **	1290.47 **	36.67 **	51.79 **	8.001 **
Varieties (G)	152.2 **	8.066 **	1.357 **	1427.05 **	39.46 **	7.714 **	0.391 **
Environments \times Varieties (G \times E)	7.744 **	11.11 **	0.239 **	533.910 **	43.20 **	3.590 **	0.201 **
Cultivations	1.446 *	18.24 **	0.090 *	5618.32 **	648.5 **	10.07 **	0.120ns
Cultivation \times Environments	28.66 **	65.49 **	0.173 **	47.1549 **	36.89 **	2.190 **	0.187 *
Cultivation× Varieties	3.256 **	4.137 **	0.202 **	115.869 **	3.491ns	1.313 **	0.395 **
Cultivation \times Varieties \times Environments	2.740 **	6.980 **	0.102 **	51.4204 **	7.492ns	1.232 **	0.482 **
Error	0.470	0.619 **	0.009	2.16051	7.942	0.249	0.087

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, number of main stems per plant, fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%) and ash content (%) of dry matter.

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

Table 2. Trait stability index across environments for two farming systems: days to 50% flowering, main stem length, number of main stems per plant, 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	Number of Stems per Plant	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content (%)	Ash Content (%) of Dry Matter
	Giannitsa	3466	215	103	202	131	89	88
	Florina	5316	318	50	204	232	98	129
Conventional	Trikala	2274	263	117	173	146	94	83
	Kalambaka	3724	312	80	112	210	126	97
	Giannitsa	2922	319	108	243	126	104	109
L orus immute	Florina	3009	175	68	206	324	96	94
Low-inputs	Trikala	3062	360	117	192	251	109	99
	Kalambaka	4920	271	192	139	259	130	78
	Giannitsa	2990	260	102	203	123	96	98
Conventional&Low- inputs	Florina	3882	223	58	197	256	98	110
	Trikala	2629	258	118	166	139	102	91
	Kalambaka	3930	292	113	120	231	125	87

	Genotypes	Days to 50% Flowering	Main Stem Length	Number of Stems per Plant	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content (%)	Ash Content (%) of Dry Matter
	Filippos	3723	192	86	129	158	102	133
	Omiros	3944	254	150	122	95	122	122
	Alexandros	3738	265	130	189	163	95	89
Conventional	Tempi	4579	299	89	164	171	85	89
	Zefyros	3124	261	68	196	176	93	79
	Pigasos	3436	197	111	249	178	108	89
	Filippos	2559	372	106	166	224	119	117
	Omiros	3264	241	122	167	181	115	115
L out inputs	Alexandros	4586	198	130	163	201	112	93
Low-inputs	Tempi	3584	260	141	180	151	110	87
	Zefyros	2553	219	84	225	370	89	78
	Pigasos	3321	256	85	250	249	101	80
	Filippos	3081	251	94	142	175	111	124
	Omiros	3613	249	136	137	119	121	120
Conventional&Low-	Alexandros	4177	230	128	169	159	100	92
inputs	Tempi	4055	281	111	146	153	97	89
*	Zefyros	2813	242	73	207	217	92	78
	Pigasos	3423	226	96	226	200	106	86

Table 3. Trait stability index across genotypes for the two farming systems: days to 50% flowering, main stem length, number of main stems per plant, 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	Number of Stems per Plant	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content (%)	Ash Content (%) o Dry Matter
					Giannitsa			
	Filippos	4895	207	164	227	321	105	79
	Omiros	4954	238	176	332	85	121	188
	Alexandros	3138	194	147	211	125	81	99
Conventional	Tempi	3306	212	134	409	59	97	58
	Zefyros	5845	182	88	293	1063	71	76
	Pigasos	3838	197	87	194	210	109	62
	Filippos	2906	374	113	295	275	161	140
	Omiros	3155	270	144	220	85	220	106
Low-inputs	Alexandros	4933	287	279	276	113	72	72
Low-inputs	Tempi	2942	232	116	342	58	106	87
	Zefyros	2750	313	71	373	1097	65	130
	Pigasos	3155	337	158	271	207	71	117
Conventional&Low-	Filippos	3447	285	121	219	248	136	101
	Omiros	3499	269	138	254	90	167	125
	Alexandros	4042	232	154	234	115	72	89
inputs	Tempi	3108	235	133	324	62	108	74
	Zefyros	3654	247	64	332	772	69	100
	Pigasos	3387	239	107	212	208	92	86
					Florina			
	Filippos	19875	501	57	310	221	119	297
	Omiros	5615	429	150	355	245	101	148
Conventional	Alexandros	19639	287	292	286	294	74	121
Conventional	Tempi	5401	343	40	144	859	88	115
	Zefyros	5985	216	63	369	172	207	113
	Pigasos	4718	315	62	232	379	98	111
	Filippos	3011	416	78	313	510	85	121
	Omiros	4523	226	106	494	284	81	118
Low-inputs	Alexandros	3232	115	213	247	490	116	92
Low inputs	Tempi	2976	197	91	414	329	83	55
	Zefyros	3768	154	54	228	746	81	70
	Pigasos	3637	141	43	145	157	120	140
	Filippos	5604	480	70	330	314	104	181
	Omiros	5332	298	132	384	274	96	140
Conventional&Low-	Alexandros	5094	163	225	284	223	96	112
inputs	Tempi	3909	263	60	143	457	86	79
	Zefyros	4955	182	58	302	287	124	86
	Pigasos	4389	209	54	179	236	112	129

Table 4. Combined trait stability index across genotypes and environments, for the two farming systems: days to 50% flowering, main stem length, number of main stems per plant, 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	Number of Stems per Plant	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content (%)	Ash Content (%) of Dry Matter
					Trikala			
	Filippos	3946	239	314	237	146	142	133
	Omiros	5708	338	195	182	100	98	121
	Alexandros	5353	304	115	422	285	84	67
Conventional	Tempi	4620	347	98	308	617	72	71
	Zefyros	3835	334	79	171	375	72	60
	Pigasos	2978	157	238	249	68	105	73
	Filippos	4895	331	191	200	132	141	78
	Omiros	6848	455	188	273	218	104	103
Low-inputs	Alexandros	8344	447	105	460	338	102	155
Low-mputs	Tempi	9366	352	236	282	220	101	147
	Zefyros	5000	273	115	266	361	75	80
	Pigasos	4510	228	95	326	552	107	59
	Filippos	4653	220	238	206	123	151	97
	Omiros	6263	241	142	207	98	108	114
Conventional&Low-	Alexandros	6866	337	95	355	182	95	99
inputs	Tempi	6478	343	148	263	208	90	102
	Zefyros	4411	291	100	201	186	78	67
	Pigasos	3805	177	145	244	120	105	70
					Kalambaka			
	Filippos	6276	261	52	295	166	84	138
	Omiros	3970	240	189	53	95	148	73
Committee of	Alexandros	4455	403	99	100	212	163	73
Conventional	Tempi	5952	397	160	81	330	140	113
	Zefyros	2976	374	50	130	210	91	75
	Pigasos	5504	327	136	379	881	133	101
	Filippos	5294	318	160	298	262	107	119
	Omiros	5423	171	348	84	269	86	108
Low-inputs	Alexandros	5326	211	220	89	129	194	78
Low niputs	Tempi	6690	292	158	128	289	148	81
	Zefyros	2759	198	193	119	261	119	52
	Pigasos	6931	509	174	329	368	109	44
	Filippos	5799	277	84	297	215	97	137
	Omiros	4133	213	160	69	146	117	90
Conventional&Low- inputs	Alexandros	4318	297	117	96	172	182	81
	Tempi	5881	329	168	95	325	148	95
	Zefyros	3055	278	84	128	244	104	66
	Pigasos	4167	426	154	329	479	117	65

Table 4. Cont.

Table 3 depicts the differences between the six varieties. (*Alexandros*) and (*Tempi*) showed the highest values for days to 50% of flowering (>4000), while (*Pigasos*) and (*Zefyros*) for fresh forage yield (>200) across environments. Combined estimations, also displayed stability for fresh forage yield of (*Pigasos*) and (*Zefyros*). Comparisons between the conventional and low-input farming systems generally showed minor differences but revealed varieties that exhibit stable performance even in low-input farming systems where stability is generally a little higher. This is a very important finding for the introduction of productive varieties in low-input farming systems.

In Table 4 stability indices combine both environmental and genotypic behavior for all traits studied, for the two cultivation systems (conventional and low-inputs). Trikala and Florina showed some extreme stability index values for the number of days to 50% of flowering, indicating a perfect environment for estimating flowering and total development period, due to the contribution of the certain environment. According to Table 4, a combination of variety-environment may exhibit a favored performance for some traits, which exhibit considerable stability. For example, for a number of days to 50% of flowering, (*Zefyros*), (*Filippos*) and (*Omiros*) showed the highest values in conventional farming systems, while Alexandros in low-inputs, and for the environmental area of (*Giannitsa*). For (*Trikala*) and (*Florina*) favored the same trait in an extreme way, especially for (*Alexandros*). For dry matter, (*Zefyros*) was favored for stability in both cultivation systems in (*Giannitsa*).

The AMMI1 and GxE biplot analysis were used to visualize the data presented in Tables 2 and 4. The AMMI1 and $G \times E$ biplot analysis on the main factor (that we may call adaptability in environments), showed different grouping of varieties and, concerning fresh forage yields, (Zefyros) outyielded all other varieties (Figure 2). Genotype and environment distribution were used to group varieties which better perform in certain environments (Figure 3). In Figures 4 and 5, it can be clearly seen that (*Zefyros*) is in the optimal position. For forage dry matter productivity G6 (*Omiros*) was the most stable variety, followed by G5 (Zefyros). The AMMI1 and GGE biplots analysis gave the same results. Regarding the crude protein content, the desirable varieties were the G2 (Omiros) and G6 (Pigasos). As for the days to 50% flowering both AMMI1 and GGE biplots analysis resulted that the varieties (G1 (Filippos), G2 (Omiros), G3 (Alexandros), G4 (Tempi), G6 (Pigasos)) formed one group which was characterized by relative stability (Figure 6). The G5 (Zefyros) was placed on the opposite side of the vector alone and near the ideal genotype and the average environment. $G \times E$ biplot may explore both environment and genotype behavior concerning yield traits stability performance over environments and years. Regarding the ash content no variety placed near the ideal one (Figure 7).

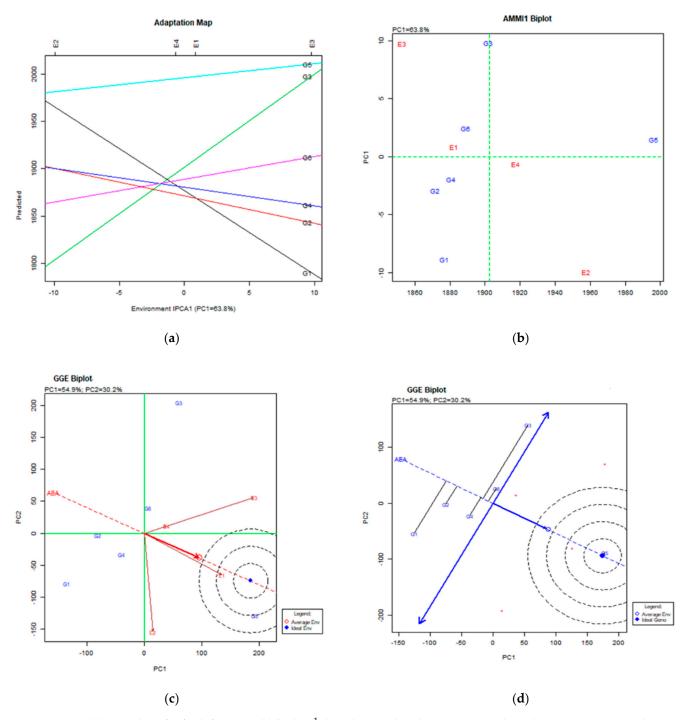


Figure 2. Stability analysis for fresh forage yield (kg ha⁻¹) based on (**a**) the adaptation map where the X-axis (PC1) visualizes the stability of varieties over environments and the Y-axis—the performance of varieties for the trait; (**b**) the AMMI1 biplot where the Y-axis is the one visualizing the trait performance and the X-axis (PC1) visualizes the stability of varieties over environments; (**c**) the GGE biplot for environments depicting the stability of the environments over years via the placement as near as possible to the ideal and average environment; (**d**) the GGE biplot for varieties depicting the stability of the varieties over environments where the productive varieties are those to the right on the AEA vector and the stable ones are those which are as close to the AEA axis as possible.

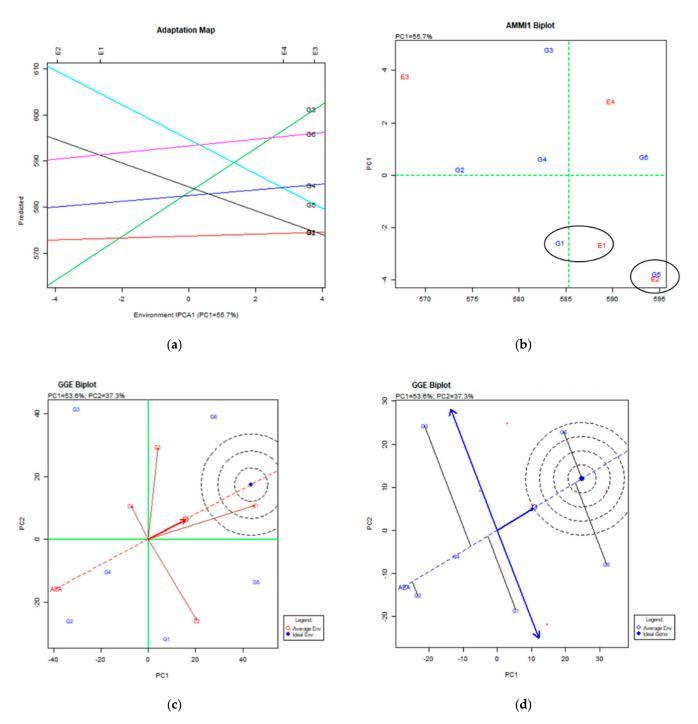


Figure 3. Stability analysis for forage dry matter yield (kg ha⁻¹) based on: (**a**) the adaptation map where the X-axis (PC1) visualizes the stability of varieties over environments and the Y-axis—the performance of varieties for the trait; (**b**) the AMMI1 biplot where the Y-axis is the one visualizing the trait performance and the X-axis (PC1) visualizes the stability of varieties over environments; (**c**) the GGE biplot for environments depicting the stability of the environments over years via the placement as near as possible to the ideal and average environment; (**d**) the GGE biplot for varieties depicting the stability of the varieties over environments where the productive varieties are those to the right on the AEA vector and the stable ones are those which are as close to the AEA axis as possible.

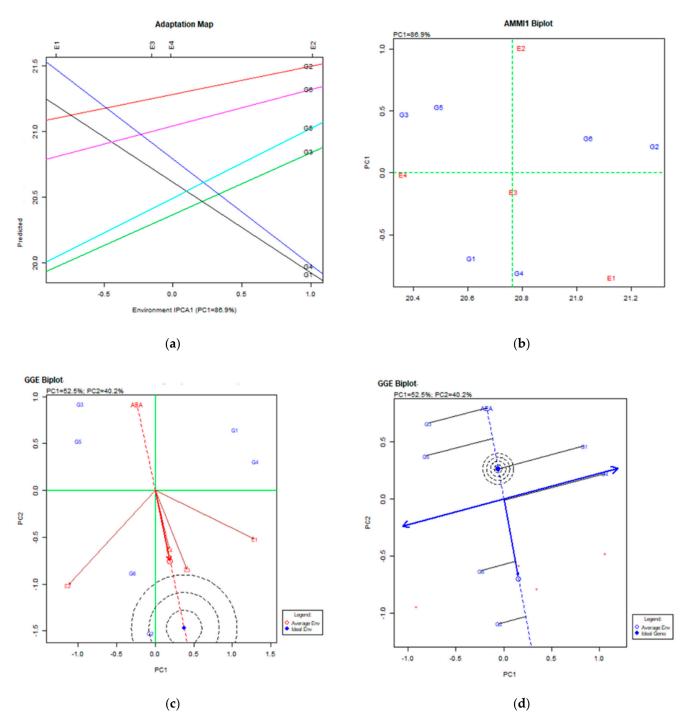


Figure 4. Stability analysis for crude protein content (%) based on: (**a**) the adaptation map where the X-axis (PC1) visualizes the stability of varieties over environments and the Y-axis—the performance of varieties for the trait; (**b**) the AMMI1 biplot where the Y-axis is the one visualizing the trait performance and the X-axis (PC1) visualizes the stability of varieties over environments; (**c**) the GGE biplot for environments depicting the stability of the environments over years via the placement as near as possible to the ideal and average environment; (**d**) the GGE biplot for varieties depicting the stability of the varieties over environments where the productive varieties are those to the right on the AEA vector and the stable ones are those which are as close to the AEA axis as possible.

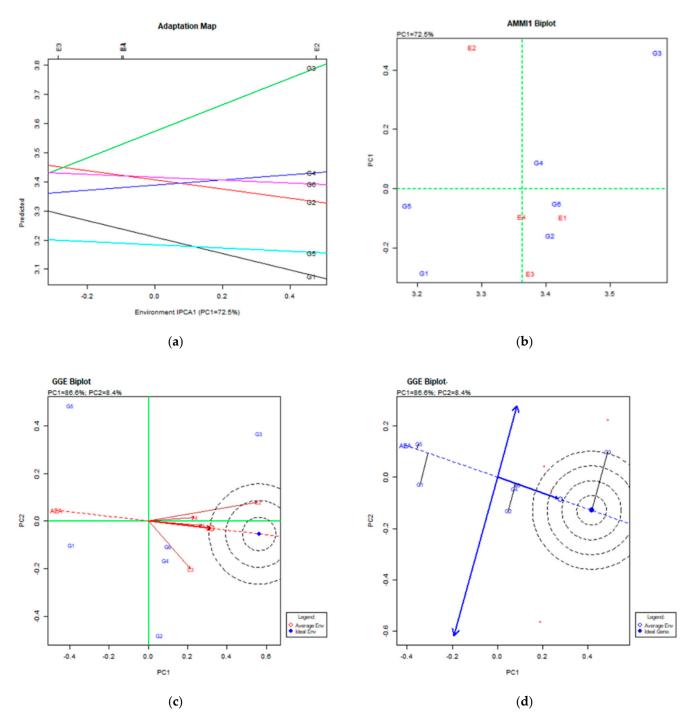


Figure 5. Stability analysis for the number of stems per plant based on: (**a**) the adaptation map where the X-axis (PC1) visualizes the stability of varieties over environments and the Y-axis—the performance of varieties for the trait; (**b**) the AMMI1 biplot where the Y-axis is the one visualizing the trait performance and the X-axis (PC1) visualizes the stability of varieties over environments; (**c**) the GGE biplot for environments depicting the stability of the environments over years via the placement as near as possible to the ideal and average environment; (**d**) the GGE biplot for varieties depicting the stability of the varieties over environments where the productive varieties are those to the right on the AEA vector and the stable ones are those which are as close to the AEA axis as possible.

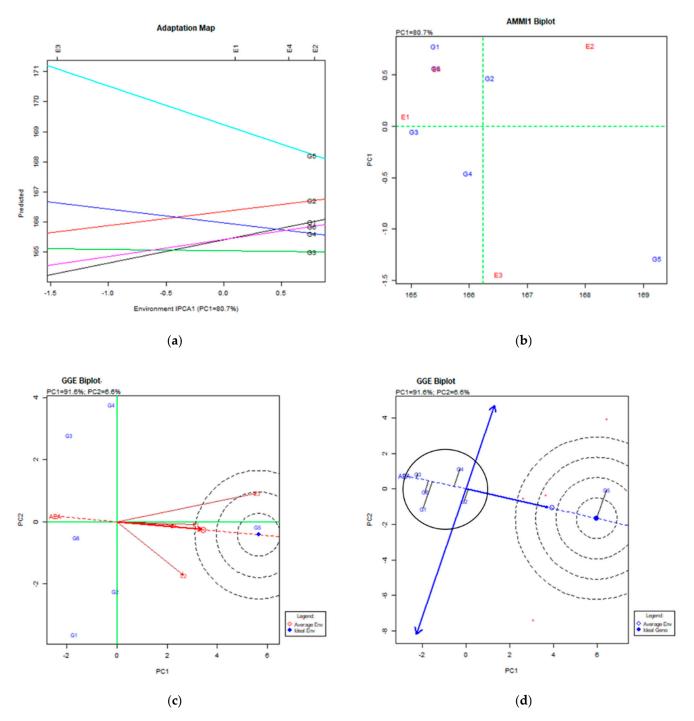


Figure 6. Stability analysis for days to 50% flowering based on: (**a**) the adaptation map where the X-axis (PC1) visualizes the stability of varieties over environments and the Y-axis—the performance of varieties for the trait; (**b**) the AMMI1 biplot where the Y-axis is the one visualizing the trait performance and the X-axis (PC1) visualizes the stability of varieties over environments; (**c**) the GGE biplot for environments depicting the stability of the environments over years via the placement as near as possible to the ideal and average environment; (**d**) the GGE biplot for varieties depicting the stability of the varieties over environments where the productive varieties are those to the right on the AEA vector and the stable ones are those which are as close to the AEA axis as possible.

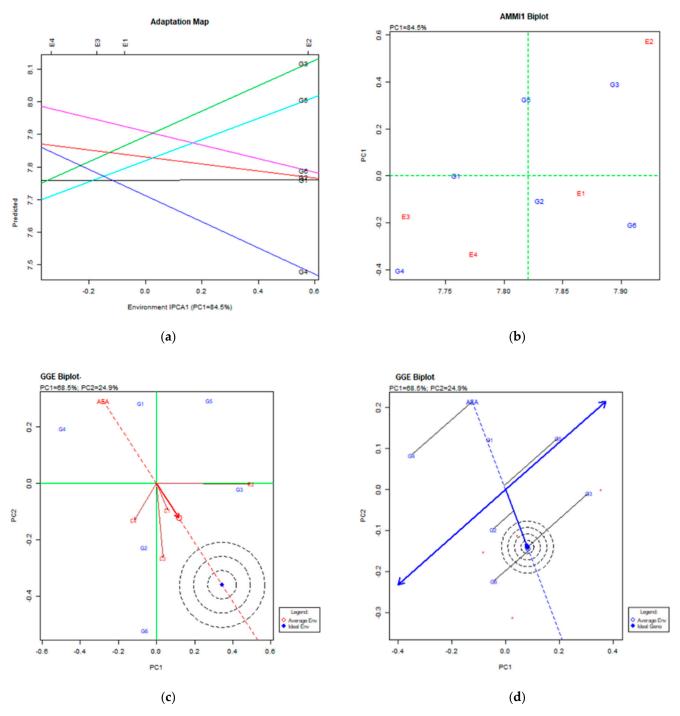


Figure 7. Stability analysis for ash content (%) based on: (**a**) the adaptation map where the X-axis (PC1) visualizes the stability of varieties over environments and the Y-axis—the performance of varieties for the trait; (**b**) the AMMI1 biplot where the Y-axis is the one visualizing the trait performance and the X-axis (PC1) visualizes the stability of varieties over environments; (**c**) the GGE biplot for environments depicting the stability of the environments over years via the placement as near as possible to the ideal and average environment; (**d**) the GGE biplot for varieties depicting the stability of the varieties over environments where the productive varieties are those to the right on the AEA vector and the stable ones are those which are as close to the AEA axis as possible.

For AMMI analysis as visualized by the adaptation map figure, the most desirable varieties 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 over the environments.

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

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

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

Correlations between Characteristics

Correlations between the traits (Table 5) displayed the positive relation of fresh forage yield with days for 50% flowering (r = 0.202) and dry forage yield (r = 0.501). A few more positive or negative correlations are also presented in Table 4.

Table 5. Correlations between all traits measured: days to 50% flowering, main stem length, a number of main stems per plant, fresh forage yield (kg ha⁻¹), dry matter yield (kg ha⁻¹), forage dry matter crude protein content (%), and ash content (%) of dry matter.

	Days to 50% Flowering	Main Stem Length	Number of Stems per Plant	Fresh Forage Yield (kg ha ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Forage Dry Matter Crude Protein Content (%)
Main stem length	0.012					
Number of stems per plant	0.083	0.038				
Fresh forage yield (kg ha^{-1})	0.202 **	0.119 *	-0.134 **			
Dry matter yield (kg ha^{-1})	0.085	0.070	0.016	0.501 **		
Forage dry matter crude protein content (%)	0.036	0.037	-0.024	0.062	0.041	
Ash Content (%) of Dry matter	-0.021	0.041	0.142 **	-0.064	0.004	0.006

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

4. Discussion

Breeders are developing varieties that must express high yield and stability of performance [7,11], especially for forage yield that is useful for livestock support [26].

In our work, the two cultivation systems (conventional and low-input) displayed differences in variety performance, but in total, the different cultivation systems did not affect the stability performance of the traits tested. In combination with GGE biplot analysis, the two farming systems revealed the most stable varieties across all environments, as well as the more stable in specific environments. Additionally, some varieties exhibited stability in the low-input farming system and that is a common practice in many cultivation areas to support the increasing livestock's nutrition needs. Generally, there were GGE interactions that may limit variety selection for cultivation. Some environment combinations may form mega-environments, in the sense of the ones referred to in other works [25].

Combined estimations, also depicted stability for fresh forage yield of (*Pigasos*) and (*Zefyros*). The availability of proper varieties is very important to stabilize forage yield performance in low-input organic farming systems [9,27].

Using GGE biplot analysis, Aydemir et al. [28] concluded that various yield components like biological yield, straw yield, forage yield, and natural plant height, resulted in highly significant variations in common vetch cultivated in three different ecological locations that may be useful to breeders. The AMMI and GGE biplots is a very useful tool for breeders for selection of the proper genotypes to be cultivated in certain environments.

Sayar [29] evaluated genotype \times environment interactions and stability of twenty common vetch (*Vicia sativa* L.) genotypes for fresh forage yield traits by using additive main effects and multiplicative interactions analysis (AMMI). Additive main effects and multiplicative interactions analysis (AMMI) showed that genotype \times environment interactions were found very high for fresh forage yield traits. Some genotypes exhibited high and stable forage yield in certain environmental conditions.

4.1. Fresh Forage Yield

The adaptation map and the AMMI1 analysis explained 63.8% of total variability for varieties' performance over all environments. Regarding the fresh forage yield and adaptation map Figure 2a the varieties tested, the G5 variety (Zefyros) was the most stable and productive over all varieties in all environments. The varieties G2 (Omiros), G4 (Tempi), and G6 (Pigasos) showed more or less an average productivity in all environments. The varieties G3 (Alexandros) and G1 (Filippos) showed environmental-specific adaptation whereas the G3 (Alexandros) favored by the E3 (Trikala) environment and the G1 (Filippos) from the E2 (Florina). The environments E3 (Trikala) and E2 (Florina) contributed to the variability for the fresh forage yield expressed by the varieties. Regarding the environments, the E1 (Giannitsa) and E4 (Kalambaka) seemed to be stable over the years of experimentation. The AMMI1 biplot analysis Figure 2b showed that the most stable and productive variety was the G5 (Pigasos). The varieties G3 (Alexandros) and G1 (Filippos) seemed to be less stable compared to the other varieties, since they were placed, far away from the center and to the positive and negative edges of the PC1 axis. The varieties G6 (Pigasos), G4 (Tempi), and G2 (Omiros) showed medium to low productivity but expressed stability. The GGE biplot for either environments Figure 2c and Varieties Figure 2d explained 85.1% (PC1: 54.9%, PC2: 30.2%) of the total variability. According to the GGE biplot for environments, there was one environment placed very close to the ideal one regarding the fresh forage productivity and it was the E1 (Giannitsa). Based on the GGE biplot analysis for the varieties tested, the G5 (Zefyros) variety was placed almost identical to the ideal variety. All other varieties appeared to be less stable, with lower productivity, as compared to the G5 (Zefyros) variety.

4.2. Forage Dry Matter Yield

Regarding the dry matter weight, the analysis via adaptation map and AMMI1 biplot explained 55.7% of the total variability. The adaptation map Figure 3a showed that the G6 (Pigasos) variety was the most stable and productive over all environments. The varieties G4 (Tempi) and G2 (Omiros) were stable but with less productivity. The varieties G3 (Alexandros), G5 (Zefyros), and G1 (Filippos) showed specific adaptation for high productivity. The G3 (Alexandros) was better adapted to the E3 (Trikala) environment whereas, in the E2 (Florina) environment, it was G5 (Zefyros) and G1 (Filippos). The AMMI1 biplot analysis Figure 3b indicated that the most stable and productive variety was the G6 (Pigasos) and equally productive but showing specific adaptation in environment E2 (Florina) was the G5 (Zefyros). The G1 (Filippos) variety showed medium productivity and selective adaptation to the E1 (Giannitsa) environment. The GGE biplot analysis for environments and varieties Figure 3c, Figure 3d explained a high amount of variability reaching 90.9% (PC1: 53.6%, PC2:37.3%). Based on the environments GGE biplot, the average environment depicted away from the ideal and the E2 (Florina) was placed near the ideal environment. The GGE biplot for the genotypes showed that the G6 (*Pigasos*) variety and G5 (*Zefyros*) tended to be near the ideal variety.

4.3. Crude Protein Content

Regarding the crude protein content, both adaptation map and AMMI1 biplot analysis explained 86.9% of the total variability. According to adaptation map Figure 4a, the most stable varieties for high protein production were the G2 (*Omiros*) and G6 (*Pigasos*), followed by the G5 (*Zefyros*) and G3 (*Alexandros*) which expressed lower protein content. The G4 (*Tempi*) and G1 (*Filippos*) showed specific adaptability and expressed very high protein content in E1 (Giannitsa) environment. AMMI1 biplot analysis Figure 4b showed that the G2 (*Omiros*) and G6 (*Pigasos*) varieties were the most productive and stable over all environments, followed by G3 (*Alexandros*) and G5 (*Zefyros*). The GGE biplot for environments appeared to be very diverse. The GGE biplot for varieties indicated that the most stable varieties were the G2 and G6, which were placed close to the average

environment dot. No variety was placed near the ideal one, which was an indication for high variability existence for this trait.

4.4. Number of Stems per Plant

The adaptation map and AMMI1 biplots analysis explained 72.5% of the total variability. The results from the adaptation map in Figure 5a showed that the G3 variety was the one with the higher number of stems per plant and quite stable. All other varieties regarding this trait showed higher stability over environments with a lower number of stems. The AMMI1 biplots analysis, Figure 5b, indicated that the G3 (*Alexandros*) variety was placed higher on the PC1 axis, expressed low stability for the trait across environments, but with a high number of stems. All other varieties expressed better stability along with an average number of stems per plant. Across the varieties, the G5 (*Zefyros*) and G1 (*Filippos*) had the lowest number of stems per plant. The GGE biplot analysis, as depicted by Figure 5c,d, explained 95% (PC1: 86.6%, PC2: 8.4%) of the total variability. In the area of the concentric circles around the ideal environment, only the E2 seemed to be placed near the ideal one Figure 4c. Regarding the varieties, the G3 (*Alexandros*) was placed near the ideal variety dot.

4.5. Days to 50% Flowering

For the days to 50% flowering, the figures of adaptation map and AMMI1 biplot analysis explained 80.7% of the total variability. The adaptation map showed that the G5 (*Zefyros*) variety needed more days for the 50% flowering stage and seemed to be less stable than all the others. All other varieties showed that need more or less the same number of days for the 50% flowering stage and expressed better stability from the G5 (*Zefyros*) variety. The same results occurred from the AMMI1 biplot analysis Figure 6b. The GGE biplot analysis for environments and varieties explained 98.2% (PC1: 91.6%, PC2:6.6%) of the total variability. Across the environments, the most stable were those placed near the dots of ideal environment and average environment and were the E3 (Trikala) and E1 (Giannitsa). Based on the GGE biplot for varieties (Figure 6d), the varieties G1 (*Filippos*), G2 (*Omiros*), G3 (*Alexandros*), G4 (*Tempi*), and G6 (*Pigasos*) formed one group which was characterized by relative stability the variety G5 (*Zefyros*) was placed on the opposite side of the varieties discriminates between early and late varieties, whereas G5 (*Zefyros*) was the late one.

4.6. Ash Content

The ash content as analyzed using the adaptation map and AMMI1 biplot figures explained 84.5% of total variability, Figure 7a,b. The varieties that showed specific adaptability for ash content where the G3 (*Alexandros*) and the G5 (*Zefyros*) varieties adapted better to E3 (Trikala) and E4 (Kalambaka) environments. The GGEbiplot analysis for environments and varieties explained 93.4% (PC1: 68.8%, PC2: 24.9%) of total variability and showed that no environment placed near the ideal one Figure 7c and no variety near the ideal variety Figure 7d. All varieties expressed relatively low stability between the environments.

4.7. Correlations between Characteristics

In our study, correlations between traits displayed the positive relation of fresh forage yield with days for 50% flowering and dry forage yield. Positive correlations may be proved useful for indirect breeding and selection of traits that show low stability through more stable ones. This is in accordance with Tiryaki et al. [30] findings and very useful to breeders. Correlations, in the same sense, were also reported for other traits in common vetch by Greveniotis et al. [31]. As Sayar [32] depicted, correlation coefficient analysis showed that yield component traits with the greatest effects on fresh forage yield were dry matter yield and main stem height, in common vetch varieties and correlations were very significant, although low correlation coefficients. This finding is in agreement with our

findings, with very significant but low correlation coefficients, indicating low linearity in our results. Thus it is expected that in-deed some traits may be correlated to fresh forage yield but in a non-linear way. Furthermore, indirect selection may improve fresh forage yield, but not at the expected level because of a lack of linearity. In other species like cotton, linearity was greater [20].

5. Conclusions

Main preconditions of all comparisons were followed and a novel approach of stability index was introduced in all measurements.

Correlations showed a significant relation between fresh forage yield and dry forage yield. Indirect yield improvement may be implemented for improving traits that generally showed high stability indices.

Comparisons between the conventional and low-input farming systems generally showed not to affect stability estimations but revealed varieties that exhibit stable performance in low-input farming systems. (*Pigasos*) and (*Zefyros*) displayed stability of performance. Low-input conditions favored forage yield performance, and that is an important agronomic result for farmers. A combination of variety-environment may exhibit a favored performance for some traits, which exhibit considerable stability. This is very useful for farmers, because they may predict with accuracy, i.e., the days to 50% of flowering, or the performance of dry matter yield, and adapt properly their cultural practice, in the specific environmental conditions. Also, a breeder may be interested in the same traits to predict pollination time and exploit indirect selection for these specific traits.

Taking into account that the interactions found significant, stability analysis was carried out via the AMMI1 and GGE biplots data processing tools. Results showed that based on the productivity expressed by the traits of fresh forage yield and forage dry matter, the most productive and stable varieties were the G5 (*Zefyros*), followed by the G6 (*Pigasos*).

In regard to the protein content, the desirable varieties were the G6 (*Pigasos*) and G2 (*Omiros*). Regarding the days to 50% flowering, the varieties were classified into two groups, the early and late, where the most productive was the late variety G5 (*Zefyros*). Based on the environments' analysis the E1 (Giannitsa) and E4 (Kalambaka) were the most stable over all traits and varieties, while the E2 (Florina) and E3 (Trikala) favored specific variety for each trait. GxE biplot analysis displayed that, for fresh forage yield, (*Zefyros*) outyielded all other varieties and was found to be in the optimal position.

If fresh forage yield stability is the main aim of both farmers and breeders, then we have to propose certain varieties for specific environments and cultivation systems. For the Trikala area, (*Alexandros*) is the best variety, for Kalambaka area (*Pigasos*), and (*Tempi*) and (*Zefyros*) for Giannitsa and Florina.

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