










Article

Analysis of Genotypic and Environmental Effects on Biomass Yield, Nutritional and Antinutritional Factors in Common Vetch

Zoi Parissi ¹, Maria Irakli ², Evangelia Tigka ³ , Panayiota Papastylianou ⁴ , Christos Dordas ⁵ , Eleni Tani ⁶ , Eleni M. Abraham ¹ , Agisilaos Theodoropoulos ⁶ , Anastasia Kargiotidou ³, Leonidas Kougiteas ⁶, Angeliki Kousta ⁴ , Avraam Koskosidis ³ , Stavroula Kostoula ⁷, Dimitrios Beslemes ³  and Dimitrios N. Vlachostergios ^{3,*}

¹ Laboratory of Range Science, Department of Forestry & Natural Environment, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; pz@for.auth.gr (Z.P.); eabraham@for.auth.gr (E.M.A.)

² Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization—DEMETER, 57001 Thermi, Greece; irakli@ipgrb.gr

³ Institute of Industrial and Forage Crops, Hellenic Agricultural Organization—DEMETER, 41335 Larissa, Greece; evitiga@yahoo.gr (E.T.); kargiotidou@elgo.gr (A.K.); makisk47@gmail.com (A.K.); dbeslemes@gmail.com (D.B.)

⁴ Laboratory of Agronomy, Department of Crop Science, Agricultural University of Athens, 11855 Athens, Greece; ppapastyl@aua.gr (P.P.); aggelikh.kousta@gmail.com (A.K.)

⁵ Laboratory of Agronomy, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; chdordas@agro.auth.gr

⁶ Laboratory of Plant Breeding & Biometry, Department of Crop Science, Agricultural University of Athens, 11855 Athens, Greece; etani@aua.gr (E.T.); agisilao@hotmail.com (A.T.); leonidaskgt@gmail.com (L.K.)

⁷ Laboratory of Genetics & Plant Breeding, Faculty of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece; staur_kostoula@yahoo.gr

* Correspondence: vlachostergios@elgo.gr



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Abstract: *Vicia sativa* L. (common vetch) is an annual legume species of high economic and ecological importance which is characterized by high nutritive value for animal feeding and its ability to adapt to various edaphic–climatic conditions. However, limited information is available about genotypic and environmental effects on agronomic, nutritional, and antinutritional traits of common vetch genotypes. Thus, in the present study, four advanced breeding lines and three commercial cultivars were evaluated for yield biomass, color assessment, fiber, crude protein (CP), and polyphenols in three locations (Spata, Larissa, and Thessaloniki) for two consecutive growing seasons (2018–2019 and 2019–2020). The effects of genotype, environment and their interaction (GXE) were significant for all the studied traits. The main source of variation for yield, color, CP, and polyphenols was the environment as it explained 71.5–89.7% of the total variation, whereas for the fibers content it was the GXE interaction. On the other hand, genotype had a much smaller effect on all the traits studied (2.9–16.6%). According to GGE biplot analysis, the ‘Alexandros’ cultivar was the most high-yielding and stable, whereas ‘Leonidas’ was the best performing in terms of nutritional and antinutritional traits. However, one advanced line combined high and stable yield biomass with high nutritive value, indicating the possibility for simultaneous improvement of both features.

Keywords: *Vicia sativa*; biomass yield; nutritional traits; phytochemical components; genotypes; locations

1. Introduction

Vicia sativa L. (common vetch) is a self-pollinating, annual legume species of high economic and ecological importance [1]. It is mainly used for animal feed as forage (grazed, hay, and silage production) [2,3] as well as grain legume [4] or intercropped with cereals [5–7]. Moreover, it is used as a cover crop and green manure [8]. Its ability to fix nitrogen as well as to restrict the incidence of crop diseases in the rotation systems [9] makes it an important crop for sustainable agriculture.

The origin of common vetch is the Middle East [10] but nowadays its cultivation has expanded to many areas of the world [11]. Its main advantage compared with other legumes is its ability to grow in various pedoclimatic conditions, even in harsh ones such as dry [12] and cold environments [13]. Furthermore, common vetch is suitable for both conventional and organic production systems [14]. In Greece, the crop occupies an area of 62,818 ha producing 112,000 tons annually [15]. Its cultivation area has consistently increased by 49% over the previous three years, mainly due to the implementation of nitrogen reduction programs and the adoption of common vetch cultivation under low-input farming systems [6,16].

The growing utilization of vetch cultivation in nonoptimal environments (low-input environments or marginal cropping zones) and the need to maintain stable productivity have led farmers to utilize improved cultivars that show performance stability under a wide range of environments. Moreover, to cope with emerging climate change issues and the deterioration of cropping areas, new breeding strategies for more resilient vetch cultivars are necessary [12]. Additionally, the exploration of the wealth of GenBank collections (i.e., the large-scale molecular characterization of a Spanish common vetch core collection) is revealing valuable genetic variation in trait-related adaptation to harsh environments and can be used in vetch breeding programs [11]. Thus, the breeding programs are focusing on the release of high-yielding cultivars that are well adapted to a variety of environments and with low yield fluctuation from year to year.

It is well documented that the performance of cultivars/genotypes is highly affected by environmental factors (temperature, precipitation), the variation of which depends on regions and years. The way that different genotypes respond to environmental variation is referred to as genotype \times environment (GXE) interaction [17] and it is a very important factor in the release of stable, well-adapted, and high-yield cultivars. Highly significant GXE interaction has been recorded for various agronomic traits of common vetch such as dry matter yield and yield components, seed yield, shattering rate, etc. [18–21].

On the other hand, limited information is available on the intra-specific variation of forage quality parameters or how the GXE interaction affects them. Generally, the main factors that affect the nutritive value of forage species are the morphology, anatomy, and biochemistry of the plants [22]. These factors depend on the genotype of the plant and on its response to the environment in which it grows, i.e., on GXE interaction. The nutritive value of common vetch is generally high, and particularly, its protein content is higher compared with other annual legumes [23]. According to Abbeddou et al. [2] the hay of common vetch is a valuable source of energy and protein for livestock in the dry areas of the Mediterranean basin. Despite its importance in livestock farming systems, there are few reports of differences in the nutritive value of green forage, hay, and straw among common vetch cultivars and genotypes [3,23,24].

Regarding the impact of GXE interaction on the forage quality of legumes, there is an assumption that the quality traits are far less sensitive to this interaction compared with agronomic traits such as herbage and seed yield [25]. In this respect, Kölliker et al. [26] demonstrated no significant GXE interactions for proanthocyanidins content of *Onobrychis viciifolia*. However, there is evidence of the significant impact of GXE interaction on the forage quality of *Medicago sativa* [27]. Furthermore, the differentiation of quality traits between common vetch cultivars of early and late maturity is indicative that GXE interaction affects its quality traits [28].

Based on the foregoing, the current study's goal was to: (i) evaluate advanced breeding lines and commercial cultivars of *V. sativa* for biomass yield, nutritional and antinutritional features in three locations with distinctly different environmental conditions for two consecutive years, (ii) assess the GXE interaction for all the traits, (iii) detect the correlations between biomass, nutritional and antinutritional traits, (iv) identify superior genotypes for specific and wide adaptation.

2. Materials and Methods

2.1. Experimental Design and Plant Material

Field experiments were carried out during November–June of 2018/2019 and 2019/2020 in Southern Greece (Spata, latitude 37°58' N, longitude 23°54' E, elevation 123 m a.s.l.), Central Greece (Larissa, latitude 39°30' N, longitude 22°42' E, elevation 77 m a.s.l.) and Northern Greece (Thessaloniki, latitude 40°32' N, longitude 22°59' E, elevation 5 m a.s.l.). The three areas are described in detail in Papastylianou et al. [29]. Meteorological data and soil parameters were recorded for each location (Tables 1 and 2). Weather data (rainfall, average, minimum, and maximum air temperatures) were collected on a daily basis from meteorological stations within 1 km of the experimental areas and given as mean monthly data for the study period. The automatic weather station consisted of a data acquisition system and a set of sensors for the measurement of the above-mentioned variables. The soil characteristics were determined according to Sparks et al. [30]. A randomized complete block design was employed, with seven common vetch genotypes replicated three times. Four advanced lines obtained by the company AGROLAND SA (namely G1: BK45; G2: BK29; G3: BK23; G4: BK27) and three commercial cultivars (namely G5: Evinos; G6: Leonidas and G7: Alexandros) developed by Industrial and Forage Crops (IIFC) were used for the experiments. A seeding rate of 170 kg ha⁻¹ was used in a plot of 5 × 8.75 m in size, with 5 rows separated by 0.25 m. Balanced basal fertilization with 160 kg ha⁻¹ (0N-46P₂O₅-0K₂O) was incorporated before sowing. No pesticides were applied during crop establishment and growth, and emerging weeds were hand-removed as needed.

Table 1. Soil features of the fields in the three experimental locations.

Environment	Sand%	Clay%	Loam%	pH	CaCO ₃ (%)	EC (μS/cm)	Organic Matter (%)
AUA, Southern Greece Env1 + Env2	50	26	24	7.9	11.6	3.0	1.43
IIFC, Central Greece Env3 + Env4	20	57	23	8.0	1.5	4.9	1.30
AUTh, Northern Greece Env5 + Env6	25	27	48	7.7	11.3	1.1	1.24

AUA: Agricultural University of Athens, IIFC: Industrial and Forage Crops, AUTh: Aristotle University of Thessaloniki, EC: electrical conductivity.

2.2. Sampling and Measurements

2.2.1. Biomass Yield and Determination of Chemical Composition

The biomass yield (BY) was determined by sampling at the stage where over 50% of the plants were in full bloom and 20% of the pods had reached their final length. In order to eliminate any boundary influence, a sample of 0.25 m² was cut to ground level with manual shears from the middle row of each replicate plot. The plant material was weighed fresh, then oven-dried for 48 h at 60 °C before being weighed again to determine the dry weights. After that, the samples were ground through a 1 mm sieve in a Wiley mill, analyzed for N using the Kjeldahl method [31], and crude protein (CP) was calculated as N content × 6.25. Moreover, the neutral detergent fiber (NDF) and the acid detergent fiber (ADF) were determined using the ANKOM 220 analyzer (ANKOM Technology Corporation, Fairport, NY, USA). The NDF was estimated without the use of alpha amylase. The ADF analysis was performed after the NDF analysis. The ADF samples were incubated with 70% sulphuric acid for the determination of acid detergent lignin (ADL).

2.2.2. Color Assessment

The color of biomass was determined using a HunterLab colorimeter, model MiniScan XE Plus (Reston, VA, USA). The tristimulus values of CIE (L*, a*, b*) readings were calibrated against standard calibration black and white plates. Brightness L* measures the whiteness value and ranges from black at 0 to white at 100, whereas a* is measured for redness when positive and greenness when negative, and b* indicates yellowness when

positive and blueness when negative. An average of three measurements for L^* , a^* , and b^* values were recorded.

Table 2. Monthly means of rainfall (mm), maximum (Tmax), minimum (Tmin), and average (Taver) temperatures ($^{\circ}\text{C}$) for 2018–2019 (Year1) and 2019–2020 (Year2) growing seasons and the 30-year average at the three locations Spata, Larissa, and Thessaloniki where the experiments were conducted.

SPATA	Year1				Year2				30-Year Average Values			
	Tmax	Tmin	Taver	Rainfall	Tmax	Tmin	Taver	Rainfall	Tmax	Tmin	Taver	Rainfall
Nov	17.6	12.3	14.8	56.8	21.3	13.9	17.2	166.6	18.2	10.4	14.7	58.8
Dec	13.5	6.8	9.8	85.6	15.3	8.9	11.8	169.6	14.2	7.1	11.1	71.2
Jan	12.3	5.5	8.7	178	12.7	5.9	9.1	55.2	12.7	5.4	9.5	54.1
Feb	13.1	6.5	9.5	98.2	15.1	6.9	10.8	63.0	13.2	5.6	10.1	55.6
Mar	17.6	9.2	13.1	16.8	16.8	8.7	12.5	73.2	15.6	7.3	12.2	43.9
Apr	19.1	10.6	14.4	107	19.2	10.2	14.5	50.0	19.6	10.2	15.7	28.1
May	25.2	14.4	19.3	2.8	26.7	15.0	20.3	21.4	24.8	14.3	20.4	19.1
June	31.4	21.2	26.0	24.0	30.3	19.2	24.1	24.6	29.7	18.8	24.9	14.8
LARISSA												
Nov	16.7	9.7	12.8	66.8	20.5	11.6	15.3	70.8	15.9	5.8	11.8	52.4
Dec	11.2	2.8	6.5	27.6	12.5	6.4	9.0	124	11.0	1.9	7.0	50.0
Jan	8.7	1.1	4.6	68.8	16.6	1.1	6.1	1.2	9.8	0.7	5.7	32.7
Feb	14.1	4.1	8.6	19.2	16.3	4.4	9.84	17.8	12.1	1.3	7.9	39.1
Mar	19.9	6.9	13.0	19.2	18.8	6.5	12.1	57.0	14.9	3.3	10.7	40.9
Apr	21.4	10.3	15.2	18.0	21.4	9.1	14.8	48.6	19.8	6.3	15.0	26.4
May	27.5	14.7	20.4	35.4	29.1	15.2	21.3	32.0	25.8	11.0	20.3	39.6
June	33.8	20.7	26.8	21.2	31.9	19.2	25.0	19.8	31.1	15.3	25.4	29.1
THESSALONIKI												
Nov	16.6	10.6	13.1	26.8	19.3	13.4	16.0	43.6	15.4	6.9	12.3	48.0
Dec	10.4	4.1	7.1	29.6	12.3	7.6	9.8	54.4	10.9	3.0	7.5	44.8
Jan	7.9	2.4	4.9	54.8	10.9	3.7	7.0	2.4	9.3	1.4	5.9	33.7
Feb	12.3	4.6	8.0	4.0	13.8	5.8	9.3	13.2	11.0	2.3	7.9	39.6
Mar	17.1	7.9	11.9	10.0	15.3	7.2	10.8	107	14.3	4.6	10.7	47.5
Apr	18.9	10.8	14.5	73.6	18.0	9.0	13.1	81.8	19.2	7.6	14.8	31.8
May	24.6	15.0	19.3	24.2	24.3	15.1	19.1	35.0	24.5	12.3	20.0	38.4
June	31.6	21.3	25.8	22.8	28.6	19.3	23.5	15.6	29.3	16.5	24.7	34.0

2.2.3. Determination of Antinutritional Composition

Phenolic Extracts

The ground material (0.4 g) was extracted with 70% aqueous acetone (10 mL), vortexed for 30 s, and then sonicated for 15 min at room temperature. The supernatant was collected after centrifugation at $2200 \times g$ for 10 min, and the extraction was repeated twice. The combined phenolic extracts were used for the determination of total phenolic content (TP), total hydrolyzed tannins content (TN), and total condensed tannins content (CT).

Total Phenolics

The TP were determined by the Folin–Ciocalteu method [32] as described below: 200 μL diluted phenolic extract was reacted with 800 μL of the Folin–Ciocalteu reagent, followed by the addition of 2 mL of 7.5% Na_2CO_3 and 7 mL distilled water. The absorption reading was taken at 765 nm following a 60-min incubation period in the dark. Gallic acid equivalents per kilogram of dry weight (g GAE kg^{-1}) were used to calculate TP.

Hydrolyzed Tannins

The TN were determined according to Makkar et al. [33]. Aliquots of phenolic extracts (400 μL) were mixed with 400 mg polyvinylpyrrolidone (PVPP), followed by centrifugation at $10,000 \times g$ at 4°C for 20 min. The supernatants were transferred into test tubes for the determination of the non-tannin phenolics as above by the Folin–Ciocalteu method. Non-tannin phenolics were subtracted from total phenolics for the estimation of TN which were expressed as g of gallic acid equivalents per kg of dry weight (g GAE kg^{-1}).

Condensed Tannins

The CT were measured according to butanol–acid assay [34], as follows: aliquots of phenolic extract (500 μL) were mixed with 3 mL of the reagent n-butanol/HCl (95:5, *v/v*), followed by 100 μL 2% ferric ammonium sulfate in 2N HCl. The absorbance of boiled mixtures for 60 min was recorded at 550 nm after cooling. The absorbance of the unheated tubes was considered blank. CT was expressed as g of procyanidin B₂ equivalents per kg dry weight (g PCBE kg⁻¹).

Oxalate Content

Oxalic acid (OA) content was determined using the titration method described by Oyeyinka et al. [35]. The milled sample (0.5 g) was extracted by continuous mechanical stirring (1 h) with 75 mL of 3 M sulfuric acid. Then, 20 mL of the filtrated extract was heated at 70 °C and titrated with freshly prepared 0.05 N potassium permanganate solution until a pink color appeared and persisted for at least 10 s. Oxalate content was calculated by the equation: Oxalate (g 100 g⁻¹) = 0.825 x titer value and was expressed on a dry basis.

2.3. Statistical Analyses

Environments were defined as a combination of location and growing season as follows: Environment 1 (Env1): AUA2018/2019, Environment 2 (Env2): AUA2019/2020, Environment 3 (Env3): IIFC2018/2019, Environment 4 (Env4): IIFC2019/2020, Environment 5 (Env5): AUTH2018/2019, Environment 6 (Env6): AUTH/2019-2020. Two-way analysis of variance (ANOVA) using the mixed model was used to analyze the data, with genotypes as a fixed variable and environments as a random effect [36]. The means were compared by Tukey's test at $\alpha = 0.05$. To establish correlations between parameters, Pearson correlation coefficients (*r*) were calculated. The statistical SPSS18 software was used to conduct all the analyses according to the experimental design. In addition, GGE biplot software [37–39] was performed for the simultaneous assessment of yield and stability across environments using GenStat software Version 21.1.

3. Results

3.1. Weather Conditions

The climate of Greece is generally Mediterranean, mainly characterized by long, hot, dry, and relatively humid summers and cold winters. The testing environments were rather different in terms of temperature and precipitation. In general, average air temperatures did not vary considerably from the long-run averages during the growing periods (Table 2). However, it should be noticed that during the second growing season, the average temperatures were higher compared with the long-run averages at all sites. Higher temperatures were observed at Spata from sowing to biomass harvest in the first year (14 °C vs. 12.6 °C, respectively). On the contrary, at Larissa and Thessaloniki, lower temperatures occurred in the first year for the same period (10.5 °C vs. 12.1 °C and 10.1 °C vs. 10.7 °C, respectively). During the growing seasons (November to June), the long-term mean rainfall mainly concentrated from November to December was 346 mm, 310 mm, and 318 mm at Spata, Larissa, and Thessaloniki, respectively. Rainfall was higher in Larissa compared with Thessaloniki, with the lowest values in January and February at both sites, notably in the second growing season. Spata had higher rainfall in comparison with the long-term average (239 mm), particularly in the winter and mid-spring (436 mm in total, averaged across growing seasons). The rainfall from sowing to biomass harvest was lower during the first growing season compared with the second at about 33%, 3%, and 27% for Spata, Larissa, and Thessaloniki, respectively. Severe winter cold (minimum temperatures were 1.1 °C and 2.4 °C, respectively, in January) was recorded in Larissa and Thessaloniki, especially in the first growing season. Furthermore, in Spata and Larissa, maximum temperatures in May during the second growing season were 26.7 °C and 29.1 °C, respectively, higher than the 30-year average.

3.2. Influence of Genotype, Environment, and Their Interaction

Genotype (G), Environment (E), and their interaction (GXE) was found to be a significant source of variation for all the analyzed compounds ($p \leq 0.001$) as presented in Table 3. It was obvious that the greatest proportion of variation for biomass, colorimetric, and antinutritional traits was attributed to E (>71.5%), whereas G had the lowest contribution, accounting for 2.6 to 10.9%. Specifically, the effect of E accounted for the majority of variance in BY, L*, a*, and b* color values, at 71.5%, 89.7%, 71.9%, and 83.8%, respectively. Similarly, the largest proportion of variation in TN, CT, TP, and OA contents was attributed to E (72.6%, 75.3%, 82.5%, and 84.3%, respectively), whereas the G was assigned to the lowest proportion, accounting for 4.1%, 5.0%, 2.6%, and 3.4%, respectively.

Table 3. Mean squares (MS) and percentage of the sum of the squares (EV%) for biomass/colorimetric, nutritional, and antinutritional traits of *V. sativa* biomass produced by seven genotypes across six environments.

	Genotype (G)		Environment (E)		GXE	
	MS	EV%	MS	EV%	MS	EV%
DF	6		5		30	
	Agronomic/Colorimetric traits					
BY	23.4 ***	7.0	283.2 ***	71.5	14.2 ***	21.5
L*	13.2 ***	3.5	406.0 ***	89.7	5.1 ***	6.8
a*	4.9 ***	10.9	39.0 ***	71.9	1.5 ***	17.2
b*	3.3 ***	2.9	113.3 ***	83.8	3.0 ***	13.3
	Nutritional traits					
NDF	2691.2 ***	12.5	8486.0 ***	32.5	2405.1 ***	55.2
ADF	1206.5 ***	7.1	10,842.1 ***	52.8	1373.4 ***	40.1
ADL	280.6 ***	14.1	798.6 ***	33.5	207.9 ***	52.4
CP	9597.1 ***	16.6	52,382.6 ***	75.7	878.8 ***	7.6
	Antinutritional traits					
TP	2.8 ***	2.6	106.2 ***	82.5	3.2 ***	14.9
TN	1.7 ***	4.1	35.6 ***	72.6	1.9 ***	23.3
CT	0.03 ***	5.0	0.5 ***	75.3	2.2 ***	19.7
OA	121.8 ***	3.4	3661.7 ***	84.3	82.1 ***	12.3

DF, degree of freedom; BY, biomass yield ($t\ ha^{-1}$); NDF, neutral detergent fiber ($g\ kg^{-1}_{dm}$); ADF, acid detergent fiber ($g\ kg^{-1}_{dm}$); ADL, acid detergent lignin ($g\ kg^{-1}_{dm}$); CP, crude protein ($g\ kg^{-1}_{dm}$); TP, total phenolics ($g\ GAE\ kg^{-1}$); TN, total hydrolyzed tannins ($g\ GAE\ kg^{-1}$); CT, condensed tannins ($g\ PCBE\ kg^{-1}$); OA, oxalic acid ($g\ kg^{-1}$); *** significant at $p < 0.001$.

However, nutritional traits such as NDF, ADF, and ADL of the common vetch biomass were equally affected by E as well as by their interaction (GXE), ranging from 32.5 to 55.2%. GXE confirmed the strongest influences of 52.4% and 55.2%, respectively, on ADL and NDF in the common vetch biomass (Table 3). In contrast, E displayed the greatest contribution to the total variance for CP in vetch biomass (75.7%), followed by G (16.6%) and GXE (7.6%).

3.3. Variation between Genotypes

Significant differences were noted in all agronomic, nutritional, and antinutritional characteristics of common vetch biomass among seven genotypes averaged across growing environments (Table 4). The 'Alexandros' cultivar (G7) had the highest BY, whereas the advanced line G3 was the lowest one among all the studied genotypes. No significant differences in BY ($p > 0.05$) were observed among the other common vetch genotypes.

Biomass from the advanced lines G1 and G4 had the highest greenness (lower a* values), whereas the biomass from the 'Leonidas' cultivar (G6) had the highest yellowness (higher b* values) and the highest lightness (higher L* values) among the studied genotypes.

Table 4. Variation in biomass/colorimetric, nutritional and antinutritional traits of *V. sativa* biomass originated from seven genotypes averaged across environments.

		Genotypes							Tukey (0.05)
		G1	G2	G3	G4	G5	G6	G7	
Biomass/colorimetric traits	BY	12.2 ^b	13.2 ^{bc}	11.3 ^a	13.1 ^{abc}	12.7 ^{ab}	11.6 ^{ab}	14.6 ^c	3.568
	L*	47.6 ^a	47.9 ^b	48.5 ^c	47.5 ^a	47.9 ^b	50.0 ^d	48.6 ^c	0.095
	a*	2.6 ^a	3.4 ^c	4.0 ^e	2.7 ^a	3.8 ^d	3.4 ^c	3.2 ^b	0.130
	b*	21.9 ^b	21.6 ^a	22.3 ^c	22.2 ^c	22.0 ^b	22.9 ^e	22.6 ^d	0.320
Nutritional traits	NDF	346.0 ^b	353.4 ^{bc}	358.5 ^c	368.1 ^d	350.6 ^{bc}	356.1 ^c	328.7 ^a	76.332
	ADF	229.2 ^b	228.7 ^b	239.2 ^c	243.5 ^c	225.7 ^{ab}	221.3 ^a	223.8 ^{ab}	30.297
	ADL	59.0 ^c	54.4 ^b	58.5 ^c	60.2 ^c	57.0 ^{bc}	50.7 ^a	50.7 ^a	13.142
	CP	229.2 ^e	190.6 ^c	170.4 ^a	217.5 ^d	185.3 ^b	167.5 ^a	184.6 ^b	15.791
	TP	9.9 ^{bc}	9.7 ^b	10.4 ^d	10.5 ^d	9.4 ^a	9.7 ^b	10.1 ^c	0.630
Antinutritional traits	TN	4.8 ^{cd}	4.7 ^{bc}	5.1 ^{de}	5.2 ^e	4.5 ^{ab}	4.3 ^a	4.7 ^{bc}	0.490
	CT	0.39 ^e	0.34 ^b	0.38 ^{de}	0.27 ^a	0.37 ^{cde}	0.35 ^{bc}	0.37 ^{cde}	0.001
	OA	39.9 ^c	41.1 ^d	43.2 ^e	43.5 ^e	39.7 ^c	36.4 ^a	38.1 ^b	1.087

G1, BK45; G2, BK29; G3, BK23; G4, BK27; G5, 'Evinos'; G6, 'Leonidas'; G7, 'Alexandros'; BY, biomass yield (t ha^{-1}); NDF, neutral detergent fiber ($\text{g kg}^{-1} \text{ dm}$); ADF, acid detergent fiber ($\text{g kg}^{-1} \text{ dm}$); ADL, acid detergent lignin ($\text{g kg}^{-1} \text{ dm}$); CP, crude protein ($\text{g kg}^{-1} \text{ dm}$); TP, total phenolics (g GAE kg^{-1}); TN, total hydrolyzed tannins (g GAE kg^{-1}); CT, condensed tannins (g PCBE kg^{-1}); OA, oxalic acid (g kg^{-1}); values followed by different letters within a row indicate significant differences according to Tukey's test ($p \leq 0.05$).

The NDF contents ranged from 328.7 to 368.1 g kg^{-1} , the contents of ADF ranged from 221.3 to 243.5 g kg^{-1} , and the contents of ADL from 50.7 to 60.2 g kg^{-1} . The highest contents of NDF, ADF, and ADL were observed in G4, whereas the 'Alexandros' cultivar (G7) had the lowest values. The range of CP contents was 170.4–229.2 g kg^{-1} , with the highest CP levels observed by G1, whereas the lowest value was monitored in the 'Leonidas' cultivar (G6) along with G3.

The advanced lines G3 and G4 presented the highest TP concentration, whereas the 'Evinos' cultivar (G5) had the lowest TP concentration. Moderate TP concentrations were found for the other genotypes. The same trend as TP concentration appeared in the TN concentrations of common vetch biomass. It was evident that the TN concentration of vetch biomass was approximately 50% of the TP concentrations. Among the common vetch genotypes, G4 had the lowest CT content, whereas no significant differences ($p \geq 0.05$) were observed among G1, G3, 'Evinos' (G5), and 'Alexandros' (G7). Moderate CT contents found in biomass originated from the G2 and 'Leonidas' (G6). Regarding OA contents, the lowest value was recorded in 'Leonidas' (G6), followed by 'Alexandros' (G7), 'Evinos' (G5), and G1, whereas G3 and G4 had the highest OA contents.

3.4. Variation between Environments

There were significant differences in vetch biomass among the six growing environments for the biomass, colorimetric, nutritional, and antinutritional traits tested ($p \leq 0.05$) averaged across genotypes (Table 5). *V. sativa* biomass grown in Spata 2018/19 (E1) exhibited the highest BY (19.6 t ha^{-1}), followed by those grown in Thessaloniki 2019/20 (E6), Spata 2019/20 (E2), and Larissa 2019/20 (E4), whereas the lowest BY (9.2 t ha^{-1}) was recorded in Thessaloniki 2018/19 (E5). Significant environmental variation existed for colorimetric values (Table 5). Vetch biomass grown in Thessaloniki 2018/19 (E5) had the highest greenness (lowest a* value; 1.6), whereas those grown in Spata 2019/20 (E2) presented the lowest greenness (highest a*; 4.8). On the other hand, vetch biomass grown in Larissa 2019/20 (E4) showed the highest yellowness (highest b* value; 24.8), whereas those grown in Thessaloniki 2019/20 (E6) had the lowest b* value (18.7).

Table 5. Variation in biomass/colorimetric, nutritional and antinutritional traits of *V. sativa* biomass originated from six environments averaged across seven genotypes.

		Environments						Tukey (0.05)
		E1	E2	E3	E4	E5	E6	
Biomass/Colorimetric traits	BY	19.6 ^d	11.5 ^b	10.9 ^{ab}	11.2 ^b	9.2 ^a	13.8 ^c	3.568
	L*	50.7 ^e	43.1 ^a	48.0 ^c	54.9 ^f	49.2 ^d	43.8 ^b	0.095
	a*	4.1 ^d	4.8 ^e	4.5 ^d	2.1 ^b	1.6 ^a	2.7 ^c	0.130
	b*	24.1 ^e	20.3 ^b	22.4 ^c	24.8 ^f	23.1 ^d	18.7 ^a	0.320
Nutritional traits	NDF	360.6 ^d	343.3 ^{bc}	387.1 ^e	350.1 ^c	331.1 ^a	337.8 ^{ab}	76.332
	ADF	229.8 ^b	209.7 ^a	269.4 ^e	242.4 ^d	216.3 ^b	213.6 ^{ab}	30.297
	ADL	51.1 ^a	51.1 ^a	53.1 ^a	52.2 ^a	64.5 ^b	62.8 ^b	13.142
	CP	161.1 ^b	256.6 ^f	128.4 ^a	169.2 ^c	193.1 ^d	244.6 ^e	15.791
	TP	6.7 ^a	10.3 ^d	8.7 ^b	13.5 ^f	10.6 ^e	9.9 ^c	0.630
	TN	2.7 ^a	5.3 ^d	3.9 ^b	6.4 ^f	4.7 ^c	5.5 ^e	0.790
Antinutritional traits	CT	0.15 ^a	0.35 ^c	0.22 ^b	0.60 ^e	0.35 ^c	0.46 ^d	0.001
	OA	33.5 ^b	54.5 ^e	52.2 ^d	26.4 ^a	49.2 ^c	25.8 ^a	1.087

E1, Spata 2018/19; E2, Spata 2019/20; E3, Larissa 2018/19; E4, Larissa 2019/20; E5, Thessaloniki 2018/19; E6, Thessaloniki 2019/20; BY, biomass yield (t ha⁻¹); NDF, neutral detergent fiber (g kg⁻¹ dm); ADF, acid detergent fiber (g kg⁻¹ dm); ADL, acid detergent lignin (g kg⁻¹ dm); CP, crude protein (g kg⁻¹ dm); TP, total phenolics (g GAE kg⁻¹); TN, total hydrolyzed tannins (g GAE kg⁻¹); CT, condensed tannins (g PCBE kg⁻¹); OA, oxalic acid (g kg⁻¹); values followed by different letters within a row indicate significant differences according to Tukey's test ($p \leq 0.05$).

There were also significant differences in the NDF, ADF, ADL, and CP contents in vetch biomass originating from the six environments (Table 5). Larissa 2018/19 (E3) produced biomass with the highest NDF and ADF contents and the lowest CP content among all the environments. On the other hand, the lowest values of both NDF and ADF contents originated in Thessaloniki 2019/20 (E6), without significant ($p > 0.05$) differences among Spata 2019/20 (E2) and Thessaloniki 2018/19 (E5). Moreover, the environment E2 produced biomass with the highest CP content, followed by those of E6, E5, E4, and E1. Concerning the ADL contents, Thessaloniki 2018/19 (E5) and Thessaloniki 2019/20 (E6) had significantly higher ADL values compared with the other environments.

Similarly, significant differences were observed in antinutritional traits among all the environments tested (Table 5). It was noticed that among the six environments, Spata 2018/19 (E1) produced vetch biomass with the lowest TP, TN, and CT contents. However, biomass produced in Larissa 2019/20 (E4) had the highest values of antinutritional parameters. Considerable differences in the OA content were noticed between the biomass of the vetch genotypes originated from the six environments, with its highest level recorded in E2 and the lowest values identified in E3 and E6.

3.5. Correlation Coefficients between Traits

Pearson correlations between the analyzed traits, e.g., agronomic/colorimetric, nutritional and antinutritional traits, are illustrated in the heatmap of Figure 1. Correlations between traits were both significantly positive and negative ($p < 0.01$ and $p < 0.05$) and the correlation coefficients varied widely, from $r < 0.1$ to $r > 0.9$.

Considering the biomass/colorimetric traits, strongest correlation (i.e., dark green denoted) and significant correlation ($r = 0.9$, $p < 0.01$) were found between color trait b* and Brightness L* ($r = 0.9$; $p < 0.01$), whereas low (i.e., light green denoted) positive correlation was detected between color trait a* and Biomass yield ($r = 0.2$; $p < 0.01$) and negative correlation color trait a* with Brightness L* ($r = -0.3$; $p < 0.01$). Regarding the nutritional traits, the strongest positive and significant correlation was found between ADF and NDF ($r = 0.7$; $p < 0.01$) and significant positive correlation was recorded between ADF and ADL ($r = 0.3$; $p < 0.01$) and NDF with CP ($r = 0.4$; $p < 0.01$). For antinutritional traits, only CT were significantly and positively correlated to TP ($r = 0.8$; $p < 0.01$) and to TN ($r = 0.7$; $p < 0.01$). On the other hand, biomass/colorimetric traits showed negative correlation with almost

all antinutritional traits, distinguishing the case of CP with color trait b^* and Brightness L^* ($r = -0.7$; $p < 0.01$).

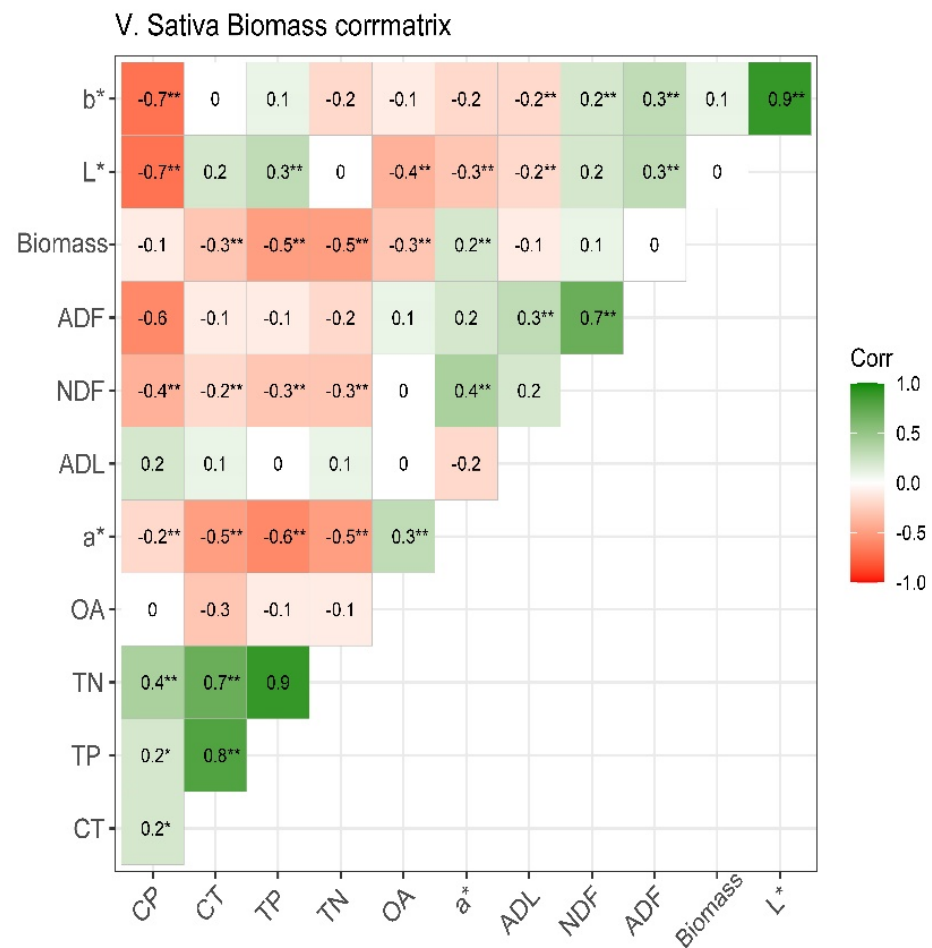


Figure 1. Pearson correlation coefficients heatmap of agronomic/colorimetric, nutritional and antinutritional traits of *V. sativa* biomass produced by seven genotypes across six environments. Dark green denotes high correlation ($r = 1$), dark red high negative correlation ($r = -1$); *, ** significant at $p < 0.05$ and $p < 0.01$, respectively.

3.6. Genotype Stability and Environment Discrimination with GGE Biplots Analysis

3.6.1. Biomass Yield

Using GGE biplot analysis for biomass yield the first two principal components explained 84.3% of the total Genotype plus Genotype by Environment (G + GE) variation. The data were not transformed (“Transform = 0”) or scaled (“Scaling = 0”), and were environment-centered (“Centering = 2”). The biplot in Figure 2 was based on environment-focused singular value partitioning (“SVP = 2”) and therefore is appropriate for visualizing the relationships among environments, whereas the biplot in Figure 3 was based on genotype-focused singular value partitioning (“SVP = 1”) and therefore is appropriate for visualizing the similarities among genotypes. The “which-won-where” view of the GGE biplot [37] showed that G7 and G1 were the highest and G3 and G5 were the poorest located at the vertexes of the polygon, responding either positively or negatively to biomass yield (Figure 2). Additionally, it revealed two mega-environments, e.g., the first mega-environment formed by environments E1, E2, E5, and E4 that coincide with E6 in which G7 is the winning genotype, and E3 with G5 as the winning genotype. The angle between environmental vectors in Figure 2 illustrates the correlation between environments. A positive correlation was recorded among all environments. It’s worth noting that E4 and E6 were highly correlated in their ranking of the genotypes, indicating that these

environments produced similar information about the genotypes. As we can see in Figure 4, the single-arranged line, “Average Environment Coordination” (“AEC”) abscissa, points to a higher mean yield across environments. Thus, across that averaged environment, cultivars G7, G5, and advanced lines G4 and G2 had the highest mean biomass yield. The double-arranged line, the “AEC” ordinate that points to greater variability (poorer stability) in either direction, indicated that G1 followed by G5 was highly unstable whereas G3 and G2 were highly stable. The genotype that is near to the “ideal” for biomass production according to Yan [40] is the ‘Alexandros’ cultivar (G7), which showed both high yield and stability.

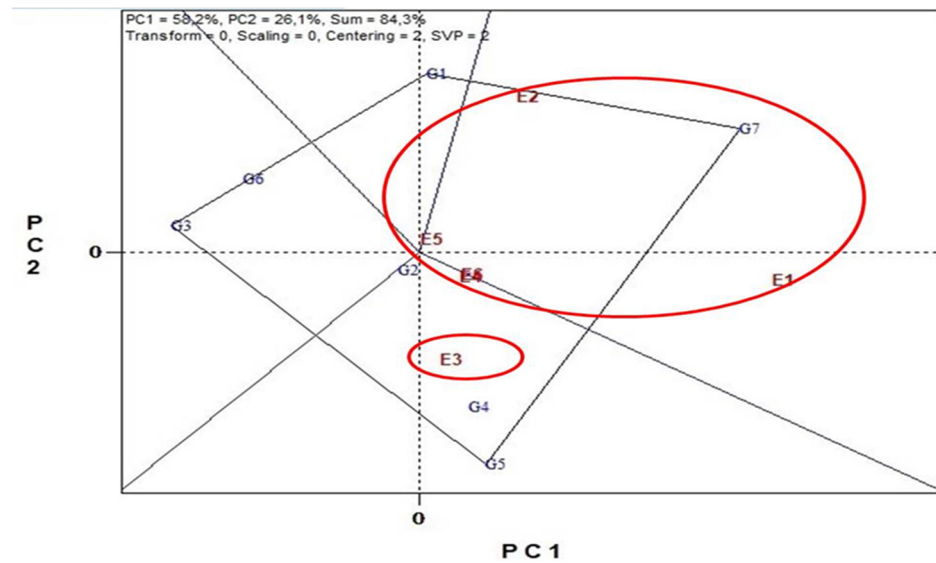


Figure 2. “Which-won-where or which is best for what” view of the GGE biplot based on biomass yield on seven *V. sativa* genotypes in six environments. Environment 4 and Environment 6 coincide.

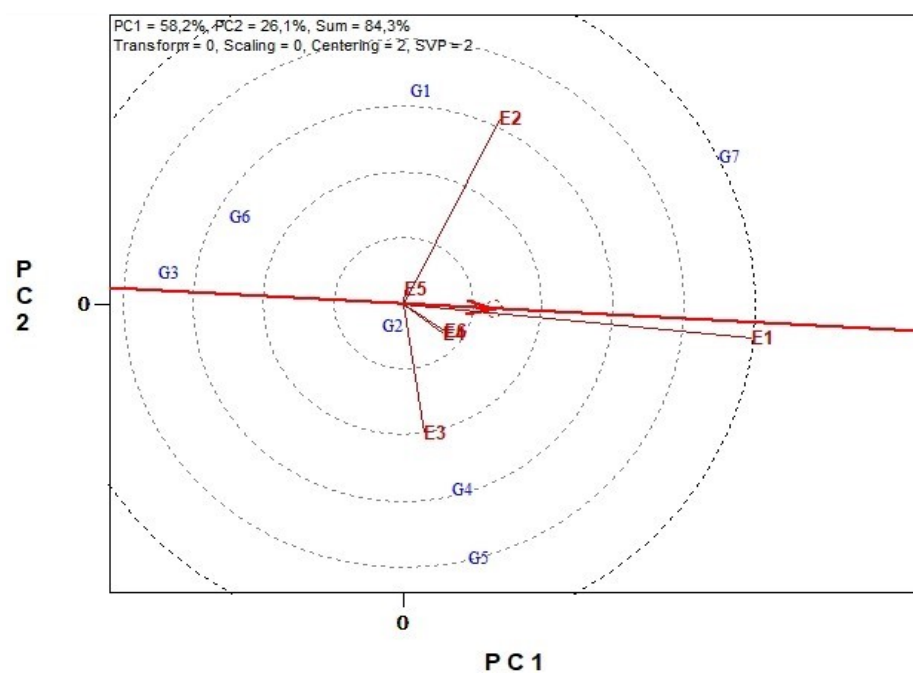


Figure 3. “Discriminating power vs. representativeness” view of the GGE biplot based on biomass yield on 7 *V. sativa* genotypes in six environments. Environment 4 and Environment 6 coincide.

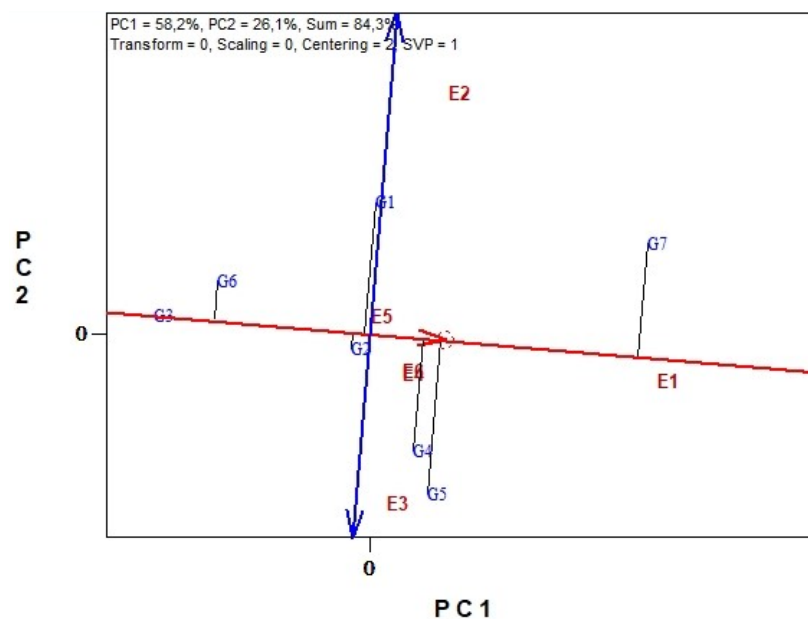


Figure 4. “Mean vs. stability” view of the GGE biplot based on biomass yield on seven *V. sativa* genotypes in six environments. Environment 4 and Environment 6 coincide.

3.6.2. Nutritional Traits

The GGE biplots in Figures 5–7 present data of seven genotypes of common vetch determined for four nutritional traits: NDF, ADL, ADF, and CP content, averaged across six environments. The biplots in Figures 5 and 6 are trait-metric preserving (“SVP = 2”) and, therefore, appropriate for visualizing the relationships among the traits, whereas the biplot in Figure 7 was based on genotype-focused singular value partitioning (“SVP = 1”) and therefore is appropriate for visualizing the similarities among genotypes. The GGE biplot analysis explained 85.8% of the total variability.

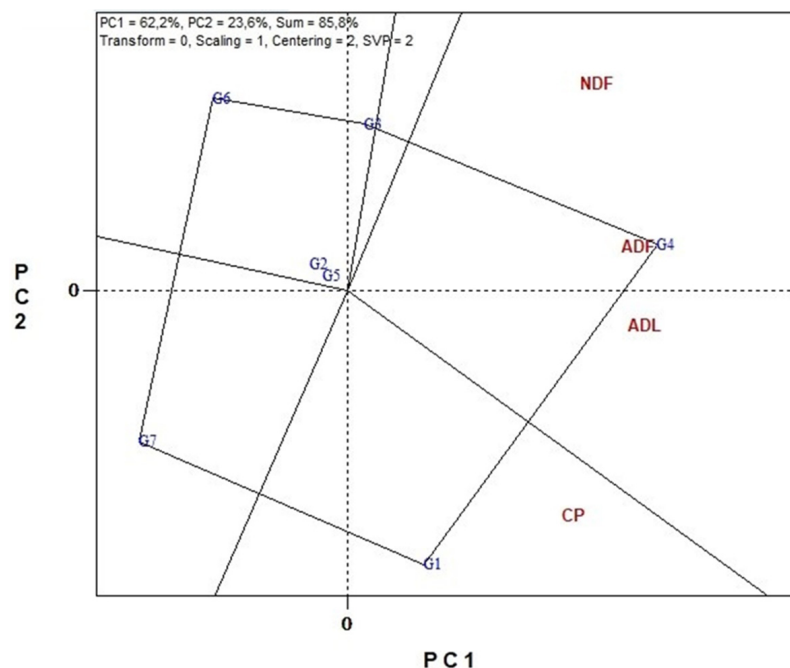


Figure 5. “Which-won-where or which is best for what” view of the GGE biplot for genotypes by nutritional traits representing seven *V. sativa* genotypes measured for four traits averaged across six environments.

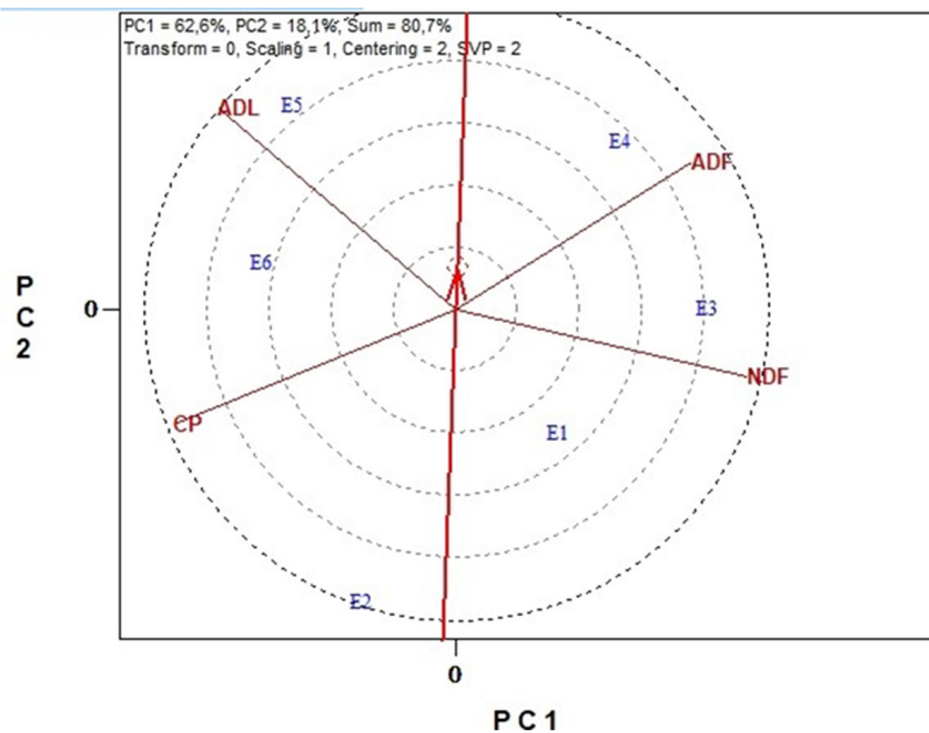


Figure 6. The “Discrimitiveness vs. representativeness” view of the GGE biplot for six testing environments of four nutritional traits, averaged across seven genotypes of *V. sativa*.

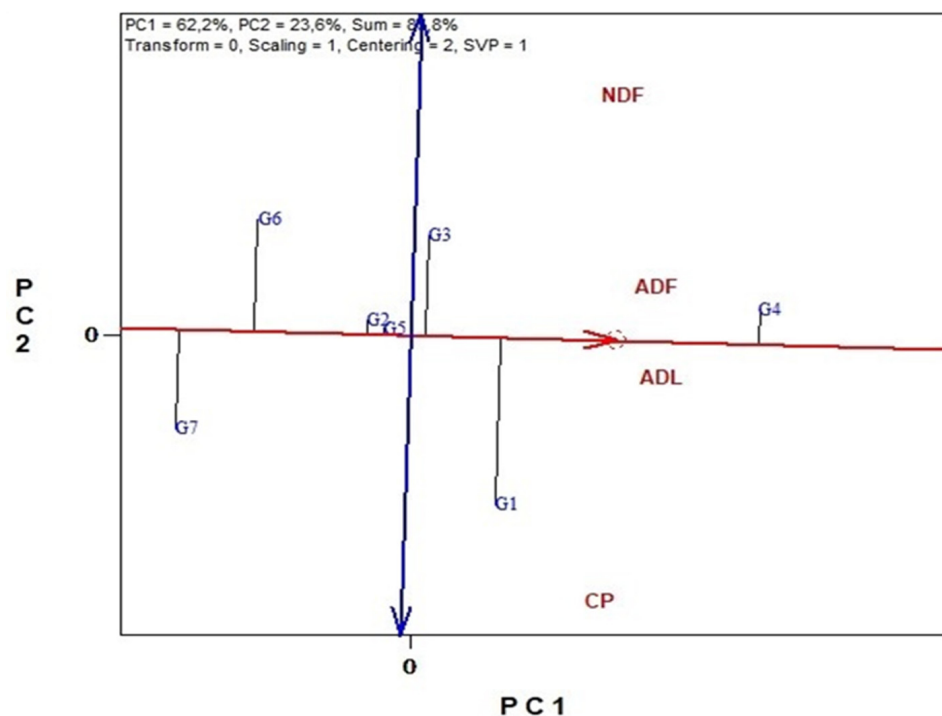


Figure 7. The “Mean vs. Stability” view of the GGE biplot for seven genotypes of *V. sativa* of four nutritional traits, averaged across six environments.

The “which-won-where” pattern of the GGE biplot (Figure 5) for the four nutritional traits fell into sectors with different winning cultivars. Specifically, the G4 genotype that is located on the right vertices of the polygon demonstrated high values for the NDF, ADL, and ADF traits thus becoming clearly the least promising genotype in terms of

nutritional traits. Genotype G1 is uniquely associated with CP. Additionally, from the trait-by-environment biplot, averaged across genotypes, (Figure 6) conclusions can be drawn on trait-by-environment interactions and environmental correlations among traits [41]. Analytically, the single-arrowed line that passes through the biplot origin; “Average Trait Axis” (“ATA”), separates the traits into two sectors based on the angles formed between the “ATA” and the trait vectors. The first sector includes ADF and NDF traits and the second sector includes ADL and CP traits. Moreover, the environment with the highest representativeness and discrimination ability in terms of nutritional traits is E2, followed by environments E5, E3, and marginally acceptable E4. According to the “Mean vs. Stability” view of the GGE biplot (Figure 7), G4 had the highest mean performance of genotypes regarding nutritional traits followed by G1 and G3, whereas G7 had the lowest mean performance. However, it should be mentioned that regarding NDF, ADF, and ADL, the desirable genotypes are the ones that obtained the lowest values. From this point of view, G7 followed by G6, G2, and G5 performed better than the others. The most stable genotype was G5, as it had a near-zero projection onto the “ATA” ordinate, followed by G2. In contrast, G7 and G6 were two of the least stable genotypes.

3.6.3. Antinutritional Traits

Four antinutritional traits, namely TP, TN, CT, and OA, averaged across six environments of seven genotypes of *V. sativa* and are presented by the GGE biplot in Figures 8–10. The biplots in Figures 8 and 9 are trait-metric preserving (“SVP = 2”) and are therefore appropriate for visualizing the relationships among the traits, whereas the biplot in Figure 10 was based on genotype-focused singular value partitioning (“SVP = 1”) and therefore is appropriate for visualizing the similarities among genotypes. The GGE biplot analysis explained 89.6% of total variability.

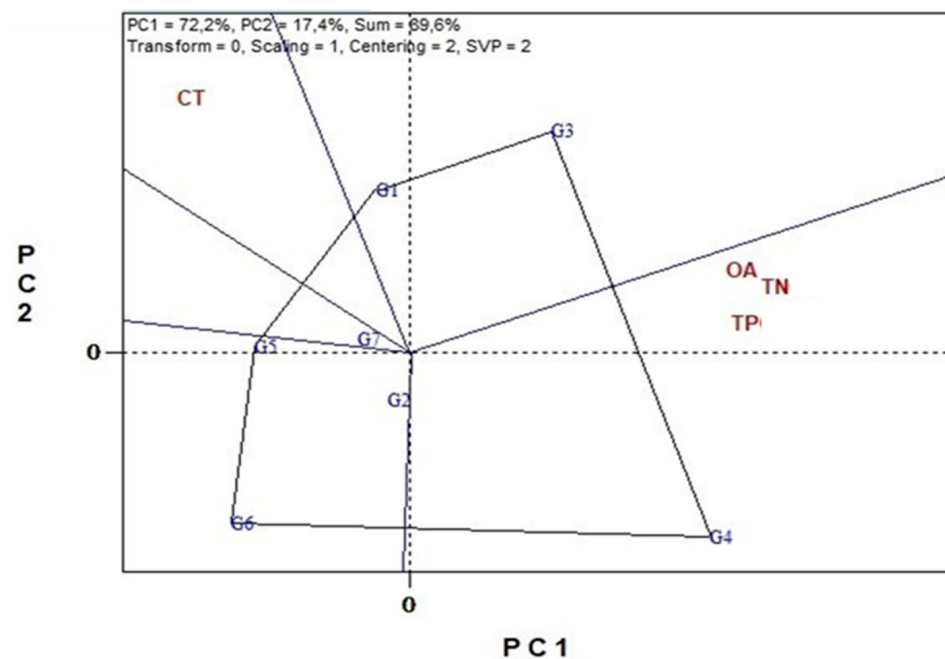


Figure 8. “Which-won-where or which is best for what” view of the GGE biplot for genotypes by antinutritional traits representing seven *V. sativa* genotypes measured for four traits averaged across six environments.

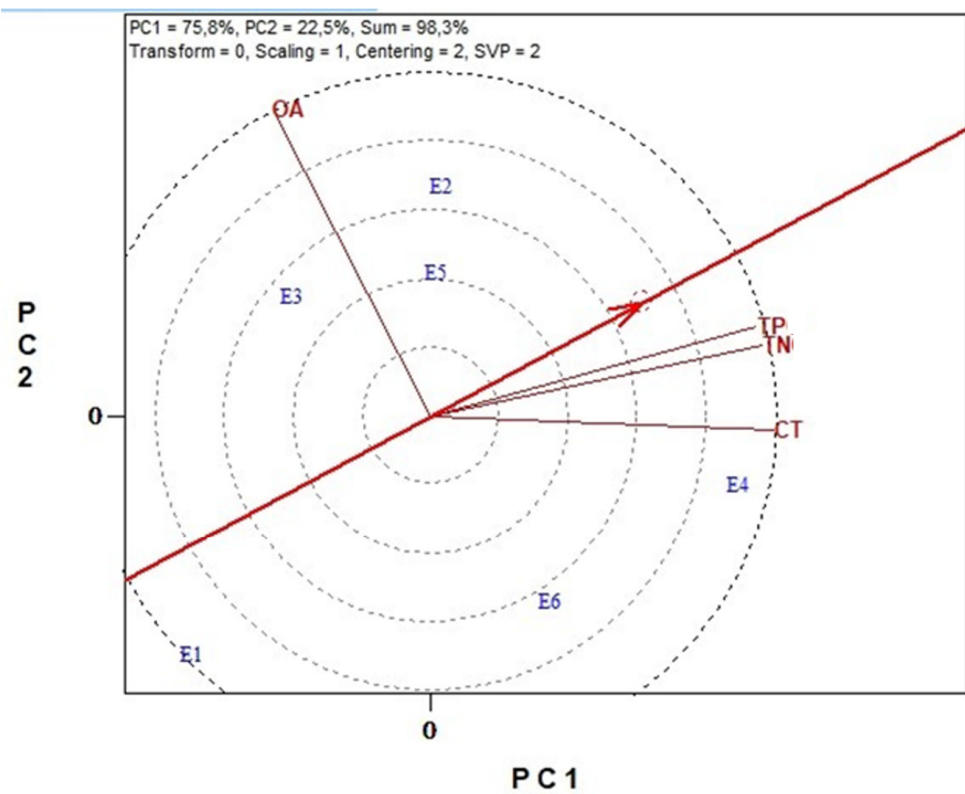


Figure 9. “Discriminativeness vs. representativeness” view of the GGE biplot for six testing environments of four antinutritional traits, averaged across seven genotypes of *V. sativa*.

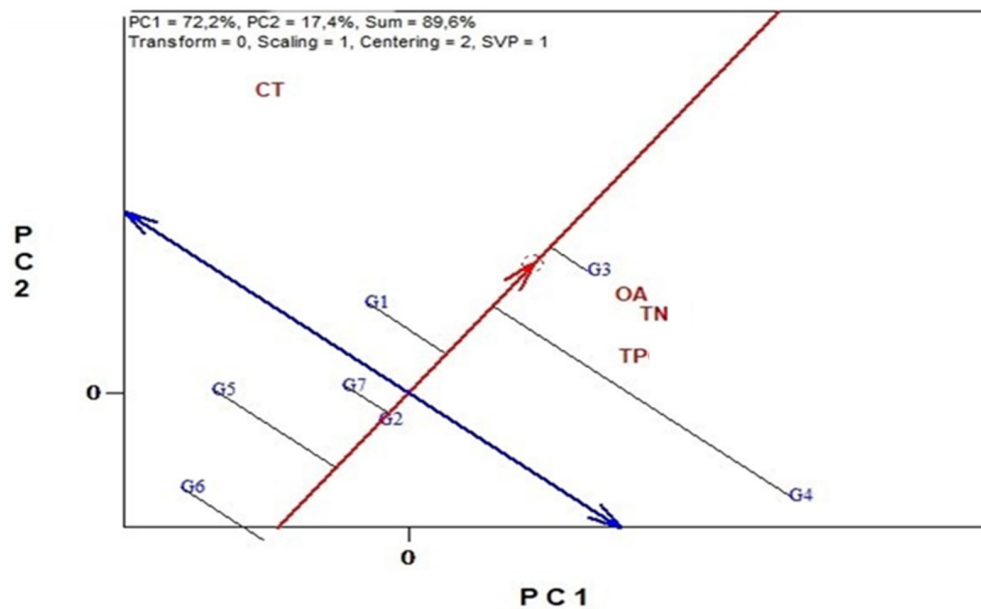


Figure 10. “Mean vs. Stability” view of the GGE biplot for seven genotypes of *V. sativa* of four antinutritional traits, averaged across six environments.

The “which-won-where” view of the GGE biplot in Figure 8 consists of an irregular polygon of genotypes that are located on the vertices of the polygon so that all other genotypes are contained within the polygon. Genotypes G3 and G4 performed well in one or more antinutritional traits and genotypes G1, G5, and G6 performed worst for these traits. The perpendicular line that starts from the biplot origin indicated G4 as the “winner” genotype for the TN, TP, and OA traits. There is no clear “winner” genotype for

the CT trait, although G1 is the strongest candidate. According to the “Discriminativeness vs. representativeness” view of the GGE biplot (Figure 9), the favorable environment for production in terms of antinutritional traits is E1, followed by environment E4, and marginally acceptable E2 and E6. The E3 and E5 are low in discrimination capability to all tested traits. In the “Mean vs. Stability” view of the GGE biplot (Figure 10), the single-arrowed line that points to the higher mean trait across environments depicted the G3 genotype as stable and with high values regarding antinutritional traits. Genotype G4 scored highly but it was not as stable as G3 or G1. The most stable genotype was G2 as it had a near-zero projection onto the ATA ordinate. Genotypes G5 and G6 had the lowest mean performance, whereas a positive connection between G1 and CT traits was detected. It is noted that genotypes with lower values of antinutritional factors are of greater quality.

4. Discussion

Common vetch can be utilized as a high-quality alternative forage legume, fodder, cover crop, hay, green manure, or for silage production in rainfed areas of the Mediterranean region mainly due to its high nutritional value and its ability for soil fertility improvement [12]. In Greece, the cultivation of common vetch and other vetches in grassland–livestock systems has expanded greatly in recent years, although its production is still limited [16]. The development of stable new vetch lines or cultivars with good adaptation and high yielding ability would greatly contribute to increasing the feed supply for livestock. Inconsistent genotypic responses to abiotic and/or to biotic factors over locations and years are attributed to GX E interactions [21].

Seven genotypes of common vetch were evaluated in six different environments in this study. A significant effect was recorded across the different environments and genotypes. The three experimental locations were in Southern, Central, and Northern Greece and exhibited considerable differences in key pedoclimatic characteristics such as air temperature, rainfall, and soil conditions, resulting in six distinct environments.

4.1. Biomass/Colorimetric Traits

The main source of variation for biomass yield was the environment, as it explained 71.5% of the total variation, followed by GXE interactions and Genotype. Generally, legumes are prone to environmental fluctuations [42,43], and this is confirmed by recent multi-environmental trials in Greece with faba bean [29] and lentil [44]. The most productive environment was E1 (19.6 t ha⁻¹) which corresponded to location Spata/2018-19, and the lowest was E5 (9.2 t ha⁻¹) which corresponded to location Thessaloniki/2018-19. This could be attributed to lower temperatures and less rainfall that prevailed during the vegetative period in the first growing season in Thessaloniki (10.1 °C vs. 10.7 °C). This is consistent with the findings of other studies [7]. However, the Spata and Thessaloniki locations were very unstable and showed significant fluctuation from year to year, in contrast to the Larissa location, which had better stability across years. According to GGE biplot analysis (Figure 2), two mega-environments were recognized. The first consisted of E1, E2, E4, E5, and E6, and the second consisted of E3. Furthermore, E1 (Spata 2018/19) followed by E2 (Spata 2019/20) were the most representative of all tested environments and thus could be considered as the optimum environments for selection and evaluation of genotypes with high biomass yield and wide adaptation capacity. This could be attributed to the climatic conditions prevailing in the Spata location (adequate humidity in the critical period of anthesis and pod filling) and to soil texture characterized by a high level of sand that ensured good drainage and deep root growth, important for vetch growth [45]. Environments E4, E5, and E6 were of low discriminating ability, whereas E3 was a discriminating environment but not representative and therefore is suitable for selecting specifically adapted genotypes. In addition, a very close association was observed between environments E4 and E6 (Figure 3). Thus, the information obtained regarding the genotypes is almost the same. According to Yan [46], in such cases, one of the two environments could be omitted in later experimentation without significant loss of data.

Genotype explained the smallest part of the total variability (7%) for biomass yield, however, significant differences were detected among genotypes tested. Biomass yield ranged from 11.3 to 14.6 t ha⁻¹, which is similar to other reports [47,48], though it is relatively high in comparison with previous studies in Greece [6]. The highest biomass yield was obtained from G7 ('Alexandros' cultivar) and the lowest from the advanced line G3. Genotype G7 demonstrated wide adaptability and high yield as it fell within the scope of the 'ideal' genotype (Figure 3), whereas G5 ('Evinos' cultivar) had specific adaptability to the pedoclimatic conditions of E3. Among the advanced lines tested, G4 was the most promising as it showed the highest yield and stability of performance.

Moreover, a clear environmental variability was observed in color values of vetch biomass, as genotypic variability was less than 10%. Previous research has pointed out that color varies notably with changes in climate during the complete cycle of leaf color variances [49]. It was obvious that the climatic variables among the environments influenced the color values; in particular, the increase in temperature in the second year in all locations (E2, E4, and E6) promoted the increase in biomass yellowness and decrease in biomass greenness. These results were similar to those of Alamu et al. [50], who reported the increase in yellowness after high-temperature exposure.

4.2. Nutritional Traits

Fiber and CP content are key parameters for the assessment of forage nutritive value. Particularly, the content of neutral detergent fiber (NDF) in the forage is negatively correlated with forage intake by animals, whereas the content of acid detergent fiber (ADF) and acid detergent lignin (ADL) are negatively related to forage digestibility [51,52]. Regarding the fiber content in ADL, NDF, and ADF, GXE interaction was the main source of variation pointing out the need for growing different genotypes with high performance and stability in different areas. The strong environmental influence on the expression of these antinutritional traits was also reported by Asekova et al. [53] for the forage of soybean based on the inconsistent detection of QTLs in different years. Similarly, Pecetti et al. [54] concluded that the environmental impact on the forage nutritive value of *M. sativa* was higher than the genetic impact. On the other hand, CP was mainly affected by the E (75.7%), which is in agreement with Vlachostergios et al. [6] who reported a 70% environmental effect on CP content, and with Rebolé et al. [55] who found significant differences between environments for forage CP. Nevertheless, Larbi et al. [3] found a small variation in CP content within varying environmental conditions.

Regarding the environmental impact on forage nutritive value, the general trend in the present study was lower fiber content and higher CP content, indicating higher nutritive value during the second experimental year, which was characterized by higher precipitation in the three locations. Inversely, Pecetti et al. [54] documented the poor nutritive value of *M. sativa* forage in the moisture favorable environment due to a low leaf-to-stem ratio and high NDF concentration in the whole plant and stems. GGE biplot analysis revealed that E2 (Spata/19-20) followed by E5 (Thessaloniki/18-19) were the most informative environments and are suggested for varietal evaluation of nutritional traits. We should note that the forage nutritive value of common vetch is less variable among genotypes from the beginning of pod filling to pod maturity [28]. In both environments (E2 and E5), the average precipitation and the average mean temperature for May and June were 23 mm and 22 °C, respectively.

The nutritional traits of forage varied among the studied genotypes of common vetch at the beginning of the pod filling stage. Similarly, varietal differences have been reported by other studies for forage, hay, and straw of common vetch [3,56,57]. The range of NDF content was 39.4 g kg⁻¹ (328.7–368.1 g Kg⁻¹), for ADF was 22.2 g Kg⁻¹ (221.3–243.5 g Kg⁻¹), and for ADL was 9.5 g Kg⁻¹ (50.7–60.2 g Kg⁻¹), and it was generally similar with other studies at the same phenological stage [28], and relatively higher than that of hay [3,57]. These traits were studied in the location of Thessaloniki by Lithourgidis et al. [5] who reported slightly higher values at the same phenological stage. The mean value of CP content

was 192.2 g kg⁻¹. Similar results have been found for vetch grown in native pasture in Turkey [58] and for cultivated vetch in Bulgaria [23]. According to our results, vetch could exceed the requirements of small ruminants grazed in semi-arid regions for maintenance (9%), gestation (11%), and lactation (13%) [59–61]. Among the studied genotypes, on average, the commercial cultivars (G5, G6, G7) were characterized by lower content of NDF, ADF, and ADL, but the advanced lines (G1, G2, G3, G4) by a higher content of CP, indicating that they could be a promising genetic material for future breeding. Additionally, G7 (the 'Alexandros' cultivar) followed by G6 (the 'Leonidas' cultivar) and advanced line G2 was the best performing genotypes in terms of nutritional traits, whereas G5 (the 'Evinos' cultivar) was the most stable.

4.3. Antinutritional Traits

In the present study, some major antinutritive components were analyzed in common vetch forage samples. Plant phenolics and tannins are physiological responses to biotic and abiotic stresses [62] and can have negative or positive effects, depending on tannin content and structure, plant source and animal species, and physiological state and nutrition. In our study, we found that the vetch forage samples under investigation are acceptable for ruminants, as they contained low CT concentrations (<50 g kg⁻¹ DM), enhancing their nutritive value, without inhibiting rumen fiber digestion or voluntary intake [63].

Oxalates are also important antinutritional compounds, which are widely distributed in plants. Oxalates are known to obstruct calcium absorption by creating insoluble calcium salts, raising the risk of renal calcium oxalate formation, which can lead to kidney stones [64]. Oxalate poisoning is a complicated process that depends on the chemical form of oxalate, the animal's age, the rate of intake, the amount and quality of other feed ingested simultaneously, the overall amount of oxalate consumed, and the animal's adaptability to an oxalate-containing diet.

Although we found high levels of oxalates in vetch forage samples (4.01%), these levels are within the range reported for the common halophytic fodder used in Egypt and the Near East region (3–5%), or for *Amaranthus* species [65,66]. However, the amounts of oxalates present in vetch forages are also much lower than in many other fodder species (3.3 to 6.6%) [67,68]. As indicated by Rhaman et al. [69], ruminants may efficiently utilize high amounts of oxalate without causing ill effects, if proper management practices will be applied.

Regarding antinutritional traits, ANOVA indicated that environment was the main source of variation ranging from 72.6% to 84.3%, whereas genotype affected at a low level the total variation (from 2.6% to 5.0%). The biosynthesis level and quality of secondary metabolites in plants are frequently affected by environmental factors [70]. Greater TN, CT, and TP content in E2, E4, and E6 environments could be related to variations in climate (temperature and precipitation) and soil conditions between environments; a higher temperature was observed in the second year in all studied sites compared with the first growing season. Similar to our results, there are reports for other forage species indicating that their growth under high temperatures had substantially greater levels of CT compared with the growth under cooler temperatures [71,72]. There are limited studies on the changes in CT content in different tissues and across different environments for Mediterranean forage species. On the contrary, higher oxalate content was noticed in E2, E3, and E5 environments. Based on the above results, it is not clear how temperature affects oxalate content in forages. It has been reported that plant tissue obtained in the early summer exhibited higher oxalate content than that obtained later in the season [73]. However, further research is required to understand the role of season or temperature on oxalate accumulation in forage plants. Therefore, the selection of the environment where common vetch is cultivated is of crucial significance when the production of high-quality dry biomass (hay) is of great priority. According to our results, environment E1 was the most suitable to discriminate genotypes for high content of antinutritional traits like TP, TN, CT, and OA. It should be highlighted that E1 was also the most informative environment for

biomass yield. Regarding genotypes, cultivars G6 ('Leonidas'), followed by G5 ('Evinos'), and G7 ('Alexandros') were characterized by a lower content of antinutritional traits. Among the advanced lines, G2 was very stable and low scored for all antinutritional traits studied, whereas G4 scored high values for certain antinutritional traits (viz. TN, TP, and OA) and also it was unstable as it showed high GXE interactions. However, it should be noted that in all cases the content of antinutritional traits in the forage of common vetch was within acceptable limits for the diet of ruminants.

Correlation between Traits

Biomass yield was almost neutrally correlated with all nutritional traits; however, it was negatively associated with all antinutritional traits. In such cases, multiple selection for biomass and low antinutritional traits could be feasible, although the efficiency of the selection is further dependent on the favorable correlation and the additive genetic effects between the target traits [74].

The available information about the relationships between both nutrient and antinutrient contents from color parameters in vetch biomass is very limited. Therefore, we could not compare our results with previous reports; however, literature data is available for vetch seed. A strongly significant negative correlation existed between CP and L* of biomass color ($p < 0.01$), i.e., vetch lines with a darker color in biomass contained higher levels of CP. Similarly, Canbolat et al. [75] suggested that the CP content is higher in dark seeded pea genotypes than in white and green seeded peas. On the contrary, Wang et al. [76] observed no correlation between CP in grass pea and L* value. Moreover, the significant negative relationship of CP with NDF means that the cultivar's selection for high CP content would lead to genotypes with lower cell wall content, which would be desirable from the perspective of forage quality. On the other hand, correlations between NDF and ADF were significant and positive as expected since the components of ADF (cellulose and lignin) are also components of NDF.

TP and CT were highly positive correlated ($p < 0.01$). Similar observations were made in grass pea seeds [76] and lentils seeds [77].

5. Conclusions

The evaluation of biomass yield and significant nutritional and antinutritional traits of seven common vetch genotypes grown in six environments in Greece for two consecutive growing seasons demonstrated that the environment exhibited a significant effect on biomass yield, CP, and antinutritional traits, whereas nutritional traits were mainly affected by GXE interactions.

Environment E1 was the most informative for both biomass yield and antinutritional traits, pointing out that this type of environment could be suggested for multiple selection or screening for both high yield and low antinutritional content. Environment E2 was the most discriminative in terms of nutritional traits (i.e., NDF, ADF, ADL, and CP) and could also be appropriate for the selection of genotypes with a high biomass yield.

Cultivar 'Alexandros' showed the highest and most stable performance for biomass yield, whereas cultivar 'Leonidas' was the best regarding nutritional and anti-nutritional traits. The advanced line 'BK-29' had the best biomass yield among advanced lines tested and stood out for its stable and low ADL, ADF, and NDF contents. Consequently, it could be a promising genetic material that might be exploited in genetic improvement efforts to generate common vetch varieties with specialized features for use as high-nutrient feed. In summarizing, further investigation is needed to better understand the environmental and genetic interactions as they are a key issue to release improved common vetch varieties and meet market needs for high-yield and high-quality feed products.

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