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RESEARCH ARTICLE

Characterisation of bitter vetch (*Vicia ervilia* (L.) Willd) ecotypes: An ancient and promising legume

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Abstract

Bitter vetch (*Vicia ervilia* (L.) Willd) is a promising legume, drought tolerant, mainly used in the Mediterranean area for its grains as a source of proteins in animal feed. However, it is an underused crop cultivated in marginal soils. Ecological, agro-morphological, and bromatological diversity evaluations were conducted to study its reintroduction potential. Seeds of seventeen ecotypes were collected in seventeen farms located in Northern Morocco in 2018. The cultivation was realised during the 2019 and 2020 growing seasons using a randomised complete block design with three replicates. Agro-morphological data were collected at the start of flowering, full flowering, and pod setting phenological stages. Yield component assessment and grain and straw bromatological characterisation were performed at maturity. The results indicated significant variations between ecotypes for almost all parameters and interesting results about yield (1 t ha^{-1}) but lower protein content (22.9% of dry matter) compared to other ecotypes of the Mediterranean region. The estimated genetic parameters could emphasise the possibility of selecting highly productive and nutritive cultivars. However, interannual variations were also detected, making the selection of the ecotypes harder. No significant correlations were observed between agro-morphological and bromatological traits of grains and geographical distances. Multivariate analyses (principal component analysis and heatmap) clustered ecotypes into five groups, where the ecotypes included in the second cluster were the most interesting candidates for developing high-yielding and nutritive varieties. That is why this plant could be considered of interest, especially in these times of climate change.

Keywords: agro-morphology; bromatology; phenology; genetic variability; *Vicia ervilia* (L.) Willd

Introduction

The aim of world food security has involved selecting highly productive plant varieties and using large amounts of chemicals and water (Tilman *et al.*, 2011). This approach led to gene and biodiversity losses and environmental pollution (Marouane *et al.*, 2015). Moreover, due to climate change, plants suffer from rising temperatures and drought (Schilling *et al.*, 2020). The use of ancient plants, neglected because of lower yields but more adapted to the local environment, must be investigated to feed animals. It could be a sustainable alternative because their reintroduction could increase plant biodiversity in farming systems (Boukrouh *et al.*, 2023a; 2023b).

Vicia ervilia (L.) Willd, or bitter vetch, is one of the earliest domesticated plants. In Morocco, bitter vetch is an underused crop mainly cultivated in marginal soils for grain and straw use in animal feeding (Larbi *et al.*, 2011; El Fatehi and Ater, 2017). However, this legume has a high capacity for fixing nitrogen (Romanyà and Casals, 2020) and is highly drought tolerant (Ghanipour Govarki *et al.*, 2019). Considering the effect of climate change on agriculture, the adaptability of bitter vetch could help farmers achieve feed security.

The analysis of genetic diversity is important for deciphering the nature and the magnitude of the variability between traits for an efficient selection of ecotypes. This evaluation was done using molecular techniques or agro-morphological traits (Larbi *et al.*, 2010; Livanios *et al.*, 2018) or combining both (El Fatehi *et al.*, 2014; Russi *et al.*, 2019). In Morocco, only two studies (El Fatehi *et al.*, 2014; El Fatehi and Ater, 2017) about genetic characterisation were realised under greenhouse conditions but without nutritional value assessment. Nevertheless, the bromatological analysis could determine better ecotypes to be used as food or feed. Indeed, Vioque *et al.* (2020) reported bitter vetch as a useful source of proteins and bioactive components with health-promoting properties. Galieni *et al.* (2020) highlighted the potential of underused species in producing sprouts as a functional food.

This study aimed thus to determine the ecology, the agro-morphology, and the nutritive value (grain and straw) of seventeen North Moroccan bitter vetch ecotypes, as well as the possible links to the original environment. This characterisation could help to revalorise performant ecotypes as feed and improve biodiversity by enriching the Moroccan bitter vetch seed bank.

Materials and Methods

All experiments and analysis methods followed relevant regulations and guidelines of Tangier's Regional Agricultural Research Center (INRA – Tangier, Morocco). All the analysis methods are described in Supplementary Material 1.

Plant material, study site, and experimental set-up

The plant material used included seventeen ecotypes of bitter vetch collected in July and September 2018, randomly from seventeen farms in the North of Morocco (Figure 1). The seeds were sown in 2019 and 2020 in the El Menzla experimental field (35°31'53"N; 5°42'36"W; 135 m), a related research station to INRA of Tangier, Morocco.

Ecological characterisation

At the collection and experimental sites, soil samples were collected at the 0–20 and 20–40 cm horizons for physico-chemical analysis (Table 1). Farm collection site coordinates (latitude, longitude) were used to extract nineteen long-term bioclimatic data (BIO 1–19) for the 1950–2000 period from major climate databases (www.worldclim.org). The data were extracted from satellite images via ArcGIS Desktop version 9.3 (Esri, Redlands, CA, USA) and were interpolated with a resolution of approximately 1 km (Hijmans *et al.*, 2005). The temperature and rainfall data for the 2019 and 2020 agronomical years at the experimental sites were collected from a climatic station 10 km nearer. The 2019 and 2020 bioclimatic variables (BIO 1–19) were determined based on these climatic data.

Experimental design

The experimental design was a randomised complete block design with three replicates. Ecotypes were sown on 17 January 2019 and 19 January 2020 in 2×3 m^2 plots with a density of 63 plants per m^2 with a spacing of 15 cm between lines and 10 cm between plants. Randomisation was consistent across the two years. To guarantee a uniform distribution, seeds were sown at a higher rate. Then, after the emergence, the seedlings were thinned out to reach the target count. One

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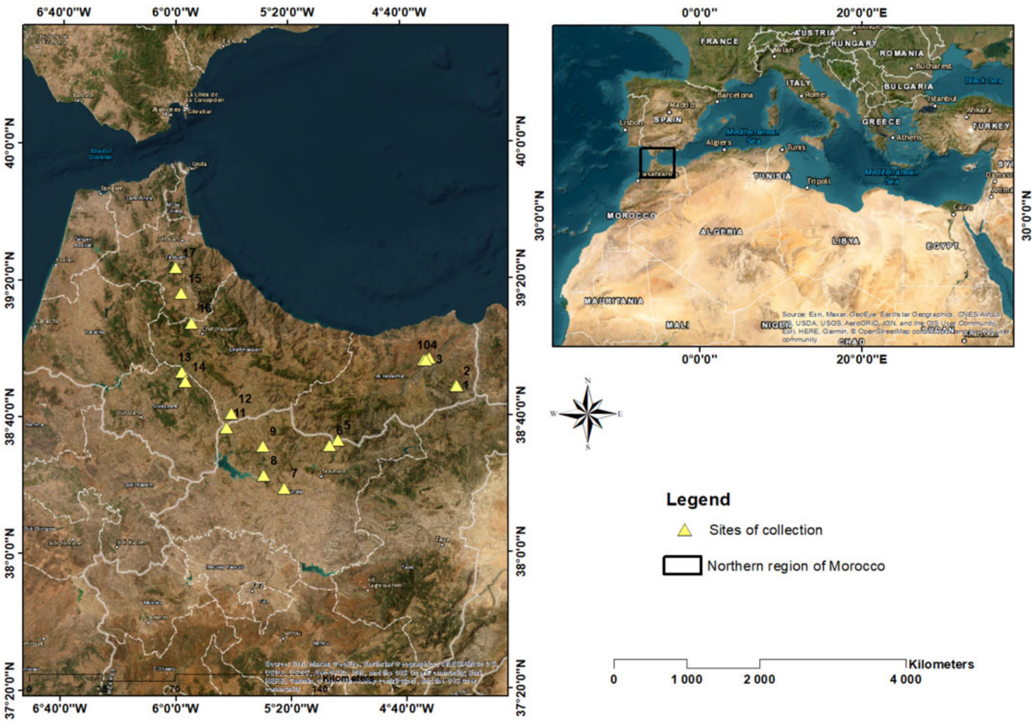

Figure 1. Collection sites of the 17 bitter vetch ecotypes in Northern Morocco. *Note*: This map was generated using ArcGIS Desktop version 10.4.1 – Esri, Redlands, CA, USA, https://en.freedownloadmanager.org/Windows-PC/Portal-for-ArcGIS.html.

meter was the distance between plots and between blocks. The trial was conducted under rainfed conditions on a fallow plot; NPK (10-30-10) fertiliser was applied at 50 kg ha^{-1} on the day of sowing. No irrigation was needed at sowing because rainfall occurred immediately after sowing. Natural weeds were removed manually during the growing season.

Statistical analysis

Analysis of variance was carried out to test the years, ecotypes, and their interactions on agro-morphological and bromatological variation. The variance components were estimated using a general linear model using the SAS 9.4 version. To summarise and visualise the relationship between morphological, phenological, and agronomic parameters, a multivariate analysis was conducted using R software version 4.2.1. Principal component analysis (PCA) was performed using 'FactoMineR and Factoextra' packages. A heatmap was created using the 'Pheatmap' package, with Euclidean distance as the similarity measure and hierarchical clustering with complete linkage. Mantel test using 'Tidyverse,' 'Vegan,' and 'Geosphere' packages to estimate the gene flow by correlating the genetic distance between ecotypes with the geographic and environmental one.

Phenotypic coefficients of variation (PCV), genotypic coefficients of variation (GCV), broad-sense heritability (H^2), genetic advance, genetic advance as a percentage of the mean (GAM), and genotypic and phenotypic correlation matrix were estimated following the formula given by Shariatipour *et al.* (2022).

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Table 1. Climatic and soil physico-chemical characteristics of the 17 collection farms and the experimental cultivation site in northern Morocco

	Collection farms							Experimental site	
	Mean	Minimum	Maximum	Standard deviation	1st quartile	Median	3rd quartile	2019	2020
Altitude (m)	527	219	1154	281	351	557	611	132	127
Edaphic parameters									
Humidity (%)	5.4	3.9	9.2	1.3	5.0	5.9	6.6	8.6	10.4
pH water	8.1	6.5	8.7	0.6	8.0	8.2	8.6	8.0	8.2
pH KCl	7.6	5.3	8.4	0.9	7.5	8.0	8.1	7.1	7.4
Electrical conductivity (mS m^{-1})	46.3	5.4	98.4	32.5	14.2	39.4	74.0	29.0	36.0
Organic matter (%)	3.3	0.9	5.4	1.6	2.0	3.4	4.5	2.2	2.6
Total limestone ($CaCO_3$, %)	2.2	0.5	6.9	2.1	0.8	1.1	2.8	7.4	3.9
Exchangeable potassium (ppm)	189	113	481	89	137	151	214	705	368
Available phosphorus (ppm)	0.7	0.4	1.2	0.3	0.4	0.5	0.8	28.6	26.9
Carbon to nitrogen ratio	11.5	3.6	20.5	5.4	6.6	12.6	15.8	6.4	8.0
Nitrogen (%)	0.2	0.1	0.2	0.0	0.2	0.2	0.2	0.2	0.2
Clay (%)	24.3	17.9	31.8	4.2	20.4	25.8	27.1	45.9	49.8
Coarse sand (%)	1.6	0.5	7.1	1.7	0.9	1.0	1.3	1.6	2
Fine sand (%)	0.7	0.4	1.0	0.2	0.5	0.6	0.7	2.3	1.2
Coarse silt (%)	62.8	50.3	75.2	8.4	58.5	61.2	72.6	37.2	34
Fine silt (%)	10.7	4.8	21.2	5.0	6.1	10.3	11.9	13	13
Bioclimatic parameters									
Average annual temperature (BIO1, °C)	17.1	13.7	19.0	1.4	16.4	17.2	17.9	18.1	18.5
Average diurnal variation (BIO2, °C)	11.4	9.8	12.2	0.6	11.4	11.5	11.8	22.9	20.4
Isothermality (BIO3 = BIO2/BIO7 × 100, %)	40.3	39.4	41.4	0.6	39.9	40.2	40.6	57.3	51.1
Temperature seasonality (BIO4, %)	589	518	618	29	567	596	612	542	520
Maximum temperature of the warmest month (BIO5, °C)	33.0	30.2	35.0	1.7	31.0	33.4	34.3	40.7	35.7
Minimum temperature of the coldest month (BIO6, C°)	4.6	1.5	6.8	1.3	4.1	4.9	5.2	0.7	1.6
Temperature annual range (BIO7 = BIO5-BIO6, °C)	28.3	24.7	30.0	1.4	27.8	28.8	29.3	40.0	39.9
Average temperature of the wettest quarter (BIO8, °C)	10.4	6.9	13.3	1.6	9.9	10.6	10.9	16.2	14.6
Average temperature of the driest quarter (BIO9, °C)	24.6	21.3	26.5	1.5	23.8	25.0	25.8	21.8	22.33
Average temperature of the warmest quarter (BIO10, °C)	24.7	21.4	26.5	1.5	23.9	25.0	25.8	24.4	25.3
Average temperature of the coldest quarter (BIO11, °C)	10.2	6.9	12.3	1.4	9.7	10.6	10.9	11.8	12.8
Annual precipitation (BIO12, mm)	701	422	947	168	614	770	837	563	737
Precipitation of the wettest month (BIO13, mm)	118	63	164	34	92	135	144	152	233
Precipitation of the driest month (BIO14, mm)	1.1	0.0	5.0	1.6	0.0	0.0	1.0	0.0	0.0
Precipitation seasonality (BIO15, %)	71.8	56.4	88.3	9.8	67.0	72.3	74.9	118	106
Precipitation of the wettest quarter (BIO16, mm)	319	175	466	99	257	358	398	130	141
Precipitation of the driest quarter (BIO17, mm)	16.5	9.0	29.0	5.4	13.0	16.0	18.0	0.1	1.5
Precipitation of the warmest quarter (BIO18, mm)	18.6	9.0	36.0	7.1	15.0	16.0	18.0	1.3	4.5
Precipitation of the coldest quarter (BIO19, mm)	317	175	466	98	257	358	398	45	52

Results

Ecological characterisation

The soil results concerning the collection and experimental sites are shown in Table 1. The collected ecotypes of bitter vetch were found on farms with altitudes varying from 219 to 1154 m. Concerning edapho-climatic parameters, bitter vetch ecotypes were sampled from neutral (6.5) soils to highly alkaline (8.7) ones on farm sites. The mean electrical conductivity varied from 5.4 to 98.4 mS m^{-1}, OM from 0.9 to 5.4%, and total limestone from 0.5 to 6.9%. For soil minerals, exchangeable potassium varied from 113.0 to 480.7 ppm, available phosphorus from 0.4 to 1.2 ppm, and nitrogen from 0.1 to 0.2%. All the parameters in the collection farms were comparable to experimental sites in the two years, except for total limestone, exchangeable potassium, and available phosphorus, which were lower.

For mean textural composition, the soil of the collection farms presented a predominance of coarse silt (62%) and a lower presence of fine and coarse sand (0.7 and 1.6%, respectively). The average annual temperatures (BIO1) of the 2019 and 2020 years were close (18.1 and 18.5°C, respectively). The long-term isothermality (BIO3) was 33.3% lower than the mean isothermality of the two experimental seasons. The long-term maximum temperature of the warmest month (BIO5) was lower than for the two studied seasons (33.0 vs. 38.2°C), while the minimum temperature of the coldest month (BIO6) was higher (4.6 vs. 1.2 °C). The temperature annual range (BIO7) of the two tested seasons increased by 41% compared to the long-term range. On the other hand, 2020 was 31% wetter than 2019 (563 and 737 mm, respectively), and they were within the defined limits of the long-term precipitation range of the collection sites (BIO12), varying between 422 and 947 mm for the 1950–2000 period.

Agro-morphological characterisation

Qualitative variables

The frequencies of the different qualitative traits of the tested ecotypes are reported in Table 2. About 82% of the ecotypes' grains in the present study showed high germination percentages. Half of the ecotypes showed a semi-erect growth habit, while only 13% were prostrate. Almost 80% of ecotypes did not have a pigmented stem. The pattern of testa was absent for almost all grains (more than 80%), while lower proportions of grains were marbled, spotted, or with no testa. The ecotypes showed a high variability of grain testa colours, with one-third having a light brown colour and less than 1% showing a greenish orange colour. Some grains had the brown or black colour of the pattern, close to 10% each. More than 80% of the grains had a pyramidal shape.

Quantitative variables

The results revealed that all the agro-morphological parameters of bitter vetch were significantly ($P < 0.01$) influenced by the ecotype (Table 3). The year had a significant effect ($P < 0.05$) on almost all parameters except for the number of primary branches, stem diameter, leaf width, and number of pods per plant. Indeed, during the second year, plant height and leaf length increased by 12% and 8%, respectively, while leaf number and root length decreased by 9 and 37%, respectively. For grain yield parameters, grain per plant and grain yield increased by 15% and 22%, respectively, in 2020. Except for the leaf width, the phenological stage influenced all parameters. So, from the start of flowering to the pod setting, the number of total branches decreased by 3%. Plant height, internode number, and leaflet number increased by almost 10%, while the stem diameter and leaf number increase exceeded 30%. The interaction effect was significant ($P < 0.05$) for many parameters.

Genetic parameters are also reported in Table 3. The phenotypic variance (PCV) was slightly higher than the genotypic variance (GCV) in all the traits. Several parameters, including flowering duration, root length, and grain yield-related parameters (grains per pod and per plant, pod

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Table 2. Frequencies of the different qualitative parameters considered to describe the 17 cultivated Moroccan bitter vetch ecotypes

Traits	Classes	Frequencies
Germination percentage	High	82.35
	Moderate	5.88
	Low	11.76
Growth habit	Prostrate	12.55
	Erect	31.76
	Semi-erect	55.69
Stem pigmentation	Absent	78.82
	Present	21.18
Pattern of testa	Absent	83.14
	Spotted	4.71
	Marbled	2.35
	Combined	9.83
Colour pattern of testa	Absent	81.57
	Black	8.63
	Brown	9.02
	Grey	0.78
Colour of testa	Dark blue	3.53
	brownish red	7.06
	Bluish grey	1.57
	Light grey	17.25
	Dark grey	2.75
	Greenish grey	8.24
	Light brown	32.16
	Dark brown	19.61
	Orange-brown	5.49
	Light orange	1.57
	Greenish-orange	0.78
Seed shape	Circular	4.31
	Conical	7.06
	Pyramidal	88.63

number, grain yield, and harvest index), were characterised by a GCV and PCV higher than 20%. Almost all studied parameters presented a heritability estimation higher than 90%.

Bromatological parameters

For grains, the difference between ecotypes was significant ($P < 0.05$) for all bromatological parameters except for DM (Table 4). The effect of year was significant ($P < 0.05$) for all parameters except for DM, ADL, NFE, and DPPH. During the second year, grain fibres decreased (NDF by 9.9%, ADF by 9.3%, and CF by 17.0%). The second year was also characterised by a decrease in ash content (40.7%), ME (1.5%), and DPPH antioxidant activity (25%). Interestingly, CP and EE increased during the second year by 13.0 and 7.1%, respectively. Grain in vitro digestibilities slightly increased during the second year (CP by 2.6%, OM by 1.6%, and true digestibility by 3.3%). For straw, the difference between the ecotypes was also significant ($P < 0.05$) for all parameters except for DM and ADL. Unlike grains, straw fibres increased during the second year (NDF by 8.3%, ADF by 14.2%, and CF by 6.8%). The nitrogen-free extract increased by 2.9%, while CP and EE decreased by 28.8 and 7.7%, respectively.

According to genetic parameters, bromatological variability was lower compared to agro-morphological one. Only GCV and PCV of DPPH and FRAP antioxidant activities were higher than 20% for grains. The heritability estimation was higher than 75% for all the parameters except for DM. The DM and IVTD traits showed the highest GAM (92.8%), while the lowest value was observed for the EE trait (1.4%). Compared to the grains, in a general way, the straw showed weaker coefficients of variation. On the other hand, like for grains, the heritability of the straw

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Table 3. Descriptive statistics and genetic parameters of agro-morphological traits of 17 cultivated Moroccan bitter vetch ecotypes across phenological stages

										Years (Y)		Phenological stages (PS)									
Traits	Mean	Min	Max	SEM	GCV	PCV	H^2	GA	GAM	2019	2020	SF	FF	PODS	E	Y	E × Y	PS	E × PS	Y × PS	E × Y × PS
Number of primary branches	3.4	3.0	3.8	0.04	19.3	19.4	99.1	1.5	39.7	3.4	3.4	3.5	3.4	3.3	**	ns	ns	**	ns	*	ns
Plant height (cm)	26.3	20.4	32.1	0.34	10.9	11.1	97.8	5.9	22.3	24.8	27.8	25.5	28.2	25.3	***	***	***	***	***	***	***
Stem diameter (cm)	2.3	2.0	2.6	0.02	9.8	11.6	71.4	0.0	17.1	0.2	0.2	0.2	0.3	0.2	***	ns	***	***	***	**	***
Internode number	3.4	3.0	3.8	0.03	5.1	5.6	82.2	0.3	9.5	3.4	3.5	3.3	3.6	3.4	***	*	**	***	***	ns	***
Leaf width (cm)	1.9	1.0	2.2	0.02	13.0	13.3	95.2	0.5	26.1	1.9	1.9	1.9	1.9	1.9	***	ns	ns	ns	***	ns	ns
Leaf length (cm)	10.1	9.6	10.9	0.05	4.1	4.2	95.9	0.8	8.2	9.7	10.5	10	10.4	10	***	***	***	***	***	ns	***
Leaflet number	23.9	21.0	26.4	0.14	5.9	5.9	97.6	2.9	12.0	23.6	24.1	22.4	24.9	24.3	***	***	***	***	***	ns	***
Leaf number	35.1	23.9	46.3	0.64	15.3	15.7	95.4	10.8	30.8	36.7	33.5	31.7	40.6	33.1	***	***	***	***	***	***	***
Start of flowering (days)	82.1	77.7	93.3	0.49	3.5	3.6	96.2	5.8	7.1	82.4	81.7				***	*	***				
Start of flowering (GDD)	813.4	790.1	836.7	6.1	6.6	6.7	98.7	110.7	13.6						***	***	***				
Full flowering (days)	91.0	86.3	97.7	0.46	3.3	3.4	94.2	6.0	6.6	91.4	90.5				***	*	***				
Full flowering (GDD)	920.9	896.5	945.3	6.1	4.0	4.1	94.8	73.1	7.9						***	***	***				
Pod setting (days)	105.7	98.0	113.5	0.47	4.1	4.1	99.5	9.0	8.5	106.5	104.8				***	***	***				
Pod setting (GDD)	1114.0	1088.6	1139.4	7.5	5.8	5.9	99.2	133.5	12.0						***	***	***				
Flowering duration (days)	23.6	12.9	28.7	0.52	20.2	20.4	98.2	9.7	41.2	24.1	23.1				***	**	***				
Flowering duration (GDD)	300.6	298.5	302.7	7.1	20.9	21.1	98.3	128.3	42.7						***	ns	***				
Root length (cm)	4.4	2.8	6.4	0.08	15.2	16.8	82.1	1.3	28.4	5.4	3.4				***	***	***				
Pod length (cm)	1.6	1.5	1.8	0.01	5.5	5.6	93.8	0.2	10.9	1.6	1.6				***	*	ns				
Grains per pod	2.8	2.1	3.2	0.02	9.5	10.0	89.8	0.5	18.5	2.7	2.8				***	***	***				
Pod number	21.3	12.8	31.3	0.32	23.7	24.8	91.3	9.9	46.6	21.1	21.4				***	ns	*				
Grains per plant	51.2	33.5	84.7	0.87	24.6	25.8	90.9	24.7	48.3	48.0	55.1				***	***	ns				
Harvest index (%)	33.6	16.3	48.1	1.01	30.2	30.5	97.9	20.7	61.5	32.7	34.5				***	*	ns				
Thousand seed weight (g)	31.6	29.4	35.0	0.20	5.2	4.8	94.6	3.3	10.5	31.1	32.1				***	***	***				
Grain yield (T ha^{-1})	1.002	0.457	1.350	0.028	25.2	25.5	97.4	0.5	51.2	0.934	1.070				***	***	**				

Min, minimum; *Max,* maximum; *SEM,* standard error of the mean; *GCV,* genotypic coefficient of variation (%); *PCV,* phenotypic coefficient of variation (%); H^2, broad-sense heritability (%); *GA,* genetic advance; *GAM,* genetic advance as a percentage of the mean (%); SF, start of flowering; FF, full flowering; PODS, pod setting; *E*, ecotype. n.s; represent non-significant.
*$P < 0.05$.
**$P < 0.01$.
***$P < 0.001$.

Table 4. Descriptive statistics and genetic parameters of bromatological traits grains and straw of 17 cultivated Moroccan bitter vetch ecotypes

Traits	Min	Max	Mean	SEM	GCV	PCV	H^2	GA	GAM	Year		E	Y	E × Y
										2019	2020			
Grains														
Dry matter (%)	92.0	94.0	92.8	0.12	0.2	0.5	40.1	0.4	92.8	92.6	93.0	ns	ns	ns
Neutral detergent fibre (% DM)	17.7	27.2	23.0	0.40	11.5	11.9	96.4	5.5	23.0	24.2	21.8	***	***	***
Acid detergent fibre (% DM)	10.7	13.8	12.8	0.18	5.3	7.1	74.9	1.4	12.8	12.9	11.7	**	***	***
Acid detergent lignin (% DM)	4.1	8.4	5.5	0.15	17.5	19.7	88.7	2.0	5.5	5.5	5.5	*	ns	ns
Crude fibre (% DM)	4.1	6.8	5.4	0.11	10.7	12.1	88.3	1.2	5.4	5.9	4.9	***	***	***
Crude protein (% DM)	18.1	26.8	22.9	0.33	8.8	9.4	93.6	4.1	22.9	21.5	24.3	***	***	***
Ether extract (% DM)	1.2	1.8	1.4	0.03	9.5	11.8	80.4	0.3	1.4	1.4	1.5	**	*	**
Ash (% DM)	3.7	5.2	4.3	0.13	10.6	10.9	97.7	0.9	4.3	5.4	3.2	***	***	***
Nitrogen-free extract (% DM)	62.6	69.0	66.0	0.30	2.7	3.0	91.3	3.7	66.0	65.9	66.1	***	ns	***
In vitro crude protein digestibility (% DM)	50.4	61.3	54.4	0.28	4.4	3.8	96.7	4.8	8.9	53.7	55.1	***	***	***
In vitro organic matter digestibility (% DM)	84.7	93.5	89.0	0.37	2.5	2.8	89.3	4.5	89.0	88.3	89.7	***	*	**
In vitro true digestibility (% DM)	88.4	95.6	92.8	0.35	2.0	2.2	87.5	3.8	92.8	91.3	94.3	***	***	*
Metabolisable energy (MJ kg^{-1} DM)	12.0	13.9	12.9	0.07	2.7	3.5	75.4	0.7	12.9	13.0	12.8	***	*	ns
Phenols (mg TAE 100 g^{-1} DM)	111.6	168.6	146.3	2.44	8.3	9.4	88.9	25.1	17.1	147.7	144.5	***	ns	***
Total tannins (mg TAE 100 g^{-1} DM)	100.6	179.4	144.7	2.61	10.7	10.7	99.8	31.7	22.0	143.5	144.3	***	ns	***
Condensed tannins (mg TAE 100g^{-1} DM)	87.6	176.7	137.5	2.84	13.7	13.7	99.9	38.8	28.2	136.2	138.7	***	ns	***
DPPH (IC50 µg mL^{-1})	0.218	0.503	0.324	0.016	20.0	20.5	97.4	0.1	41.1	0.352	0.298	***	***	***
FRAP (mg $FeSO_4$ g^{-1} DM)	0.094	0.319	0.207	0.006	26.1	26.2	99.6	0.1	53.7	0.202	0.208	***	ns	***
Straw														
Dry matter (%)	88.4	90.8	89.4	0.15	0.3	0.7	37.1	0.5	89.4	89.6	89.3	ns	ns	ns
Neutral detergent fibre (% DM)	58.2	70.7	64.3	0.60	4.7	5.3	89.2	6.3	64.3	61.8	66.9	***	***	***
Acid detergent fibre (% DM)	44.4	56.7	50.5	0.63	5.6	6.5	86.5	5.9	50.5	47.1	53.8	***	***	***
Acid detergent lignin (% DM)	15.8	19.9	18.6	0.20	2.5	5.2	47.1	0.9	18.6	18.5	18.6	ns	ns	ns
Crude protein (% DM)	8.7	16.6	10.7	0.29	16.1	17.0	94.6	3.5	10.7	12.5	8.9	***	***	**
Crude fibre (% DM)	43.3	56.1	51.4	0.47	7.2	7.7	93.9	7.7	51.4	49.7	53.1	***	***	***
Ether extract (% DM)	0.9	1.5	1.2	0.02	8.9	12.6	70.7	0.2	1.2	1.3	1.2	*	*	*
Ash (% DM)	4.1	9.1	6.5	0.20	16.5	19.4	85.3	2.2	6.5	6.8	6.3	***	ns	*
Nitrogen-free extract (% DM)	23.1	40.3	30.2	0.62	16.0	17.0	94.2	10.0	30.2	29.8	30.6	***	**	***
In vitro enzymatic organic matter digestibility (% DM)	22.7	37.0	30.0	0.46	10.8	11.7	92.7	6.7	30.0	30.0	30.1	***	ns	ns
In vitro true digestibility (% DM)	28.8	47.7	40.9	0.68	10.2	11.4	89.2	8.6	40.9	40.9	41.0	***	ns	*
Metabolisable energy (MJ kg^{-1} DM)	2.6	5.0	3.2	0.06	14.2	15.4	92.2	0.9	3.2	3.1	3.3	***	ns	ns

Min, minimum; *Max*, maximum; *SEM*, standard error of the mean; *GCV*, genotypic coefficients of variation (%); *PCV,* phenotypic coefficients of variation (%); H^2, broad-sense heritability (%); *GA*, genetic advance; *GAM*, genetic advance as percentage of the mean (%); *DPPH,* 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity; *FRAP,* ferric reducing ability of plasma; *Phenols*, total phenols; *CT,* condensed tannins. n.s; represent non-significant.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

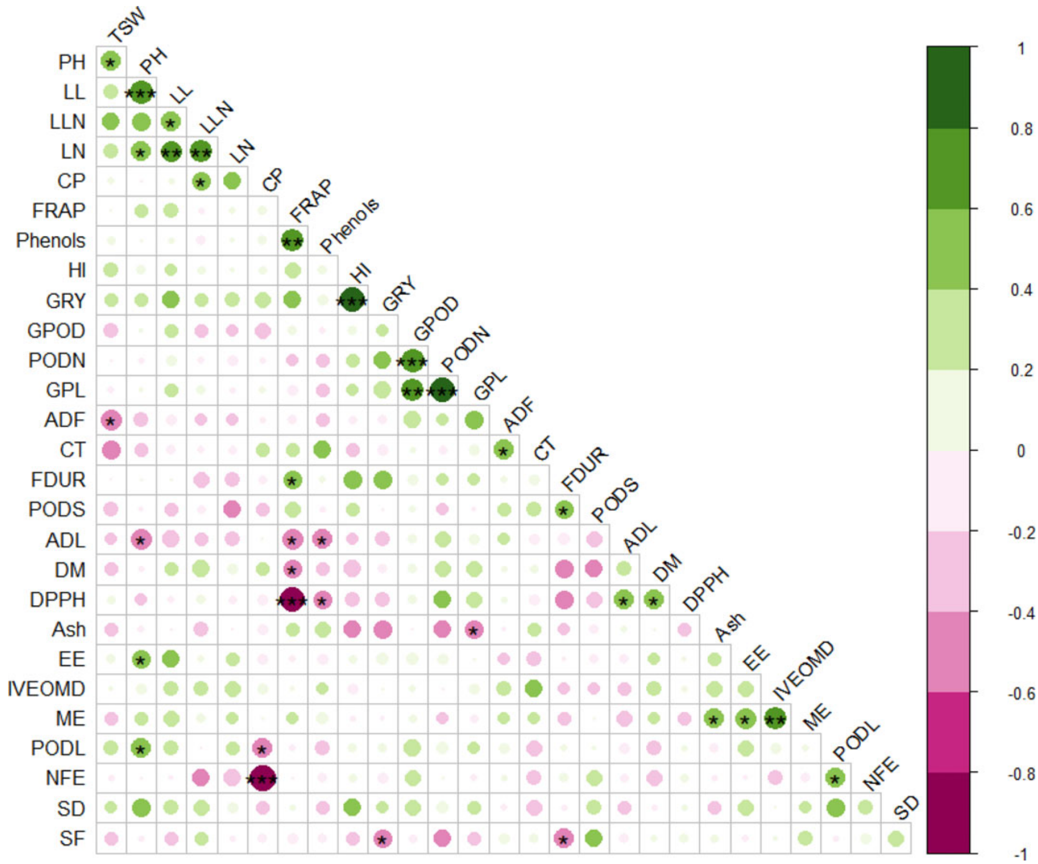

Figure 2. Correlation matrix of agro-morphological and bromatological traits evaluated in 17 Moroccan bitter vetch ecotypes. *Note*: The positive correlations are displayed in green and the negative ones in purple. The colour intensity and the size of the circle are proportional to the correlation coefficients. On the right side of the correlogram, the legend colour shows the correlation coefficients and the corresponding colours. *PH*, plant height (cm); *SD*, stem diameter (mm); *LL*, leaf length (cm); *LLN*, leaflet number per leaf; *LN*, leaf number; *FDUR*, flowering duration (days); *SF*, days to start of flowering (days); *PODS*, days to pod setting (days); *GPL*, grains per plant; *GPOD*, grains per pod; *PODN*, pod number per plant; *PODL*, pod length (cm); *HI*, harvest index (%); *GRY*, grain yield (T ha^{-1}); *DM*, dry matter (%); *ADF*, acid detergent fibre (% DM); *ADL*, acid detergent lignin (% DM); *CP*, crude protein (% DM); *EE*, ether extract (% DM); ash, (% DM); *NFE*, nitrogen-free extract (% DM); *IVEOMD*, in vitro enzymatic organic matter digestibility (% DM); *ME*, metabolisable energy (MJ kg^{-1} DM). *Phenols,* total phenols (mg TAE g^{-1} DM); *FRAP*, ferric-reducing antioxidant power (mg FeSO4g DM); *DPPH*, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (EC50 μg mL^{-1}); *CT*, condensed tannins (mg 100 g^{-1} DM).

bromatological parameters was higher than 70%, except for DM and ADL, and the highest and lowest GAM were also DM (89.4%) and EE (1.2%), respectively.

The significant correlation coefficients for the vegetative parameters, yield components, and bromatological values of bitter vetch grains are shown in Figure 2 and Supplementary Table 1.

Principal component analysis

The PCA was calculated for 38 quantitative agro-morphological and bromatological parameters of the grains. The first three components explained 60.01% of the variability between bitter vetch ecotypes. Figure 3 and Supplementary Table S2 represent the distribution of variables and individuals in the first two dimensions. The first dimension (Dim1) explained 26.9% of the total

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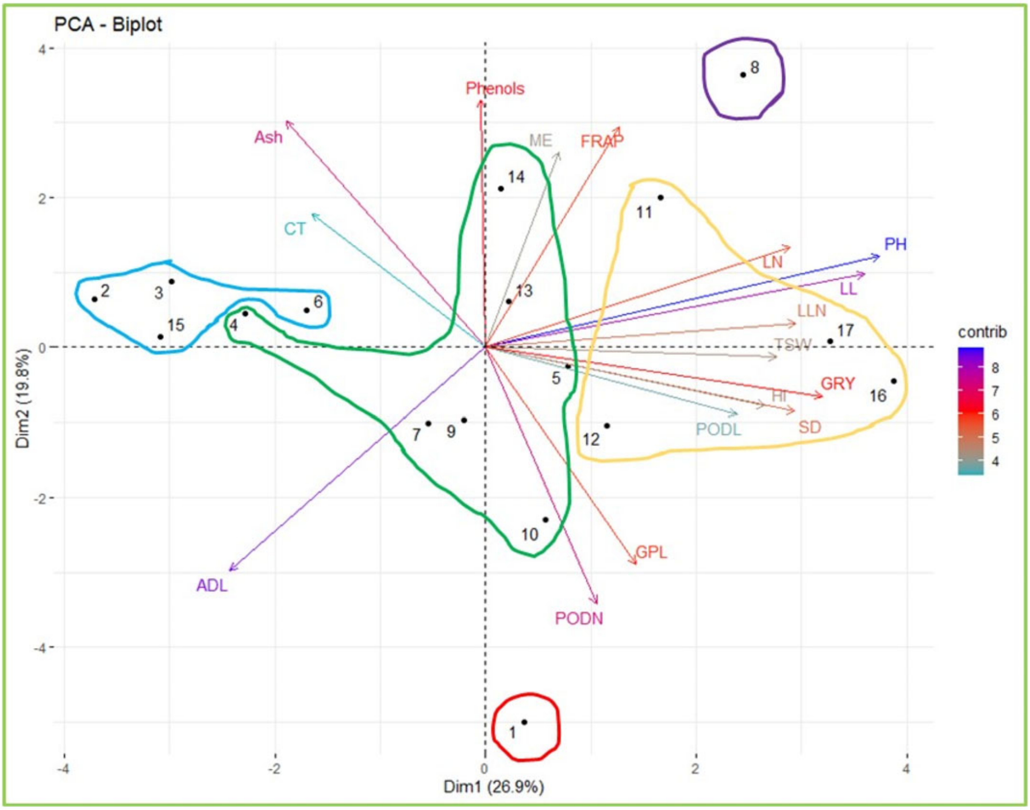

Figure 3. Graph of the variables and individuals of the principal component analysis. *Note*: *PH*, plant height (cm); *SD*, stem diameter (mm); *LL*, leaf length (cm); *LLN*, leaflet number per leaf; *LN*, leaf number; *GPL*, grains per plant; *PODN*, pod number per plant; *HI*, harvest index (%); *TSW*, thousand seed weight (g); *GRY*, grain yield (T ha^{-1}); *ADL*, acid detergent lignin (% DM); Ash, (% DM); *ME*, metabolizable energy (MJ kg^{-1} DM); *Phenols*, total phenols (mg TAE g^{-1} DM); *FRAP*, ferric-reducing antioxidant power (mg $FeSO_4$ g^{-1} DM); *DPPH*, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (IC50 mg mL^{-1}); *CT*, condensed tannins (mg $100g^{-1}$ DM). The arrow colours indicate the importance of the contribution to the two components. Five clusters were determined by the cluster heatmap analysis and represented via the five coloured circles (red, green, purple, yellow, and blue) on this figure.

variation with a maximal contribution of plant height (0.78), leaf length (0.77), leaf (0.60) and leaflet number (0.60), stem diameter (0.60), and grain yield (0.71). The second dimension (Dim2) accounted for 19.8% of the total variation, and the traits with the greatest weight on this component were pod number (–0.76), grains per plant (–0.62), FRAP (0.57), phenol (0.63), and ash (0.62) contents.

Heatmap analysis

A heatmap was conducted to cluster the ecotypes based on the agro-morpho-phenological and bromatological parameters (Figure 4). The heatmap analysis structured the dendrogram on the left side of the figure according to bitter vetch ecotypes, and the second dendrogram at the top side showed the parameters that contributed to the clustering. The heatmap described five clusters in Figure 3 by the five coloured circles. The variables were divided into four groups. The first group was related to yield parameters (grain yield, harvest index, grains per plant, pod number, and length) and stem diameter, the second one to vegetative parameters (leaf length, leaf and leaflet

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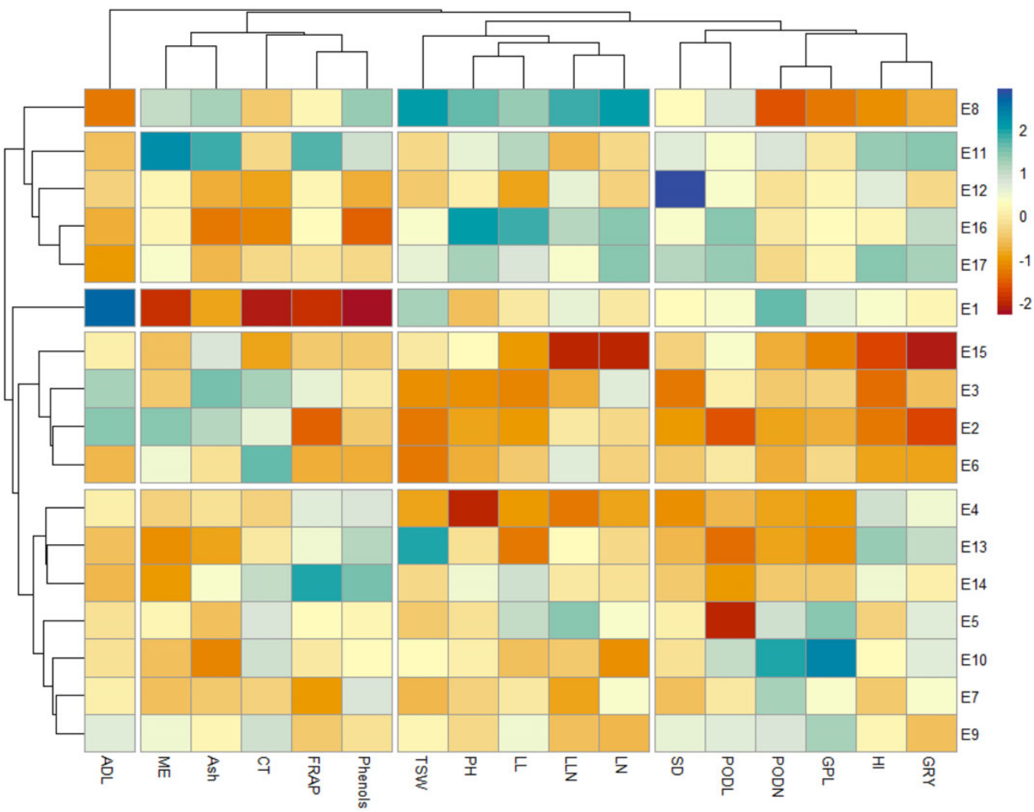

Figure 4. Heatmap and two-dimensional dendrogram of 17 Moroccan bitter vetch ecotypes. *Note*: The heatmap plot describes the relative abundance of each bitter vetch ecotype (rows) within each trait (column). The colour code (blue to dark red) displays the values of the parameters: blue colour indicates high values, while red indicates low values. The dendrogram (on the left) shows the hierarchical clustering of bitter vetch ecotypes based on the Euclidian distance and Ward's clustering method. *Phenols*, total phenols (mg TAE $100g^{-1}$ DM); *FRAP*, ferric-reducing ability of plasma (mg $FeSO_4$ g^{-1} DM); *CT*, condensed tannins (mg TAE 100g DM); $^{-1}$ash (% DM); *ME*, metabolisable energy (MJ kg^{-1} DM); *GRY*, grain yield (T ha^{-1}); *HI*, harvest index (%); *GPL*, grains per plant; *PODN*, pod number per plant; *PODL*, pod length (cm); *SD*, stem diameter (mm); *LN*, leaf number; *LLN*, leaflet number per leaf; *LL*, leaf length (cm); *PH,* plant height (cm); *TSW*, thousand seed weight (g); *ADL*, acid detergent lignin (%).

number, and plant height) and thousand seed weight, the third one to some bromatological parameters (phenols, FRAP, CT, ash, ME), and the fourth one to the ADL.

Mantel test

The Mantel test was conducted using edapho-climatic, agro-morphological, and bromatological data. It revealed that no significant correlations were found between morpho-pheno-agronomic and bromatological parameters and geographical data of the collection sites of the ecotypes ($r = -0.06$, $P = 0.69$), nor with environmental data ($r = 0.16$, $P = 0.14$). The geographic distance and environmental data were also not correlated ($r = 0.04$, $P = 0.31$).

Discussion

Ecological evaluation

The environmental evaluation is an important tool for formulating conservation policies that naturally lead to more effective exploitation and utilisation of the targeted ecotypes (Engels and

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Thormann, 2020). The tolerance to a particular temperature range is one of the most important traits used to explain the geographic distribution of a species (Willi and Van Buskirk, 2022). During the trial, the annual temperature was comparable to the optimal long-term average required for the growth and development of the bitter vetch ecotypes. Isothermality quantifies how large the day-to-night temperatures oscillate relative to the annual oscillations (O'Donnell and Ignizio, 2012). Some species were reported to be influenced by larger or smaller temperature fluctuations within a month relative to the year (Renzi *et al.*, 2023). The long-term isothermality was 33.3% lower than the mean isothermality of the two growing seasons. It is obviously due to climate change effects and could influence the cropping of bitter vetch ecotypes in the long term. On the other hand, the second year was 31% wetter than the first, but they both stayed within the long-term precipitation range of the collection sites for the 1950–2000 period. The rainfall recorded in the sampling and cultivation sites is 300 mm higher than the ones reported for some bitter vetch fields in other Mediterranean countries (Abd El Moneim and Ryan, 2004). The collected ecotypes were found on altitudes varying from 219 to 1154 m. Hassanpour and Sahhafi (2020) reported crops reaching an altitude of 2496 m in Iranian landraces. Some authors observed that ecotypes at high altitudes might exhibit frost and cold tolerance (Abd El Moneim, 1993).

The large pH range of the sampling sites (6.5–8.7) was similar to the results reported for different *Vicia* species in Algeria (Issolah *et al.*, 2022). No study reported the effect of soil pH on symbiosis with rhizobium bacteria of bitter vetch. However, for *Vicia* species, this soil range supports the survival and activity of rhizobia, promoting effective nodulation and nitrogen fixation (Basu and Kumar, 2020).

In the same study of Issolah *et al.* (2022), *Vicia* species were found on saline soils, while in the present study, the mean electrical conductivity shows that bitter vetch can grow on a large scale of salinity ranging from low saline (5.4 mS m^{-1}) to highly saline (98.4 mS m^{-1}) soils.

The other chemical edaphic parameters were either lower, or in the range, or higher than values reported in the literature, showing great adaptability of bitter vetch. For mean textural composition, the soil of the collection farms had a predominance of coarse silt (62%). The related distribution of bitter vetch ecotypes to coarse silt could be because it promotes water retention and reduces air circulation (Bünemann *et al.*, 2018). The variability between collection sites regarding edapho-climatic parameters provided an opportunity to study the impact of the agro-climatic conditions of the collection sites on the pheno-agro-morphological variability of the ecotypes.

Agro-morphological characterisation

Evaluating agro-morphological variation in bitter vetch ecotypes is decisive in determining the adaptation, agronomic potential, and breeding value of the local ecotypes. About 82% of the cultivated ecotypes in the present study showed high germination percentages (>90%), and half had a semi-erect growth habit (Table 2). Similar results were reported for Greek and Italian landraces (Livanios *et al.*, 2018; Russi *et al.*, 2019). The predominance of the erect and semi-erect habit (86%) could be due to the effect of selection pressure for the adaptation to harvesting machines. Nearly 80% of plants did not have a pigmented stem, which could indirectly indicate lower tannin content (Smykal, 2014). The ecotypes showed a high variability of grain testa colours, with one-third having light brown colour and less than 1% showing greenish orange colour. Some ecotypes had a brown or black colour of the pattern, close to 10% each. Stem pigmentation and grain colour were reported as indicators of some plant secondary components, including tannins (Mirali *et al.*, 2016). More than 80% of the ecotypes have no pattern of testa and pyramidal shape of grains. The predominance of a characteristic for most of the parameters could be due to the exchange of seeds between regions or to the farmer selection.

In the present study, the mean values for plant height were below those mentioned for some Mediterranean ecotypes cultivated under greenhouse conditions (26.3 vs. 52.6 and 50.4 cm) (El Fatehi *et al.*, 2014; Russi *et al.*, 2019). Also, the mean stem diameter was below the values reported

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for Italian ecotypes (2.3 vs. 3.5 mm) (Russi *et al.*, 2019), probably for the same reason. However, it was in the range of 2.0 mm values reported for Moroccan ecotypes (El Fatehi *et al.*, 2014).

Mean days to the start of flowering were lower than values reported for Syrian and Italian ecotypes (82 vs. 93 and 183 days, respectively) (Larbi *et al.*, 2011; Russi *et al.*, 2019). Nonetheless, the present ecotypes were late compared to other early Moroccan ecotypes included in studies conducted under greenhouse conditions (El Fatehi *et al.*, 2014), possibly because higher temperatures for the latter ones could accelerate their start of flowering (Tun *et al.*, 2021). Moreover, the present ecotypes had lower GDD to flowering than Spanish bitter vetch germplasms (Gonzalez-Verdejo *et al.*, 2020). It indicates that, possibly due to genetic differences between the ecotypes, they require less heat accumulation to flower. Moreover, flowering duration was below 48 days recorded for the same study. The increased temperature under greenhouse conditions could also shorten the flowering duration (Nagahama *et al.*, 2018). However, the present observations were above 5–19 days recorded for 49 populations in Greece (Livanios *et al.*, 2018), showing an adaptation of the crop to diverse agroecological environments. The variability in the days to start flowering among the tested ecotypes being low, breeders could rather use the high variability for flowering durations to help them select cultivars adapted to different regions of Northern Morocco and neighbouring Mediterranean regions. Indeed, flowing duration is especially an adaptation to drought parameters (Kumari *et al.*, 2021).

In the present study, yield parameters were intermediate between values reported for Moroccan and Greek ecotypes for pod number and number of grains per pod (El Fatehi *et al.*, 2014; Livanios *et al.*, 2018). Pods were longer in the second year, seemingly explained by the higher grain yield. The pod number did not change over the two years, but grains per pod were higher in the second year. It corroborates the fact that the genotype of the plants influences pod number, while the environment influences grains per pod. Low annual rainfall in an arid environment can lead to high rates of abortion of pods after fecundation (Larbi *et al.*, 2010). Consequently, grain yield, thousand seed weight, and harvest index were higher in the rainier second year than in the first.

Except for plant height, which increased by 12% in the second year, the other morphological parameters did not exceed an 8% increase. Similarly, phenological parameters varied little between the first and second years. Moreover, an increase of 15% in grains per plant and 22% in grain yield was recorded in the second year. The slower increase in these yield parameters despite the differences in rainfall (175 mm) is manifested by the higher heritability and could be due to the resistance of the ecotypes to a slight lack of water during the first year and, thus, to a low influence of the environment on these parameters.

PCV and GCV of the studied parameters offer an opportunity to select phenotypes based on these traits. GCV and PCV were categorised as low (0–10%), moderate (10–20%), and high (>20%), as indicated by Chithra *et al.* (2022). Flowering duration, root length, and grain yield-related parameters (grains per pod and per plant, pod number, grain yield, and harvest index) were characterised by high GCV and PCV. The wide genetic diversity for these parameters provides an opportunity to improve grain yield potential, which is the main objective of breeding programs. On the other hand, those genetic parameters were low for all the other morphological parameters. This result is most likely due to bitter vetch's self-pollination, which lowers its diversity (Zohary and Hopf, 2000). Moreover, the small differences between GCV and PCV in almost all parameters indicate that phenotypic variability is a reliable measure of genotypic variability and that selection for improvement of all traits is possible and could be effective on a phenotypic basis. Similar results were reported in Iranian ecotypes about lower GCV and PCV of days to start flowering, plant height, and thousand seed weight (Hassanpour and Sahhafi, 2020).

Heritability is categorised as low (0–30%), moderate (30–60%), and high (60% and above) (Chithra *et al.*, 2022). The heritability estimates were high for almost all parameters in the present study. High heritability indicates a low influence of the environment on the expression of the characters. Therefore, breeders could select superior genotypes based on phenotypic performance for these traits. However, heritability alone does not indicate the amount of genetic improvement

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that would result from selecting individual ecotypes. High heritability and high genetic advance (GA) are considered more accurate in predicting the gains via the selection of the parameters as they may be controlled by additive gene action in their expression (Sardana *et al.*, 2007). According to Chithra *et al.* (2022), genetic advance as a percentage of the mean (GAM) is classified as low (<10%), moderate (10–20%), and high (>20%). Therefore, flowering duration, pod number, grains per plant, grain yield, and harvest index had moderate GAM and are probably governed by additive gene action (Sardana *et al.*, 2007). Hence, simple selection could be effective for improving those characters. Similar results were reported for 210 pea germplasms (Sardana *et al.*, 2007). However, the heritability estimates in the present study were higher than the values reported for Iranian ecotypes (Hassanpour and Sahhafi, 2020), presumably due to the lower effect of the environment on the expression of the traits.

Bromatological parameters

Analysing the influence of genotype and environment on bitter vetch bromatological characteristics is important to help identify the most suitable conditions for accumulating beneficial compounds in the grain. Following descriptive statistics, a decrease in fibres and ash contents, an increase in CP and EE contents, and different digestibilities were reported for grains in the second year. It could be due to the higher rainfall in the second year that promoted the accumulation of carbohydrates instead of fibres (Panozzo and Eagles, 1999). However, the decrease in the ash content could be due to a dilution effect caused by the competition for minerals induced by the higher yield in the second year (Murphy *et al.*, 2008). The nutritive value of the straw showed the opposite trend of grains, with an increase in different fibres and a decrease in CP, CF, EE, ash, and NFE contents. It is presumably due to the intensive exportation of organic matter from leaves and stems to grains, as grain yield and harvest index were high in the second year.

According to several studies in the Mediterranean area, anti-nutritional factors can be the first limitation to bitter vetch grain use (Sadeghi *et al.*, 2009). In the present study, the phenols and condensed tannins (CT) in the grains were lower than those reported for Iranian bitter vetch grains (Golchin-Gelehdooni *et al.*, 2014) (0.15 vs. 0.20% DM and 0.14 vs. 0.23% DM, respectively). Due to these very low values, tannins are not the anti-nutritional factors responsible for the non-use of bitter vetch. For the nutritive value of the grains, the ecotypes in the present study had lower CP (22.9% DM) compared to other ecotypes from the Mediterranean area (26.6–28.0% DM) (Larbi *et al.*, 2011; Sadeghi *et al.*, 2009). However, a maximal value of 26.8% was reported for one ecotype, highlighting the importance of selection. Interestingly, EE, NFE, and ash contents were higher than values reported for Iranian ecotypes (1.4 vs 0.4, 66.0 vs 58.9, and 4.3 vs 3.4% DM, respectively) (Sadeghi *et al.*, 2009). For the studied straw, even if CP content was slightly higher than values reported for Syrian ecotypes (10.7 vs. 9.1% DM), the OM digestibility was much lower (30.0 vs. 52.3%) (Larbi *et al.*, 2011). All these parameters are important for characterising bitter vetch as animal feed, but they could also be used to exploit bitter vetch seeds and sprouts as functional foods.

The genetic variability of the bromatological parameters of the grains and straw was lower than that of agro-morphologic parameters. Most of the bromatological parameters of the grains straw presented medium to low variability. Otherwise, traits with reasonable variations present a wide opportunity for improvement (Olanrewaju *et al.*, 2021). Thus, traits exhibiting low GCV and PCV show low variability; hence, they cannot be used to discriminate among the ecotypes for crop improvement. However, considering heritability and genetic advance, almost half of the grain and straw bromatological in this study showed both high heritability and genetic advance, which implies that a direct selection could be recommended because of the highly addictive gene effect.

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Correlation analysis, principal component analysis, and heatmap

The positive correlation between plant height, leaf length, and leaf number suggests the possible orientation of some ecotypes towards hay and fodder production. Similar correlations were reported for other species as those are photosynthesis-improving parameters (Amitrano *et al.*, 2021). Crude protein is the first component looked for in a legume. The positive correlation between leaflet number and CP was also supported by several authors who reported a higher concentration of CP in leaves (Hakl *et al.*, 2016). This latter strong correlation showed that future ecotype selection could use leaflet numbers during preliminary evaluation, as this trait is easier to measure than crude protein, which needs laboratory analysis using expensive chemicals and materials. Concerning phenology parameters, the negative correlation between flowering duration and the start of flowering was also expected, as it implies that late ecotypes have a shortened flowering duration, probably as an adaptation strategy which allows them to escape drought at grain filling period, as soil humidity helps to improve the translocation of organic matter to grains (Murphy *et al.*, 2008). The negative correlation between the start of flowering and grain yield makes it clear that this adaptation could not be enough to avoid yield losses and that phenology is an important parameter to consider when choosing the suitable ecotype for a particular region. Similarly, in several Mediterranean legumes (Livanios *et al.*, 2018), grain yield was expected to be positively correlated to the harvest index, as ecotypes in Morocco are cultivated solely for grain production (Enneking *et al.*, 1995). The ME was positively correlated to EE and OM digestibility. These results are logical, as fat is an important energy source (Singh *et al.*, 2017).

The principal component analysis is a multivariate statistical tool that aims to summarise and analyse the relationships between many variants and reduce the data's dimensionality while retaining all crucial information from the original genotype data set (Price *et al.*, 2006). In the present study, 60.01% of the variability was explained by the first three components. In characterising Greek ecotypes of bitter vetch, the first three components explained only 46.5% of the variability (Livanios *et al.*, 2018). The difference could possibly be due to the lower inter-population and higher intra-population diversity levels of their collection. In the present study, yield contributing parameters, including grain yield, thousand seed weight, and pod length, are related to the first principal component, as in various legume studies (Hassanpour and Sahhafi, 2020; Livanios *et al.*, 2018). According to this analysis, the first component concerned agronomical (plant morphology) and grain yield at plot level parameters. The second component was positively related to bromatological parameters (phenols, ash, and FRAP) and negatively to grain at plant level dimension (grains per plant and pod number). The distribution of the ecotypes on the axis will contribute to easily choosing the ecotypes for preliminary selection. The ecotypes E2, E3, E4, E6, and E15 placed on the negative side of the first component should be avoided as they combine low vegetative and yield parameters.

The heatmap is a data imagining practice that displays the extent of a phenomenon as colour in two dimensions. The colour variation gives the reader noticeable visual indications about how the phenomenon is clustered or varies over space. In genetic studies, the heatmap visualises the relative patterns of high-concentrated parameters against a background of mostly low-concentrated or absent parameters. Heatmap is a multi-information tool that allows plant breeders to develop varieties for specific agroecological zones and different purposes. In the present study, the first cluster composed of one ecotype (E8) was characterised by high vegetative parameters and nutritive value and low grain yield components. This ecotype could be destined for hay production. Its high thousand seed weight makes it an interesting candidate to improve this parameter. However, its low grain yield and harvest index could hinder the selection. The second cluster had intermediate to high grain yield and vegetative parameters, while except for ecotype E11, they had low bromatological parameters. This interesting grain yield and vegetative parameters could classify this cluster as a dual-purpose group. Ecotype E11 of this group is probably better as it had high ash, metabolisable energy, phenols, and antioxidant activity. It appears to be more promising as it combines high-yield and high-nutritive value. The third group was only composed of ecotype E1, characterised by a low nutritive value and a medium yield. The four ecotypes of the fourth cluster (E15, E3, E2, and E6) were the least interesting according to all the variable groups, except E2, which had high ME. Intermediate values for all the parameters characterised the fifth cluster, which was subclustered into two groups. Ecotypes E4, E13, and E14 were characterised by high grain yield, harvest index, phenol contents, FRAP, and low grain-yield components parameters, making them also important for selection. The opposite characterised the rest of the ecotypes.

The absence of correlation between morpho-pheno-agronomic and bromatological parameters, geographical data of the collection sites of the ecotypes, and environmental data could be due to the highly variable altitudes in the Rif Mountains and neighbouring regions where the ecotypes were sampled. Moreover, the clustering of the ecotypes was independent of the region from which they were collected; it could be explained by the free exchange of seed materials between farmers, which led to gene flow among different regions. Scientists have discussed genetic drift, selection pressure, and environment as other major factors that could cause greater diversity than geographical distance (Star and Spencer, 2013). Therefore, more emphasis must be directed to the ecotype level rather than the geographical level as a source of diversity in this collection.

Conclusion

The results of the present study indicate that the cultivated ecotypes of bitter vetch showed higher variability for grain yield components compared to morphological and bromatological parameters. Despite a relatively lower protein content, the grains presented more interesting digestibility and metabolisable energy than ecotypes from other Mediterranean countries. The calculated genetic parameters emphasised the possibility of selecting highly productive and nutritive cultivars. However, interannual variations were also detected, making the selection of the ecotypes harder. Despite the low variability, the multivariate analyses (principal component analysis and heatmap) allowed the clustering of interesting ecotypes for animal production and bitter vetch selection programs. The multivariate analyses allowed to highlight the second cluster, showing interesting grain yield and vegetative parameters, particularly the ecotype E11 with a better nutritive value. In addition to agro-morphological and bromatological characterisation, future molecular analysis of this ecotype collection could help discern similarities and prevent duplication of similar genetic ecotypes during the selection program. This study was repeated in two years. Therefore, multi-locational and multi-environmental trials should be used to evaluate and give precise locational responses for better crop improvement for the environment-dependent traits, especially in drought conditions, which should be of interest in these times of climate change.

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Data availability statement. The datasets presented in this study can be sent by a request to the authors.

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