

Original Research

# Agro-Morphological, Yield Components and Nutritional Quality Attributes of *Vicia faba* L. var. *Minor* Cropped in Tunisian Arid Regions

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## Abstract

Faba bean (*Vicia Faba* var. *Minor*) is of great importance as it is commonly used as an excellent protein source in food and feed. In Tunisia, the wide variability among local genetic resources might be valorized by preservation and breeding programs. For that, the knowledge of the diversity within this crop and its distribution across the oasis could be of great help in managing and improving its germplasm. The objectives of the present study were to assess the phenotypic diversity within a germplasm of 23 populations of local faba bean cropped in Tunisian arid regions. Characterization was undertaken based on 29 parameters related to seeds, plant growth, flowers, and pods characteristic. This agro-morphological characterization was carried out based on UPOV and Bioversity International descriptors. Crude protein (CP), neutral and acid detergent fibers (NDF, ADF), and *in vitro* dry/organic matter digestibility (*IVDMD/IVOMD*) parameters were also analyzed. Results revealed a considerable genetic variability for most of the agro-morphological parameters. In fact, significant differences ( $p < 0.05$ ) were revealed by ANOVA for the majority of the analyzed quantitative traits. The coefficient of variation, used as a homogeneity index, was above 1.44 % for all characters, which ensures the predominance of genetic components in the differences among populations. The overall variability was analyzed *via* multivariate and dimension reduction approaches relatively using hierarchical clustering and PCA methods, in order to classify populations into relatively homogenous groups after the identification of the major traits contributing to the overall diversity. The superior populations with the best precocity (95 DAS), with high total yielding per plant (500 g), and having the highest digestibility (96.5%), the highest CP content (29.6%), and the least NDF content (44.5%) were identified.

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This assessment of traits diversity can assist breeders to manage and to valorize populations with desirable characteristics to be used in various breeding programs.

**Keywords:** germplasm, feed, breeding, multivariate analysis, *Vicia faba* var. *minor*

## Introduction

Faba bean (*Vicia faba* L. var. *minor*) is the most important grain legume for direct human consumption. In recent years, cultivation of this crop has received large attention in Canada, USA and Europe [1-3]. As a legume, faba bean has the ability to fix atmospheric nitrogen *via* symbioses with rhizobia bacteria. Thus, improving soil quality and saving nitrogen fertilizer [4-6].

In addition to being high in protein 19-39% dry weight [7, 8] with a high level of lysine, Faba bean flour had a high content of starch (27-50% dry weight) [9]. Furthermore, the beans, whose outer seed coat is removed, can be eaten raw or cooked in several regions such as North Africa, West Asia, and Ethiopia. Moreover, faba bean has a valuable potential as animal feed, particularly as an alternative to soybean (*Glycine max* (L.) Merr.) [10, 11].

Nevertheless, the majority of seed legume crops are facing a lack of performance due to low yields and yield instability over seasons and environments; inducing insufficiency and unprofitability for farmers [12, 13].

In Tunisia, this problem increases with aridity and the sustainable use of saline water in irrigated agriculture of the southern region environment. Native faba bean germplasm is imperatively being more valorized for an efficient use in feed production. Several studies have been carried out on faba bean and other legume species [14], considering mainly genetic [15], physiological [16] Phyto-pathological [17] and biochemical [18] assessments.

Actually, according to the last statistics provided by the Tunisian ministry of agriculture, faba bean is

cropped on 52 100 ha where the production reaches about 67 500 tons [19].

To cope with the rising cost of raw materials used for the animal feed industry (soybean and maize), researchers in Tunisia are increasingly interested in developing performing local fodder protein sources such as faba bean [20-22].

These genetic resources exhibit a wide diversity that would be useful to perform breeding programs promising genotypes providing high performance (yield, feed quality, etc.), and ensuring an acceptable genetic fitness and stability under varying environmental conditions [23].

In this context, a breeding program which concerns a collection of faba bean genetic resources collected from different Tunisian oases in order to preserve this germplasm and develop new high-performance synthetic varieties. This breeding program had been undertaken in the Arid Lands Institute aiming to create new varieties adapted to a changing climate of arid regions [24]. The present study was performed as a part of this program. Twenty-three faba bean genotypes were evaluated for their agro-morphological performance, chemical composition, and *in vitro* digestibility. The high-performing plants and the most interesting traits will be used for selection and breeding program.

## Experimental

### Plant Material and Experimental Design

Twenty-three faba bean (*Vicia faba* L. var. *minor*) populations were originally collected and assembled

Table 1. Climatic data during trial period (from October 2015 to May 2016) along the experimental period under open field conditions.

Months	T (°C)	TM (°C)	Tm (°C)	H(%)	PP (mm)	V (km/h)	VM (km/h)
October	24.70	30.20	20.30	58.10	59.18	8.10	14.70
November	17.20	21.50	13.30	69.70	54.11	7.60	13.70
December	13.30	19.20	8.40	65.20	0.51	4.00	9.70
January	14.50	19.70	9.30	49.30	1.27	7.60	16.50
February	15.80	21.10	10.00	44.50	1.02	10.60	16.70
March	17.40	23.70	10.60	37.70	0.25	11.40	17.90
April	20.60	27.30	14.90	51.00	1.52	11.50	18.50
May	23.60	30.20	17.80	45.60	1.78	12.50	20.40

T : Average Temperature (°C), TM : Maximum Temperature (°C), Tm : Minimum Temperature (°C), H : Average relative humidity(%), PP : Total rainfall and/or snowmelt(m), V: Average wind speed(km/h), VM : Maximum sustained wind speed(km/h)

by Arid Lands Institute researcher from the southern Tunisia's oasis.

These populations were grown in the experimental field of the Arid Lands Institute of Medenine located in southern Tunisia (33°29'57.80"N, 10°38'32.96"E, Altitude 16 m). The trial was conducted in a randomized complete block design (RCBD) with three replications. Seeds of faba bean genotypes were planted on 12<sup>th</sup> of October 2015. Monthly climate data were recorded during the trial period (Table 1). Seeds of faba bean populations were planted in rows with 60 × 60 cm of inter-plants distance. Each of the 23 populations was represented by ten plants per row on each block. Soil of experimental research station is loamy sand with low organic matter.

The plots were immediately irrigated after sowing and then subsequently irrigated twice weekly with equal quantities during the trial period and supplied with a mineral fertilizer (NPK 20-20-20). Weeds were hand-controlled, while chemical pesticides are often used to control diseases and pests.

### Agro-Morphological Characterization

These populations were characterized according to the descriptors established by the International Union for the Protection of New Varieties of Plants [25]. A total of 29 agro morphological traits (Table 2) were used. Time of flowering and pod maturity (days) were recorded from visual observations on each planted row (population). At maturity, 10 randomly selected plants from each population (10 samples per population for each block) were used to measure agro-morphological traits on individual plant basis (i.e., plant height was measured from soil surface to the upper most tip of the plant, number of pods bearing branches/plant, number of pods/plants, number of seeds/plant and seed yield/plant). Seed compounds traits were assessed based on 10 randomly sampled pods per plant.

### Chemical Composition and *in vitro* Digestibility

#### *Chemical Composition*

Three representative samples of seeds per population were subjected to chemical composition surveys. Each sample, composed by 200 g of seeds, was divided into two parts for independent analysis. The first subsample was dried at 105°C to determine the dry matter (DM) content. The second was dried at 65°C for 48 h to determine the chemical composition. Ash was determined by incinerating samples in a furnace at 550°C for 6 h and the crude protein (CP) was determined by Kjeldahl method [26]. Analysis of neutral and acid detergent fibers (NDF, ADF) was performed according to the method described by [27].

### *In vitro* Digestibility

The *in vitro* digestibility was estimated using the first step of [28]; this method involves incubating the feed sample for 48 hours in the presence of microbial rumen flora in conditions similar to those of the animal's natural rumen. The incubation was conducted in 100 mL flasks, adding in each 0.5 g of each faba sample (ground to 1 mm) 40 mL of artificial saliva (mix of 6 solutions) (Table S1) and 10 mL of rumen fluid collected by oral esophageal rumen recovery by using a soft and flexible rubber and a vacuum pump (Alcatel, Annecy, France) from three adult goats. Flasks were then transferred to a water bath oven at 39°C. Simultaneously, a series of blank flasks (without rumen fluid) were prepared. All samples were incubated for 48 h then saturated with CO<sub>2</sub>; the pH was adjusted to 6.8. After incubation, samples were filtered; the residue obtained was dried at 105°C, then incinerated at 550°C and weighted.

*In vitro* digestibility was calculated as follows:

$$IVDMD (\%) = \frac{I-F1}{I} \times 100 \quad (1)$$

$$IVOMD (\%) = \frac{I-F2}{I} \times 100 \quad (2)$$

*IVDMD* : *in vitro* dry matter digestibility;  
*IVOMD* : *in vitro* organic matter digestibility;  
*I* : sample % DM: the intake;  
*F1* : residual dry weight (105°C);  
 and *F2* : residual ash weight (550°C).

### Statistical Analysis

Statistical analyzes were performed using One-way ANOVA ( $\alpha = 0.05$ ) on the centered and standardized values for each assessed trait, then population means' comparisons were given by Duncan multi-range test ( $p < 0.05$ ). The structure of genetic variability among populations based on agro-morphological, yield compound, and nutritional quality traits was analyzed using hierarchical cluster analysis (HCA) and Principal Component Analysis (PCA) on the basis of Pearson bi-variate correlations.

### Ethics Statement

Rumen fluid extracts from goats were used in the section of *in vitro* digestibility experiment. Extractions were approved by the veterinary unit of the Arid Lands Institute (IRA) of Médenine on behalf of the Tunisian committee of ethics in animal experimentation. They were collected, from goats bred in the Wildlife and Livestock Laboratory- IRA, according to protocols approved in the relevant Animal Ethics approvals by sampling through a fistula or via stomach tubing.

Table 2. Agro-morphological traits of the studied *Vicia faba var minor* populations.

Code	Trait	Method /Unit
Pheno-morphological traits		
STEMCOLOR	Stem color	3: low; 5: medium; 7: high
GROWHABIT	Growth habit	1: undetermined; 2: determined
PLANTHGT	Plant height	cm
STEMNBR	Stem number	Count
NODNBR	Nodes number by pod	Count
LENGTHLEALET	Leaflet length	cm
LEAFLETSH	Leaflet shape	Leaflet shape observed on middle leaflet of fully expanded leaf at the intermediate flowering node of the plant. 1: narrow; 2: intermediate; 3: round
LEAFCOL	Color of leaflet	1: light green; 2: medium green; 3: dark green
FLWPERINF	Flowers per inflorescence	Count
MELCOL	Melanin color	1: brown; 2: black; 3: yellow
PIGANTHO	Anthocyanin pigmentation	1: absent, 9: present
WINGPETCOL	Wing petal color	Wing petal color taken on newly opened flowers. 1: purple; 2: white; 3: light purple
STREAKINT	Intensity of streaks	Intensity of streaks on standard petal 0: absent; 3: slim ; 5: moderate; 7: intense
PODANGLE	Pod angle	Pod angle / attitude at maturity on the second or third pod bearing node. 1: very weak; 3: weak; 5: medium; 7: high
COLORPOD	Color green of pod	3: weak; 5: medium; 7: high
PODHIGHT	Pod height	Height of the lowest pod_ cm
PODWIDTH	Pod width	Pod width in mature pods from the lowest insertion point on the primary inflorescence cm
DAYSEMER- GENCE	Seedling emergence	Number of days from sowing to first observed leaf.
DAYSFLOWER	Days of flower	Number of days from sowing to first observed flower.
PODMAT	Pod maturity	Days from sowing until 90% of the pods have dried.
Yield components traits		
TOTALWEIGHT	Plant weight	g
PODPLANT	Pods per plant	count
WEIGHTPOD	Weight of 10 pods	g
SEEDSPOD	Seeds per 10 pods	Count: Average number of seeds per pod
WGHTSEEDPOD	Weight of seed by 10 pods	g
SEEDCOLOR	Color of seed	1: beige; 2: green; 3: purple; 4: violet-brown
SEEDSHAPE	Seed shape	1: circular 2: elliptical; 3: irregular
HILUMCOLOR	Hilium color	Color of hilum of mature seed. 0: colorless; 1: black
SEEDWGT	100 Seeds weight	g

### Results and Discussion

#### Pheno-Morphological Characterization of Faba Bean Populations

The survey of phenological growth stages revealed significant differences among studied faba bean populations in terms of seedling emergence date ( $p < 0.001$ ), flowering ( $p < 0.001$ ) and pod maturity ( $p < 0.05$ ) periods (Table 3). The timing of seedling emergence averages varied from 9.67 days (Populations *Vf14*, *Vf18* and *Vf23*) to 15 days after sowing (Populations *Vf6*, *Vf8*, *Vf11*, *Vf15*, *Vf19* and *Vf22*).

Flowering period averages ranged from 48.67 days (Population *Vf2*) to 100.33 days (Population *Vf22*). Whereas, averages of pod maturity period varied from 95 days for *Vf2* to 120.67 days for *Vf22*.

These values show that our populations are later than those evaluated by [29] with flowering days ranging from 33 to 78 days.

Descriptive statistics (min, max, average and variances) of the agro-morphological parameters assessed were presented in Table 4. Significant differences ( $p < 0.05$ ) were identified among the 23 populations for all morphological traits. These results largely correspond to those found by [29] for a similar study on faba bean genotypes for the same traits.

The population *Vf23* had the lowest coefficient of variation, 9.25% and 8.12%, respectively for the number of flowers by inflorescence and Plant height at maturity with values greater than the average recorded; while the population *Vf20* showed the highest number of stems per plant (12.86 stems), Leaflet length (14.47cm) and Pod height (13 cm).

The population *Vf22* exhibited the highest average of Plant height at maturity (143.25 cm). However, *Vf6* had the lowest average among the populations studied. On the other hand, the average Plant height of all populations (122.24 cm) was lower than that recorded by [29] (139.5 cm). Variations, in terms of pod width, were 37.71% lower for *Vf16* compared with *Vf7* which recorded an average of 1.75 cm. while nodes number per stem varied from 19.40 to 27.86 obtained for *Vf6* and *Vf13*, respectively.

The lowest coefficient of variation was observed for Plant height at maturity with the population *Vf20* (4.17%), while the Pod width and Pod height parameters showed the most important variability represented by the population *Vf8* with CV = 67.59 % and CV = 87.18%, respectively.

The averages of pod lengths (9.43 cm) were higher than those recorded in cultivar NEB404 from Beja (7.86 cm) [15] while those of number of seeds per pod (3.19 seeds) measured for the studied populations are similar to those found by [15] in the local Beja commercial cultivar (3 seeds) and the local cultivar "Hara" from Kef (2.9 seeds).

The different qualitative traits were shown in Fig. 1. The majority of populations exhibited the

Table 3. Pheno-morphological traits of the studied Vicia faba var minor populations.

Populations	DAYS-EMERGENCE	DAYSFLOWER	PODMAT
<i>Vf1</i>	13.67 <sup>bc</sup>	76.33 <sup>b-c</sup>	110.33 <sup>a-d</sup>
<i>Vf2</i>	<b>10.33<sup>a</sup></b>	<b>48.67<sup>a</sup></b>	<b>95.00<sup>a</sup></b>
<i>Vf3</i>	11.00 <sup>ab</sup>	76.67 <sup>b-c</sup>	100.00 <sup>a-c</sup>
<i>Vf4</i>	12.33 <sup>a-c</sup>	65.00 <sup>a-c</sup>	104.67 <sup>a-d</sup>
<i>Vf5</i>	12.33 <sup>a-c</sup>	77.33 <sup>b-c</sup>	115.33 <sup>b-d</sup>
<i>Vf6</i>	15.00 <sup>c</sup>	82.67 <sup>c-f</sup>	112.67 <sup>a-d</sup>
<i>Vf7</i>	12.33 <sup>a-c</sup>	66.00 <sup>a-c</sup>	102.33 <sup>a-d</sup>
<i>Vf8</i>	15.00 <sup>c</sup>	93.33 <sup>d-f</sup>	111.33 <sup>a-d</sup>
<i>Vf9</i>	13.67 <sup>bc</sup>	96.33 <sup>ef</sup>	117.67 <sup>cd</sup>
<i>Vf10</i>	13.67 <sup>bc</sup>	79.67 <sup>b-f</sup>	102.33 <sup>a-d</sup>
<i>Vf11</i>	15.00 <sup>c</sup>	91.00 <sup>d-f</sup>	107.00 <sup>a-d</sup>
<i>Vf12</i>	13.67 <sup>bc</sup>	60.67 <sup>a-c</sup>	96.33 <sup>ab</sup>
<i>Vf13</i>	12.33 <sup>a-c</sup>	75.33 <sup>b-c</sup>	96.33 <sup>ab</sup>
<i>Vf14</i>	<b>9.67<sup>a</sup></b>	74.67 <sup>b-c</sup>	110.67 <sup>a-d</sup>
<i>Vf15</i>	15.00 <sup>c</sup>	73.33 <sup>b-d</sup>	98.67 <sup>a-c</sup>
<i>Vf16</i>	12.33 <sup>a-c</sup>	64.00 <sup>a-c</sup>	98.67 <sup>a-c</sup>
<i>Vf17</i>	13.67 <sup>bc</sup>	82.67 <sup>c-f</sup>	115.33 <sup>b-d</sup>
<i>Vf18</i>	<b>9.67<sup>a</sup></b>	60.00 <sup>ab</sup>	96.33 <sup>ab</sup>
<i>Vf19</i>	15.00 <sup>c</sup>	82.00 <sup>b-f</sup>	109.00 <sup>a-d</sup>
<i>Vf20</i>	11.00 <sup>ab</sup>	89.67 <sup>d-f</sup>	108.33 <sup>a-d</sup>
<i>Vf21</i>	12.33 <sup>a-c</sup>	80.67 <sup>b-f</sup>	108.33 <sup>a-d</sup>
<i>Vf22</i>	15.00 <sup>c</sup>	100.33 <sup>f</sup>	120.67 <sup>d</sup>
<i>Vf23</i>	<b>9.67<sup>a</sup></b>	82.00 <sup>b-f</sup>	107.00 <sup>a-d</sup>
ANOVA	3.96 ***	3.67 ***	1.68 *

ANOVA test is represented by F values followed by significance levels: ns ( $P \geq 0.05$ ), \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ); mean values are followed by Duncan post-hoc multirange groups.

medium green leaf color intensity with an intermediate leaf shape, a very weak pod angle and a black melanin color.

All populations presented anthocyanin pigmentation, medium green stem color, weak green pod color, and indeterminate growth.

The intensity of streaks in faba bean flowers was rather absent, slight, moderate or intense. The highest percentage of slight and moderate streaks was about 62.5% presented by *Vf9* and *Vf8*, respectively. While, *Vf11* showed the highest percentage of intense streaks with 71.4%.

The color of petals was light purple for most populations except *Vf13*, *Vf15*, *Vf19* with 100% purple

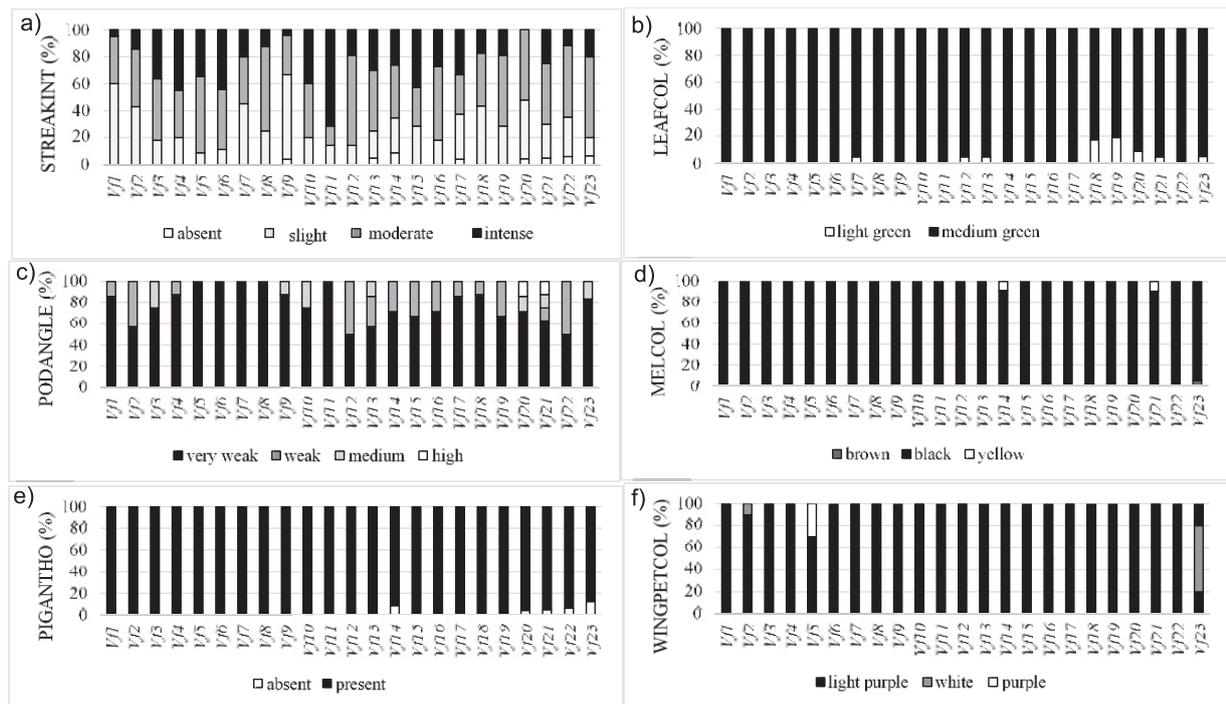


Fig. 1. Qualitative morphological traits of the studied *Vicia faba var. minor* populations; a) Intensity of streaks; b) Color of leaflet; c) Pod angle; d) Melanin color; e) Anthocyanin pigmentation; f) Wing petal color.

petals and Vf22 with 100% of white petals. According to [30], the variations in this trait (WINGPETCOL) are correlated with some chemical components such as tannin, phenolics, etc. and could be considered as an indicator to classify faba bean genotypes.

Agro-morphological traits have been considered by several researchers [15, 31-33] as the most commonly used tool for breeding and assessment of genetic resources in many countries.

Matrix of correlation (Table 7) showed different levels of significance between traits. Moderately high positive associations were detected between traits. To avoid spurious significant results due to the examination of a large number of correlations, only the correlations with  $p < 0.05$  were given.

Therefore, the highest positive correlations were detected for pod width with pod height (0.855) and stem number (0.797), followed by the correlation between days of flowering and pod maturity (0.784). Negative correlations were significant between days of seedling emergence and leaflet length (-0.649), number of flowers per inflorescence (-0.586) and node numbers (-0.430).

#### Yield Compounds of Faba Bean Populations

Six quantitative yield components traits were assessed and the results are presented in Table 5. High significant variability among populations for all traits ( $p < 0.001$ ) was obtained. The highest coefficient of variation (34.72%) was observed for the number of pods

per plant followed by the total weight of plant (33.75%); and the 100 seed weight showed the lowest coefficient of variation (10.32%).

The number of pods were determined and weighted on the basis of a single plant. The averages ranged from 33.23 to 171 pods/plant and producing from 87.27 to 500.78 g/plant for Vf22 and Vf23, respectively. Pod characteristics were assessed on the basis of 10 pods and related results revealed that highest averages were obtained by Vf20 for a weight of 10 pods (54.96 g), the number of seeds (43.57 seeds/10pods) and weight of seeds (44.25 g). Weight of 100 seeds varied from 80.17 g to 130.71 g which recorded for Vf22 and Vf8 respectively. Our results are in agreement with those reported by [18] who ranged the values from 42.70 (Saber 02 commercial cultivar to 106.40 g per 100 seeds for the cultivar NEB 404.

Within-population variability revealed that the most homogenous population was Vf18 presenting the lowest CV (<10%) for the six yield components traits (Table 5).

Table 7 shows that the most significant correlations were between the weight of pods and those of seeds per pod ( $r = 0.991$ ), as well as between the number of pods per plant and the total weight ( $r = 0.963$ ).

Three qualitative yield components were assessed (Fig. 2). Related results revealed that the majority of the populations had black hilum and elliptic shape of seed. While Vf5, Vf12, Vf13 and Vf22 were the most homogenous populations regarding seed color with 100% of violet-brown seeds, while Vf14 exhibited 100% of beige seed color.

Table 4. Quantitative agro morphological traits of the studied *Vicia faba* var *minor* populations.

Populations	FLWPERINF		LENGHTHLEALET		PLANTHGT		STEMNBR		PODHIGHT		PODWIDT		NODNBR	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
Vf1	5.02 <sup>cd</sup>	14.29	11.19 <sup>e</sup>	24.13	99.36 <sup>ef</sup>	20.59	8.57 <sup>ac</sup>	49.43	17.95	9.57 <sup>bd</sup>	1.31 <sup>a</sup>	36.87	26.43 <sup>ab</sup>	28.30
Vf2	5.86 <sup>a-c</sup>	47.09	12.57 <sup>a-c</sup>	16.32	115.93 <sup>b-c</sup>	19.81	9.71 <sup>ac</sup>	32.40	13.86	10.14 <sup>ac</sup>	1.47 <sup>a</sup>	25.34	26.57 <sup>ab</sup>	22.77
Vf3	5.81 <sup>a-d</sup>	22.39	13.08 <sup>a-c</sup>	21.59	118.25 <sup>a-c</sup>	14.36	9.00 <sup>ac</sup>	66.40	49.55	8.81 <sup>ac</sup>	1.29 <sup>a</sup>	53.56	25.25 <sup>ab</sup>	31.17
Vf4	5.43 <sup>a-d</sup>	21.90	12.35 <sup>c-e</sup>	25.37	112.19 <sup>c-f</sup>	27.62	11.38 <sup>ac-c</sup>	53.15	35.73	9.56 <sup>ac-c</sup>	1.49 <sup>a</sup>	34.74	21.13 <sup>ab</sup>	24.72
Vf5	5.37 <sup>a-d</sup>	18.44	12.36 <sup>b-c</sup>	20.88	98.57 <sup>ef</sup>	16.68	9.00 <sup>ac-c</sup>	72.86	45.00	8.29 <sup>b-d</sup>	1.14 <sup>ab</sup>	54.61	20.43 <sup>ab</sup>	29.49
Vf6	5.56 <sup>a-d</sup>	13.66	12.01 <sup>ab</sup>	18.97	91.20 <sup>f</sup>	51.41	11.20 <sup>ac-c</sup>	56.96	36.19	9.30 <sup>ac-c</sup>	1.50 <sup>a</sup>	40.82	19.40 <sup>b</sup>	36.41
Vf7	5.42 <sup>a-d</sup>	14.29	11.88 <sup>c-e</sup>	19.06	118.83 <sup>a-c</sup>	15.31	12.67 <sup>ab</sup>	31.05	19.09	10.92 <sup>ab</sup>	1.75 <sup>a</sup>	22.20	23.50 <sup>ab</sup>	24.78
Vf8	5.33 <sup>a-d</sup>	21.09	12.40 <sup>a-c</sup>	23.24	98.67 <sup>ef</sup>	11.04	5.33 <sup>c</sup>	<b>18.75</b>	67.59	4.50 <sup>d</sup>	0.50 <sup>b</sup>	87.18	19.67 <sup>b</sup>	19.64
Vf9	5.46 <sup>a-d</sup>	13.91	11.38 <sup>e</sup>	21.02	110.63 <sup>d-f</sup>	17.35	9.75 <sup>ac-c</sup>	46.44	23.20	9.50 <sup>ac-c</sup>	1.29 <sup>a</sup>	32.54	22.75 <sup>ab</sup>	24.05
Vf10	5.60 <sup>a-d</sup>	19.36	12.56 <sup>b-c</sup>	21.24	118.50 <sup>a-c</sup>	11.10	10.75 <sup>ac-c</sup>	50.65	30.66	10.31 <sup>ac-c</sup>	1.61 <sup>a</sup>	14.99	24.00 <sup>ab</sup>	12.60
Vf11	5.47 <sup>a-d</sup>	16.21	12.08 <sup>c-e</sup>	<b>14.36</b>	126.42 <sup>a-d</sup>	10.98	7.17 <sup>ac-c</sup>	49.46	11.83	10.00 <sup>ac-c</sup>	1.25 <sup>a</sup>	33.47	23.33 <sup>ab</sup>	19.48
Vf12	4.91 <sup>d</sup>	31.18	12.14 <sup>c-e</sup>	26.32	134.58 <sup>a-d</sup>	23.20	8.00 <sup>ac-c</sup>	60.26	19.56	9.00 <sup>ac-c</sup>	1.58 <sup>a</sup>	23.77	23.33 <sup>ab</sup>	24.08
Vf13	6.09 <sup>ab</sup>	19.73	12.38 <sup>b-c</sup>	22.37	135.93 <sup>a-c</sup>	<b>7.34</b>	11.14 <sup>ac-c</sup>	31.62	31.18	10.14 <sup>ac-c</sup>	1.60 <sup>a</sup>	39.31	27.86 <sup>a</sup>	21.17
Vf14	5.98 <sup>a-c</sup>	18.33	14.34 <sup>ab</sup>	21.92	133.79 <sup>a-d</sup>	<b>8.17</b>	11.29 <sup>ac-c</sup>	39.25	42.13	8.21 <sup>b-d</sup>	1.44 <sup>a</sup>	44.09	22.43 <sup>ab</sup>	<b>12.27</b>
Vf15	5.32 <sup>a-d</sup>	25.35	13.02 <sup>a-c</sup>	24.46	122.67 <sup>a-c</sup>	19.38	9.33 <sup>ac-c</sup>	56.36	52.28	6.25 <sup>cd</sup>	1.15 <sup>ab</sup>	45.57	20.83 <sup>ab</sup>	40.13
Vf16	5.21 <sup>b-d</sup>	17.83	12.27 <sup>c-e</sup>	30.82	117.79 <sup>b-c</sup>	14.83	6.29 <sup>ac-c</sup>	54.23	41.07	7.50 <sup>b-d</sup>	1.09 <sup>ab</sup>	57.20	22.57 <sup>ab</sup>	19.60
Vf17	5.54 <sup>a-d</sup>	25.41	12.88 <sup>a-c</sup>	23.09	127.93 <sup>a-d</sup>	10.01	8.57 <sup>ac-c</sup>	71.31	40.52	9.04 <sup>ac-c</sup>	1.23 <sup>a</sup>	56.08	21.14 <sup>ab</sup>	27.46
Vf18	6.26 <sup>a</sup>	18.39	13.79 <sup>a-d</sup>	17.94	133.50 <sup>a-d</sup>	12.78	11.13 <sup>ac-c</sup>	57.05	24.12	9.75 <sup>ac-c</sup>	1.56 <sup>a</sup>	34.54	22.75 <sup>ab</sup>	23.26
Vf19	5.14 <sup>cd</sup>	29.83	12.16 <sup>c-e</sup>	20.36	139.67 <sup>ab</sup>	11.88	12.50 <sup>ab</sup>	40.90	<b>9.57</b>	11.17 <sup>ab</sup>	1.65 <sup>a</sup>	16.11	24.83 <sup>ab</sup>	26.27
Vf20	5.64 <sup>a-d</sup>	18.43	14.47 <sup>a</sup>	19.22	140.64 <sup>ab</sup>	<b>4.17</b>	12.86 <sup>a</sup>	43.54	38.30	13.00 <sup>a</sup>	1.60 <sup>a</sup>	41.99	23.57 <sup>ab</sup>	13.19
Vf21	6.00 <sup>a-c</sup>	23.77	13.82 <sup>a-c</sup>	22.92	138.13 <sup>ab</sup>	<b>4.65</b>	11.50 <sup>ac-c</sup>	53.00	46.60	11.50 <sup>ab</sup>	1.63 <sup>a</sup>	42.35	24.25 <sup>ab</sup>	25.40
Vf22	5.63 <sup>a-d</sup>	21.26	12.51 <sup>a-c</sup>	31.83	143.25 <sup>a</sup>	20.04	5.67 <sup>bc</sup>	59.76	37.74	8.67 <sup>ac-c</sup>	1.25 <sup>a</sup>	53.65	21.83 <sup>ab</sup>	17.78
Vf23	5.59 <sup>a-d</sup>	<b>9.26</b>	14.37 <sup>ab</sup>	23.53	135.08 <sup>a-d</sup>	<b>8.12</b>	10.67 <sup>ac-c</sup>	49.61	13.42	11.67 <sup>ab</sup>	1.70 <sup>a</sup>	<b>12.20</b>	27.50 <sup>a</sup>	26.10
Max	6.26		14.47		143.25		12.86		13.00		1.75		27.86	
Min	4.91		11.19		91.20		5.33		4.50		0.50		19.40	

Table 4. Continued.

Average	5.55	12.70	122.24	9.72	9.43	1.39	23.28
Variance	0.11	0.83	231.41	4.71	3.26	0.08	5.58
Mean square	2.39	16.71	1408.60	27.57	17.57	0.37	34.38
F	1.63*	2.39***	3.99***	1.03*	1.61*	1.21*	1.07*

ANOVA test is represented by F values followed by significance levels: ns ( $P \geq 0.05$ ), \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ); mean values are followed by Duncan post-hoc multirange groups.

Table 5. Quantitative yield component traits of the studied *Vicia faba* var *minor* populations.

Populations	PODPLANT		TOTALWEIGHT		WEIGHTPOD		SEEDSPOD		WGHTSEEDPOD		SEEDWGT	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Vf1	57.63 <sup>bc</sup>	14.42	186.01 <sup>c-e</sup>	15.28	40.11 <sup>c-e</sup>	4.48	29.74 <sup>b-d</sup>	4.26	31.65 <sup>cd</sup>	4.66	107.35 <sup>c-f</sup>	4.02
Vf2	85.52 <sup>bc</sup>	12.82	274.47 <sup>bc</sup>	14.58	45.69 <sup>b-d</sup>	3.20	33.62 <sup>b</sup>	1.72	36.64 <sup>bc</sup>	3.14	109.08 <sup>b-e</sup>	2.71
Vf3	85.00 <sup>bc</sup>	16.53	245.47 <sup>b-d</sup>	16.31	42.73 <sup>d-e</sup>	5.55	29.73 <sup>b-d</sup>	3.80	34.00 <sup>b-d</sup>	5.54	113.10 <sup>b-e</sup>	3.31
Vf4	61.11 <sup>b-e</sup>	14.78	177.68 <sup>c-e</sup>	15.08	39.98 <sup>c-e</sup>	4.91	31.16 <sup>b-d</sup>	4.72	32.35 <sup>b-d</sup>	5.12	104.25 <sup>b-e</sup>	2.61
Vf5	66.30 <sup>b-e</sup>	10.78	205.13 <sup>b-d</sup>	11.87	41.12 <sup>b-e</sup>	3.32	29.65 <sup>b-d</sup>	2.58	33.28 <sup>b-d</sup>	3.31	112.27 <sup>b-e</sup>	2.09
Vf6	59.76 <sup>b-e</sup>	16.83	171.42 <sup>c-e</sup>	15.99	39.32 <sup>c-e</sup>	4.97	28.88 <sup>b-d</sup>	3.49	31.93 <sup>cd</sup>	5.37	112.61 <sup>b-e</sup>	6.28
Vf7	79.70 <sup>b-d</sup>	12.33	241.35 <sup>b-d</sup>	13.78	41.22 <sup>b-e</sup>	5.01	32.60 <sup>bc</sup>	3.44	33.60 <sup>b-d</sup>	5.03	102.50 <sup>d-f</sup>	4.31
Vf8	66.69 <sup>b-e</sup>	25.13	218.25 <sup>b-d</sup>	21.60	46.02 <sup>b-d</sup>	10.02	28.19 <sup>cd</sup>	7.00	37.16 <sup>bc</sup>	10.48	130.71 <sup>a</sup>	6.23
Vf9	63.21 <sup>b-e</sup>	10.50	213.35 <sup>b-d</sup>	10.59	42.63 <sup>b-e</sup>	3.80	33.50 <sup>b</sup>	2.67	33.46 <sup>b-d</sup>	3.99	100.34 <sup>e</sup>	3.58
Vf10	73.60 <sup>b-d</sup>	17.43	203.87 <sup>b-d</sup>	15.69	39.86 <sup>c-e</sup>	6.15	32.90 <sup>bc</sup>	5.95	33.50 <sup>b-d</sup>	6.26	103.65 <sup>b-e</sup>	4.42

Table 5. Continued.

Vf11	44.57 <sup>de</sup>	15.32	136.79 <sup>de</sup>	15.00	39.23 <sup>c-e</sup>	6.70	31.29 <sup>b-d</sup>	5.04	31.26 <sup>cd</sup>	6.68	101.16 <sup>ef</sup>	6.00
Vf12	81.65 <sup>b-d</sup>	13.87	214.94 <sup>b-d</sup>	14.32	38.37 <sup>d-f</sup>	4.94	30.35 <sup>b-d</sup>	4.08	31.23 <sup>cd</sup>	5.33	102.98 <sup>d-f</sup>	3.61
Vf13	66.82 <sup>b-e</sup>	13.02	227.20 <sup>b-d</sup>	12.63	45.91 <sup>b-d</sup>	3.31	32.64 <sup>bc</sup>	3.35	37.05 <sup>bc</sup>	3.79	113.92 <sup>b-e</sup>	3.09
Vf14	88.61 <sup>bc</sup>	10.80	249.52 <sup>b-d</sup>	10.15	44.68 <sup>b-d</sup>	3.43	32.74 <sup>bc</sup>	2.66	35.73 <sup>bc</sup>	3.74	109.64 <sup>b-e</sup>	3.41
Vf15	52.95 <sup>c-e</sup>	14.48	156.28 <sup>c-e</sup>	15.81	35.59 <sup>ef</sup>	8.59	27.76 <sup>d</sup>	5.37	28.52 <sup>de</sup>	8.30	99.27 <sup>e</sup>	5.23
Vf16	72.73 <sup>b-d</sup>	17.75	222.10 <sup>b-d</sup>	17.07	40.61 <sup>c-e</sup>	5.70	29.91 <sup>b-d</sup>	4.39	32.10 <sup>cd</sup>	5.79	106.71 <sup>b-e</sup>	3.14
Vf17	67.04 <sup>b-e</sup>	13.31	222.61 <sup>b-d</sup>	15.73	42.63 <sup>b-e</sup>	5.69	28.70 <sup>cd</sup>	5.28	33.91 <sup>b-d</sup>	6.17	116.66 <sup>bc</sup>	3.13
Vf18	89.35 <sup>bc</sup>	7.16	247.48 <sup>b-d</sup>	8.70	41.33 <sup>b-e</sup>	2.48	32.74 <sup>bc</sup>	1.67	32.59 <sup>b-d</sup>	2.48	99.50 <sup>e</sup>	1.76
Vf19	72.56 <sup>b-d</sup>	21.47	225.98 <sup>b-d</sup>	18.15	49.27 <sup>ab</sup>	11.04	32.56 <sup>bc</sup>	5.05	39.15 <sup>ab</sup>	10.07	118.21 <sup>b</sup>	5.00
Vf20	69.91 <sup>b-e</sup>	10.25	275.63 <sup>bc</sup>	10.38	54.96 <sup>a</sup>	7.35	43.57 <sup>a</sup>	5.05	44.25 <sup>a</sup>	7.46	103.75 <sup>b-e</sup>	6.09
Vf21	93.14 <sup>b</sup>	13.09	305.85 <sup>b</sup>	12.15	47.58 <sup>bc</sup>	3.38	32.82 <sup>bc</sup>	2.67	37.77 <sup>bc</sup>	3.51	115.62 <sup>b-d</sup>	3.05
Vf22	33.23 <sup>e</sup>	24.18	87.27 <sup>e</sup>	23.77	31.28 <sup>a</sup>	10.20	30.54 <sup>b-d</sup>	9.93	24.30 <sup>e</sup>	11.29	80.17 <sup>f</sup>	6.35
Vf23	171.00 <sup>a</sup>	16.55	500.78 <sup>a</sup>	15.64	40.45 <sup>b-d</sup>	3.54	40.18 <sup>a</sup>	3.54	31.82 <sup>cd</sup>	3.91	81.14 <sup>f</sup>	6.05
Average	74.00		226.50		42.20		31.99		33.79		106.29	
SD	25.70		76.45		4.80		3.62		3.92		10.97	
CV (%)	34.72		33.75		11.38		11.32		11.59		10.32	
F	4.74 <sup>***</sup>		4.47 <sup>***</sup>		3.53 <sup>***</sup>		7.92 <sup>***</sup>		3.45 <sup>***</sup>		5.10 <sup>***</sup>	

ANOVA test is represented by F values followed by significance levels: ns ( $P \geq 0.05$ ), \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ); mean values are followed by Duncan post-hoc multirange groups.

Table 6. Nutritive quality traits of the studied *Vicia faba* var minor populations.

Populations	DM (%)		ASH (%)		CP (%)		NDF (%)		ADF (%)		ADL (%)		IVDMD		IVOMD	
	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Vf1	89.15 <sup>a</sup>	0.01	17.22 <sup>a</sup>	25.39	26.66 <sup>d-g</sup>	1.73	46.25 <sup>ef</sup>	0.24	36.91 <sup>c</sup>	0.17	3.91 <sup>fg</sup>	0.91	80.96 <sup>b-d</sup>	1.92	77.73 <sup>bc</sup>	1.50
Vf2	90.75 <sup>a</sup>	0.10	12.20 <sup>b</sup>	9.35	26.77 <sup>d-g</sup>	3.21	46.97 <sup>ab</sup>	0.10	38.70 <sup>a</sup>	0.09	4.00 <sup>d-g</sup>	1.59	96.52 <sup>a</sup>	4.88	95.74 <sup>a</sup>	5.66
Vf3	90.78 <sup>a</sup>	0.62	11.37 <sup>b</sup>	14.96	28.22 <sup>a-d</sup>	3.05	46.90 <sup>b</sup>	0.24	36.77 <sup>d</sup>	0.12	3.85 <sup>gh</sup>	2.20	75.84 <sup>d</sup>	9.65	72.71 <sup>c</sup>	11.36
Vf4	90.42 <sup>a</sup>	8.46	12.90 <sup>ab</sup>	17.31	28.00 <sup>a-d</sup>	3.54	45.85 <sup>hi</sup>	0.13	36.42 <sup>g-j</sup>	0.02	3.73 <sup>h</sup>	0.76	81.07 <sup>d</sup>	1.20	78.10 <sup>bc</sup>	0.21
Vf5	79.65 <sup>a</sup>	2.40	11.52 <sup>b</sup>	44.14	26.87 <sup>d-f</sup>	2.94	45.94 <sup>gh</sup>	0.14	36.43 <sup>f-i</sup>	0.16	4.04 <sup>e-f</sup>	0.18	79.81 <sup>b-d</sup>	3.00	88.40 <sup>ab</sup>	17.45
Vf6	88.19 <sup>a</sup>	2.38	10.20 <sup>b</sup>	13.51	27.69 <sup>b-e</sup>	3.32	46.08 <sup>h</sup>	0.05	36.29 <sup>i-k</sup>	0.12	4.09 <sup>b-d</sup>	0.35	80.26 <sup>b-d</sup>	0.70	78.50 <sup>bc</sup>	2.00
Vf7	88.13 <sup>a</sup>	0.58	11.64 <sup>b</sup>	21.06	29.14 <sup>ab</sup>	2.69	46.17 <sup>fg</sup>	0.08	36.44 <sup>f-i</sup>	0.19	3.85 <sup>gh</sup>	0.55	79.28 <sup>b-d</sup>	1.91	75.88 <sup>c</sup>	2.32
Vf8	90.43 <sup>a</sup>	0.16	13.37 <sup>ab</sup>	41.78	25.56 <sup>d-g</sup>	1.81	46.22 <sup>e-g</sup>	0.14	36.56 <sup>e-g</sup>	0.04	4.00 <sup>d-g</sup>	0.71	80.16 <sup>b-d</sup>	0.58	77.60 <sup>bc</sup>	0.68
Vf9	90.29 <sup>a</sup>	1.25	9.62 <sup>b</sup>	4.40	27.34 <sup>e-c</sup>	1.42	45.84 <sup>hi</sup>	0.28	36.40 <sup>b-j</sup>	0.19	4.46 <sup>a</sup>	0.48	84.01 <sup>b</sup>	5.11	81.28 <sup>bc</sup>	6.18
Vf10	91.42 <sup>a</sup>	13.02	9.45 <sup>b</sup>	3.31	28.62 <sup>b-c</sup>	3.45	46.49 <sup>de</sup>	0.91	36.61 <sup>e</sup>	0.27	4.19 <sup>bc</sup>	0.34	83.17 <sup>bc</sup>	1.49	80.84 <sup>bc</sup>	1.75
Vf11	88.55 <sup>a</sup>	3.39	14.17 <sup>ab</sup>	1.72	26.84 <sup>d-f</sup>	3.15	47.19 <sup>a</sup>	0.06	35.76 <sup>m</sup>	0.12	4.12 <sup>b-d</sup>	0.00	80.80 <sup>b-d</sup>	0.11	78.47 <sup>bc</sup>	0.12
Vf12	89.50 <sup>a</sup>	0.50	10.99 <sup>b</sup>	13.55	27.74 <sup>b-e</sup>	0.47	45.30 <sup>kl</sup>	0.22	36.17 <sup>k</sup>	0.41	4.09 <sup>b-d</sup>	0.35	80.07 <sup>b-d</sup>	1.33	77.75 <sup>bc</sup>	1.38
Vf13	90.78 <sup>a</sup>	1.34	11.27 <sup>b</sup>	1.27	26.09 <sup>e-g</sup>	3.77	45.27 <sup>kl</sup>	0.02	36.45 <sup>f-h</sup>	0.12	4.02 <sup>d-f</sup>	3.87	80.22 <sup>b-d</sup>	0.54	76.66 <sup>c</sup>	5.38
Vf14	87.45 <sup>a</sup>	30.65	9.29 <sup>b</sup>	0.99	25.25 <sup>f-h</sup>	3.65	45.60 <sup>jl</sup>	0.62	36.28 <sup>jk</sup>	0.14	3.96 <sup>d-g</sup>	0.18	80.92 <sup>b-d</sup>	0.30	77.50 <sup>bc</sup>	0.21
Vf15	79.32 <sup>a</sup>	3.33	9.92 <sup>b</sup>	0.09	28.77 <sup>b-e</sup>	2.29	44.88 <sup>m</sup>	0.02	35.92 <sup>l</sup>	0.14	3.92 <sup>e-g</sup>	0.54	80.43 <sup>b-d</sup>	0.80	76.34 <sup>c</sup>	0.22
Vf16	78.84 <sup>a</sup>	22.98	13.53 <sup>ab</sup>	1.60	22.39 <sup>i</sup>	2.09	45.03 <sup>lm</sup>	0.11	36.19 <sup>k</sup>	0.10	3.99 <sup>d-g</sup>	0.71	83.29 <sup>bc</sup>	4.08	75.48 <sup>c</sup>	1.87
Vf17	83.64 <sup>a</sup>	10.97	17.16 <sup>a</sup>	1.29	26.90 <sup>d-f</sup>	1.70	45.93 <sup>gh</sup>	0.18	36.62 <sup>e</sup>	0.06	3.99 <sup>d-g</sup>	1.06	78.56 <sup>b-d</sup>	0.31	75.66 <sup>c</sup>	6.26
Vf18	83.83 <sup>a</sup>	11.80	9.36 <sup>b</sup>	0.89	29.60 <sup>a</sup>	0.26	45.30 <sup>kl</sup>	0.06	36.27 <sup>jk</sup>	0.19	4.05 <sup>b-f</sup>	2.27	80.91 <sup>b-d</sup>	3.86	76.58 <sup>c</sup>	2.75
Vf19	81.02 <sup>a</sup>	9.84	9.92 <sup>b</sup>	0.09	26.69 <sup>d-g</sup>	1.50	45.35 <sup>jk</sup>	0.19	36.71 <sup>de</sup>	0.35	4.08 <sup>b-c</sup>	2.08	81.13 <sup>b-d</sup>	0.92	76.63 <sup>c</sup>	5.49
Vf20	83.00 <sup>a</sup>	3.41	9.43 <sup>b</sup>	1.73	25.15 <sup>g-h</sup>	3.91	45.16 <sup>kl</sup>	0.09	36.58 <sup>ef</sup>	0.06	4.02 <sup>d-f</sup>	0.35	80.95 <sup>b-d</sup>	0.00	73.91 <sup>c</sup>	7.03
Vf21	84.54 <sup>a</sup>	8.21	13.84 <sup>ab</sup>	1.65	26.89 <sup>d-f</sup>	0.98	46.26 <sup>ef</sup>	0.15	37.15 <sup>b</sup>	0.10	4.07 <sup>b-f</sup>	1.22	79.29 <sup>b-d</sup>	4.65	76.40 <sup>c</sup>	0.19
Vf22	90.10 <sup>a</sup>	1.26	13.67 <sup>ab</sup>	6.94	21.99 <sup>i-l</sup>	3.01	46.79 <sup>bc</sup>	0.27	36.41 <sup>b-j</sup>	0.24	4.21 <sup>b</sup>	1.51	76.59 <sup>d</sup>	2.44	75.40 <sup>c</sup>	6.75
Vf23	92.00 <sup>a</sup>	0.24	9.57 <sup>b</sup>	5.47	23.93 <sup>h</sup>	0.83	46.58 <sup>cd</sup>	0.08	36.69 <sup>de</sup>	0.13	4.11 <sup>b-d</sup>	5.16	77.36 <sup>cd</sup>	3.91	73.20 <sup>c</sup>	1.74
MIN	78.84		9.29		21.99		44.88		35.76		3.73		75.84		72.71	
MAX	92.00		17.22		29.60		47.19		38.70		4.46		96.52		95.74	

Table 6. Nutritive quality traits of the studied *Vicia faba var minor* populations.

Mean	87.03	11.81	14.42	45.97	36.55	4.03	80.94	78.12
Variance	18.45	5.55	0.68	0.44	0.31	0.02	15.25	24.75
CV (%)	4.94	19.95	5.73	1.44	1.52	3.58	4.82	6.37
F 0.05	0.54 <sup>NS</sup>	2.63*	862.50***	55.30***	145.94***	8.70***	4.65***	2.33*

ANOVA test is represented by F values followed by significance levels: ns ( $P \geq 0.05$ ), \* ( $P < 0.05$ ), \*\* ( $P < 0.01$ ) and \*\*\* ( $P < 0.001$ ); mean values are followed by Duncan post-hoc multirange groups.

The significant variability observed among faba beans populations could offer an important genetic basis for plant breeders to develop high yields of faba bean genotypes under marginal environmental conditions [32, 34-36].

Although morphological and agronomic traits are commonly used to access genetic diversity, they are not sufficient to cover the genome, they are affected by environmental factors and they depend on the stage of development [29]. In fact, studied populations showed no significant correlation (with  $r = 0.181$ ) of total weight with flowering date (negative). However, the studies of [23] reported positive correlation between the yield and the whole flowering period.

In the line of the present study, *Vf23* produced the highest yield value and high value of plant weight between populations and highest number of pods per plant. As reported in [37], phenotypic and genotypic correlations between seed yield and number of pods per plant were positive and significant; this result is similar to the aforementioned current study and the population *Vf23* proves this point. This indicates that the selection based on seeds per pod increases seed yield. In the current research, the practice of selecting the next generation will be very successful through selection based on seeds per pod and the number of pods per plant.

The populations *Vf14*, *Vf18*, *Vf21* and *Vf23* showed high performance in arid oasis environments, so they are highly recommended to be used in breeding programs and released as synthetic varieties. Increasing yield is the main breeding goal of most faba bean crop improvement programs. The grain yield of faba beans depends on many related traits. Determining the degree and nature of the relationship between traits, especially the impact of each trait on yield, is the first step in selecting the best plant breeding components. The interrelationship between features helps to develop a multi-character selection scheme. The success of an inbred plant breeding program depends on the selection of the genotype that can produce offspring with the desired combination of traits. Like other crops, the yield of faba bean is a complex feature consisting of many interrelated morphological and physiological characteristics.

Plant height, number of stems and pods per plant, 100-seed weight; days to flowering and maturity are the most important traits in faba bean improvement for increasing seed yield due to direct and indirect correlation with seed yield [38, 39].

Moreover, the relationship between agro-morphological characteristics and the chemical composition of faba beans must be known and studied [40, 41] to better understand their effect on the determination of quality parameters in varieties adapted to local conditions [16, 42, 43].

Table 7. Correlations between agro morphological, yield components and nutritive quality traits of the studied *Vicia faba var minor*.

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
1 PODPLANT	1																								
2 TOTALWEIGHT	<b>0.963</b>	1																							
3 WEIGHTPOD	0.232	<b>0.429</b>	1																						
4 SEEDSPOD	<b>0.527</b>	<b>0.641</b>	<b>0.537</b>	1																					
5 WGHITSEEDPOD	0.213	0.403	<b>0.991</b>	<b>0.521</b>	1																				
6 SEEDWGT	-0.204	-0.110	<b>0.555</b>	-0.376	<b>0.583</b>	1																			
7 NDF	0.069	0.034	-0.223	-0.034	-0.223	-0.149	1																		
8 ADF	0.269	0.352	0.370	0.209	0.359	0.189	0.393	1																	
9 ADL	-0.022	-0.010	-0.076	0.200	-0.099	-0.280	0.108	-0.083	1																
10 IVDMD	-0.033	0.022	0.231	0.103	0.245	0.137	0.072	<b>0.658</b>	0.095	1															
11 IVOMD	-0.119	-0.102	0.032	-0.111	0.063	0.167	0.259	<b>0.591</b>	0.138	<b>0.805</b>	1														
12 DM	0.175	0.118	-0.160	0.118	-0.135	-0.189	<b>0.613</b>	0.247	0.134	0.059	0.057	1													
13 Ash	-0.340	-0.287	-0.177	<b>-0.441</b>	-0.208	0.215	0.323	0.148	-0.262	-0.067	-0.008	0.009	1												
14 CP	-0.068	-0.118	0.010	-0.222	0.068	0.255	-0.049	-0.019	-0.211	0.082	0.162	0.034	-0.213	1											
15 FLWPERINF	0.236	0.260	0.248	0.286	0.230	-0.031	0.137	0.241	0.021	0.070	0.029	0.162	-0.262	0.076	1										
16 LENGTHLEALET	<b>0.565</b>	<b>0.586</b>	0.352	<b>0.570</b>	0.331	-0.177	-0.130	0.057	-0.124	-0.178	-0.286	-0.157	-0.376	-0.174	<b>0.585</b>	1									
17 PLANTHGT	0.239	0.248	0.178	<b>0.478</b>	0.139	-0.374	-0.189	-0.060	0.140	-0.197	-0.399	-0.097	-0.199	-0.257	0.337	<b>0.564</b>	1								
18 STEMNBR	0.337	0.390	<b>0.499</b>	<b>0.536</b>	<b>0.523</b>	0.025	-0.261	0.151	-0.153	0.064	-0.046	-0.062	<b>-0.524</b>	<b>0.442</b>	0.350	0.335	0.226	1							
19 PODHIGHT	0.349	<b>0.431</b>	0.406	<b>0.751</b>	0.398	-0.296	0.119	0.272	0.139	0.060	-0.066	0.115	-0.192	0.074	0.252	0.257	<b>0.454</b>	<b>0.697</b>	1						
20 PODWIDTH	0.402	0.379	0.172	<b>0.551</b>	0.189	-0.351	-0.065	0.162	0.016	0.039	-0.081	0.137	-0.394	0.260	0.274	0.247	<b>0.475</b>	<b>0.795</b>	<b>0.855</b>	1					
21 NODNBR	<b>0.481</b>	<b>0.521</b>	0.275	<b>0.481</b>	0.249	-0.194	0.201	<b>0.466</b>	0.013	0.200	0.004	0.354	-0.039	-0.088	0.216	0.110	0.387	0.282	<b>0.577</b>	<b>0.528</b>	1				
22 DAYSIFTING	<b>-0.677</b>	<b>-0.680</b>	-0.343	<b>-0.552</b>	-0.321	0.169	0.028	-0.351	0.257	-0.179	-0.076	-0.076	0.315	0.023	<b>-0.586</b>	<b>-0.649</b>	-0.256	-0.412	-0.357	-0.394	<b>-0.430</b>	1			
23 DAYSFLOWER	-0.261	-0.181	0.009	0.095	-0.020	-0.072	0.225	-0.347	<b>0.523</b>	<b>-0.487</b>	-0.359	0.104	0.079	-0.358	-0.078	-0.021	0.044	-0.231	-0.031	-0.317	-0.231	<b>0.488</b>	1		
24 PODMAT	-0.256	-0.201	-0.070	-0.043	-0.107	-0.033	0.236	-0.156	0.395	-0.338	-0.068	-0.011	0.247	-0.354	-0.165	-0.112	-0.168	-0.181	-0.057	-0.293	<b>-0.418</b>	0.369	<b>0.784</b>	1	

Values in bold are different from 0 with a significance level  $\alpha=0.05$

### Chemical Composition and Nutritive Value of Faba

Results related to the chemical composition of studied populations were shown in Table 6. With the exception of DM, analysis of variances revealed highly significant differences ( $p<0.05$ ) among populations for the entire assessed traits. Variability determined by the coefficient of variation showed that the lowest value (1.44%) was observed for the NDF followed by the ADF (1.52%); while ASH exhibited the highest coefficient of variation (19.95%).

Faba bean populations assessed by chemical composition presented averaged levels of DM and ash of about 87.03% and 11.81%, respectively. The variation in mineral composition may be due to the soil composition, harvesting area, and the nature of the soil corresponding to each population in our study. Moreover, crude proteins contents varied from one population to another, they ranged from 21.99 (*Vf22*) to 29.60% (*Vf18*) (Table 6). Our results are similar to those reported by several authors [30, 41] and it is in the global average of 29% mentioned by [44]. Compared with other pulses, such as pea, which have CP contents from 15 to 30% dry weight [45], it was found that *Vicia faba var minor* have acceptable levels [46]. On the other hand, compared to forages such as barley and straw which have low CP levels from 6 to 10 %, faba bean have the advantage of providing a ration richer in CP, it can therefore be associated with these poor forages in nitrogen to relatively improve CP level in animal feed [47, 48]. Furthermore, NDF averages varied from 44.88 (*Vf15*) to 47.19% (*Vf11*) which is higher than that found for lucerne hay (41.4%) by [49]. While the ADF averaged from 35.76 (*Vf11*) to 38.70% (*Vf2*) The lowest average of ADL was recorded by *Vf4* (3.73%) versus *Vf9* which presented the highest mean value (4.46%). Compared with the results reported by [49] on lucerne hay, our studied *Vicia faba var minor* populations

represented higher rates of ADF and ADL than those of lucerne hay with respectively (29%) and (0.9%).

The average fiber content is higher than that found by [50], with NDF (12.6 to 16.5% DM) and ADF levels (10.1 to 13.7% DM). The high value can be explained by the climatic conditions of the cultivation of our faba bean. [30] mentioned that climatic factors

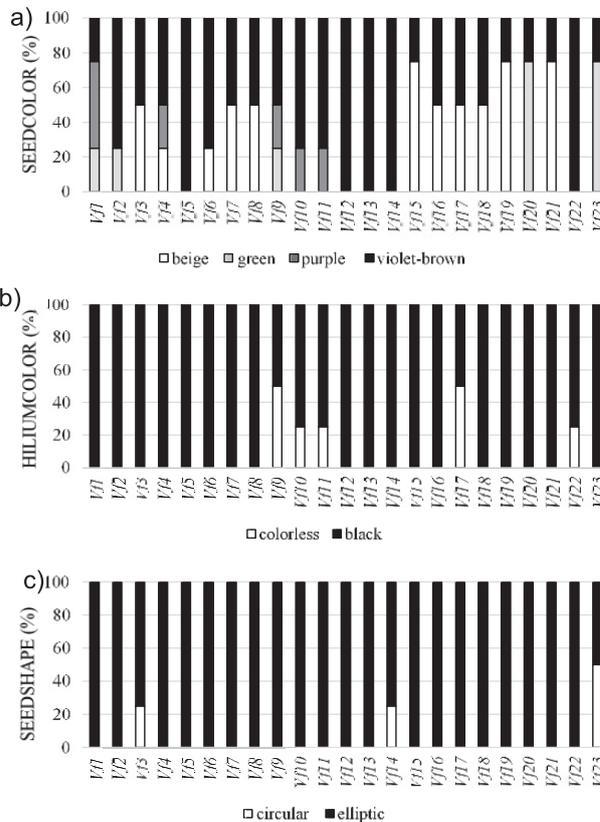


Fig. 2. Qualitative yield component traits of the studied *Vicia faba var minor* populations; a) Color of seed; b) Hilum color; c) Seed shape

influence the content and composition of walls via the morphological composition, so that high temperatures stimulate rapid lignifications of supporting tissues. In addition, other feed quality traits related particularly to ruminant in vitro digestibility were evaluated. The digestibility of faba beans differs from one population to another; *Vf3* recorded the lowest value in IVDMD (75.84 %) and IVOMD (72.71 %), while *Vf2* exhibited the highest values in IVDMD (96.52%) and IVOMD (95.74%). According to [51], proteins can form an insoluble complex with the presence of anti-nutritional factors, mainly tannins, and consequently, reducing its digestibility. In general, digestibility is influenced by several factors such as climatic conditions, harvesting, and storage conditions and the interactions between these factors [52]. Other sources of variation may also affect in vitro digestibility such as fineness of grinding, incubation time, proportion of rumen / saliva juice, and in some cases, the quality of rumen juice [53]. In our study, digestibility variation is correlated with fiber and CP content that varies between populations (Tables 6 and 7). Pearson's Correlation Matrix shows significant correlations between multiple pairs of variables (Table 7). There is therefore a linear relationship between the variables. In addition, IVDMD was positively correlated with ADF, IVOMD with ADF and IVDMD, then DM with NDF.

### Multivariate Analysis and Clustering of the Assessed Faba Bean Populations

Breeding schemes are widely performed to valorize diverse genetic resources via the crucial steps

of developing a Distinct, Homogenous, and Stable material serving as effective and suitable varieties regarding several selection criteria. In this context, several studies defined various traits providing a valuable representation of the existing diversity related to phenotypic classification as it is one of the crucial steps toward the germplasm clustering [54, 55]. For this, an appropriate selection of the superior genetic material needed for the breeding program which requires a good understanding of the genetic pool structure, the identification of more relevant variables, the detection of the relationships among traits and genotypes, as well as the identification of possible groups [56].

The cluster analysis for agro-morphological qualitative traits classified the populations into two groups (Fig. 3b). However, the PCA analysis from the same traits obtained by principal components showed that populations did not form distinct groups, with a wide dispersion among the populations (Fig. 3a). The first and second components represented 25.48% and 17.20% of the total variation observed among populations, respectively. A total of 8 duplicates were found in the cluster analysis (Fig. 3b), including the following populations: *Vf12- Vf4*, *Vf18- Vf7*, *Vf23- Vf14*, *Vf20- Vf19*, *Vf8- Vf5*, *Vf15- Vf6*, *Vf22- Vf11* and *Vf17- Vf1*.

The first group G1 was represented by the populations: *Vf12*, *Vf4*, *Vf10*, *Vf18*, *Vf7*, *Vf2*, *Vf23*, *Vf14*, *Vf3*, *Vf20*, *Vf19*, *Vf21*, and *Vf13*. These populations are more efficient and represent a better precocity (earliness regarding the three phenological stages studied). We noted here that among the materials of this cluster, *Vf2* was distinguished by the highest digestibility and

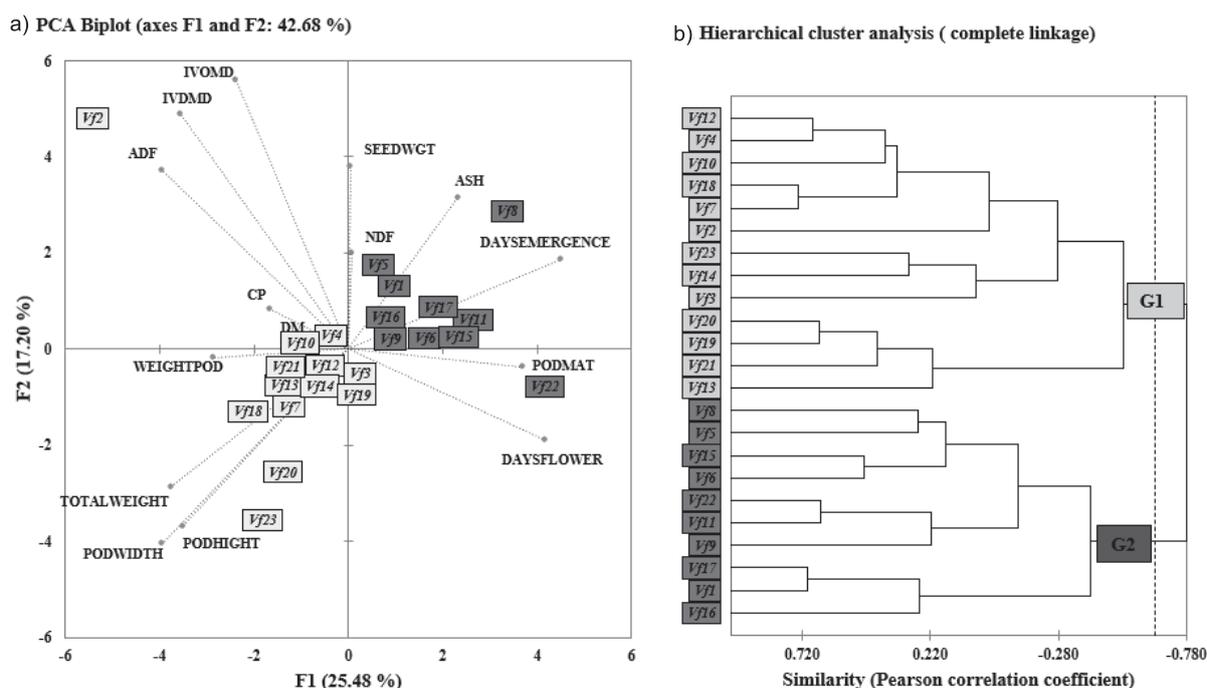


Fig. 3. Multivariate classification of the studied *Vicia faba var minor* populations based on the most discriminative traits; a) PCA Biplot b) Hierarchical cluster analysis.

ADF content, while *Vf20* and *Vf23* exhibited the highest scores regarding pod dimensions and total yield.

The second group G2 including the following populations: *Vf16*, *Vf1*, *Vf17*, *Vf9*, *Vf11*, *Vf22*, *Vf6*, *Vf15*, *Vf5*, and *Vf8* which are less performant than those of G1 except *Vf8* which had the highest mineral composition (ash content).

This could help in genotype selection on the basis of PCA and hierarchical cluster analysis for phenol-morphological characterizations, yielding performances, and nutritional quality. The results also suggest the importance of these traits in the development of high yielding varieties.

Similar studies reporting these approaches were undertaken by [56].

The PCA analysis was widely applied as a key-approach to study the genetic structure in collections of genetic resources defining many crops [56, 57]. In our study, we used this approach to identify the traits that were the main source of the variability based on morphological parameters (Fig. S1a, Table S2-A), yield components traits (Fig. S1b, Table S2-B), and those related to nutritional quality (Fig. S1c, Table S2-C).

Regarding the pheno-morphological traits, the two PCs, F1 and F2, together account for 60.33% of the variability among the populations studied (Fig. S1a). We noted that the most discriminating traits were: the pod width, pod height and lifting period, which contributed respectively with 16.43%, 13.52% and 13.14% of the total variance over F1 axis versus flowering period and pod maturity period which defined the F2 axis with 40.69% and 33.71% of variances (Table S2-A).

On the other hand, the first two PCs, F1 and F2, performed on the basis of yield compound traits explained 86.93% of the variability (Fig. S1b) and revealed that pod weight and total weight were the main traits responsible for 22.08% and 21.57% of the variability described by the F1 axis while seed weight was the most representative trait on the F2 axis with 40.07% of the variability (Table S2b).

Except ADL, all the traits related to nutritional quality contributed to 52.86% of the variability on both F1 and F2 axes (Fig. S1c, Table S2-C).

In order to define the most important traits for faba bean breeders, [58] and [23] reported in their studies that there is a compromise between the precocity of flowering and the high number of pods per node and seeds per pod as yield components.

Based on the main traits chosen from each of the previous parts, a global PCA was carried out in order to classify our faba bean populations according to the gathered breeding criteria. This approach was considered to serve as a tool to select performant populations candidate to pursue the current breeding scheme. The analysis of the obtained PCs revealed 42.68% of the total variability. Our findings showed a narrower variability among *Vicia faba* var *minor*

populations than those of [23] who found that both PCs accounted for 74.5% of the total variability among *V. faba* major and minor genotypes. This portion of the variation resulted mainly from the variation in the number of seeds per pod, number of pods per node, generative period, protein percentage, hundred seed weight, vegetative period, plant height and flowering period.

## Conclusion

The knowledge of genetic diversity plays an important role in breeding strategies. Therefore, it is imperative to evaluate the variability of the local germplasm used in genetic improvement and breeding of high-yielding varieties that adapt to the agro-climatic drought conditions and small-scale farming systems.

Twenty-three faba bean populations of arid regions of Tunisia are used in this study. This active germplasm was collected from the agro-ecosystem of arid oasis. Future selections may potentially contribute to the wider use of traditional grain legume crops in agricultural systems throughout the arid regions of the Tunisian oasis. However, the level of genetic diversity of those populations that can be used in breeding programs has not yet been quantified.

This study was carried out to determine the extent of genetic diversity among *Vicia faba* var *minor* populations. They were studied in the experimental field of the arid regions institute, based on the assessment of their agro-morphological, chemical composition and *in vitro* digestibility differences.

The Obtained results revealed differences between all populations for all agro-morphological traits (qualitative and quantitative ones) as shown by multivariate analyses. The parameters have different correlations between them, some are correlated positively, others negatively. Thus, the cluster analysis distinguishes the differences between populations: It showed two groups with a total of 8 duplicates.

The populations *Vf12*, *Vf4*, *Vf10*, *Vf18*, *Vf7*, *Vf2*, *Vf23*, *Vf14*, *Vf3*, *Vf20*, *Vf19*, *Vf21*, and *Vf13* are more performant and represent a better precocity, having the highest digestibility, ADF content and high total yielding.

Overall, we can assume that Faba bean could be one of the most important livestock *fodder* crops in Tunisian arid regions, improving the livelihood of smallholder farmers. The present study confirmed the existence of a wide phenotypic variation in this arid germplasm in many traits assessed. This genetic diversity is very useful for increasing the opportunities for genetic improvement of the yield potential in the faba bean crop through the development of synthetic variety in open-pollinated conditions to increase genetic gains.

In fact, this agro-morphological characterization, chemical composition and *in vitro* digestibility of Tunisian arid faba bean is a preliminary study which

can be reinforced by other more specific markers as biochemical and molecular markers.

There is also a need to start a breeding program to create new varieties for arid-oasis, which can tolerate diverse abiotic stresses and that guarantee a wide suitability to different cropping systems.

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### Conflict of Interest

The authors declare no conflict of interest.

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## Supplementary Material

Table S1. Composition of the Artificial saliva solution used for *in vitro* digestibility analysis.

Solutions	Buffers	Quantity (g/l)
Solution (a)	Na Cl	2.35
	K Cl	2.25
	MgCl <sub>2</sub> 6H <sub>2</sub> O	0.5013
	CaCl <sub>2</sub>	0.275
Solution (b)	NaHCO <sub>3</sub>	46.2
Solution (c)	Na <sub>2</sub> HPO <sub>4</sub>	28.2
Solution (d)	FeSO <sub>4</sub> 7H <sub>2</sub> O	7.5
	CuSO <sub>4</sub> 5H <sub>2</sub> O	0.2
	ZnSO <sub>4</sub> 2H <sub>2</sub> O	0.01
Solution (e)	Blue methylene	0.1
Solution (f)	Cysteine HCl	0.625
	Distilled H <sub>2</sub> O	95 ml
	NaOH (1N)	4 ml

Table S2. Contribution of the assessed traits on the variability based on the PCA.

A. pheno-morphological traits			B. yield compounds traits			C. Nutritional quality traits		
	F1 (%)	F2 (%)		F1 (%)	F2 (%)		F1 (%)	F2 (%)
FLWPERINF	8.06	1.77	PODPLANT	15.13	16.08	NDF	12.34	<b>29.84</b>
LENGTHLEALET	8.31	5.22	TOTALWEIGHT	<b>21.57</b>	9.79	ADF	<b>27.44</b>	0.01
PLANTHGT	8.32	7.43	WEIGHTPOD	22.08	13.00	ADL	0.55	0.01
STEMNBR	12.73	1.23	SEEDSPOD	18.66	6.66	IVDMD	<b>25.21</b>	12.91
PODHIGHT	<b>13.52</b>	6.64	WGHTSEEDPOD	21.40	14.39	IVOMD	<b>26.96</b>	8.18
PODWIDTH	<b>16.43</b>	0.50	SEEDWGT	1.15	<b>40.07</b>	DM	6.67	<b>21.80</b>
NODNBR	9.95	0.91				ASH	0.62	<b>19.00</b>
DAYSEMERGENCE	<b>13.14</b>	1.90		<b>CP</b>	0.21	<b>8.27</b>		
DAYSFLOWER	4.18	<b>40.69</b>						
PODMAT	5.37	<b>33.71</b>						

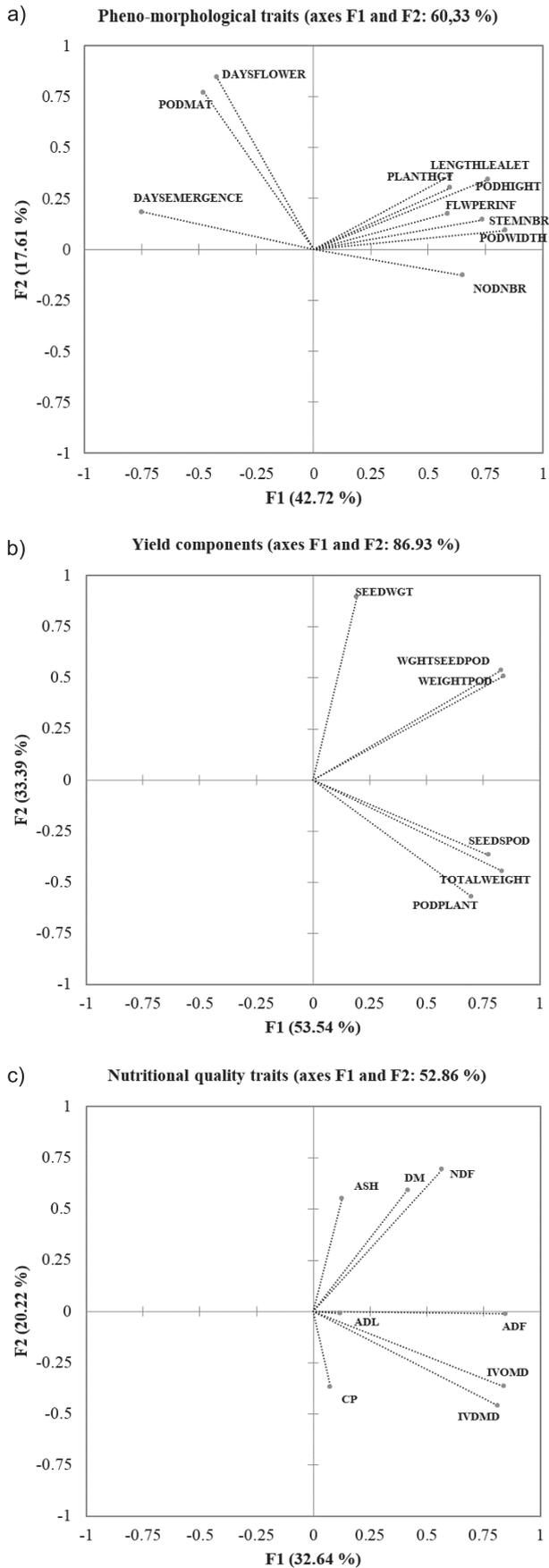


Fig. S1. Principal Component Analysis (PCA) related to pheno-morphological, a) yield components, b) and nutritional quality, c) traits.