Hindawi
Journal of Food Quality
Volume 2021, Article ID 9493607, 10 pages
https://doi.org/10.1155/2021/9493607

Research Article

Genetic Variability of Tunisian Faba Beans (*Vicia faba* L.) Based on Seeds' Morphophysical Properties as Assessed by Statistical Analysis

Khalil Khamassi[1] **Elyes Babay**,[2,3] **Mustapha Rouissi**,[3] **Abir Dakhlaoui**,[1] **Rayda Ben Ayed**,[4] **and Mohsen Hanana**[5]

[1]*University of Carthage, Institut National de la Recherche Agronomique de Tunisie (INRAT), Field Crop Laboratory (LR16INRAT02), Rue Hédi Karray, CP 1004, Menzah 1, Tunis, Tunisia*
[2]*University of Carthage, Institut National de la Recherche Agronomique de Tunisie (INRAT), Agricultural Applied Biotechnology Laboratory (LR16INRAT06), National Gene Bank of Tunisia, Street Yasser Arafet, Tunis 1080, Tunisia*
[3]*University of Carthage, Institut National de la Recherche Agronomique de Tunisie (INRAT), Agricultural Applied Biotechnology Laboratory (LR16INRAT06), Rue Hédi Karray, CP 1004, Menzah 1, Tunis, Tunisia*
[4]*Laboratory of Molecular and Cellular Screening Processes, Genomics and Bioinformatics Group, Centre of Biotechnology of Sfax, P.B. 1177, Sfax 3018, Tunisia*
[5]*Laboratory of Extremophile Plants, Center of Biotechnology of Borj-Cédria, B.P. 901, Hammam-Lif 2050, Tunisia*

Correspondence should be addressed to Rayda Ben Ayed; raydabenayed@yahoo.fr

Received 2 June 2021; Revised 24 August 2021; Accepted 14 September 2021; Published 16 November 2021

Academic Editor: Nesibe E. Kafkas

Faba bean (*Vicia faba* L.) is a legume crop cultivated for its nutritious seeds that are an important worldwide source of human food and feed. Seeds characterization is a prerequisite step for faba bean quality improvement. The morphophysical characterization of the seeds of twenty-four local faba bean accessions following the UPOV descriptors and the AOAC International standards was carried out and assessed with an approach based on Euclidean statistical model. "205 Bulk" was the unique accession harboring white hilum color seed which is linked to low convicine grain content. Irregular seed shape was the most observed character among the studied accessions except "Badii" displaying an elliptical seed shape; therefore, seed shape did not allow discrimination within our Tunisian germplasm. Interestingly, the physical characters of the seeds showed significant diversity between the accessions for all the measured parameters. A highly significant variability was observed for axial, length, and width dimensions of seeds, with "Memdouh" being the longest and largest seed accession, whereas "01-02" was the shortest and narrowest. Classification of the studied faba bean germplasm accessions based on morphophysical characters using clustering by Euclidean distance revealed three different groups. Moreover, multivariate PCA analysis further classified the faba bean accessions into four main clusters. Correlation study performed by using Spearman's test established positive correlations within physical parameters of seeds such as between mean length and mean width of seeds. Therefore, using morphophysical parameters screening, valuable phenotypes have been selected for deeper physiological characterization and further breeding programs.

1. Introduction

Faba bean, *Vicia faba* L., a diploid ($2n = 12$) Leguminosae species, also known as broad bean, is the third most important food grain legume after soybean and pea [1, 2]. It is mainly cultivated all over the world for its rich protein content, and therefore it serves for both food and feed use [3]. Moreover, introduced in intercropping system with cereals, it contributes to nitrogen fixation from soil, soil fertility improvement, and thus crop yield increase [1]. Faba bean is cultivated in almost seventy countries, on 2.58 million ha with a yield of 5.43 million tons [4]. In Tunisia, faba bean with its two seed sizes (large = broad bean or small = field bean) is the most cultivated Fabaceae with 60 thousand hectares representing 70% of the total grown grain legume area [5]. Furthermore, in 2018, the Tunisian Ministry of Agriculture accorded a budget to buy 150.000 tons of field bean from farmers to re-wholesale them later to fodder manufactures. This is a first step of the five-year plan national strategy to promote faba bean and increase the acreage up to double. Indeed, this approach is focusing on encouraging farmers to introduce faba bean into the cropping system as this crop plays a key role in agriculture sustainability. However, this strategy needs other alternatives to develop a value chain for this crop, which could create a demand market for highlighting the flour of faba bean in food industry. Consequently, a focus on physical properties and seed quality traits is the key for that purpose. Moreover, diversity study through the analysis of the physical properties of seeds is a preliminary step for faba bean germplasm characterization and improvement. Several attempts to analyze the morphological diversity and to characterize the physical properties of faba bean seeds have been realized, whether concerning varieties, accessions, cultivars, line, or even mutants [6–13]. However, no one paid great attention to the Tunisian germplasm that is rich in autochthonous accessions scattered among farmers. Therefore, in our present study, we initiated an analysis of the genetic variability of Tunisian faba bean germplasm based on seeds physical parameters. Our work aims ultimately to highlight the technological value of faba bean grains of Tunisian origin and to valorize their nutraceutical potentialities. The results of our study were further validated through powerful statistical assessment using Euclidean clustering, PCA multivariate analysis, and Spearman's correlation to verify the significance and meaningfulness of each seed character.

2. Materials and Methods

2.1. Plant Material. Twenty-four Tunisian accessions of *Vicia faba* L. were used in this study. Seeds were obtained from three different origins: local populations and old landraces (16) provided by farmers and conserved "*ex situ*" in Tunisian National gene bank; Tunisian germplasm (4) collected during the seventies and preserved in the German Gene Bank (Institute of Plant Genetics and Crop Research); and improved varieties (4) as reference.

2.1.1. Local Populations. Part of the genetic material used in this work was collected from farmers' own seed stock from different localities in northwestern of Tunisia. Indeed, only two farmers (Farm 1 and Farm 2) who are still growing their old own local landraces were found in "Amdoun" region from "Béja" governorate with 400 m altitude and average annual rainfall of 600 mm. Seeds were collected from Farm 1 and Farm 2. Therefore, a third farmer (Farm 3) was found in "Fernana" region from Jendouba governorate (altitude 220 m and 700 mm of average rainfall). The collection took place in spring 2018 from a raw seed sample of *faba bean* from the previous cropping year (2017-2018). It concerned only the farmers who cultivate the local varieties or landraces if the selection was not been done yet on that material. The size of the collected sample was sufficiently large (1 kg/local population/farmer) to represent the existing variability. The seed sample was divided into subgroups (homogeneous lots) distinguished according to seed morphology or seed type (field bean "small type" or broad bean "large seed type"). Table 1 shows details of *Vicia faba* seeds collections, subpopulations and origins.

2.1.2. Collection from IPK Genebank. The Genebank of the Leibniz Institute of Plant Genetics and Crop Plant Research at the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK, Germany) provided the other part of seeds material (Table 1). This material corresponds to accessions collected in Tunisia in the 1970s, kept and conserved by *ex situ* conservation in the IPK Genebank.

2.1.3. Improved Local Varieties. In addition to these local populations, four Tunisian improved cultivars were considered as references and for comparison study (Table 1).

2.2. Phenotypic Variability of Vicia faba L. Based on Seeds Traits. The phenotypic characterization of the seeds was carried out according to several morphological and physical parameters related to the technological quality of the beans following UPOV [15] and the agri-food industry AOAC international standards [16].

2.2.1. Morphological Parameters. The following morphological parameters were considered:

(i) Shape of the seed: The characterization of the seed shape is based on the standards described in the universal descriptors according to UPOV [15] (Figure 1(a)).

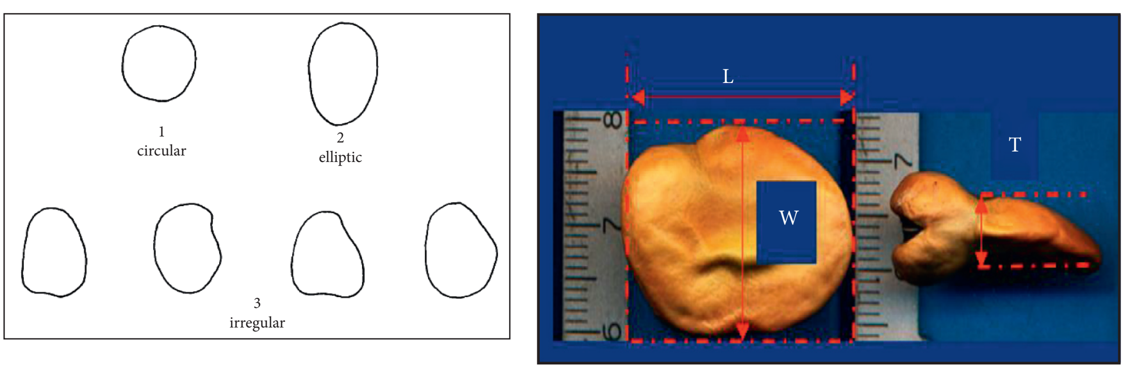
(ii) Hilum pigmentation: The pigmentation of the hilum is observed on the dry seed and scored as follows: black (1), white, or pigment-less (0) according to Khamassi et al. [14].

(iii) Seeds dimension: The three axial dimensions of the seed (length, width, and thickness) were measured using a Vernier caliper (Gilson Tools, Japan) with accuracy of 0.05 mm (Figure 1(b)). Then, 10 randomly selected seeds (blindly taken from their bags of conservation) for each variety, landrace or local population and subgroup within each of them were used to estimate seeds' dimensions.

2.2.2. Physical Properties of Seeds. The arithmetic mean of the seed diameter (Ad, mm) was calculated according to the formula given by Joshi et al. [17, 18]. This formula is based on three dimensions: length (L, mm), width (W, mm) and thickness (T, mm) as follows:

Table 1: List of collected landraces and local populations of *Vicia faba* L. and their origins.

N°	INRAT id	Type	Seed size	Governorate	Origin	Observations
1	01_02	Fieldbean	Small	Jendouba	Farm 1	Subgroup 2
2	02_06	Fieldbean	Small	Beja	Farm 2	Subgroup 3
3	03_05	Fieldbean	Small	Jendouba	Farm 3	Subgroup 5
4	03_06	Fieldbean	Small	Jendouba	Farm 3	Subgroup 6
5	79_5'	Fieldbean	Small	Beja	[14]	Selection from local landrace F75
6	202 lot 2	Fieldbean	Small	Beja	NA	Selection from local landrace F75
7	202 lot 3	Fieldbean	Small	Beja	NA	Selection from local landrace F75
8	FAB 6434	Fieldbean	Small	Unknown	Genebank	Genebank
9	FAB 6641	Fieldbean	Small	Unknown	Genebank	Genebank
10	Chourouk	Fieldbean	Small	Na	Breeding	INRAT registered variety
11	Najeh	Fieldbean	Small	Na	Breeding	INRAT registered variety
12	Badii	Fieldbean	Small	Na	Breeding	INRAT registered variety
13	03_04	Horsebean	Medium	Jendouba	Farm 3	Subgroup 4
14	FAB 5042	Horsebean	Medium	Unknown	Genebank	Genebank
15	01_05	Broadbean	Large	Jendouba	Farm 1	Subgroup 1
16	02_04	Broadbean	Large	Beja	Farm 2	Subgroup 1
17	02_05	Broadbean	Large	Beja	Farm 2	Subgroup 2
18	03_01	Broadbean	Large	Jendouba	Farm 3	Subgroup 1
19	03_02	Broadbean	Large	Jendouba	Farm 3	Subgroup 2
20	03_03	Broadbean	Large	Jendouba	Farm 3	Subgroup 3
21	03_07	Large	Large	Jendouba	Farm 3	Subgroup 7
22	205 bulk	Broadbean	Large	Beja	NA	Selection from local landrace F75
23	FAB 7111	Broadbean	Large	Unknown	Genebank	Genebank
24	Memdouh	Broadbean	Large	Na	Breeding	INRAT registered variety



(a) (b)

Figure 1: Different shapes and morphophysical parameters of faba bean seeds. L: length, W: width, and T: thickness.

$$\mathrm{Ad} = \frac{L + W + T}{3}. \tag{1}$$

The geometric mean of the seed diameter (Gd, mm) was calculated using the formula reported by Rotimi [19]:

$$\mathrm{Gd} = (L * W * T)^{1/3}. \tag{2}$$

The degree of seed sphericity (Φ, %) is an indicator of seed shape. According to Rotimi [19] and Mohsenin [20], the degree of sphericity was calculated based on the mean values in mm of major diameter (a), minor diameter (b), and intermediate diameter (c) of the seed, according to the following formula:

$$\Phi = \left(\frac{\mathrm{Gd}}{L}\right) * 100. \tag{3}$$

The elongation (E) and the degree of flattening (A) of the seeds (dimensionless) are also a seed shape indicators. These parameters were determined as follows:

$$E = \frac{L}{W},$$
$$A = \frac{W}{T}. \tag{4}$$

The seed aspect ratio (Ra, %) parameter, expressed as percentage, was determined according to Hara et al. [21] and Omobuwajo et al. [22]:

$$\text{Ra} = \left(\frac{W}{L}\right) * 100. \tag{5}$$

The seed volume (V, mm^3) was calculated according to the below formula [20]:

$$V = \left(\frac{\pi}{6}\right) * \text{Gd}^3. \tag{6}$$

The surface area (Sa, mm^2) was determined by the following formula [20]:

$$\text{Sa} = \pi(\text{Ad})^{1/2}. \tag{7}$$

In order to determine the one hundred seed weight (OHSW, g), a sample consisting of 100 seeds of each accession was weighed using a precision scale with three random replicates.

2.2.3. Statistical Analyses. To test the existence of significant differences between the local accessions studied, a single-factor analysis of variance (ANOVA) was applied using the SAS software. The comparison of the average morphological characteristics of the seed was made by means of the LSD test at the alpha threshold of 5%. Genetic diversity among cultivars based on biological and physicochemical data was examined using the clustering analysis with Euclidean distance matrix and the principal components analysis (PCA). Diversity among the 18 faba bean genotypes was assessed by estimating Euclidean distance as the formula suggested by Shifriss and Sacks [23]:

$$\text{Ed} = \sum_{k-1}^{7}\left(\frac{(Xik - Xjk)}{Sk}\right)^2, \tag{9}$$

where Xik = performance of the ith parent for kth character, Xjk = Performance of the jth parent for kth character, Sk = standard deviation of the kth character, and Ed: Euclidean distance.

The analysis of the relationship between all studied parameters was performed using several statistical techniques. Principal component analysis (PCA) of 18 faba bean seeds' physical properties data was performed with the R language. An absolute value of 0.50 was used in the loading matrices to select the characteristics in a particular principal component (PC). Correlations between variables were calculated with Spearman's correlation coefficients. All tests were considered as statistically significant when P is less than 0.05. All analyses were carried out by using the computer R programming software.

3. Results and Discussion

3.1. Morphological Seeds Features. The black pigmentation of the hilum (Figure 2(b)) is present in all accessions evaluated except the seeds of "205 Bulk" accession, which are characterized by a white (pale or colorless) hilum (Figure 2(a)). This white pigmentation of the hilum is a recessive trait with a maternal effect [14, 24]. According to Khamassi et al. [14], this trait of interest would serve as morphological marker for the selection of lines carrying the zero vicine and convicine (VC-). The variety carrying the VC- gene is of interest for monogastric feeding and as food for human deficient G_6PD enzyme allowing the avoidance of metabolic disease called favism [25]. Although faba bean seeds harbor several nutritional benefits, their consumption is restricted and limited by the vicine and convicine compounds that are responsible for favism disease [26–28]. Therefore, discovering a morphological trait associated with low VC phenotype would be an easy and simple marker for farmers, but also a considerable gain of time and energy in breeding programs for detecting low/free CV faba bean phenotypes [26–28]. Indeed, in German winter faba beans, the hilum color has been used as morphological marker in breeding works [29]. Recently, a linkage of 5 cM has been found between Hc gene (responsible for colorless hilum) and VC- gene only in the genitor line that carries VC- gene [26–28] and this is applicable only to lines where one of the parent is carrying both VC- in linkage with Hc gene. Nevertheless, not all colorless hilum faba bean genotypes are VC-, it should not be considered as pleiotropic linkage [14]. In any case, the discovery of genetic sources of low VC are an important result from our study. Actually, low VC grain content cultivars are being registered under several catalogues and databases, and their cultivation is growing in many countries [28]. Irregular seed form (Figures 2(c) and 2(d)) was the main morphology observed in all accessions, except the variety "Badii" whose seeds harbor elliptic form (Figure 2(e)). The seed shape in this case did not allow discrimination between the studied subpopulations.

3.2. Physical Properties of Seeds. The axial dimensions of seeds analyzed by accession are shown in Table 2. Analysis of variance for all axial dimensions showed a highly significant difference between the evaluated accessions ($P < 0.001$). The lengths of the studied *Vicia faba* seeds vary between 9 and 22.80 mm; the average widths vary from 6.5 to 17 mm and the average thicknesses from 4.20 to 6.20 mm. Those results are quite similar to those observed in previous studies that dealt with local either small or large *Vicia faba* germplasm collections. Indeed, seeds length varied between 11.80 and 15 mm; width ranged between 7.5 and 10 mm in an indigenous Omani faba bean collection [9]. The study carried out on Turkish faba bean varieties showed bigger values of length, width, and thickness, ranging respectively from 18 to 22 mm, 13 to 16 mm, and 13 to 16 mm [30]. The seeds of the "Memdouh," "01_05," and "02_05" accessions are the longest (22.45 mm) while the seeds of the accessions "202_lot 2," "01_02," and "FAB 6334" with a length around 9 mm are

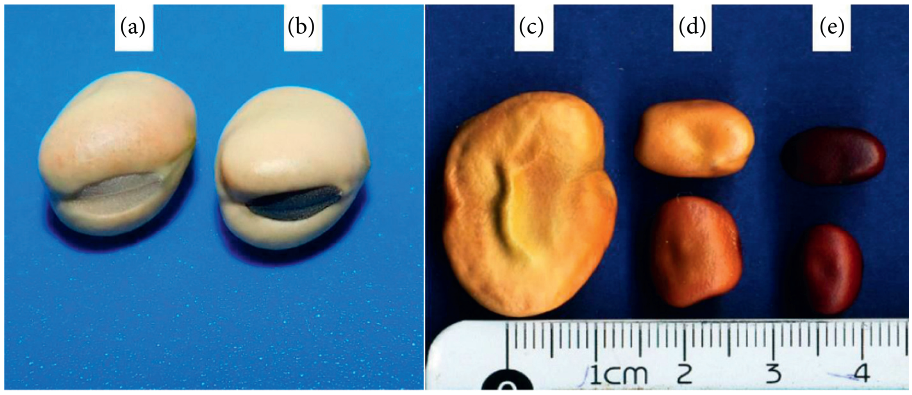


Figure 2: a) Black; (b) pale white pigmentation of the hilum; (c, d) irregular form of seed in "02_04" and "79_5"; (e) elliptical shape of "Badii."

the shortest (Figure 3). However, the average length of "Chourouk" seeds is statistically similar to those of "03_04" and "02_06." Also, the accessions "03_05" and "202_lot 3" presented an average grain length similar to that of the Najeh reference (10.70 mm). The "FAB 6434" and "Badii" accessions produced seeds ranging in length from 10 to 10.20 mm. The width of the seeds varies depending on the accession of *Vicia faba* (Table 2). "Memdouh" and "FAB 7111" accessions produced the largest seeds (16.7 mm), while "Badii," "01_02," and "205 bulk" accessions gave the narrowest seeds (6.6 mm). In addition, "02_06" and "202_lot3" accessions had an average seed width similar to those of the "Chourouk" and "Najeh" references. Statistical analysis for the arithmetic (Ad) and geometric (Gd) diameters of the seeds showed highly significant differences ($P < 0.001$) between *Vicia faba* accessions. Table 2 shows the average values of the arithmetic and geometric diameter recorded for the different accessions. It shows that the average arithmetic diameter and the average geometrical diameter vary respectively from 6.87 to 14.93 mm and from 6.67 to 12.64 mm. The seeds of accession "Memdouh" are characterized by the highest arithmetic and geometric diameters, while the seeds of accession "01_02" are characterized by smaller arithmetic and geometric diameters compared to other accessions. The axial dimensions (length, width, and thickness) allowed deducing the shape of the seeds, characterized by their degree of flattening, elongation, average index of sphericity, and aspect ratio. Analysis of variance for the various form parameters (E, A, Φ, and Ra) showed highly significant differences between the evaluated accessions ($P < 0.001$). Table 2 shows the average values of *Vicia faba* seed shape. The average values of the degree of flattening are between 1.22 and 3.40. "Memdouh" accession is the most flattened with a degree of flattening of 3.40. On the other hand, "205_Bulk-2" accession has the most convex seed which is characterized by a relatively weak flattening index (1.22) (Table 2). Average values of seed elongation range from 1.16 to 1.63. The seeds of accession "01_05" are the most elongated with an average elongation of the order of 1.63, whereas the seeds of "FAB 7111" are the least elongated ($E = 1.16$). The "03_05," "205_Bulk," and "03_04" accessions have an average elongation of 1.53, which is statistically similar to that of "Badii" (1.57) (Table 2). The seed aspect ratio connects the width to the length of the seeds. The average values of this aspect ratio ranged from 0.61 to 0.87 for "01_05" and "FAB 7111" accessions, respectively (Table 2). As a result, the seeds of "01_05" have a strong tendency towards the oblong form. These results confirm the elongated shape of the seeds of accession "01_05" characterized by an average elongation of 1.63. Mean spherical indices ranged from 54.40 to 80.64% (Table 2). This gives information on the strong tendency of *Vicia faba* seed form to a spherical shape. The seeds of the "202_lot 2" accession are the most spherical with a mean sphericity index of 80.64%, whereas the seeds of the "Memdouh," "01_05," and "02_04" accessions are less spherical (54, 40%) than the other accessions. The average sphericity index (67.86%) of the "03_06," "FAB 6641," and "79_5" accessions are statistically similar to that of the "Chourouk" and "Badii" references. Some Indian faba bean varieties reached 80–83% of sphericity index [31]. Indeed, the study of sphericity remains necessary to deduce the shape of the seed for the design of transport and storage equipment. Ixtaina et al. [32] and Mpotokwane et al. [33] showed that a high sphericity of seeds allows them to roll rather than slide during transport. The average area of the *Vicia faba* seed in the studied accessions varies between 140.10 and 488.60 mm^2 (Table 2). The "Memdouh," "01_05," and "02_05" accessions are characterized by the largest area of seeds. On the other hand, the accessions "01_02" and "FAB 6434" are characterized by a weak Sa (140.10 mm^2), while the other varieties recorded intermediate values. The average volume of the *Vicia faba* seeds for the studied accessions is between 156 and 1020 mm^3 (Table 2). The accessions "01_05," "02_05" and the reference variety "Memdouh" display the highest average volume. On the other hand, a relatively low average volume characterized the "01_02" and "FAB 6434" accessions compared to the other accessions that recorded intermediate values. By using an artificial neural network-based method, Aboukarima et al. [13] have shown that seeds volumes of Egyptian faba bean varieties ranged from 700 to 930 mm^3. The weight of one hundred seeds is a very important parameter for the estimation of yield in *Vicia faba*. The weight of one hundred seeds of the accessions studied varies from 47 to 248.5 g

Table 2: Morphophysical parameters of 24 faba bean seed accessions.

Accession	*L* (mm)	*W* (mm)	*T* (mm)	Ad (mm)	Gd (mm)	Φ (%)	*E*	*A*	Sa (mm^2)	*V* (mm^3)	Ra (%)	OHSW (g)
01_02	9.0 K	6.6 I	5.0 EFGH	6.8 M	6.6 I	74.3 BC	1.36 DEFGHI	1.32 IJ	140 K	156 K	73 DEFGH	47
02_06	12.6 FG	8.6 FG	4.8 FGHI	8.6 HI	8.0 FGH	63.9 EF	1.47 BCDE	1.82 G	201 HI	271 GHIJK	68 FGHIJK	6
03_05	11.0 HIJ	7.0 HI	5.0 EFGH	7.6 JKLM	7.2 HI	66.1 EF	1.57 AB	1.40 IJ	166 IJK	201 IJK	63 JK	180
03_06	12.0 GH	9.0 F	5.0 EFGH	8.6 HI	8.1 FG	67.8 DE	1.33 FGHIJ	1.80 G	208 GHI	282 FGHIJK	75 CDEF	160
79_5′	11.8 GHI	8.0 FGH	5.2 DEFG	8.3 HIJK	7.8 GH	67.0 DE	1.48 BCD	1.54 GHI	195 HIJ	257 GHIJK	68 FGHIJK	50
202_lot 2	9.0 K	7.6 GHI	5.6 ABCD	7.4 LM	7.2 HI	80.6 A	1.18 KL	1.36 IJ	165 IJK	201 IJK	84 A	47
202_lot 3	10.6 HIJ	8.6 FG	6.2 A	8.4 HIJ	8.2 FG	78.1 AB	1.24 HIJKL	1.38 IJ	214 FGHI	297 EFGHIJ	81 ABC	56
FAB 6434	10.0 JK	6.9 HI	4.5 HI	7.1 M	6.7 I	67.6 DE	1.45 BCDE	1.54 GHI	143 K	162 JK	69 EFGHIJ	170
FAB 6641	13.5 F	11.0 E	5.0 EFGH	9.8 FG	9.0 DE	67.1 DE	1.22 IJKL	2.20 F	257 EFG	388 EFG	81 ABC	94
Chourouk	12.8 FG	8.7 FG	5.9 ABC	9.1 GH	8.6 DEF	67.9 DE	1.47 BCDE	1.47 HIJ	237 EFGH	344 EFGH	67 GHIJK	77
Najeh	10.7 HIJ	8.2 FG	5.3 CDEF	8.0 IJKL	7.7 GH	72.6 C	1.30 GHIJK	1.55 GHI	189 HIJK	247 HIJK	76 BCD	57
Badii	10.2 JK	6.5 I	5.0 EFGH	7.2 LM	6.9 I	67.8 DE	1.57 AB	1.30 IJ	150 JK	173 JK	63 JK	50
03_04	13.2 FG	8.8 F	5.2 DEFG	9.0 GH	8.4 EFG	64.1 EF	1.51 AB	1.70 GH	223 FGH	315 EFGHI	67 HIJ	175
FAB 5042	16.9 DE	13.9 BC	4.9 FGH	11.9 D	10.4 C	62.1 FGH	1.21 JKL	2.85 BCD	344 D	603 D	82 AB	140
01_05	22.8 A	14.0 BC	6.0 AB	14.2 AB	12.4 A	54.4 J	1.63 A	2.34 EF	485 A	1014 A	61 K	191
02_04	20.4 B	14.3 BC	4.8 FGHI	13.1 C	11.1 BC	54.7 J	1.43 BCDE	2.99 B	392 CD	734 D	70 DEFGHIJ	157
02_05	22.1 A	14.7 B	5.7 ABCD	14.1 AB	12.2 A	55.6 IJ	1.50 ABC	2.60 DE	473 A	972 A	66 IJK	173
03_01	19.2 BC	14.2 BC	5.4 BCDE	12.9 C	11.3 B	59.2 GHI	1.35 DEFGHIJ	2.64 CDE	408 BC	782 BC	74 DEFG	138
03_02	18.2 CD	13.4 C	4.6 GHI	12.0 D	10.3 C	57.0 IJ	1.36 CDEFGH	2.91 BC	342 D	604 D	73 DEFGHI	248
03_03	16.2 E	12.2 D	4.2 I	10.8 E	9.3 D	58.0 HIJ	1.32 FGHIJ	2.93 BC	277 E	435 E	75 CDE	182
03_07	15.8 E	11.6 E	4.2 I	10.5 EF	9.1 DE	57.9 HIJ	1.36 CDEFGH	2.82 BCD	265 EF	414 E	74 HEFG	157
205 bulk	10.4 IJK	6.8 I	5.6 ABCD	7.6 KLM	7.3 HI	70.7 D	1.52 AB	1.21 J	170 IJK	211 HIJK	65 JK	60
FAB 7111	19.0 BC	16.4 A	5.4 BCDE	13.6 BC	11.8 AB	62.5 FG	1.15 L	3.08 B	444 AB	887 AB	86 A	122
Memdouh	22.8 A	17.0 A	5.0 EFGH	14.9 A	12.4 A	54.7 J	1.34 EFGHIJ	3.40 A	488 A	1020 A	74 CDEFG	184

L: length, *W*: weight, *T*: thickness, Ad: arithmetic diameter, Gd: geometric diameter, Φ: sphericity, *E*: elongation, *A*: flattening, Sa: seed area, *V*: volume, Ra: aspect ratio, and OHSW: one hundred seeds weight.

(Table 2). The obtained results showed that accession "01_02" has the lightest seeds (OHSW = 47 g), whereas "03-02" had the heaviest seeds (248.5 g) which is almost twice the one obtained by Hacıseferoǧulları et al. [30] for Turkish varieties. A Palestinian landrace of faba bean could reach 239 g in terms of OHSW [34]. Upadhyaya et al. [35] have argued that the OHSW parameter is highly heritable and often used for the distinction between genotypes. Several authors have reported that OHSW is necessary for the design of aerodynamic cleaning equipment [32]. In addition, this parameter is very useful for calculating the sowing dose. There was variability in the measured dimensions between the accessions. This parameter makes it possible to have an idea about the varietal genetic diversity and could be a determining parameter in the selection. Moreover, Koura et al. [36] have shown that physical properties vary

Figure 3: a, b) shortest seeds of the studied accessions of *Vicia faba* L. ("202 lot2" and "01-02"); (c, d) longest seeds of the studied accessions of *Vicia faba* L. ("Memdouh" and "01-05").

according to variety. These characteristics are very useful in the different separation, sorting, and conditioning processes [37].

3.3. Classification of the Studied Faba Bean Germplasm Accessions Based on Morphological Characters Using Clustering by Euclidean Distance and Principal Component Analysis

3.3.1. Hierarchical Classification Using Matrix of Euclidean Distances. The genetic similarity dendrogram is an effective means for evaluating the genetic relationship between the different studied accessions. Euclidean distances were calculated between all pairs of individual trees based on standardized values of eleven quantitative seed traits and the matrix of Euclidean distance was used for cluster analysis in R language, as shown in Figure 4. The graphical representation of the matrix of Euclidean distances, which lies between the studied twenty-four faba bean genotypes based on morphological characteristics, revealed three different groups. Group 1 included ten faba accessions ("03_03," "03_07," "0.3_02," "03_01," "FAB 5042," "FAB 7111," "Memdouh," "02_04," "02_05," and "01_05"). Group 2 combined the two faba landraces ("202 lot 2" and "202 lot 3"). Group 3 contained twelve faba accessions ("205 Bulk," "Najeh," "01_02," "02_06," "03_04," "Chourouk," "03_06," "FAB 6641," "FAB 6434," "Badii," "79-5," and "03_05").

3.3.2. Multivariate Analysis by PCA Method. The interaction between eleven quantitative seed traits were studied by the PCA which serves to appreciate which combination of seed traits could leads to high quality of the landraces, varieties, and local populations of faba bean germplasm in terms of their significance and value in marketing or for adoption by farmer according to seed size or suitability for industrial use. The multivariate PCA analysis was performed by using R language as illustrated in Figure 5. The first two components of the PCA explained 87.38% of the total variation among a subset of 24 of faba beans germplasm included in the analysis. The first component (axis 1) explained 67.36% of the variation, followed by 20.02% for the second components (axis 2). Axis 1 was positively correlated with the arithmetic diameter (0.997), seed length (0.989), geometric diameter (0.988), grain area (0.984), seed width (0.977), and seed volume (0.975) and negatively correlated with sphericity index with a value of −0.847. On the other hand, axis 2 was positively correlated with the percentage of aspect ratio (0.992) and negatively correlated with the seed elongation with a value of −0.992. Moreover, we showed that the PCA method classified the studied faba accessions into four main clusters (Figure 4), where cluster1 was composed by "202 lot 2," "202 lot 3," "Najeh," "01_02," "03_06," and "FAB 6641," whereas cluster 2 grouped "FAB 7111," "FAB 5042," "03_03," "03_06," "0.3_01," "03_02," and "Memdouh." Cluster 3 combined "FAB 6434," "205 Bulk," "79-5," "03_04," "03_05," "02_06," "Badii," and "Chourouk." Finally, cluster 4 grouped "02_04," "02_05," and "01_05." The regrouping of the latter accessions in the same cluster 4 by PCA analysis was essentially related to a remarkable similarity of their large seed size, aspect ratio, and seed elongation, while the eight faba accessions composing the cluster 3 had similarity of the two quantitative seed traits (sphericity index and seed elongation). On the other hand, cluster 1 grouped six faba accessions that had resemblance in numerous seed trait parameters such as seed elongation, arithmetic diameter, seed length, geometric diameter, grain area, seed width, and seed volume. Finally, the seven faba accessions included in cluster 2 had high similarity in the aspect ratio, arithmetic diameter, seed length, geometric diameter, grain area, seed width, and seed volume.

3.4. Correlation Study between the Physical Characteristics of the Studied Seeds. The association between the different measured parameters has been established by Spearman's correlation test. The correlation matrix showed that there was a highly positive significant correlation ($r = 0.948$, $P < 0.001$) between mean length and mean width. The accessions of *Vicia faba* with the longest seeds are also the largest. In addition, the average length was high positively related to other physical parameters such as arithmetic diameter ($r = 0.986$, $P < 0.001$), geometric diameter ($r = 0.973$, $P < 0.001$), surface area

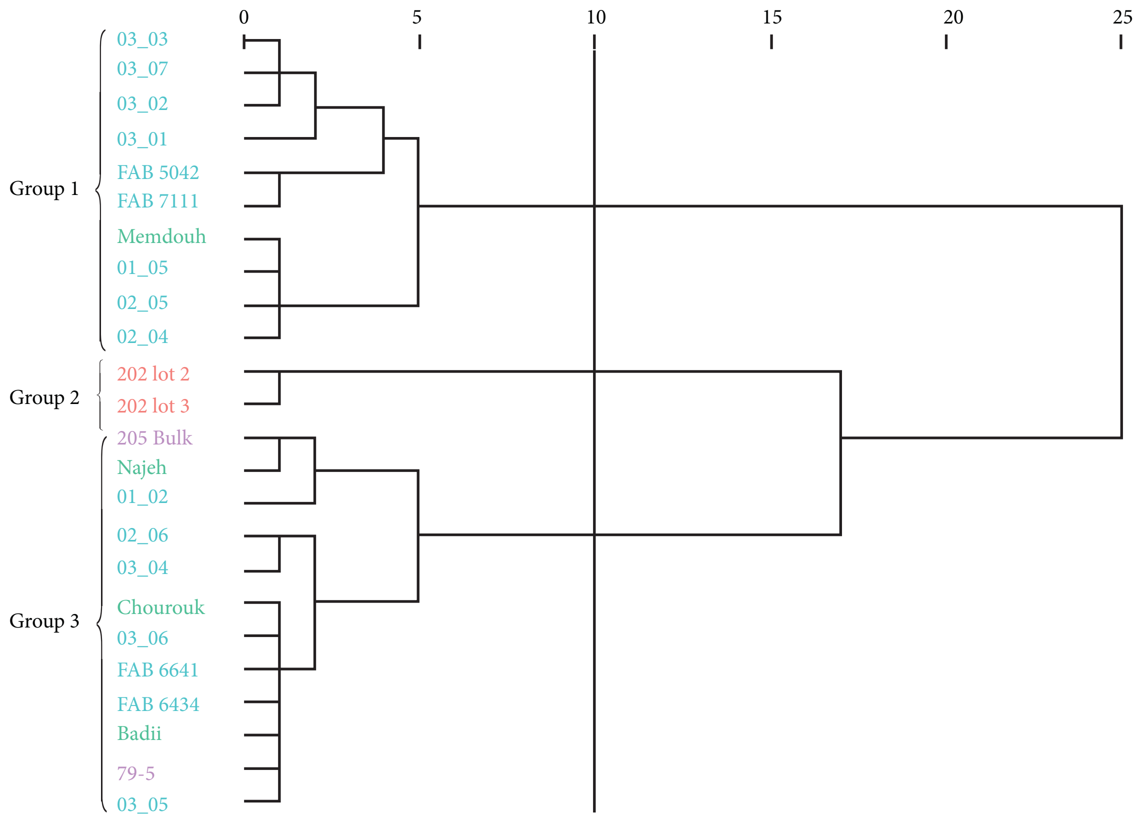


Figure 4: Classification of the studied faba bean germplasm accessions based on morphological characters using clustering by Euclidean distance.

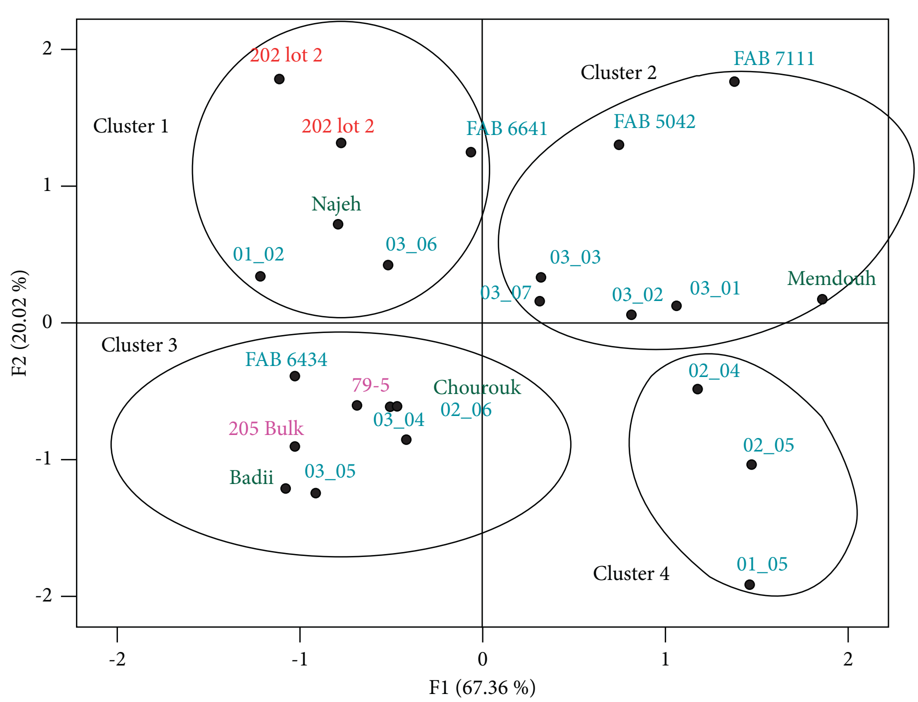


Figure 5: Principal component analysis performed by using 18 quantitative seed traits and 24 faba bean germplasm accessions.

($r = 0.973$, $P < 0.001$), degree of flattening ($r = 0.878$, $P < 0.001$), and volume ($r = 0.969$, $P < 0.001$), and high negatively influenced by the sphericity index ($r = -0.903$, $P < 0.001$). On the other hand, the average width was high positively correlated with arithmetic diameter ($r = 0.973$, $P < 0.001$), geometric diameter ($r = 0.970$, $P < 0.001$), surface area ($r = 0.970$, $P < 0.001$), degree of flattening ($r = 0.908$, $P < 0.001$), and volume ($r = 0.970$, $P < 0.001$), and high negatively influenced by the sphericity index ($r = -0.788$, $P < 0.001$). Therefore, this showed that the axial dimensions had a positive effect on certain physical properties and that the longest and widest seeds have a low sphericity index. Furthermore, it was noted that there was not a significant connection of the average thickness with all the studied parameters like the average width ($r = -0.01$, $P = 0.998$) and the average length of the seeds ($r = -0.32$, $P = 0.883$). Moreover, the aspect ratio parameter was high negatively correlated with the elongation ($r = -0.990$, $P < 0.001$). Additionally, the average volume of the seeds was positively associated with the parameters length ($r = 0.969$, $P < 0.001$), width ($r = 0.970$, $P < 0.001$), arithmetic diameter ($r = 0.994$, $P < 0.001$), geometric diameter ($r = 0.998$, $P < 0.001$), flattening ($r = 0.853$, $P < 0.001$), and surface area ($r = 0.998$, $P < 0.001$), and negatively correlated with sphericity ($r = -0.809$, $P < 0.001$). However, there was no correlation between thickness and the flattening index ($r = -0.368$, $P = 0.077$). It has been also found that the OHSW is positively correlated with certain physical parameters such as length, width, arithmetic diameter, geometric diameter, surface, and volume. On the other hand, this parameter is negatively correlated with the sphericity index ($r = -0.7637$, $P < 0.001$). It follows from all the foregoing that the seeds of the accessions "Memdouh," "01_05," "02_05," "02_04," "03_01," "FAB 7111," "03_02," "FAB 5042," "03_03," and "03_07" are correlated to lengths. The highest averages are the widest, the most flattened with the largest arithmetic and geometric diameters and also they possess the largest surface area and the highest volume. Nevertheless, they are characterized by the lowest sphericity index.

4. Conclusions

Even the morphological characters (shape of seeds and hilum color) of seeds did not allow significant discrimination among the Tunisian faba bean germplasm, our results highlighted the presence in our studied germplasm of one accession ("205 bulk") with low VC grain content. Indeed, the research of low VC content genotypes started from the 1970s by screening hundreds of lines, and the discovery of the first low VC genotype was in 1989 by Duc et al. [38]. Particularly, the "205 bulk" accession that could a valuable candidate genitor for potential breeding programs aiming to improve nutritional value and food quality of faba bean cultivars by crossing with low VC genotype. Moreover, with almost 250 g of OHSW, the "03_02" accession is suitable for horticultural practices and very interesting for consumers. Overall, the physical properties of seeds appeared to be interesting markers for Tunisian faba bean germplasm discrimination. Moreover, several seed characters were positively correlated one to each other, helping in screening for better faba bean quality phenotype. Our work led to the identification of some Tunisian faba bean accessions with desirable traits, which constitutes a valuable source for breeding material with the aim of improving agronomic and nutritional traits as well as adaptative characters. From all the foregoing, this work has provided useful data for the technological treatment of seeds, food quality improvement, elaboration of strategies for the conservation and sustainable management of the genetic heritage, and better use of germplasm and for global genetic improvement of *Vicia faba*.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors wish to thank the Ministry of Agriculture, Hydraulic Resources and Maritime Fisheries and the Ministry of Higher Education and Scientific Research for their financial support to this work. They are also thankful to the IPK Genebank for free donation of the Tunisian faba beans conserved in their genebank.

References

[1] E. Carrillo-Perdomo, A. Vidal, J. Kreplak et al., "Development of new genetic resources for faba bean (*Vicia faba* L.) breeding through the discovery of gene-based SNP markers and the construction of a high-density consensus map," *Scientific Reports*, vol. 10, no. 1, p. 6790, 2020.

[2] S. Sharan, G. Zanghelini, J. Zotzel et al., "Fava bean (*Vicia faba* L.) for food applications: from seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color," *Comprehensive Reviews in Food Science and Food Safety*, vol. 20, no. 1, pp. 401–428, 2021.

[3] X. Li and Y. Yang, "A novel perspective on seed yield of broad bean (*Vicia faba* L.): differences resulting from pod characteristics," *Scientific Reports*, vol. 4, no. 1, p. 6859, 2014.

[4] FAOSTAT, (n.d.), 2021, http://www.fao.org/faostat/fr/#data/QC.

[5] E. Babay, K. Khamassi, W. Sabetta et al., "Serendipitous in situ conservation of faba bean landraces in Tunisia: a case study," *Genes*, vol. 11, no. 2, p. 236, 2020.

[6] N. Yilmaz, "Morphological and molecular characterization of local faba bean (*Vicia faba* L.) accessions using inter-simple sequence repeat (issr) markers," *Fresenius Environmental Bulletin*, vol. 29, pp. 3756–3763, 2020.

[7] S. S. A. Nurmansyah, S. S. Alghamdi, and H. M. Migdadi, "Morphological diversity of faba bean (*Vicia faba* L.) M2 mutant populations induced by gamma radiation and diethyl sulfate," *Journal of King Saud University Science*, vol. 32, no. 2, pp. 1647–1658, 2020.

[8] G. Avola, F. Gresta, and V. Abbate, "Diversity examination based on physical, technological and chemical traits in a locally grown landrace of faba bean (*Vicia faba* L. var. major)," *International Journal of Food Science and Technology*, vol. 44, no. 12, pp. 2568–2576, 2009.

[9] N. AlSaady, S. Nadaf, A. Al Lawati, and S. Al Hinai, "Faba bean (*Vicia faba* L.) germplasm collection and its diversity in Oman," *Agricultural Sciences & Agronomy*, 2018.

[10] Ş. Göl, S. Doganlar, and A. Frary, "Relationship between geographical origin, seed size and genetic diversity in faba bean (*Vicia faba* L.) as revealed by SSR markers," *MGG Molecular & General Genetics*, vol. 292, 2017.

[11] A. Attia, M. EL-Abady, and H. AL-Agamy, "Morphological identification of some faba bean genotypes," *Journal of Plant Production*, vol. 10, no. 11, pp. 911–915, 2019.

[12] Ö. Sozen and U. Karadavut, "Determination of morphological and phenological properties of faba beans grown in Eastern Mediterranean Region of Turkey," *Jornal of Central Research Institute for Field Crops*, vol. 25, no. 2, 2016.

[13] A. Aboukarima, M. El-Marazky, H. Elsoury, M. Zayed, and M. Minyawi, "Artificial neural network-based method to identify five varieties of egyptian faba bean according to seed morphological features," *Engenharia Agrícola*, vol. 40, no. 6, pp. 791–799, 2020.

[14] K. Khamassi, F. Ben Jeddi, D. Hobbs et al., "A baseline study of vicine-convicine levels in faba bean (*Vicia faba* L.) germplasm," *Plant Genetic Resources*, vol. 11, no. 3, pp. 250–257, 2013.

[15] Compilation of the 2002 & 2003 Joint Symposia Documents of the World Intellectual Property Organization (WIPO) and the International Union for the Protection of New Varieties of Plants (UPOV), (n.d.), 2021, https://www.upov.int/meetings/en/doc_details.jsp?meeting_id=4687&doc_id=285580.

[16] AOAC International, *Official Methods of Analysis*, AOAC International, Rockville, MD, USA, 2019, https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/, 21st edition.

[17] D. C. Joshi, S. K. Das, and R. K. Mukherjee, "Physical properties of pumpkin seeds," *Journal of Agricultural Engineering Research*, vol. 54, no. 3, pp. 219–229, 1993.

[18] View of Some Physical Properties of Jackbean Seed (Canavalia Ensiformis), 2021, https://cigrjournal.org/index.php/Ejounral/article/view/896/890.

[19] D. Rotimi, "Some physical properties of groundnut grains," *Research Journal of Applied Sciences, Engineering and Technology*, vol. 1, no. 2, 2009.

[20] N. N. Mohsenin, *Physical Properties of Plant and Animal Materials. Structure, Physical Characteristics and Mechanical Properties*, Gordon & Breach, New York, NY, USA., 1st edition, 1978.

[21] M. Hara, B. Criner, G. Brusewitz, and J. Solie, "Selected physical characteristics and aerodynamic properties of cheat seed for separation from wheat," *The GIGR Journal of Scientific Research and Development*, vol. 2, 2000.

[22] T. O. Omobuwajo, L. A. Sanni, and J. O. Olajide, "Physical properties of ackee apple (*Blighia sapida*) seeds," *Journal of Food Engineering*, vol. 45, no. 1, pp. 43–48, 2000.

[23] C. Shifriss and J. M. Sacks, "The effect of distance between parents on the yield of sweet pepper x hot pepper hybrids, *Capsicum annuum* L. in a single harvest," *Theoretical and Applied Genetics*, vol. 58, no. 6, pp. 253–256, 1980.

[24] G. R. A. Crofton, "D. A. National I. of A. B. Bond, A review of the genetics of seed coat colour and hilum colour in field beans (*Vicia faba* L. (Partim)) with comments on some implications for national listing and certification, Plant Varieties and Seeds (United Kingdom)," 1998, https://agris.fao.org/agris-search/search.do?recordID=GB1997053722.

[25] L. Luzzatto and P. Arese, "Favism and glucose-6-phosphate dehydrogenase deficiency," *New England Journal of Medicine*, vol. 378, no. 1, pp. 60–71, 2018.

[26] H. Khazaei, D. M. O'Sullivan, F. L. Stoddard et al., "Recent advances in faba bean genetic and genomic tools for crop improvement," *Legume Science*, vol. 3, no. 3, p. e75, 2021.

[27] H. Khazaei, R. W. Purves, M. Song et al., "Development and validation of a robust, breeder-friendly molecular marker for the vc - locus in faba bean," *Molecular Breeding*, vol. 37, no. 11, p. 140, 2017.

[28] H. Khazaei, R. W. Purves, J. Hughes et al., "Eliminating vicine and convicine, the main anti-nutritional factors restricting faba bean usage," *Trends in Food Science & Technology*, vol. 91, pp. 549–556, 2019.

[29] S. Gasim, S. Abel, and W. Link, "Extent, variation and breeding impact of natural cross-fertilization in German winter faba beans using hilum colour as marker," *Euphytica*, vol. 136, no. 2, pp. 193–200, 2004.

[30] H. Hacıseferoğulları, I. Gezer, Y. Bahtiyarca, and H. O. Menges̡, "Determination of some chemical and physical properties of Sakız faba bean (*Vicia faba* L. Var. major)," *Journal of Food Engineering*, vol. 60, pp. 475–479, 2003.

[31] P. Sundaram, A. Singh, and S. Kumar, "Studies on some engineering properties of faba bean seeds," *Journal of Agri-Search*, vol. 1, pp. 4–8, 2014.

[32] V. Y. Ixtaina, S. M. Nolasco, and M. C. Tomás, "Physical properties of chia (*Salvia hispanica* L.) seeds," *Industrial Crops and Products*, vol. 28, no. 3, pp. 286–293, 2008.

[33] S. M. Mpotokwane, E. Gaditlhatlhelwe, A. Sebaka, and V. A. Jideani, "Physical properties of Bambara groundnuts from Botswana," *Journal of Food Engineering*, vol. 89, no. 1, pp. 93–98, 2008.

[34] T. Barri, M. Shtaya, and J. Shtaya, "Phenotypic characterization of faba bean (*Vicia faba* L.) landraces grown in Palestine," *Journal of Agricultural Science*, vol. 5, pp. 110–117, 2013.

[35] H. D. Upadhyaya, R. P. S. Pundir, C. L. L. Gowda, K. N. Reddy, and S. Singh, "Geographical patterns of diversity for qualitative and quantitative traits in the pigeonpea germplasm collection," *Plant Genetic Resources*, vol. 3, no. 3, pp. 331–352, 2005.

[36] K. Koura, P. Ouidoh, P. Azokpota, J. Ganglo, and D. Hounhouigan, "Caractérisation physique et composition chimique des graines de *Parkia biglobosa* (Jacq.) R. Br. en usage au Nord-Bénin," *Journal of Applied Biosciences*, vol. 75, no. 1, pp. 6239–6249, 2014.

[37] M. Cetin, "Physical properties of barbunia bean (*Phaseolus vulgaris* L. cv. 'Barbunia') seed," *Journal of Food Engineering*, vol. 80, no. 1, pp. 353–358, 2007.

[38] G. Duc, G. Sixdenier, M. Lila, and V. Furstoss, *Search of Genetic Variability for Vicine and Convicine Content in Vicia faba L.: a First Report of a Gene Which Codes for Nearly Zero-Vicine and Zero-Convicine Contents*Pudoc, La Union, Philippines, 1989.