

# Genetic Relationship and Diversity Analysis of Faba Bean (*Vicia Faba L. var. Minor*) Genetic Resources Using Morphological and Microsatellite Molecular Markers

Ghassen Abid · Dominique Mingeot · Sripada M. Udupa ·  
Yordan Muhovski · Bernard Watillon · Khaled Sassi ·  
Mahmoud M'hamdi · Fatma Souissi · Khediri Mannai ·  
Fathi Barhoumi · Moez Jebara

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**Abstract** Assessment of genetic diversity is an essential component in germplasm characterization and utilization. Molecular markers serve as a valuable tool to assess the genetic variation and germplasm identification, which play a key role for faba (*Vicia faba* L.) bean breeding. In this study, we analyzed the genetic diversity of faba bean accessions based on simple sequence repeats (SSRs) and morphological traits. Forty-six faba bean accessions, originating from different countries and from the ICARDA breeding program, were evaluated by using 15 morphological and agronomic traits and 17

simple sequence repeat (SSR) loci. Significant differences among accessions for the 15 morphological descriptors were observed. Analysis by SSR markers showed a high genetic diversity among the accessions: All SSRs showed polymorphism, and 101 alleles were revealed for all accessions. The number of alleles at each locus ranged from 2 to 10, with an average of 5.94 alleles per marker, and the polymorphic information content (*PIC*) values ranged from 0.38 to 0.84 with a mean of 0.69. Expected heterozygosity (*He*), observed heterozygosity (*Ho*), unbiased expected heterozygosity (*UHe*), and Shannon's information index (*I*) showed existence of high genetic variation between accessions from different pedigree. Analyses of genetic distance of the accessions separated the accessions into two groups and seven and five subgroups according to morphological and SSR analysis, respectively. Principal component analysis (PCA) of the SSR markers showed that the first two principal components (PCs) explained a total of 43.90 % of the genetic variation and allowed to distinguish three groups of accessions. Regardless of the method of analysis, Tunisian cultivars are grouped together. For the rest of the accessions, the geographical origin did not seem to be the main factor for structuring the variability of the studied accessions. Elite accessions from the ICARDA faba bean program differed from others and clustered together. The results obtained suggested that the faba bean microsatellite markers can be used to efficiently distinguish faba bean genotypes and to estimate their genetic diversity.

G. Abid (✉) · F. Souissi · K. Mannai · F. Barhoumi · M. Jebara  
Laboratory of Legumes, Center of Biotechnology of Borj Cedria,  
University of Tunis El Manar, 901, 2050 Hammam-Lif, Tunisia  
e-mail: gha\_abid@yahoo.fr

D. Mingeot · Y. Muhovski · B. Watillon  
Unit of Biological Engineering, Department of Life Sciences,  
Walloon Agricultural Research Centre, Chaussée de Charleroi, 234,  
5030 Gembloux, Belgium

S. M. Udupa  
ICARDA-INRA Cooperative Research Project, International Center  
for Agricultural Research in the Dry Areas (ICARDA), B.P.  
6299Rabat Instituts Rabat, Morocco

K. Sassi  
Laboratory of Agronomy, Department of Agronomy and Plant  
Biotechnology, National Agronomy Institute of Tunisia (INAT),  
University of Carthage, Avenue Charles Nicolle, 43,  
1082 Tunis-Mahrajène, Tunisia

M. M'hamdi  
Higher Agronomic Institute of Chott Mariem, BP 47, 4042, Chott  
Mariem Sousse, Tunisia

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## Introduction

Faba bean (*Vicia faba* L.) is one of the ancient cultivated crops widely believed to have originated in the Mediterranean-West Asia region (Tanno and Willcox 2006). In the Mediterranean countries, this crop is used as source of protein in both human and animal nutrition because of the high nutritional value of its seeds (Mejri et al. 2012). Faba bean includes 13–14 % of cell wall slightly lignified, 3 % of soluble sugars, 6.5 % of oligosaccharides, 27–34 % of proteins, and around 45 % of starch of total dry matter (Ayed 2011). Besides being an important food crop, it contributes to feed and fodder supply for livestock and affects positively the soil productivity for the cereal crops grown in rotation through biological nitrogen fixation (Duc 1997). Faba bean is a diploid with  $2n=2x=12$  chromosomes, possesses one of the largest genomes among crop legumes (~13,000 Mb), and is partially cross-pollinated ranging from 4 to 84 % (Ellwood et al. 2008). Despite the partial allogamous status of this crop, Perrino et al. (1991) suggested that the genetic variability of faba bean species is quite large. Although most researchers refer to four botanical varieties, namely, *Vicia faba major*, *Vicia faba equina*, *Vicia faba minor*, and *Vicia faba paucijuga*, in practice, a continuous variation for most seed, pod, plant, floral biology, and chemical and physiological traits, and also tolerance to several biotic and abiotic stresses, has been observed (Duc et al. 2010). North Africa is one of the top-producing regions of the faba bean in the world (Aouar-sadli et al. 2008). The average seed yield of faba bean in Africa (1.3 t/ha) is below world average (1.5 t/ha), while the average yields obtained in Asia (1.7 t/ha) and Europe (2.2 t/ha) are higher. Exceptionally high yields are obtained in Egypt and Sudan where the crop is irrigated (3.0 and 2.3 t/ha, respectively), whereas the national average yield of faba bean in Tunisia is low (0.7–0.8 t/ha) and characterized by wide fluctuations (Kharrat et al. 1991). The instability of the yield is mainly due to the lack of suitable genetic material with stable yield and reasonable resistance/tolerance to the prevalent diseases, pests, and abiotic stress. High yield and resistance/tolerance to both biotic and abiotic stresses are the prime objectives across faba bean breeding programs. It is therefore imperative to develop new cultivars that are able to overcome the biotic and abiotic factors responsible for low faba bean productivity in Tunisia. The introduction of new genetic resources into breeding programs may further improve agronomic traits and increase genetic variability and thereby lead to the development of high-yield cultivars (Hwang et al. 2008). In this context, a proper characterization of the genetic variation in the available local and Mediterranean faba bean germplasm resources is necessary for further improvement of crop yield and to impart tolerance to biotic and abiotic stress. Using morphological and agronomic traits for studying genetic diversity was previously achieved for many plants including faba bean (Mustafa 2007). Terzopoulos and Bebeli (2008)

suggested that using only morphological/agronomic traits is not adequate for the development of gene pools since either these traits are influenced by environmental factors and stage of plant development or they reveal only limited variation. The rapid development of molecular markers has provided new tools for evaluating germplasm and assessing the genetic diversity and population structure of plant species (Baranski et al. 2012; Boczkowska et al. 2012; Cao et al. 2012; Mir et al. 2012; Roy et al. 2012; Sardos et al. 2012; Wang and Li 2012). Different types of markers such as isozymes, random amplification of polymorphic DNAs (RAPDs), restriction fragment length polymorphisms (RFLPs), target region amplification polymorphisms (TRAPs), sequence-specific amplification polymorphisms (SSAPs), and amplified fragment length polymorphisms (AFLPs) have already been used for the assessment of genetic variability and diversity and to perform fingerprinting of *Vicia* species and *V. faba* L. accessions (Van de Ven et al. 1990; Przybylska et al. 1998; Gresta et al. 2010; Kwon et al. 2010; Liu and Hou 2010; Ouji et al. 2012; Waly et al. 2012).

Simple sequence repeats (SSRs), also called microsatellites, which correspond to tandemly repeated DNA sequences with a very short repeat unit, have been introduced as powerful genetic markers in plant improvement studies (Smith et al. 1997). Moreover, SSRs have been used as DNA markers for genomic fingerprinting and phylogenetic analysis, because they are economical to score, highly abundant in the genome, and co-dominant, they have high allelic diversity, and they are usually selectively neutral (Achtar et al. 2010). Some SSRs, derived from transcripts (known as expressed sequence tag (EST)-SSR markers), have become a powerful tool in plant genetic analysis and breeding research. Moreover, these markers are more closely connected with known function genes and possess higher transferability to related species (Ellis and Burke 2007). SSRs and EST-SSRs have been isolated, characterized, and reported in various species, including *V. faba* L. (Zeid et al. 2009; Gong et al. 2010; Ma et al. 2011).

In Mediterranean countries such as Tunisia, *V. faba* represents a crucial source of protein for foods, especially *V. faba* var. *minor* for animal feed. Most diversity studies on cultivated *V. faba* published by now have focused on Tunisian *V. faba* var. *major* germplasm, but only a few genotypes of *V. faba* var. *minor* have been represented among germplasm studied (Ouji et al. 2012; Yahia et al. 2012).

In order to develop *V. faba* var. *minor* cultivars, a preliminary characterization and evaluation of the genetic variability among local and cultivated *V. faba* var. *minor* accessions originating from Mediterranean countries and a comparison with widely grown commercial varieties could be useful for the exploitation of this genetic diversity in breeding programs.

The aim of this work was to assess the genotypic variability and relationship among local *V. faba* var. *minor* genetic

resources distributed in Tunisia, commercial cultivars and cultivated and improved genotypes from different geographic origins based on agronomic and morphological characters, and SSR molecular markers previously developed in *V. faba*. In addition, the results of this study provide guidelines and a scientific base to better explore *V. faba* L.var. *minor* germplasm resources and facilitate local cultivar improvement.

## Materials and Methods

### Plant Materials

Plant materials of faba bean used in this study totaled 46 accessions and were received from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, or directly collected by the authors (Table 1). This set includes ICARDA elite faba bean accessions used in the ICARDA faba bean breeding program and samples from eight countries, encompassing three different geographical regions (Fig. 1) such as Northern Africa (Algeria, Tunisia, Libya, and Egypt), Eastern Africa (Ethiopia, and Sudan), and Near East (Syria, and Turkey). Eight seeds per accession were germinated, and seedlings were transferred to individual pots into a greenhouse. Leaves were collected after about 3 weeks on the eight individual plants, pooled to represent a bulk DNA, frozen in liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  prior to DNA extraction.

### Morphological Characterization

The 46 accessions were evaluated in greenhouse for their morphological and agronomic traits. Morphological characteristics from eight plants per accession were recorded using 15 descriptors including qualitative characters (seed color, flower color, seed texture, seed shape, standard anthocyanin coloration, and standard extent of anthocyanin coloration) and quantitative plant and seed traits (100-seed weight, number of pod per node, number of seeds per pod, seed length, seed width, number of leaflets per leaf, plant height, pod length, and duration (number of days from sowing to 50 % of flowering)) chosen from faba bean descriptors of the International Board for Plant Genetic Resources (IBPGR 1985).

### Molecular Characterization

#### DNA Extraction

Total genomic DNA was extracted from the young leaves (0.5 g) which were powdered in liquid nitrogen, mixed with 700  $\mu\text{l}$  CTAB extraction buffer and 20 mg PVP 40000, and incubated at  $65^{\circ}\text{C}$  for 30 min with slow shaking every 5–10 min. The mixture was treated two times with 700  $\mu\text{l}$

chloroform-isoamyl alcohol (24:1). DNA was precipitated by adding (1/10) volume acetate sodium (3 M, pH 5.2) and (2/3) volume ice-cold isopropanol. The tubes were placed on ice for 20 min, after which the samples were centrifuged for 15 min at 13,800 rpm. The DNA pellet was washed in 70 % ethanol and dissolved in 50  $\mu\text{l}$  sterilized water. The quality of the DNA was estimated on an agarose gel (0.8 %) stained with ethidium bromide.

#### PCR Amplification and SSR Analysis

Twenty-one primer pairs were tested. These standard loci have been used in characterization of faba bean genetic diversity in several germplasm collections (Gong et al. 2011; Ma et al. 2011). After optimization of the PCR conditions, 17 primer pairs were selected on the basis of the reproducibility and the polymorphism of the generated bands (Table 3). PCRs were performed in a final volume of 25  $\mu\text{l}$  containing 20 ng of faba bean DNA template,  $1\times$  PCR buffer (10 mM Tris-HCl (pH 8.5), 50 mM KCl, 2.5 mM  $\text{MgCl}_2$ ), 0.2 mM of each dNTP, 0.2  $\mu\text{M}$  of each primer, and 1 U of *Taq* polymerase (Vivantis, USA). Reactions were performed in a Minicycler (Bio-Rad iCycler) thermal cycler with the following conditions:  $94^{\circ}\text{C}$  for 5 min, followed by 35 cycles at  $94^{\circ}\text{C}$  for 30 s,  $55\text{--}60^{\circ}\text{C}$  depending on the primer pair for 30 s, and  $72^{\circ}\text{C}$  for 1 min. The last step was  $72^{\circ}\text{C}$  for 5 min. A volume of 5  $\mu\text{l}$  of stop solution (10 mM NaOH, 95 % formamide, 0.05 % bromophenol blue, 0.05 % xylene cyanol) was added to the product of the PCR amplification before denaturation for a period of 5 min at  $94^{\circ}\text{C}$ . Four microliters of the reaction mixture was loaded onto denaturing gels of polyacrylamide at 6 % (w/v; 19:1 acrylamide-bisacrylamide) with 8 M urea. Electrophoresis was performed at 75-W constant power for about 3 h (Sequi-Gen GT sequencing cell, Bio-Rad). The products of the amplification were visualized by silver staining according to the protocol of Benbouza et al. (2006). Gels were dried at room temperature and scanned. The reproducibility of PCR experiments was confirmed by repeating the procedure at least two times.

#### Data Analysis

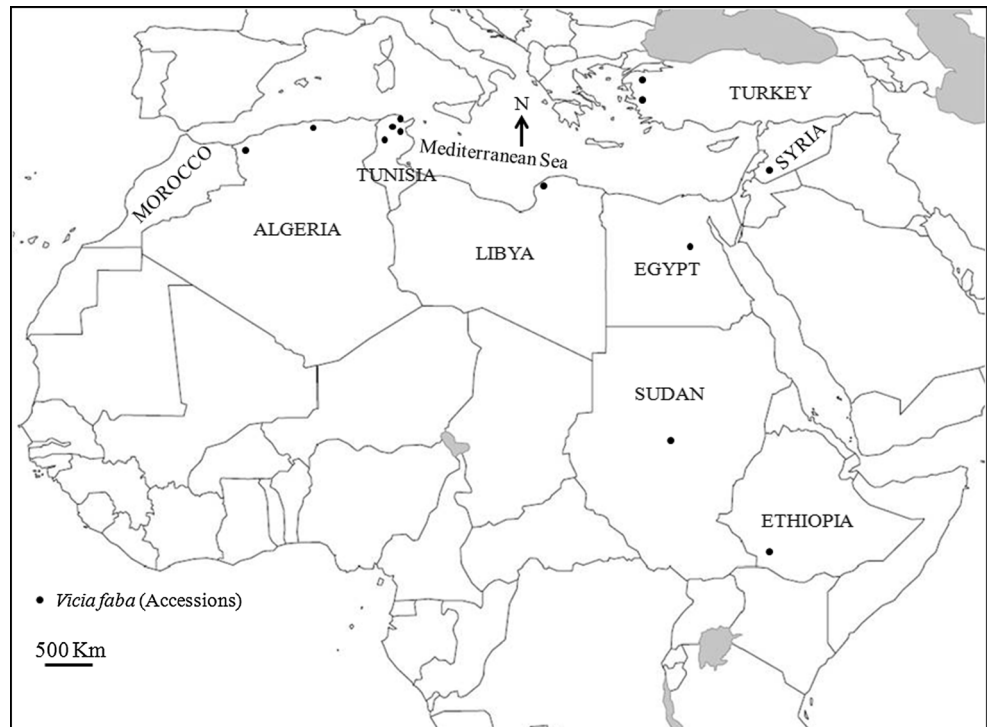
For quantitative morphological characteristics, an ANOVA analysis was performed; homogeneous groups (Newman and Keuls) were determined by using SYSTAT 8.0 software. The data of morphological characteristics were scored according to Table 2 and used to determine distance matrices for morphological characteristics based on Gower general similarity coefficient (Gower 1971). Unweighted Pair-Group Method using Arithmetic

**Table 1** Passport data of *Vicia faba* L. var. *minor* accessions provided by ICARDA and collected by the authors (\*)

Accession number	Country	Province	Latitude	Longitude
<i>Northern Africa (21 accessions)</i>				
NEB 97	Egypt	Asyut	N27 14	E31 07
NEB 397	Tunisia	Zaghwan	N36 20	E09 54
NEB 404	Tunisia	Beja	N36 43	E09 13
NEB 453	Egypt	–	–	–
F. 390	Egypt	Aswan	N24 26	E32 57
Misri	Tunisia	Beja	N36 43	E09 13
Misri 45	Tunisia	Beja	N36 43	E09 13
250	Egypt	–	–	–
Giza 3	Egypt	–	–	–
VIR 1960	Tunisia	–	–	–
VIR 2036	Egypt	–	–	–
VIR 2037	Libya	–	–	–
VIR 2040	Libya	–	–	–
VIR 2064	Libya	–	–	–
VIR 2128	Libya	–	–	–
VIR 2163	Libya	–	–	–
VIR 490	Algeria	–	–	–
Saber 02 commercial cultivar	Tunisia	Beja	–	–
Local Beja commercial cultivar	Tunisia	Beja	–	–
Local cultivar* (Hara)	Tunisia	Kef	–	–
Local cultivar* (Mahasen)	Tunisia	Kef	–	–
<i>Eastern Africa (3 accessions)</i>				
NEB 118	Ethiopia	Gamo Gofa	N05 51	E36 32
NEB 482	Ethiopia	–	–	–
Triple white	Sudan	–	–	–
<i>Near East (3 accessions)</i>				
NEB 99	Turkey	Bursa	N40 12	E29 04
NEB 102	Syria	Damascus	N33 31 15	E36 17 39
Malti medium	Syria	Damascus	N33 31	E36 17
<i>ICARDA faba bean breeding program (19 accessions)</i>				
FLIP 03-35FB	ICARDA faba bean breeding program	–	–	–
FLIP 03-52FB	–	–	–	–
FLIP 03-53FB	–	–	–	–
FLIP 03-55FB	–	–	–	–
FLIP 03-56FB	–	–	–	–
FLIP 03-60FB	–	–	–	–
FLIP 03-62FB	–	–	–	–
FLIP 03-67FB	–	–	–	–
FLIP 03-72FB	–	–	–	–
FLIP 03-76FB	–	–	–	–
FLIP 03-80FB	–	–	–	–
FLIP 03-82FB	–	–	–	–
FLIP 05-01FB	–	–	–	–
FLIP 05-02FB	–	–	–	–
FLIP 05-03FB	–	–	–	–
FLIP 05-04FB	–	–	–	–
FLIP 05-05FB	–	–	–	–
FLIP 06-001FB	–	–	–	–
FLIP 06-003FB	–	–	–	–

ICARDA International Center for Agricultural Research in the Dry Areas

**Fig. 1** Geographical distribution of faba bean accessions from eight countries



average (UPGMA) analysis was performed with the program MVSP 3.1 (Multi-Variate Statistical Package; Kovach 2007).

For molecular analysis by SSR markers, allele sizes were estimated using gel\_analyzer software. The summary statistics including the number of alleles per locus ( $N_a$ ), number of effective alleles ( $N_e$ ), unbiased expected heterozygosity ( $UHe$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $He$ ), Shannon's information index ( $I$ ), and fixation index ( $F_{st}$ ) were determined using GenAlEx6 software (Peakall and Smouse 2006). The polymorphism information content ( $PIC$ ) value was calculated following the formula described by Botstein et al. (1980).

For genetic distance analysis based on SSR results, the allelic size data were transformed to binary data: presence (1) versus absence (0) of each allele. A similarity matrix was generated using the Nei's genetic distance (Nei and Li 1979). Additionally, a matrix of genetic distance (Nei 1972) was calculated from the three main geographical groups and ICARDA elite faba bean accessions included in this study. Similarity data were processed through the UPGMA cluster analysis conducted using the MVSP 3.1 program. To assess further the genetic relationships of faba bean accessions as individual plants, a principal component analysis (PCA) was conducted based on the SSR variation patterns using MVSP 3.1 software program.

## Results

### Morphological Characterization

Morphological comparison was performed between 46 faba bean accessions (Table 1) from different geographic origins. Among these accessions, nine originated from Tunisia such as Saber 02, Mahasen, Hara, and Beja which represent the major faba bean accessions currently grown in various regions of the country. All genotypes did flower under our trial conditions, and their morphological characterization could be completed. The results are summarized in Table 2. Each quantitative characteristic was compared among accessions by using ANOVA. Data show significant differences ( $P 0.05$ ) between accessions for the nine quantitative agronomic characters considered.

The 46 accessions showed significant difference in days to 50 % flowering. Five different mean groups were found according to the Newman and Keuls comparison denoted by alphabets from a to e (Table 2). Triple white, FLIP 06-003FB, and F.390 had the earliest flowering date, whereas NEB 397, FLIP 03-76FB, and local cultivar (Beja) had the latest. There was a significant difference between accessions for hundred-seed weight. Using the Newman and Keuls procedure, the mean result allowed separation into eight statistically different groups. Accessions NEB 404, Malti, Giza 3, FLIP 03-52FB, FLIP 03-67FB, FLIP 03-82FB, FLIP 05-03FB, FLIP 05-05FB, FLIP 06-001FB, and FLIP 06-003FB

**Table 2** Morphological characters of *Vicia faba* accessions

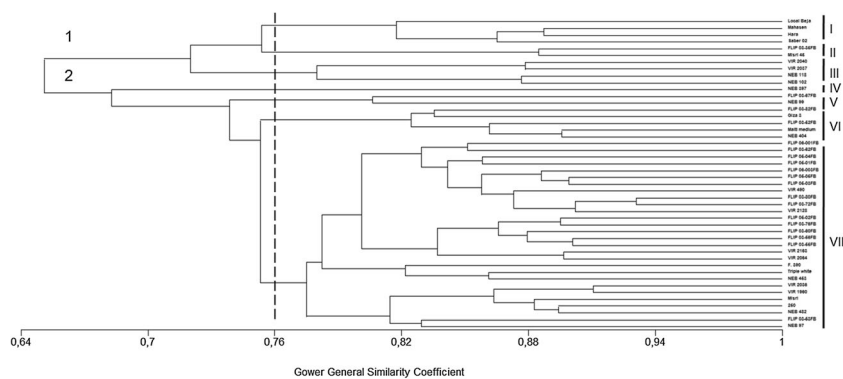
Ac	Quantitative characters											L/N/L			
	SC	SSH	ST	FC	SEAC	SAC	FD	WHS	PH	SL	SW		PL	PN/N	SN/P
NEB 97	Dark reddish-brown	Round	Smooth	White Large	1	38.33±0.51 <sup>ab</sup>	55.50±1.20 <sup>bc</sup>	57.10±3.16 <sup>b</sup>	1.35±0.07 <sup>bc</sup>	0.94±0.05 <sup>bc</sup>	6.93±0.86 <sup>de</sup>	1.19±0.17 <sup>ab</sup>	1.78±0.30 <sup>cd</sup>	4.77±0.19 <sup>c</sup>	
NEB 99	Brown	Cuboid	Smooth to rough	White Large	1	46.16±2.16 <sup>cd</sup>	77.80±2.36 <sup>cde</sup>	50.53±2.87 <sup>a</sup>	1.76±0.11 <sup>d</sup>	1.25±0.10 <sup>ef</sup>	7.20±0.96 <sup>de</sup>	1.29±0.05 <sup>b</sup>	1.33±0.24 <sup>b</sup>	3.99±0.33 <sup>c</sup>	
NEB 102	Dark reddish-brown	Round	Smooth to rough	White Medium	1	37.00±0.63 <sup>a</sup>	42.750±1.20 <sup>b</sup>	60.35±3.60 <sup>c</sup>	1.13±0.10 <sup>ab</sup>	0.83±0.09 <sup>b</sup>	6.06±0.74 <sup>bc</sup>	1.27±0.09 <sup>b</sup>	1.64±0.28 <sup>c</sup>	3.80±0.17 <sup>bc</sup>	
NEB 118	Dark reddish-brown	Round	Smooth	Pink Small	1	37.16±0.40 <sup>ab</sup>	48.12±2.36 <sup>bc</sup>	59.80±3.39 <sup>c</sup>	1.25±0.15 <sup>b</sup>	0.82±0.04 <sup>b</sup>	5.90±0.80 <sup>bc</sup>	1.55±0.07 <sup>cd</sup>	2.48±0.25 <sup>cde</sup>	4.02±0.29 <sup>c</sup>	
NEB 397	Dark reddish-brown	Round	Smooth	White Medium	1	53.14±2.47 <sup>c</sup>	59.42±1.09 <sup>c</sup>	57.05±4.03 <sup>b</sup>	1.40±0.07 <sup>bc</sup>	1.02±0.05 <sup>cd</sup>	7.43±0.61 <sup>de</sup>	1.07±0.08 <sup>a</sup>	1.35±0.21 <sup>b</sup>	3.30±0.33 <sup>a</sup>	
NEB 404	Cream	Cuboid	Smooth	White Medium	1	41.88±0.92 <sup>bc</sup>	106.40±2.33 <sup>g</sup>	73.15±4.73 <sup>de</sup>	1.76±0.08 <sup>d</sup>	1.29±0.07 <sup>fg</sup>	7.86±0.94 <sup>def</sup>	1.16±0.05 <sup>ab</sup>	1.62±0.17 <sup>c</sup>	5.08±0.36 <sup>ef</sup>	
NEB 453	Cream brown	Round	Smooth	White Small	1	36.10±0.87 <sup>b</sup>	64.15±1.90 <sup>c</sup>	67.73±3.32 <sup>d</sup>	1.36±0.10 <sup>bc</sup>	0.98±0.08 <sup>bc</sup>	6.40±0.50 <sup>cd</sup>	1.18±0.17 <sup>ab</sup>	2.61±0.28 <sup>cde</sup>	4.36±0.12 <sup>d</sup>	
NEB 482	Brown	Cuboid	Smooth to rough	White Medium	1	36.25±0.70 <sup>b</sup>	70.90±3.18 <sup>cd</sup>	58.50±3.76 <sup>b</sup>	1.53±0.15 <sup>c</sup>	1.08±0.05 <sup>cd</sup>	5.50±0.55 <sup>b</sup>	1.10±0.14 <sup>ab</sup>	1.32±0.10 <sup>b</sup>	4.80±0.17 <sup>c</sup>	
F. 390	Cream brown	Cuboid	Smooth to rough	White Small	1	33.77±0.83 <sup>a</sup>	63.05±2.40 <sup>c</sup>	65.96±3.64 <sup>d</sup>	1.58±0.08 <sup>cd</sup>	1.11±0.05 <sup>de</sup>	8.33±0.60 <sup>defg</sup>	1.13±0.12 <sup>ab</sup>	2.49±0.23 <sup>cde</sup>	3.82±0.16 <sup>c</sup>	
Misri	Brown ochre	Cuboid	Smooth	Pink Medium	1	43.00±1.05 <sup>c</sup>	74.62±2.22 <sup>cd</sup>	67.60±4.27 <sup>d</sup>	1.55±0.14 <sup>cd</sup>	1.09±0.12 <sup>cd</sup>	6.30±0.45 <sup>c</sup>	1.09±0.10 <sup>ab</sup>	1.16±0.13 <sup>a</sup>	4.38±0.34 <sup>d</sup>	
Misri 45	Brown ochre	Cuboid	Smooth	White Large	1	48.80±1.30 <sup>d</sup>	60.30±0.61 <sup>c</sup>	73.36±3.38 <sup>de</sup>	1.34±0.13	0.94±0.08 <sup>bc</sup>	6.23±0.30 <sup>c</sup>	1.11±0.09 <sup>ab</sup>	2.35±0.21 <sup>cde</sup>	4.52±0.21 <sup>d</sup>	
Malti medium	Brown	Cuboid	Smooth to rough	White Medium	1	46.75±1.03 <sup>cd</sup>	96.85±5.51 <sup>cdef</sup>	73.16±2.30 <sup>d</sup>	1.80±0.07 <sup>de</sup>	1.26±0.11 <sup>ef</sup>	8.03±0.47 <sup>def</sup>	1.08±0.14 <sup>a</sup>	2.09±0.33 <sup>cde</sup>	5.30±0.32 <sup>fg</sup>	
250	Reddish-brown	Cuboid	Smooth to rough	White Medium	1	37.55±0.72 <sup>ab</sup>	71.95±2.89 <sup>cd</sup>	71.55±4.45 <sup>de</sup>	1.51±0.10 <sup>c</sup>	1.10±0.10 <sup>de</sup>	6.23±0.70 <sup>c</sup>	1.13±0.12 <sup>ab</sup>	1.76±0.22 <sup>cd</sup>	4.66±0.30 <sup>de</sup>	
Giza 3	Brown ochre	Cuboid	Smooth to rough	White Medium	1	36.11±1.16 <sup>b</sup>	94.17±1.30 <sup>cdef</sup>	78.70±3.34 <sup>defg</sup>	1.64±0.30 <sup>cd</sup>	1.14±0.08 <sup>de</sup>	6.53±0.65 <sup>cd</sup>	1.05±0.10 <sup>a</sup>	2.07±0.23 <sup>cde</sup>	5.19±0.17 <sup>f</sup>	
Triple white	Beige	Cuboid	Smooth to rough	White Small	0	33.63±0.67 <sup>b</sup>	61.50±0.42 <sup>c</sup>	69.73±3.19 <sup>de</sup>	1.40±0.06 <sup>bc</sup>	1.00±0.07 <sup>cd</sup>	6.60±0.43 <sup>cde</sup>	1.66±0.28 <sup>cd</sup>	2.33±0.38 <sup>cde</sup>	3.94±0.29 <sup>c</sup>	
VIR 1960	Brown	Cuboid	Smooth to rough	White Large	1	41.81±1.94 <sup>bc</sup>	77.77±1.44 <sup>cde</sup>	80.00±3.70 <sup>defg</sup>	1.50±0.03 <sup>c</sup>	1.06±0.05 <sup>cd</sup>	5.96±0.51 <sup>bc</sup>	1.07±0.08 <sup>a</sup>	1.56±0.20 <sup>c</sup>	4.91±0.14 <sup>c</sup>	
VIR 2036	Brown ochre	Cuboid	Smooth	White Large	1	36.87±1.80 <sup>ab</sup>	66.20±0.56 <sup>cd</sup>	80.66±4.05 <sup>defg</sup>	1.44±0.11 <sup>c</sup>	0.96±0.05 <sup>bc</sup>	5.85±0.75 <sup>bc</sup>	1.09±0.07 <sup>ab</sup>	1.65±0.21 <sup>c</sup>	5.02±0.29 <sup>ef</sup>	
VIR 2037	Cream brown	Round	Smooth	White Small	1	38.40±1.14 <sup>ab</sup>	31.87±0.21 <sup>a</sup>	79.16±4.20 <sup>defg</sup>	1.04±0.10 <sup>b</sup>	0.69±0.04 <sup>a</sup>	4.80±0.50 <sup>a</sup>	1.29±0.11 <sup>b</sup>	1.61±0.19 <sup>c</sup>	4.45±0.52 <sup>d</sup>	
VIR 2040	Cream brown	Round	Smooth	White Medium	1	36.30±1.25 <sup>a</sup>	47.55±0.80 <sup>bc</sup>	81.93±3.1 <sup>defgh</sup>	1.13±0.07 <sup>ab</sup>	0.75±0.07 <sup>ab</sup>	5.25±0.77 <sup>bc</sup>	1.17±0.16 <sup>ab</sup>	1.78±0.12 <sup>cd</sup>	4.80±0.17 <sup>c</sup>	
VIR 2064	Brown	Cuboid	Smooth to rough	Pink Medium	1	44.09±1.51 <sup>c</sup>	64.72±3.35 <sup>c</sup>	77.83±3.50 <sup>def</sup>	1.43±0.12 <sup>c</sup>	1.03±0.08 <sup>cd</sup>	6.06±0.68 <sup>bc</sup>	1.12±0.05 <sup>ab</sup>	2.08±0.20 <sup>cde</sup>	4.19±0.17 <sup>cd</sup>	
VIR 2128	Cream brown	Cuboid	Smooth	White Large	1	38.81±1.07 <sup>b</sup>	73.9±2.68 <sup>cd</sup>	71.76±3.85 <sup>de</sup>	1.59±0.09 <sup>cd</sup>	1.15±0.10 <sup>de</sup>	7.08±0.52 <sup>de</sup>	1.25±0.16 <sup>ab</sup>	2.10±0.32 <sup>cde</sup>	4.30±0.30 <sup>cd</sup>	
VIR 2163	Cream brown	Cuboid	Smooth to rough	White Medium	1	42.90±1.85 <sup>c</sup>	77.95±3.39 <sup>cde</sup>	73.26±4.16 <sup>de</sup>	1.57±0.11 <sup>cd</sup>	1.06±0.05 <sup>cd</sup>	6.20±0.65 <sup>c</sup>	1.11±0.10 <sup>ab</sup>	2.37±0.27 <sup>cde</sup>	3.80±0.17 <sup>bc</sup>	
VIR 490	Brown	Cuboid	Smooth to rough	White Medium	1	37.11±1.53 <sup>ab</sup>	77.85±2.31 <sup>cde</sup>	67.53±3.25 <sup>d</sup>	1.73±0.17 <sup>d</sup>	1.15±0.11 <sup>de</sup>	6.93±0.90 <sup>de</sup>	1.01±0.02 <sup>a</sup>	2.06±0.35 <sup>cdef</sup>	3.41±0.21 <sup>ab</sup>	
Saber 02	Brown	Round	Smooth	White Medium	1	44.11±1.83 <sup>c</sup>	42.70±1.73 <sup>b</sup>	88.16±3.75 <sup>defghi</sup>	1.16±0.05 <sup>ab</sup>	0.92±0.08 <sup>bc</sup>	6.03±0.41 <sup>bc</sup>	1.68±0.16 <sup>d</sup>	2.47±0.26 <sup>cde</sup>	4.80±0.17 <sup>c</sup>	
Local Beja	Brown ochre	Cuboid	Smooth	White Medium	1	50.00±2.05 <sup>de</sup>	50.15±2.45 <sup>bc</sup>	89.86±4.4 <sup>defghi</sup>	1.23±0.06 <sup>b</sup>	0.84±0.05 <sup>b</sup>	6.86±0.45 <sup>de</sup>	1.85±0.13 <sup>de</sup>	3.00±0.25 <sup>cde</sup>	3.88±0.19 <sup>bc</sup>	
Hara	Brown	Cuboid	Smooth	Pink Medium	1	41.91±1.62 <sup>bc</sup>	54.93±1.80 <sup>bc</sup>	91.16±3.82 <sup>defghi</sup>	1.35±0.05 <sup>bc</sup>	0.88±0.02 <sup>b</sup>	7.50±0.52 <sup>de</sup>	1.63±0.15 <sup>cd</sup>	2.90±0.23 <sup>cde</sup>	4.69±0.05 <sup>de</sup>	

**Table 2** (continued)

Qualitative characters		Quantitative characters													
Mahasen	Brown	Cuboid	Smooth	Pink	Medium	1	43.90±1.72 <sup>c</sup>	51.36±1.25 <sup>bc</sup>	86.40±3.50 <sup>defghi</sup>	1.24±0.11 <sup>b</sup>	0.80±0.07 <sup>ab</sup>	6.93±0.51 <sup>de</sup>	1.43±0.20 <sup>c</sup>	2.71±0.15 <sup>cdefghijk</sup>	4.74±0.28 <sup>de</sup>
FLIP 03-35FB	Dark green	Round	Smooth	White	Medium	1	49.10±1.96 <sup>de</sup>	46.72±0.45 <sup>b</sup>	76.96±2.55 <sup>def</sup>	1.25±0.14 <sup>b</sup>	0.85±0.05 <sup>b</sup>	4.83±0.30 <sup>a</sup>	1.12±0.12 <sup>ab</sup>	2.17±0.31 <sup>cdefg</sup>	4.63±0.12 <sup>de</sup>
FLIP 03-52FB	Brown ochre	Cuboid	Smooth to rough	White	Medium	1	42.00±1.58 <sup>bc</sup>	94.57±2.93 <sup>cd</sup>	74.63±4.30 <sup>def</sup>	1.71±0.08 <sup>d</sup>	1.16±0.06 <sup>ef</sup>	8.20±0.78 <sup>de</sup>	1.31±0.13 <sup>b</sup>	2.22±0.09 <sup>cdefgh</sup>	4.66±0.14 <sup>de</sup>
FLIP 03-53FB	Cream brown	Cuboid	Smooth to rough	White	Large	1	38.88±1.69 <sup>ab</sup>	68.25±1.2 <sup>cd</sup>	57.26±3.73 <sup>b</sup>	1.49±0.10 <sup>c</sup>	1.04±0.11 <sup>cd</sup>	4.86±0.55 <sup>a</sup>	1.38±0.04 <sup>bc</sup>	1.81±0.26 <sup>cde</sup>	4.27±0.04 <sup>cd</sup>
FLIP 03-55FB	Brown ochre	Truncate	Smooth to rough	White	Medium	1	48.12±1.95 <sup>d</sup>	74.22±2.72 <sup>cd</sup>	64.10±3.1 <sup>d</sup>	1.55±0.07 <sup>cd</sup>	1.06±0.04 <sup>cd</sup>	7.06±0.47 <sup>de</sup>	1.34±0.15 <sup>bc</sup>	2.83±0.24 <sup>cdefghijk</sup>	3.91±0.15 <sup>bc</sup>
FLIP 03-56FB	Brown ochre	Truncate	Smooth to rough	White	Medium	1	47.90±1.44 <sup>d</sup>	81.75±0.98 <sup>cde</sup>	68.33±3.75 <sup>d</sup>	1.51±0.12 <sup>c</sup>	1.04±0.06 <sup>cd</sup>	7.66±0.55 <sup>def</sup>	1.13±0.08 <sup>ab</sup>	2.71±0.19 <sup>cdefghijk</sup>	4.19±0.39 <sup>cd</sup>
FLIP 03-60FB	Brown	Cuboid	Smooth to rough	Pink	Large	1	48.00±2.13 <sup>d</sup>	80.82±2.32 <sup>cde</sup>	66.90±3.63 <sup>d</sup>	1.67±0.18 <sup>cd</sup>	1.09±0.11 <sup>cd</sup>	5.83±0.49 <sup>b</sup>	1.17±0.04 <sup>ab</sup>	2.47±0.38 <sup>cdefgh</sup>	4.27±0.25 <sup>cd</sup>
FLIP 03-62FB	Brown	Cuboid	Smooth to rough	White	Medium	1	41.88±1.61 <sup>bc</sup>	85.45±2.75 <sup>cde</sup>	60.00±3.46 <sup>c</sup>	1.54±0.06 <sup>c</sup>	1.08±0.08 <sup>cd</sup>	8.76±0.61 <sup>de</sup>	1.15±0.06 <sup>ab</sup>	2.46±0.19 <sup>cdefgh</sup>	4.49±0.16 <sup>d</sup>
FLIP 03-67FB	Brown	Cuboid	Smooth to rough	White	Large	1	49.25±1.75 <sup>de</sup>	91.45±3.88 <sup>cd</sup>	68.06±3.35 <sup>d</sup>	1.73±0.19 <sup>d</sup>	1.24±0.15 <sup>ef</sup>	4.83±0.30 <sup>a</sup>	1.18±0.02 <sup>ab</sup>	1.41±0.12 <sup>b</sup>	4.30±0.29 <sup>cd</sup>
FLIP 03-72FB	Brown	Cuboid	Smooth to rough	White	Medium	1	38.20±1.39 <sup>ab</sup>	82.07±2.22 <sup>cde</sup>	71.13±3.8 <sup>de</sup>	1.62±0.10 <sup>cd</sup>	1.18±0.08 <sup>de</sup>	6.50±0.45 <sup>cd</sup>	1.07±0.07 <sup>a</sup>	2.1±0.32 <sup>cdefg</sup>	4.41±0.21 <sup>d</sup>
FLIP 03-76FB	Brown ochre	Cuboid	Smooth to rough	White	Small	1	50.10±1.85 <sup>de</sup>	78.30±0.74 <sup>cde</sup>	56.63±2.59 <sup>b</sup>	1.52±0.05 <sup>c</sup>	1.09±0.09 <sup>de</sup>	6.10±0.47 <sup>c</sup>	1.02±0.03 <sup>a</sup>	2.49±0.23 <sup>cdefgh</sup>	3.72±0.25 <sup>b</sup>
FLIP 03-80FB	Brown	Cuboid	Smooth to rough	White	Medium	1	37.00±1.58 <sup>ab</sup>	84.72±1.3 <sup>cde</sup>	68.80±3.55 <sup>d</sup>	1.75±0.12 <sup>d</sup>	1.19±0.05 <sup>de</sup>	6.40±0.70 <sup>c</sup>	1.12±0.02 <sup>ab</sup>	2.53±0.18 <sup>cdefgh</sup>	4.36±0.12 <sup>d</sup>
FLIP 03-82FB	Dark green	Cuboid	Smooth to rough	White	Medium	1	37.22±1.48 <sup>ab</sup>	97.42±2.36 <sup>cd</sup>	70.83±3.78 <sup>de</sup>	1.96±0.11 <sup>e</sup>	1.27±0.06 <sup>fg</sup>	6.66±0.56 <sup>cde</sup>	1.22±0.03 <sup>bc</sup>	1.87±0.29 <sup>cde</sup>	4.60±0.09 <sup>de</sup>
FLIP 05-01FB	Brown	Cuboid	Smooth to rough	White	Medium	1	38.14±1.67 <sup>ab</sup>	79.07±3.99 <sup>cde</sup>	66.70±3.37 <sup>d</sup>	1.56±0.10 <sup>cd</sup>	1.03±0.09 <sup>cd</sup>	7.46±0.51 <sup>de</sup>	1.27±0.05 <sup>b</sup>	2.25±0.38 <sup>cdefgh</sup>	5.13±0.55 <sup>ef</sup>
FLIP 05-02FB	Brown ochre	Truncate	Smooth to rough	White	Medium	1	49.00±2.07 <sup>de</sup>	83.62±4.77 <sup>cde</sup>	56.46±2.55 <sup>b</sup>	1.62±0.09 <sup>cd</sup>	1.13±0.04 <sup>de</sup>	7.56±0.90 <sup>de</sup>	1.11±0.03 <sup>ab</sup>	2.32±0.25 <sup>cdefgh</sup>	3.88±0.19 <sup>bc</sup>
FLIP 05-03FB	Brown	Truncate	Smooth to rough	White	Medium	1	38.12±2.23 <sup>b</sup>	94.92±3.00 <sup>cd</sup>	57.85±2.47 <sup>b</sup>	1.63±0.04 <sup>cd</sup>	1.21±0.07 <sup>ef</sup>	6.02±0.48 <sup>c</sup>	1.26±0.02 <sup>b</sup>	2.37±0.32 <sup>cdefgh</sup>	4.19±0.17 <sup>cd</sup>
FLIP 05-04FB	Brown	Cuboid	Smooth to rough	White	Medium	1	38.87±1.88 <sup>b</sup>	81.00±1.27 <sup>cde</sup>	64.63±4.02 <sup>d</sup>	1.66±0.13 <sup>cd</sup>	1.14±0.05 <sup>de</sup>	7.13±0.45 <sup>de</sup>	1.27±0.12 <sup>b</sup>	3.33±0.46 <sup>m</sup>	4.55±0.09 <sup>de</sup>
FLIP 05-05FB	Brown ochre	Cuboid	Smooth to rough	White	Medium	1	37.28±1.79 <sup>ab</sup>	91.40±1.34 <sup>cd</sup>	59.43±4.40 <sup>c</sup>	1.70±0.17 <sup>d</sup>	1.15±0.04 <sup>de</sup>	7.36±0.41 <sup>de</sup>	1.34±0.08 <sup>bc</sup>	2.36±0.32 <sup>cdefgh</sup>	4.52±0.21 <sup>d</sup>
FLIP 06-001FB	Brown ochre	Cuboid	Smooth to rough	White	Medium	1	41.87±1.35 <sup>bc</sup>	93.65±0.77 <sup>cd</sup>	61.03±3.07 <sup>c</sup>	1.78±0.13 <sup>d</sup>	1.16±0.05 <sup>de</sup>	7.60±0.75 <sup>de</sup>	1.04±0.07 <sup>a</sup>	1.83±0.24 <sup>cde</sup>	4.30±0.04 <sup>cd</sup>
FLIP 06-003FB	Brown	Cuboid	Smooth to rough	White	Small	1	34.83±1.60 <sup>a</sup>	95.85±2.19 <sup>cd</sup>	61.60±3.67 <sup>cd</sup>	1.66±0.08 <sup>cd</sup>	1.06±0.05 <sup>de</sup>	6.50±0.40 <sup>cd</sup>	1.07±0.06 <sup>a</sup>	2.35±0.21 <sup>cdefgh</sup>	4.38±0.34 <sup>e</sup>

Quantitative characters are presented as mean of eight individuals per accession. Superscript letters represent homogenous groups following the Newman and Keuls test *SD* standard deviation of the ANOVA study. *Ac* accessions. *FD* duration from sowing to 50 % of flowering, *WHS* weight of hundred seeds, *PH* plant height, *SL* seed length, *SW* seed width, *PL* pod length, *PN/V* number of pods per node, *SN/P* seed number per pod, *LN/L* number of leaflets per leaf. Qualitative characters: *SC* seed color, *SSH* seed shape, *ST* seed texture, *FC* flower color, *SEAC* standard extent of anthocyanin coloration, *SAC* standard anthocyanin coloration (0, absent; 1, present)

**Fig. 2** UPGMA dendrogram for morphological traits of 46 faba bean accessions. Genetic distances were based on Gower general similarity coefficients



had the highest hundred-seed weight, whereas VIR 2037, NEB 102, NEB 118, VIR 2040, Saber 02, and FLIP 03-35FB had the lower hundred-seed weight.

Surprisingly, the four currently cultivated genotypes in Tunisia showed late flowering dates, and lower hundred-seed weight compared to the average of all accessions (41.46 and 72.81, respectively) and have a higher number of seeds per pod, and a higher number of pods per node compared to the average of all accessions (2.14 and 1.22, respectively).

The qualitative and quantitative data were used to evaluate genetic distances among accessions and to construct a

dendrogram (Fig. 2). The 46 analyzed accessions were classified according to the 15 selected descriptors, indicating two main groups. At a coefficient of 0.76, accessions of the first group was divided into three subgroups in which the first subgroup (cluster I) comprised only the currently four cultivated genotypes in Tunisia. The second group was divided into four subgroups (clusters IV to VII). The cluster IV comprised only one accession (NEB 397) while the cluster VII comprised the majority of the accessions from the ICARDA elite faba bean breeding programs. The cluster V comprised two accessions NEB 99 and one from ICARDA elite accessions (FLIP 03-67FB). Although this accession share similar qualitative morphological traits with other accessions (particularly with those from the ICARDA elite faba bean breeding program), it differed significantly for quantitative traits such as seed number per pod (SN/P) from most of the other ICARDA elite accessions.

**Table 3** Data summary for 17 microsatellite markers across 46 *Vicia faba* accessions

SSR locus	Na	Ne	Ho	He	UHe	I	$F_{st}$	PIC
M9	7	6.21	0.78	0.83	0.84	1.88	0.06	0.81
M14	5	4.09	0.45	0.75	0.76	1.49	0.39	0.71
M22	5	3.37	0.96	0.70	0.71	1.35	-0.39	0.65
M25	4	2.87	0.76	0.65	0.65	1.13	-0.16	0.6
M27	8	5	0.93	0.80	0.80	1.76	-0.16	0.78
M36	9	6.04	0.95	0.83	0.84	1.94	-0.17	0.81
M41	10	6.11	1	0.83	0.82	1.94	-0.19	0.82
M43	2	1.98	0.86	0.49	0.50	0.68	-0.75	0.38
M46	6	4.12	0.69	0.75	0.76	1.58	0.08	0.73
M57	7	4.60	0.58	0.78	0.79	1.67	0.25	0.76
M64	4	3.25	0.97	0.69	0.70	1.26	-0.41	0.64
M66	3	2.10	0.71	0.52	0.53	0.90	-0.36	0.47
P11	6	3.59	0.97	0.72	0.72	1.48	-0.35	0.69
P27	5	3.87	0.91	0.74	0.75	1.46	-0.23	0.7
P28	4	2.82	0.55	0.64	0.65	1.17	0.19	0.59
P43	8	5.60	0.93	0.82	0.83	1.85	-0.13	0.8
P121	8	6.74	0.97	0.85	0.86	1.98	-0.14	0.84
Mean	5.94	4.26	0.82	0.73	0.73	1.50	-0.14	0.69

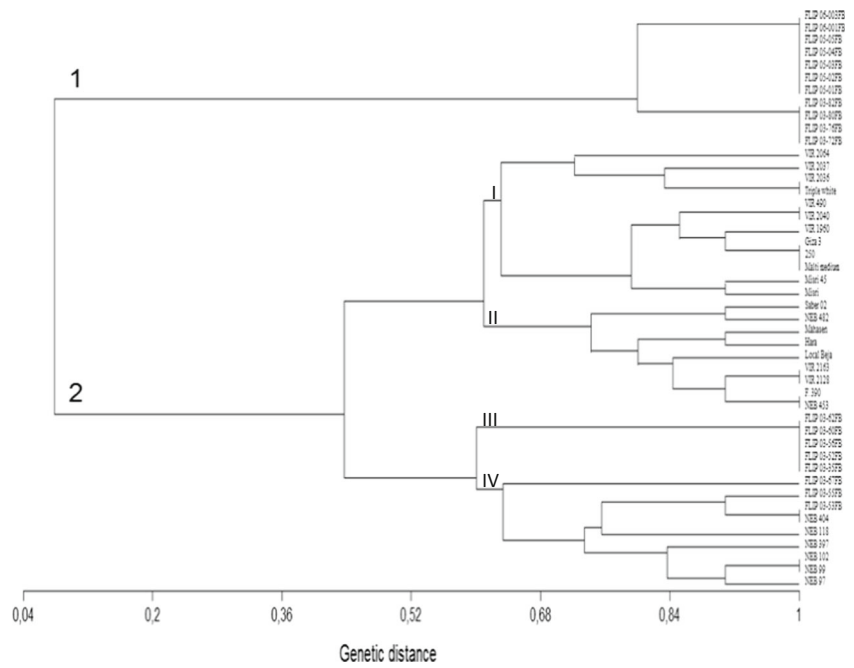
Na number of alleles, Ne number of effective alleles, Ho observed heterozygosity, He expected heterozygosity, UHe unbiased expected heterozygosity, I Shannon's diversity index,  $F_{st}$  fixation index, PIC polymorphism information content

### Molecular Characterization

Seventeen SSR markers were used for the characterization and genetic diversity analysis of the 46 faba bean accessions (Table 3). A total of 101 alleles were detected; the number of alleles per locus ranged from 2 (M43) to 10 (M41), with an average of 5.94 alleles across the 17 loci. The polymorphism information content (PIC) values ranged from 0.38 (M43) to 0.84 (P121), with an average of 0.69. The frequency of the most common allele at each locus ranged from 21 % (P121) to 64 % (M66). On average, 35.35 % of the 46 faba bean accessions shared a common major allele at any given locus. The He mean was 0.73, ranging from 0.49 in M43 to 0.85 in P121, and Ho ranged from 45 % of heterozygous individuals in M14 to 100 % in M41. The Wright's fixation index ( $F_{st}$ ) compares He with Ho, estimating the degree of allelic fixation, and ranged from 0.75 in M43 to 0.39 in M14 with an average value of 0.14. Finally, Shannon-Weaver's information indices ranged from 0.68 to 1.98 and averaged at 1.5. The results showed an abundant genetic diversity



**Fig. 3** Dendrogram of the 46 faba bean accessions included in this study generated by UPGMA cluster analysis from the similarity matrix obtained using Nei's genetic distance (Nei and Li 1979) based on the results of 17 SSR markers



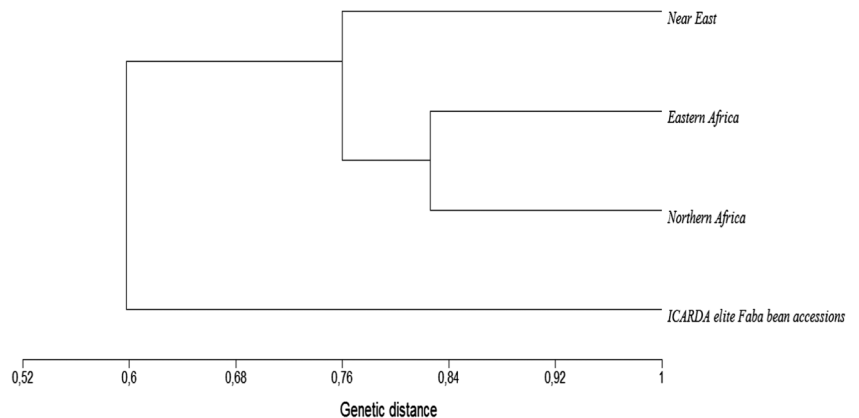
among the 46 accessions, and the most polymorphic loci, P121 and M41, revealed a high level of genetic variation.

**Cluster Analysis**

In order to understand the genetic relationships of faba bean accessions from different sources and origins, the Nei's genetic distances were calculated. UPGMA cluster analysis of the similarity matrix obtained from 17 SSR markers resulted in a dendrogram of genetic relationships (Fig. 3) producing two major clusters. The first group included 11 among the 19 accessions originating from ICARDA elite faba bean accessions. The second cluster was subdivided into four subclusters, one of which included currently cultivated Tunisian varieties along with some genotypes originate from Egypt, Libya, and Ethiopia such as VIR2163, VIR 2128, NEB 482, F.390, and NEB 453. The second subcluster comprised accessions of different countries of origins. The third subcluster

included five accessions originating from ICARDA elite faba bean accessions, and finally, the fourth subcluster comprised of six NEB accessions and three accessions originating from ICARDA elite faba bean accessions that the register data available assigned tentatively to an ICARDA improved genotypes origin probably derived from NEB. Interestingly, a number of traditional and improved genotypes originating from ICARDA elite faba bean accessions did not form a well and defined group. This dendrogram shows that the members of the same geographic origin were not assigned to the same group. However, the four currently cultivated Tunisian cultivars belonged to the same group. The cluster analysis performed using the similarity data between groups including accessions that belong to each geographical area (Fig. 4) showed that North Africa and Eastern Africa accessions were very similar ( $D=0.82$ ). Near East accessions seemed to be as closely related to the Eastern Africa as to the Northern Africa group ( $D=0.74$ ). Finally, accessions originating from ICAR

**Fig. 4** Dendrogram of the four main originate groups included in this study generated by UPGMA cluster analysis from the similarity matrix obtained using Nei's unbaised genetic distance coefficients (Nei 1972)





diversity and characterize crop resources (Keneni et al. 2012; McClean et al. 2012; Suresh et al. 2013).

In this study, we utilized SSR analysis to characterize genetic diversity in 46 faba bean accessions. The average number of alleles per locus (5.94) was higher compared to AFLP (1.45) and SSAP markers (1.68) (Liu and Hou 2010; Ouji et al. 2012). These suggest that SSR markers are a very suitable tool for assessing genetic diversity of faba bean.

The molecular analysis was used to tentatively classify the 46 accessions by different ways: UPGMA cluster analysis and principal components analysis.

Based on the UPGMA cluster analysis, the 46 accessions were divided into two major groups (Fig. 3). Group 1 included 11 among 19 ICARDA elite faba bean accessions. The remaining eight ICARDA elite faba bean accessions were clustered in group 2. Within this group, four subgroups were observed. Interestingly all ICARDA elite faba bean accessions observed in group 1 and group 2 were clustered together. Moreover, PCA analysis showed that all ICARDA elite faba bean accessions were clustered separately from the genotypes of other origin but simultaneously formed three subgroups of points. These data suggested that ICARDA elite faba bean accessions differ from other accessions and grouped together regardless of the type of analysis. This probably means that they were selected according to the same characters. Interestingly, SSR provide more information than morphological analysis distinguishing some subgroups that morphological analysis did not distinguish. Moreover, subgroups observed in ICARDA elite faba bean accessions could suggest a common pedigree. According to the dendrogram analysis (Fig. 3), six accessions—NEB 97, NEB 404, NEB 99, NEB 102, NEB 397, and NEB 118—from Egypt, Turkey, Syria, Tunisia, and Ethiopia, respectively, were classified with ICARDA elite faba bean accessions into group 2. This may suggest that accessions from these regions could have been used as a basis for the development of the ICARDA elite faba bean accessions. NEB accessions have been utilized in faba bean breeding programs, especially at ICARDA, in order to introduce genes to confer tolerance to abiotic and biotic stress, and these faba bean germplasms have been successfully introgressed into faba bean cultivars (Stoddard et al. 1999).

Duc et al. (2010) described main ecogeographical groups; three of them were represented in this study (ICARDA elite faba bean accessions, Eastern Africa, and Northern Africa), and we performed a cluster analysis on this base using the similarity data between groups including accessions that belong to each geographical area and ICARDA elite faba bean accessions. This cluster analysis showed the most diverging set of accessions to be from ICARDA elite faba bean accessions (Fig. 4) and showed a lower level of diversity between the other regions. The second most distinct set was from Near East (Fig. 4). On the other hand, Fig. 3 shows that accessions were clustered into two groups but that the groups do not

correspond to the ecogeographical origin of the accessions. This clustering may reflect the pedigree relations and commonality of important agricultural traits in accessions from different regions. Moreover, the observed genetic diversity of SSR loci was subjected to principal component analysis. The multivariate analyses classified the 46 accessions into groups consisting of ICARDA elite faba bean accessions, VIR genotypes, and other accessions from different pedigree. Figure 5 allows assessment of the genetic relationship among accessions. There is no obvious relationship between geographical origin and grouping based on genetic similarities. Both UPGMA cluster analysis and PCA showed relatively distant relationship of ICARDA elite faba bean accessions with other accessions from different origin. On the other hand, the correlation between genetic and geographical distances among faba bean accessions needs to be further studied using more accessions across greater geographic.

SSR and morphological analyses showed significant differences between Tunisian local and commercial faba bean cultivars (Mahasen, Hara, local Beja, and Saber 02) and the remaining accessions. The Tunisian accessions are characterized by late flowering dates, weak 100-seed weight, higher number of seeds per pod, and a higher number of pods per node compared to the average of all accessions. The molecular and morphological differentiation of the Tunisian cultivars suggests a common genetic pool as well as an adaptation to local agro-climatic conditions.

Elite faba bean cultivars were clearly discriminated from accessions originating from different geographical origin. Indeed, the genetic distances among some elite faba bean accessions were relatively high suggesting the existence of an interesting diversity among these elite accessions. On the other hand, we found that the genetic diversity between the Tunisian faba bean genotypes is narrow and that the Tunisian faba bean genotypes are closer to the other faba bean germplasm sources than to the ICARDA elite faba bean accessions. Therefore, we suggest the importance of introducing elite faba bean accessions to enrich the genetic foundation of breeding of faba bean in Tunisia. Similar results were approved by Liu and Hou (2010) which suggested that introducing elite foreign germplasm to enrich genetic foundation of breeding of faba bean in Qinghai should be necessary.

Although the 46 accessions could not be obviously divided into several unattached groups, some valuable information about the genetic relationship of some accessions was obtained from the dendrogram based on SSRs. Indeed, breeders might introduce some heterogeneity in their material from other breeding centers (Li et al. 2008). It is interesting to note that PCA analysis placed all VIR accessions in one group showing a high level of genetic relatedness and close parentage. This analysis suggests that the collection for the in situ conservation of VIR accessions was, may be, made on the

basis of specific criteria which allowed us to distinguish them from most of the other accessions.

Our study confirmed the high potential of SSRs as excellent molecular markers for faba bean genotype identification, differentiation, and evaluation of genetic variation.

## Conclusion

In conclusion, our data showed significant variation in morphological traits and microsatellite DNA polymorphisms among 46 faba bean accessions. The relatively high average number of alleles per locus and allelic diversity observed in the set of genotypes analyzed indicate that the genetic base of the material is relatively wide. The allelic richness of the total accessions suggested the presence of potential unique and rare alleles. ICARDA elite faba bean accessions had clustered together versus the other accessions from different origins suggesting a common pedigree and the similarity of the selective traits. Morphological and molecular analysis showed that local and commercial Tunisian genotypes seem to be different from all the other accessions, suggesting an adaptation to local conditions but also a narrow genetic base. Results from this study show that SSRs markers are a useful tool for faba bean diversity studies, which is a key in conducting faba bean breeding programs. Indeed, knowledge of the genetic diversity of a species is important for the choice of crossing parents in hybrid breeding (Tams et al. 2004). To ensure sustaining breeding progress in the future, the introduction of new germplasm into national faba bean breeding programs, especially by the aid of molecular markers, is recommended.

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