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Biodiversity of cultivated *Trigonella foenum-graecum* L. in Tunisia, North Africa

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Abstract

In agricultural systems, biodiversity includes diversity within species and among species and provides many benefits for production, resilience and conservation. This article aims to study the diversity of *Trigonella foenum-graecum* L., an annual herb of *Leguminosae* with a worldwide distributed culture, among thirty eight local populations collected from different Tunisian regions. The variability analysis was based on morphological characters, compositions of minerals in leaves and vitamins in seeds, and molecular profile. Morphological parameters consisted of vegetative and reproductive characters. Mineral analysis concerned sodium, potassium, iron, calcium and magnesium leaves contents. The analyzed vitamins contents in seeds were B1, B9 and C vitamins and molecular study was carried out by ISSR technique. The structure of the studied populations was established by the principal compound analysis (PCA) and by the unweighted pair group method using arithmetic means (UPGMA). Both analyses based on the combination of morphological, chemical and biochemical parameters and on ISSR molecular study, presented a binary clustering of populations; but the one was different from the other. The combination of morphological, chemical and biochemical parameters allowed distinguishing the populations 2 and 4 of Menzel Temime, 11 of Mateur and 13 of Beja from the remaining populations. These four populations had the highest yields in biomass and seeds and, compared to the rest of populations, they were the best forages and seed producers. Molecular study showed that population 26 of Menzel Temime was genetically far away from the rest of studied fenugreek populations.

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Introduction

Humans have been cultivating plants and developing agricultural systems since thousands of years. Several species have gradually been adapted to meet people's needs. They have become domesticated. When plants became so called cultivated plants, they lost the characteristics which made them specially adapted to their natural environment. With the help of humans they have instead developed other characteristics (Vigouroux *et al.*, 2011). In agricultural systems, biodiversity includes diversity within species and among species and provides many benefits for production, resilience and conservation (Enjalbert *et al.,* 2011). Studies on crop genetic diversity are not as yet very widespread and sharply focused. However, studies on genetic diversity of wild crop relatives and domesticated animals have been carried out (Malik and Singh, 2006). Such studies within and among populations of species can provide important basic information for breeding programs to conserve genetic resources (Bruschi *et al.,* 2003). Different methods of estimating the genetic diversity were used in plant breeding and conservation programs. Among these methods, morphological and chemical markers were used in germplasm management, however they have a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith, 1992; Statti *el al.,* 2004; Beyene *et al.,* 2005). Alternatively, DNA markers have not such limitations. They can be used to detect variation at the DNA level and have proven to be effective tools for distinguishing between closely related genotypes (Beyene *et al.,* 2005).

Trigonella foenum-graecum L., vernacular name fenugreek, is an annual herb of *Leguminosae* (Ramesh *et al.,* 2001) which is used as spice and forage (Vats *et al.,* 2002) and known also as medicinal plant rich in proteins, vitamins and amino acids (Hidvegi *et al.,* 1984). Culture of fenugreek is worldwide distributed and this reflects its adaptation to varying climatic conditions. Duke (1986) indicated that the best development of the plant is obtained in regions with fresh and temperate climate with annual rainfall between 100 and 600 mm / year. Culture of *T. foenum-graecum* does not require specific conditions of soil. However, one of the most important features to ensure proper growth of the plant is the ability of soil to provide adequate moisture throughout the growing period (Petropoulos, 2002). Actually, fenugreek is widely cultivated in Asia, Europe, America and North Africa. In Tunisia, it is especially cultivated in the regions of the North (Marzougui *et al.,* 2007).

Tunisian populations diversity of cultivated *T. foenum-graecum* has been previously studied by our team and that based separately on morphological and chemical parameters (Marzougui *et al.,* 2007), on biochemical (Marzougui *et al.,* 2009A) and molecular data (Marzougui *et al.,* 2009B). In this study we aim to combine morphological, chemical and biochemical parameters to get an idea about the structure of the thirty-eight collected populations of fenugreek, and compare this structure with that obtained starting from the molecular data and to distinguish the best populations on the basis of the considered parameters.

Materials and methods

Plant material and culture

The seeds of thirty eight (38) fenugreek local populations were collected from different Tunisian regions (Fig. 1). Populations were selected according to the abundance of this culture in the corresponding region and the period of cultivation (seeds cultivated for at least 10 years in the same region), as well as other factors such as geographical distance separating populations from one another (2 to 316 km), bioclimate, altitude, longitude and edaphic substrate (Table 1). The collected seeds were cultured in Elfejeh in the South-East of Tunisia. This region belongs to the lower arid bioclimatic stage. It has a sandy muddy and limestone ground which rests on a gypsy layer. The culture of each population was repeated three times and for each repetition one plant was sampled for our studies. Sowings were carried out manually in

December and harvest was done in June (Marzougui *et al.,* 2007).

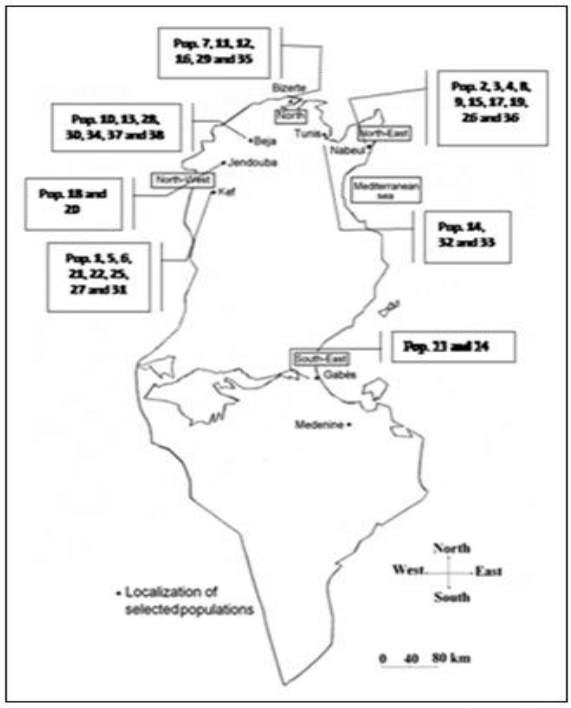

Fig. 1. Collection region of thirty eight selected populations of *Trigonella foenum-graecum* L. Pop.

Morphological analysis

Morphological analysis for each population was assured by the study of their vegetative and reproductive characters (Table 2). Length, width and diameter measurements were done by an electronic slide calliper. The first measurement was one month after the rising, the second two months after the first one and the third two months after the second one. Number, length and width of leaf and leaflet values for each plant corresponded to the average of three measurements.

Mineral analysis

For determination of sodium, potassium, iron, calcium and magnesium 4 g of grinded leaves were gently heated on a hot plate. Heating was continued until enough water was driven off for partial carbonization to occur. Samples were placed at 100°C in an electronic furnace for 1 h and weighed. Then they were ashed for 4 h at 500°C, 2 ml of double distilled water was added to ashes and 5 ml of conc. HCl was added to dissolve the salts. Double distilled water was used to prepare fixed volumes of measurement solution (25 ml). A Shimadzu AA 6800 was used to determine the sodium, potassium, iron, calcium and magnesium contents with flame atomic absorption method. The element concentration was calculated according to the equation: % Na, % K, %Ca, %Mg and %Fe = C x V /(10^4 x m) x % MS, where C featured value (ppm), V extract volume (ml), m mass (g), % MS Percentage of dry material. For determination of phosphorus content, 1 g of grinded leaves was ashed for 4 h at 550°C. After cooling, ashes were mixed with 4 ml of distilled water and 1 ml of conc. HCl. The solution was taken to boiling, then filtered and completed to 100 ml with distilled water. A Secomam spectrophotometer was used to determine the phosphorus concentration. Standard solutions of P 0, 2, 4, 8 mg/ l were prepared by diluting basic solution of P 100 mg/l, and 10 ml of each standard and the diluted extract were placed in 25 ml tubes with 10 ml of the vanadomolybdic reagent (200 ml of a 10% ammonium heptamolybdate solution obtained with dissolving 100 g of ammonium heptamolybdate in 10 ml of ammonium hydroxide and adding distilled water until volume of 1 litre; 200 ml of ammonium monovanadate solution obtained with 2.35 g of ammonium monovanadate in 400 ml of hot distilled water, 20 ml of 35% diluted nitric acid and distilled water until 1 litre, 135 ml of concentrated nitric acid and distilled water until 1 litre). After 10 min, the absorbances were measured at 430 nm. Extracts were diluted if needed. P concentrations were calculated according to the formula: % P = (C x DF) / (100 x m), where C P content (mg/l), DF Dilution factor, m extract mass (g).

Vitamins analyses

Preparation of samples for vitamin B analyses

We developed a fenugreek extraction protocol of the various categories of vitamins (Marzougui *et al.*, 2009A). Fenugreek seeds (1 g) were cooked during 30 min at 95°C in 25 ml of 0.1 N H_2SO_4 solution. After cooling, the contents were adjusted into pH 4.5 with 2.5 M sodium acetate. Then 0.05 g of the enzyme takadiastase was added (Vinas *et al.*, 2003). The

preparation was kept one night at 35°C, filtered through a micropore filter (0.45 µm) and diluted with 50 ml of distilled water. A volume of 20 µl of this filtrate was injected into the injector (Toma and Tabekhia, 1976). The recovery percentage of the used extraction method reached 79.45% for B6, 73.41% for B1 and 83.67% for B9.

Preparation of samples for vitamin C analysis

Samples (1 g) were diluted in 30 ml of 1% orthophosphoric acid. The mixture was agitated with vortex during 1 min. After centrifugation with 1740 rpm during 15 min at 20°C, the supernatant was filtered through a micropore filter (0.45 µm) and diluted with 25 ml of orthophosphoric acid. Handling was quickly carried out to avoid the degradation of the vitamin C (Arella *et al.*, 1997). The recovery percentage of the used extraction method was 74.86%.

Chromatographic determination

The Knauer apparatus was equipped with two pumps, a control system, a reversed phase, a curl injector of 20 µl and an ultraviolet absorbance detector (254 nm). The used column was Eurospher 100 C-18.5 (250 × 4.6 mm i.d., 5 µm) (Colmer and Davies, 1974). The used mobile phase was a mixture of methanol 9 %, 10 ml of crystalline acetic acid and 600 ml of distilled water. This solvent was added with sulfonic acid pentane and sulfonic acid octane; the whole was filtered through a 0.45 µm filter and deaerated by agitation. The prepared solvent was pumped through the column with a programmed flow between 0.5 and 2.1 ml/min. Peak surfaces were calculated using an electronic integrator. The quantitative values were obtained against vitamin standard injections containing vitamins (mg/50 ml) as follows: 9.7 B6, 4.0 B1, 0.25 B9 and 1.0 C.

Molecular analysis

Fresh young fenugreek leaves were harvested for each cultivar. Plant DNA was extracted by the Doyle and Doyle method with minor modifications (Doyle and Doyle, 1987). DNA concentration was determined by spectrophotometry at 260 nm. A set of 12 anchored microsatellite primers was used in this study. PCR amplification of 100 ng of DNA was performed in PCR buffer (10x), 25 mM $MgCl_2$, 10 mM dNTPs, 20 uM of ISSR primer and 5 U/µl of Taq DNA polymerase (Fermentas) in a 20 µl reaction using GeneAmp PCR System 9700 thermocycler. The PCR program was 5 min at 94°C for initial denaturation, followed by 35 cycles of 54 s at 94°C, 45 s at 45°C or 50°C (temperature hybridization varied from 45 °C to 50°C according to primer variation) (Marzougui *et al.*, 2009B), 2 min at 72°C, and a final 5 min extension at 72°C. Amplified products were electrophoresed for 2 h under constant voltage (60 V) on 1% agarose gel using 1xTBE buffer (10 mM Tris-boric acid and 1 mM EDTA pH 8.0) and visualized by ethidium bromide staining. The gels were observed under ultraviolet light and photodocumented using the image capturing system Bioprint. The Jules DNA ladder was used as standard molecular weight marker. PCR was carried out 3 times, only the repeatable bands were considered for diversity assessment.

Statistical analyses

The structure of the 38 studied populations of *T. foenum-graecum* was performed by principal component analysis (PCA) and the unweighted pair group method using arithmetic means (UPGMA). PCA analysis was done using StatBox version 6.4 (Grimmersoft, Paris, France). Mahalanobis distances matrices for morphological parameters, minerals compositions and vitamins contents were generated using XLSTAT version 2008 (Addinosoft SARL, Paris, France). Concerning the molecular analysis, the genetic similarity among populations was determined by Nei's genetic distance (Nei, 1978). The similarity matrix was generated using Nei and Li's coefficient (Nei and Li, 1979). All matrices values were entered to MVSP 3.1 (Kovach Computing Services, Wales, UK) to establish the UPGMA dendrogram. Correlations between Mahalanobis distances and geographic distances between populations were evaluated by the test of Mantel (Mantel, 1967) using XLSTAT version 2008 (Addinosoft SARL, Paris, France). To estimate

the geographical structure of populations, we have grouped them into seven sets, depending on the area of collection (Kef, Jendouba, Beja, Bizerte, Mhamdia, Menzel Temim and Menzel Lahbib) and performed a PCA on the average characters for each group.

Results

Structure of populations based on the combination of morphological, chemical and biochemical parameters

In our previous works, the structure of the thirty-eight collected populations of *T. foenum-graecum* was based on morphological and chemical parameters (Marzougui, 2010), biochemical parameters (Marzougui *et al.*, 2009A) and molecular data separately (Marzougui *et al.*, 2009B) (Fig. 3). Whereas in this study, we are interested in the combination of morphological, chemical and biochemical parameters to get an idea about change in Tunisian cultivated fenugreek structure and compare this structure with that obtained starting from the molecular data. PCA showed that the first three axes account for 46.79 % of the total variability (Table 3). The population projection on the planes defined by axes 1-2 and 1-3 of PCA absorbs respectively 36 % and 34 % of the total inertia (Fig. 4 A, B), and shows the existence of two identical groups. The first group (G1), located on the negative side of axis 1, is formed by the populations 2 and 4 of Menzel Temime ; 11 of Mateur and 13 of Béja. The second group (G2), distributed on the positive and the negative sides of axis 1, contains the rest of the considered populations (Fig. 4 A, B).

Table 1. Location and main ecological traits for the 38 Tunisian *Trigonella foenum-graecum* populations.

Bioclimamic stage and alternative	Locality	Population code	Edaphic substrate	Altitude (m)	Latitude (N)	Longitude (E)	Rainfull (mm/year)
Upper-Semi arid[s]	Menzel Temime	2, 3, 4, 8, 9, 15, 17, 19, 26, 36	Argillaceous	22	36°47’	10°59’	390-630
	Bizerte	7	Limestone	4	37°16’	9°52’	300-800
	Mater	11, 12, 16, 29, 35		37	37°02’	9°41’	300-800
	Mhamdia	14, 32, 33	Clay/Limestone	64	36°40’	10°09’	275-515
	Sidi Hamed	18	Carbonated	143	36°29’	8°46’	450-1500
	Ben Bechir	20	Limestone	143	36°29’	8°46’	450-1500
	Nefza	30, 37	Marl/Limestone	213	36°43’	9°11’	350-1000
Upper-Semi arid [m]	Sidi Khiar	1, 25	Marl/Clay	650	36°11’	8°43’	400-700
	Nebeur	5		524	36°17’	8°44’	400-700
	Elkef	6, 22		654	36°10’	8°42’	400-700
	Tel Elgozlan	21, 31		657	36°11’	8°43’	400-700
	Borj Berrzig	27		648	36°11’	8°43’	400-700
Sub humid[m]	Beja	10, 13, 28, 34, 38	Sandy	213	36°43’	9°11’	350-1000
Lower arid [m]	Menzel Habib	23, 24	Sundy Loam	84	34°27’	9°44’	184,4

[s] the soft alternative. [m] the moderate alternative.

To better visualize the structure of populations and verify that achieved by PCA, we conducted the UPGMA classification. And this leads to obtaining a dendrogram with two great groups, denoted a and b (Fig. 5). The first (a) includes four populations and can be divided into two subgroups. The second (b) collects the rest of the populations and can also be subdivided into two subgroups. The dendrogram can therefore distinguish four subgroups.

The first subgroup (G1) contains the populations 1 and 25 of Sidi Khiar, 3, 19 and 36 of Menzel Temime, 7 of Bizerte, 12 of Mateur, 14 and 32 of Mhamdia, 18 of Sidi Hamed, 21 and 31 of Tell Elghozlan , 20 of Ben Bechir, 30 of Nefza, 22 of Kef, 27 of Borj berrzig, 34 of Beja, 23 and 24 of Menzel Lahbib. The second (G2) consists of the populations 17, 9, 15, 8 and 26 of Menzel Temime, 16, 29 and 35 of Mateur, 33 of Mhamdia, 5 of Nebeur, 6 of Kef, 37 and 10 of Nefza,

28 and 38 of Beja. The third (G3) comprises the populations 2 of Menzel Temime, 13 of Beja and 11 of Mateur. And the fourth (G4) is formed by the population 4 of Menzel Temime.

Table 2. Examined morphological characters for the 38 studied populations of *Trigonella foenum-graecum*.

Vegetative characters		Reproductive characters	
Code	character	Code	character
% G	Germination percent	FC	Flowers standard colour
LN	Leaves number at the flowering beginning	FN	Flowers number at the flowering end
CFl	Leaflets colour	PN	Pods number by stem
LL*	Central leaflet length (cm)	PL	Pods length (cm) at the green maturity stage
LW*	Central leaflet width (cm)	SN	Seeds number by pod
SL	Stem length (cm) at the flowering end	SW	200 collected seeds weight (g)
SD	Stem diameter (cm) at the flowering end		
BN	Branch number by stem		

* Fig. 2.

For geographical groups of populations, PCA based on the combination of morphological, chemical and biochemical parameters showed that the first three axes account for 77.87 % of the total variability (Table 4). The projection of average points representing geographical groups, on the planes defined by axes 1-2 and 1-3 (respective percentages of total inertia 62 % and 53 %) showed two groups (Fig. 6 A and B).

Table 3. Variability between the 38 fenugreek populations based on the combination of morphological, chemical and biochemical parameters: absorption of variability by the first three PCA axes and their significance in relation to variables.

Axes	Eigenvalue	Proportion (%)	Cumulated (%)	Parameters most correlated with axes
1	5.38	22.40	22.4	LL (+), PN (-),SN (-), Na (+), K(+), Ca (+)
2	3.17	13.21	35.61	LN (+), CFl (-), LW (+), LL (+)
3	2.68	11.18	46.79	LN (+), CFl (+), PN (+), PL (+)

LL: Central leaflet length, PN: Pods number by stem, SN: Seeds number by pod, LN: Leaves number, CFl: Leaflets colour, LW: Central leaflet width, PL: Pods length, K: Potassium content, Ca: Calcium content, Na: Sodium content, (+) and (-): Positive and negative correlations of variables with the PCA axes.

The first (G1), located on the negative side of axis 1, contains the groups of Kef (LK), Jendouba (J) and Menzel Lahbib (MH), with the lowest values for the characters Leaves number, Branch number by stem and Pods length, and the highest levels of iron. The second geographical group (G2), located on the positive side of axis 1, consists of the groups of Beja (Bj), Bizerte (Bz), Mhamdia (M) and Menzel Temime (MT). These groups are characterized by the highest values for the characters Leaves number, Branch number by stem and Pods length and the lowest levels of iron. This structure does not operate according to the distance between the geographical groups of populations. Indeed in G1 the groups of Kef and Jendouba are located northwest of Tunisia (Fig. 1) while Manzel Lahbib is localized southeast. And G2 consists of the groups of populations of northern Tunisia.

UPGMA dendrogram of geographical groups confirmed their PCA structure and showed two groups (Fig. 7). The first (G1) was formed by the groups of Kef (LK), Jendouba (J) and Menzel Lahbib (MH). And the second (G2) contained the groups of

Béja (Bj), Bizerte (Bz), Mhamdia (M) and Menzel Temime (MT). Once again the obtained structure didn't operate according to the distances between the geographical groups. Indeed in G1 the groups of Kef and Jendouba are located northwest of Tunisia (Fig. 1) while Manzel Lahbib is localized southeast. And G2 consists of the groups of populations of northern Tunisia.

Table 4. Variability between the 7 geographical groups based on the combination of morphological, chemical and biochemical parameters: absorption of variability by the first three PCA axes and their significance in relation to variables.

Axes	Eigenvalue	Proportion (%)	Cumulated (%)	Parameters most correlated with axes
1	8.59	37.36	37. 36	% G (-), SN (+), PL (+)
2	5.67	24.65	62.00	SN (-), Na (+), K (+), Ca (+), P (+
3	3.65	15.86	77.86	Mg (+), B9 (-), C (+)

% G: Germination percent, SN: Seeds number by pod, PL: Pods length, K: Potassium content, Ca: Calcium content, Na: Sodium content, P: phosphorus content, Mg: magnesium content, B9: vitamin B9 content, C: vitamin C content, (+) and (-): Positive and negative correlations of variables with the PCA axes.

Table 5. Molecular variability between the 7 geographical groups: absorption of variability by the first three PCA axes and their significance in relation to variables.

Axes	Eigenvalue	Proportion (%)	Cumulated (%)	Parameters most correlated with axes
1	4,667	66,672	66,672	$(GAG)_3GC$ (+)
2	1,742	24,879	91,551	$(GA)_6CC$ (+)
3	0,546	7,803	99,355	$(CA)_6GT$ (+), $(CA)_6AG$ (+)

$(GAG)_3GC$, $(GA)_6CC$, $(CA)_6GT$ and $(CA)_6AG$: ISSR primers; (+): Positive correlation of variables with the PCA axes.

Table 6. Correlation coefficients between matrices of the used parameters and their significance by the Mantel test ($p<0.05$).

Parameters	r	p	Sig.	Per.
Morphological/chemical	0.428	0.0001	**	1000
Morphological/biochemical	0.11	0.003	**	1000
Morphological/molecular	-0.21	0.0001	**	1000
Molecular/chimiques	-0.07	0.062	ns	1000
Molecular/biochemical	0.015	0.015	*	1000
Chemical/biochemical	0.056	0.138	ns	1000

r: correlation coefficient, p: probability of Mantel test, sig.: significance, per.: permutations, **: significant at $p<0.01$, *: significant at $p<0.05$ et ns: no significant.

Comparison between the structure obtained from combination of the different studied parameters and the molecular data structure

The grouping of the populations based on the combination of morphological, chemical and biochemical parameters made it possible to structure them in two groups. The first group is formed by the populations 2 and 4 of Menzel Temime, 11 of Mateur and 13 of Béja. And the second group contained the rest of the studied populations (Fig. 4 and 5). Whereas referring to molecular results, the grouping of the studied populations, made by PCA and UPGMA method (Fig. 3 D), appeared to be consisted by two other structures of groups

(Marzougui *et al.*, 2009B). The first group was formed by the population 26 of Menzel Temime and the second group contained all the rest of the considered populations.

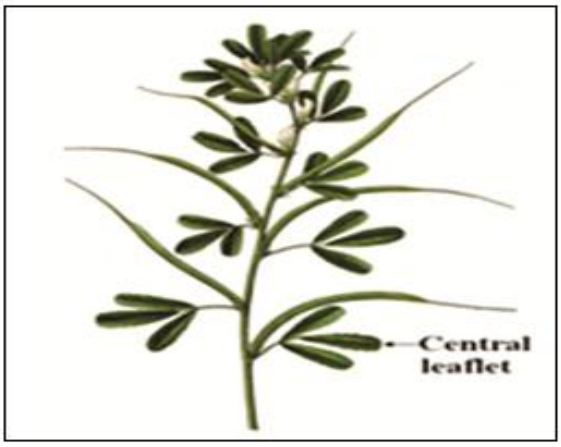

Fig. 2. Sampling location on the leaf used to measure the length (LL) and width (LW) of the central leaflet.

For geographical groups of populations, the molecular data made it possible, using PCA, to classify them in two groups different from those obtained by the combination of the other studied parameters (Fig. 7 A and B). Based on molecular parameters, PCA showed that the first three axes account for 99.34 % of the total variability (Table 5) and allowed to join together the populations of the regions of Jendouba (J), Bizerte (Bz), Menzel Témime (MT), Menzel Lahbib (MH), Le Kef (LK) and Béja (Bj) in the first group (G1). These groups of populations were characterized by a number of bands which varies between 0 and 7, and a molecular weight ranging between 1700 and 0 Pb. The second geographical group (G2), located on the positive side of axis 1, consists of Mhamdia (M). This structuring does not made according to the geographical distance between the groups of populations.

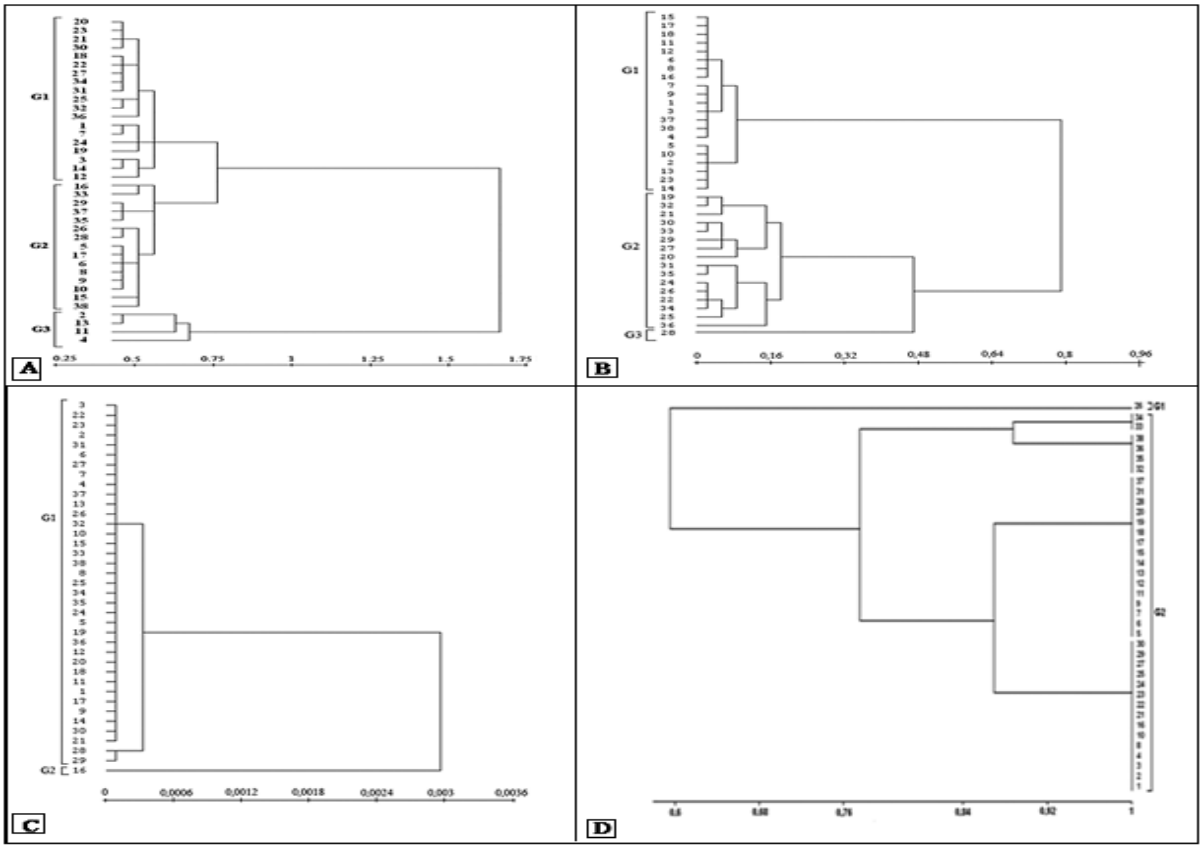

Fig. 3. Dendrograms of the 38 studied populations of *Trigonella foenum-graecum*. A, B and C: dendrograms established from Mahalanobis distances for morphological, chemical, and biochemical parameters respectively. D: from Nei genetic distances for the molecular parameters established by ISSR technique. G1: group 1, G2: group 2, G3: group 3.

Correlations between the different studied parameters

The Mantel test was used to evaluate the correlations between the different studied parameters, taken in pairs (Table 6). Morphological parameters are correlated with all types of studied parameters. Molecular parameters show significant correlations with biochemical parameters, and no significant correlations with chemical ones. Latter two have not significant correlations between them.

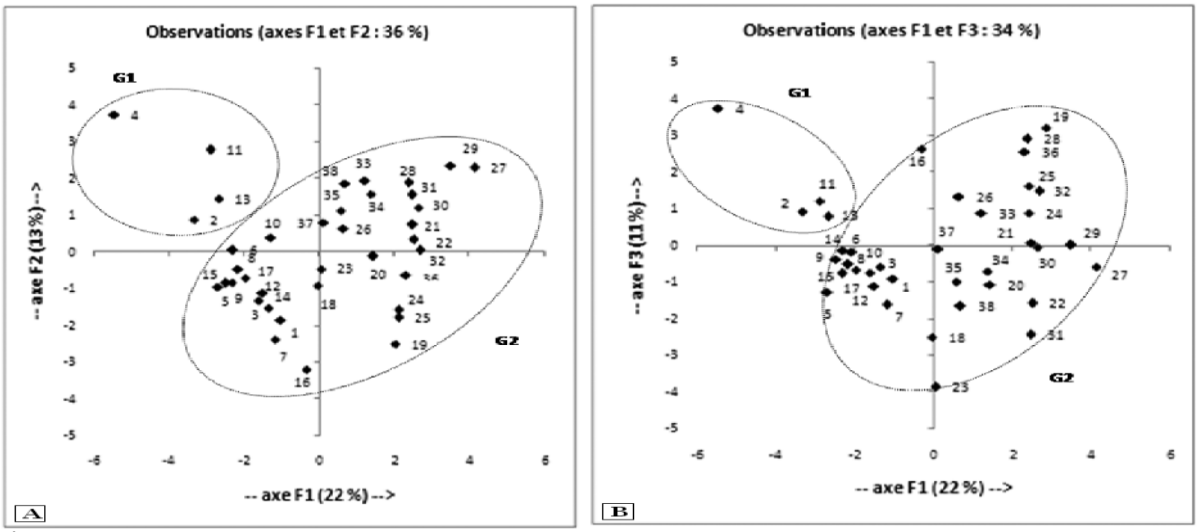

Fig. 4. Principal component analysis based on the combination of morphological, chemical and biochemical parameters, and performed for thirty-eight analyzed populations. A: Population projection on the plane defined by the axes 1-2, B: axes 1-3. G1: group 1, G2: group 2.

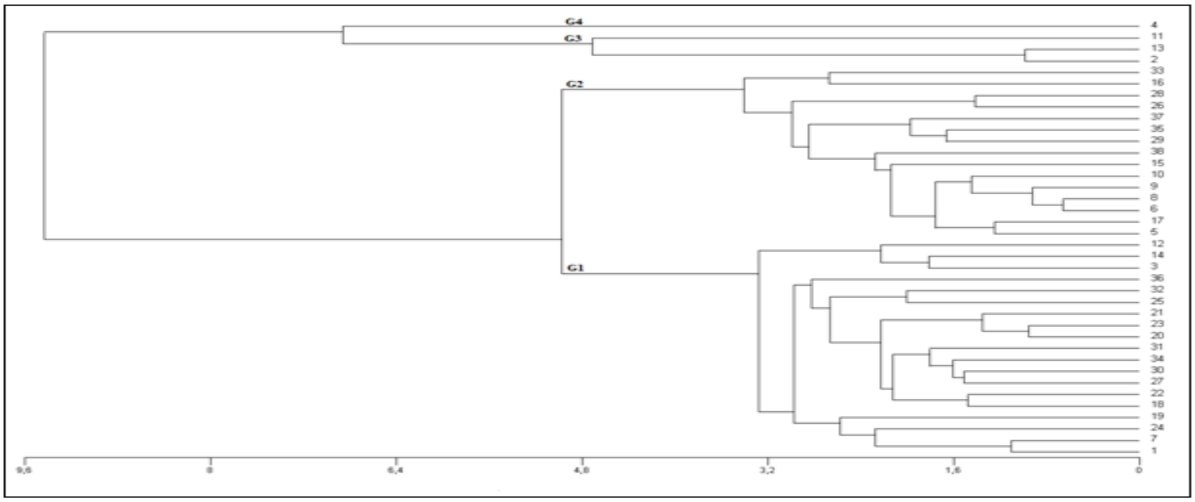

Fig. 5. Dendrogram of 38 populations of Tunisian *Trigonella foenum-graecum*, built according to the UPGMA method on the basis of the combination of morphological, chemical and biochemical parameters; a: group 1, b: group 2, G1: subgroup 1, G2: subgroup 2, G3: subgroup 3, G4: subgroup 4.

Discussion

Biodiversity within plant species has often been analyzed on the basis of the combination of different parameters. Tucak *et al.*, (2008) studied the genetic diversity of forty cultivated populations and one spontaneous population of alfalfa (*Medicago* spp.) collected from different countries. This study was based on a combination of morphological and RAPD molecular parameters. The established structure allowed to distinguish between the spontaneous

population (*M. falcata*) and the cultivated populations (*M. sativa* and *M. media*) grouped into two subgroups. They did not detect significant correlations between morphological and molecular distances, and they found that RAPD markers were most useful for the assessment of genetic diversity in germplasm of alfalfa. Chahidi *et al.*, (2008) used morphological, physiological and molecular parameters to assess the genetic diversity of three populations of Clementine. They observed a relatively high phenotypic diversity, which contrasts with the low molecular polymorphism. Basha *et al.*, (2009) were based on the molecular and biochemical traits for assessing genetic relationships among seventy-two accessions of *Jatropha curcas* L. representing thirteen countries around the world. They found high genetic diversity in the Mexican germplasm and low genetic variation among accessions from other countries.

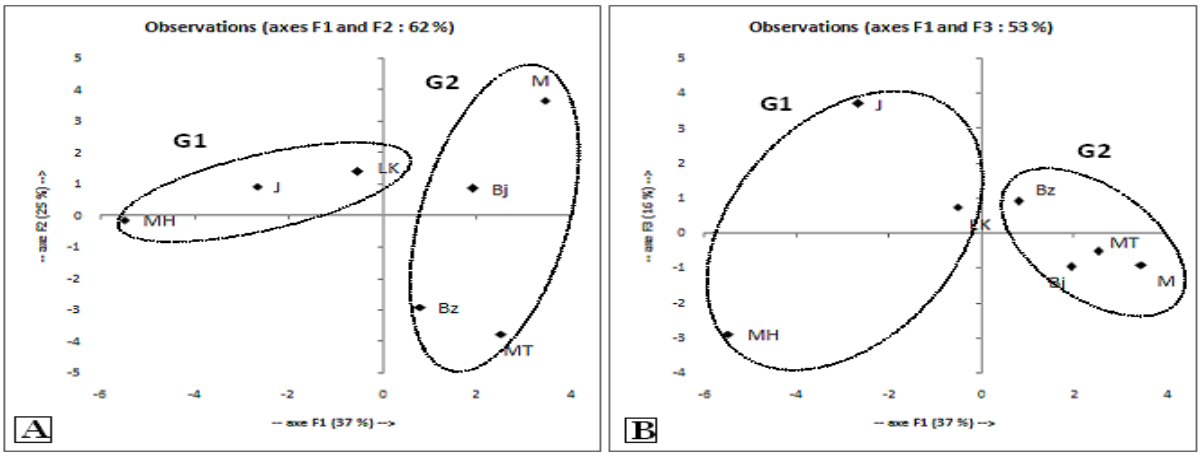

Fig. 6. Principal component analysis based on the combination of parameters, and performed for 7 geographical groups. A: Groups projection on the plane defined by the axes 1-2, B: axes 1-3. G1: group 1, G2: group 2. LK: Kef, J: Jendouba, B: Beja, Bz: Bizerte, M: Mhamdia, MT: Menzel Temim and MH: Menzel Lahbib.

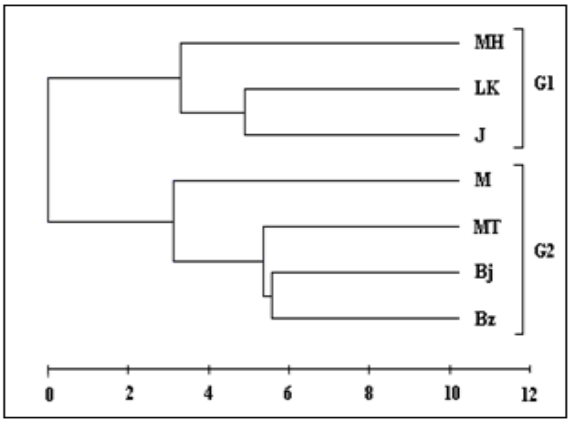

Fig. 7. Dendrogram of 7 geographical groups of Tunisian *Trigonella foenum-graecum* populations, built according to the UPGMA method on the basis of combination of morphological, chemical and biochemical parameters. G1: group 1, G2: group 2. LK: Kef, J: Jendouba, B: Beja, Bz: Bizerte, M: Mhamdia, MT: Menzel Temim and MH: Menzel Lahbib.

Mkaddem (2007) evaluated the variability between ten natural Tunisian populations of *Mentha pelegium* L. on the basis of phenotypic and enzymatic characteristics and composition of essential oils in terpenes. The combination of these parameters allowed him to structure the collected populations of *M. pelegium* into three groups, finding that this structure recalls that revealed by terpene markers and that the three types of approaches have no significant correlations between them. In our case, PCA structure of the thirty eight *T. foenum-graecum* populations using jointly morphological, chemical and biochemical parameters resulted in a binary clustering. UPGMA analysis was identified the same two great groups that those identified by PCA. Geographical groups were divided into two clusters by both PCA and UPGMA method. The obtained structures of populations and geographical groups

were not correlated with their geographic distributions and recalled those revealed by morphological parameters (Marzougui, 2010). The diversity analysis of the thirty eight studied fenugreek populations based on ISSR molecular data allowed to classify them in two groups different from those obtained by the combination of morphological, chemical and biochemical parameters.

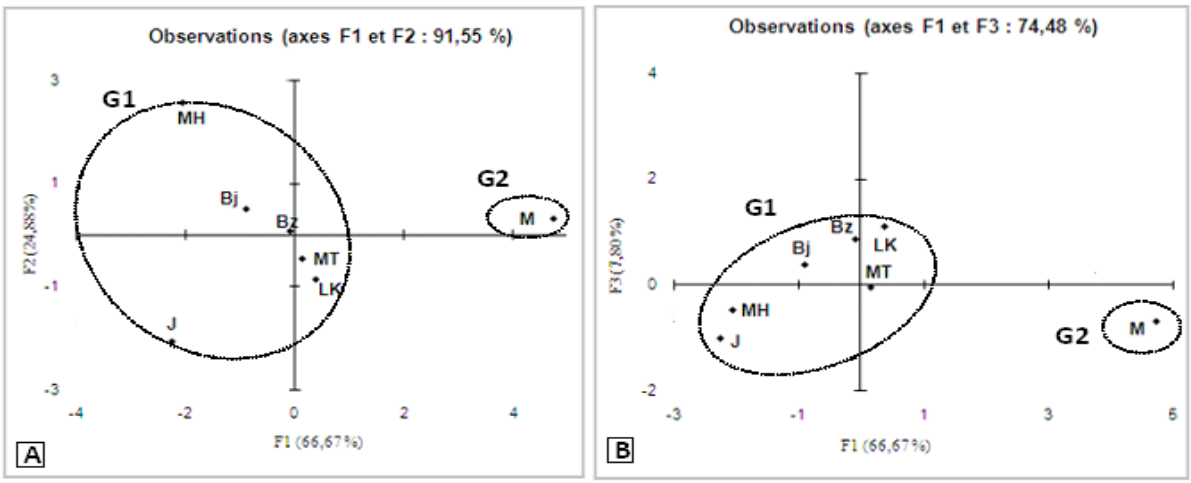

Fig. 8. Principal component analysis based on molecular data for 7 geographical groups. A: Groups projection on the plane defined by the axes 1-2, B: axes 1-3. G1: group 1, G2: group 2. LK: Kef, J: Jendouba, B: Beja, Bz: Bizerte, M: Mhamdia, MT: Menzel Temim and MH: Menzel Lahbib.

The structure of geographical groups on the basis of molecular parameters showed also a different grouping from this obtained by the combination of the other studied parameters and was not correlated with their geographic distribution. This absence of correlation between the obtained structure and the geographical distances separating the groups of populations testifies to an excessive domestication of *T. foenum-graecum* in Tunisia, disadvantages the genetic drift and causes the impoverishment of the genetic base of this species. Indeed, the study of correlations between morphological descriptors on the one hand and chemical, biochemical and molecular parameters, taken separately by pairs on the other hand, showed significant correlations. Molecular data showed significant correlations with morphological and biochemical data and no significant correlations with chemical data. Such absence of correlation may be due to that chemical characteristics and molecular markers correspond to different levels of complexity, which act on different evolutionary forces. In other studies, molecular markers like RAPD, SSR, ISSR, AFLP and RFLP showed no significant correlation with morphological descriptors for several plants such as *Pisum sativum* L., *Momordica charantia* L. and *Vasconcellea* (Dey *et al.*, 2006; Tar'an *et al.*, 2005; Kyndt *et al.*, 2005). In contrast, studies on pea accessions by SRAP markers suggested that the molecular and morphological systems provide similar assessments of genetic relationships (Espósito *et al.*, 2007). Molecular study showed that population 26 of Menzel Temime was genetically far away from the other populations (Marzougui, 2010). Results of the combination of morphological, chemical and biochemical parameters showed that the thirty eight collected populations of *T. foenum-graecum* belong to two groups. Populations 2 and 4 of Menzel Temime, 11 of Mateur and 13 of Beja were distinguished from the rest of the populations. The population 4 presented high number of leaves; the highest brunch number, high pod length, high number of seeds per pod, the highest content of Mg in leaves and the highest seed content in vitamin B1. The population 11 was characterized by the highest number of leaves, high number of branches, high pod length and the highest number of

seeds per pod. Populations 2 and 13 showed high values in number of branches and length of pods. Thus, these four populations have the highest yields in biomass and seed number, compared to the rest of the populations; they are the best forage and have the best seed production. These populations will prove to be interesting to replace alfalfa in crop rotations in the short term, because unlike alfalfa, fenugreek does not cause bloating, and contains diosgenin, a steroid. Similarly, in Canada, Acharya *et al.*, (2007) studied two local cultivars of fenugreek, one named Tristar and grown in Alberta and the other, named Amber and taken as control in their study. They indicated that Tristar yield in biomass exceeds that of Amber and claimed that Tristar will prove interesting to replace alfalfa in crop rotations in the short term, because this cultivar gives as much biomass as two cuts of alfalfa.

Conclusion

Analysis of Tunisian fenugreek biodiversity based on the combination of morphological, chemical, biochemical and molecular data, allowed distinguishing populations 2 and 4 of Menzel Temime, 11 of Mateur and 13 of Beja from the rest of populations. These four populations had the highest yields in biomass and seeds and compared to the rest of studied populations, they were the best forages and seed producers.

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