

Fabaceae Diversity Assessment: Distribution, Richness and Similarity in Northern Tunisia

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Abstract

The study focused on the evaluation and characterization of plant genetic resources of fodder legumes in northern Tunisia for the purpose of biodiversity conservation. The study was carried out on the biodiversity of legumes (*Fabaceae*) and their geographical distribution as well as on the ecological characterization of their natural environment using different ecological indices. Nine sites distributed in the governorates of Bizerte, Beja and Jendouba were visited to collect information. In this context, an inventory was carried out showing the presence of 40 different species of forage legumes divided into 15 genus, some of which exist in the three governorates prospected at the same time. The genus *Trifolium* and *Medicago* was the most diversified by the presence of 9 and 8 species. Thus, eight less diversified monospecific genus have been listed such as (*Anthyllis*, *Calicotome*, *Hippocrepis*, *Lathyrus*, *Lupinus*, *Melilotus*, *Scorpiurus* and *Retama*). We have also identified species with widespread distribution such as *Trifolium campestre* and *Trifolium tomentosum* and species with restricted geographical distribution such as *Anthyllis vulneraria* at Rimel in the governorate of Bizerte and *Hippocrepis unisiliquosa* in the governorate of Beja in Medjez El Bab. Shannon index showed differences between and within governorate. The three governorates studied have an average of 1.883 bits in Bizerta, 2.052 bits in Beja and 1.601 bits in Jendouba. This shows that diversity index is significantly higher in Béja compared to that of Bizerte and Jendouba. Jaccard similarity index calculated for the different studied localities.

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Introduction

Biodiversity has been at the center of scientific interest for about twenty years (Wilson, 1988, Lubchenco *et al.*, 1991) as well as that of the international political community (CNUED, Rio de Janeiro, Brazil in 1992). This biodiversity has become a "common concern for humanity", and its conservation a global issue. Thus, Tunisian flora extremely rich in various spontaneous species includes *Fabaceae* family. This important family contains 36 genus and 216 species in Tunisia and is considered one of the most abundant botanical families (Nabli, 1989). Economically, legumes (*Fabaceae*) represent the second most important family of crop plants after the grass family, *Poaceae*. Grain legumes are a source of proteins used for human and animal nutrition and their cultivation did not require nitrogen fertilizers. For the evaluation and valorization of plant genetic resources of legumes interest in northern Tunisia, a study was conducted on the ecological characterization of the natural habitat of *Fabaceae*.

Biodiversity is defined as sum of characteristics present in an individual. It has been observed that greater biodiversity within ecological zone help as a buffer against deterioration of the ecosystem. Moreover, greater biodiversity helps to carry out various ecological services such as protection against runoff, collapse of ecosystem due to spread of diseases. In contrast to biodiversity, genetic diversity is presence of variation at particular locus. Genetic diversity improve the sustainability of species.

The main objective of this study is to enrich and update knowledge on forage legumes of northern Tunisia, to identify their geographical distribution and their organization in an ecological community and to preserve germplasm of major legume.

Material and methods

Study Area

Three governorates in northern Tunisia were visited (Bizerte, Beja and Jendouba) and three sites per governorate were studied. The governorate of Bizerta is the northern point of Africa. It is bordered in the north by the Mediterranean Sea (250 km), by the governorate of Beja in the southwest, and by the governorates of Manouba and Ariana in the south-east. Its climate is humid and the average temperature is 22.8°C. Three governorates were visited and three sites per governorate were studied (Table 1).

The first site in this governorate concerns the Rimel forest, located in the south of Bizerte. Its altitude decreases from the south-east where it varies from 180 to 150 meters, towards the north-west where it borders the beach of Rimel between Menzel Jemil and Zarzouna. Cap Blanc, is the second site in Bizerta governorate, located at the end of northern Bizerta. This region has a specific biodiversity, such as the plant association of *Quercus coccifera*, *Juniperus phoenicea* and *Juniperus oxycedrus*. The weather is characterized by an average temperature equal to 17.1°C and by an annual precipitation average equal to 614 mm year⁻¹.

Table 1. Different prospected localities

Location	Latitude N	Longitude E	Altitude (m)
Rimel	37°15'16''	9°55'14''	150
Manzel abderrahmen	37°14'15''	9°53'01''	35
Cap blanc	37°19'57''	9°51'04''	261
Medjez El Bab	39°39'31''	9°33'38''	52
Chott Zouraa	37°01'42''	8°54'39''	6
Oued Zarga	36°40'19''	9°25'47''	125
Ain Draham	36°47'44''	8°41'11''	22
Tabarka	36°55'40''	8°47'21''	717
Bou Salem	36°36'15''	9°58'28''	127

The third site, Menzel Abderrahman is located about sixty kilometers from the north of Tunis. Climate is warm, the annual average temperature is 17.9°C and the average annual precipitation is 527 mm year⁻¹.

The governorate of Beja is the second governorate of north-western Tunisia. The precipitation is high in winter than in summer. The average annual rainfall is 662 mm year⁻¹. Oued Zarga is the first site visited in this governorate. The average annual temperature is 17.9°C and the average annual rainfall is around 511 mm year⁻¹.

Medjez El Bab is the second province of the governorate of Beja, located at 36°39' north latitude and 9°33' east longitude. This site is recently exploited by the cultivation of some olive trees. Vegetation cover is marked by the predominance of certain species such as *Ebunus pinnata*, *Ampelodesma mauritanica*, *Scolymus hispanicus*, *Hyparrhenia hirta* and *Leontodon tuberosus*. The average annual temperature is 18.1°C and the average annual rainfall is 443 mm in Medjez El Bab. Chott Zourâa is the third site in Beja governorate located at 37°1' north latitude and at 8° 54' east longitude. This site presents a very particular landscape consisting of a dune field that extends over a cork oak forest reaching the village of Ouechtata at an altitude varying between 6 and 57 meters (Mehat, 2005). Plant biodiversity is very rich, with a dominance of dune plants such as *Sporobolus pungens* and *Ammophila arenaria*. Chott Zourâa receives a significant annual contribution in terms of rainfall, ranging from 800 to 1500 mm year⁻¹. Governorate of Jendouba is the third governorate visited in the north-west of the country, on the Tuniso-Algerian border, its covers an area of 3102 km². The climate is warm and the average temperature varies between 5-10°C in winter and between 25-30°C in summer.

Tabarka is a coastal town in north-western Tunisia at 36°55' north latitude and 8°47' east longitude, 180 km from Tunis and 65 km from Jendouba. It falls around 865 mm year⁻¹. The average annual temperature in Tabarka is 18.2°C. The studied site is located in front

of the Sylvo-Pastoral Institute of Tabarka and is 5 km from the Tabarka beach. This site corresponds to agricultural fields of wheat and barley. It is crossed by a small valley which constitutes the source of potential water of these fields and its altitude is around 22 meters. The second site of this governorate is located in Ain Drahem. It is a small town at the north-west of Tunisia located at 36° 47' north and 8° 41' east. In winter, there is much more rainfall in Ain Drahem than in summer. The average annual precipitation is 1389 mm year⁻¹ and the average annual temperature is 15.0°C. The altitude of the site visited is about 717 meters. The plant layer is dominated by undergrowth abundant and dense such as *Phillyrea angustifolia*, *Myrtus communis*, *Erica arborea*, *Pistacia lentiscus*, *Populus alba* and *Urginea maritima*. The third site in this latest governorate is in Bou Salem which is a city in north-western Tunisia at 36°36' north latitude and 8°56' east longitude. The average annual temperature in Bou Salem is 18.0°C. The site is located in a small village called Graïia, at an altitude of 127 meters. Sampling was carried out on the bank of the wadi Medjerda, characterized by the dominance of certain species such as *Cynodon dactylon*, *Phragmites australis*, *Leontodon tuberosus* and *Taraxacum sp.*

Data Collection

The ground data allowed validating the classification of different type of vegetation in primary forest and secondary and also crop fields. The sampling for the data collection was carried out in 10 plots measuring 256 m² of 16 m × 16 m each one placed in different strata of the study area: primary forest; secondary forest and crop fields. The GPS coordinates of the zones were recorded in a GPS. Ground data validated the classification. Thus, for each site, the number at each plot is essential to analyze the ecological indices by collecting some samples of the plants for the identification of non-recognized species on the ground while using Tunisian flora (Pottier-Alapetite, 1979).

Measuring Biodiversity

A number of measures have been used in order to measure species diversity. Thus we measured species richness (S) which is the number of different species present in an area (Boulinier et al., 1998). Species richness as a measure on its own takes no account of the number of individuals of each species present. It gives equal weight to those species with very few individuals and those with many individuals.

Simpson's index (D) was calculated. It is a measure of diversity, which takes into account both species richness, and an evenness of abundance among the species present. It measures the probability that two randomly selected individuals belong to the same species (Simpson, 1949). This index, on the other hand, can underestimate the diversity in a sample in case of dominance of one species relative to the others. This is why we based our interpretations on the combination

of the specific richness analysis and Simpson's formula index.

The formula for calculating (D) is presented as:

$$D = \frac{\sum n_i(n_i - 1)}{N(N - 1)}$$

Where n_i = the total number of organisms of each individual species, N = the total number of organisms of all species

The value of D ranges from 0 to 1. With this index, 0 represents infinite diversity and, 1, no diversity. That is, the bigger the value the lower the diversity. We apply also the Shannon diversity index (H') as a measure of species abundance and richness to quantify diversity of species. This index takes both species abundance and species richness into account. Its formula is as follows:

$$H' = - \sum_{i=1}^s p_i \ln p_i$$

The Pielou equitability index (J') (Pielou, 1969) was used to measure the distribution of individuals within species, regardless of species richness. Its value varies from 0 (dominance of a species) to 1 (equi-distribution of individuals in species). This index is linked to the Shannon-Weaver index and its formula is as follows:

$$J' = H' / H'_{\max}$$

$H'_{\max} = \log S$ (S = total number of species),

H' : Shannon diversity index, N_i : number of individuals of a given species, i : ranging from 1 to S (total number of species), N : total number of individuals.

Similarity

The Jaccard index was used to calculate the degree of similarity between two lists of species. The similarity of two samples (floristic sample) is based on the presence or absence of certain species in the two samples (Legendre and Legendre, 1984). It is calculated by dividing the number of species common to both surveys by the total number of species present (Jaccard, 1912). For the calculation of the diversity indices, PAST software (Paleontological Statistic, version 2.4) (Hammer et al., 2001) was used.

Results

Forage species identified in northern Tunisia

The taxonomic lists of *Fabaceae* recorded and reported in Tunisia in the old works identified 251 species divided into 41 genera belonging to the following subfamilies: Papilionoideae, Caesalpinioideae and Mimosoideae (Le Floch et al., 2008). However, the examination of our legume samples revealed 40 species belonging to 15 genera belonging to the subfamily of Papilionoideae. Species identification was based on the key based on determination and morphological descriptions of Tunisian flora (Pottier-Alapetite, 1979). The new classification of species updated in the catalog entitled the flora of Tunisia (Le Floch et al., 2008) has also been revised. Figures (1a, 1b and 1c) summarize all the taxa encountered during sampling at the nine stations visited in northern Tunisia.



Figure 1a. Illustration of the different Fabaceae collected different stations (a1: *Anthyllis vulneraria*, a2: *Astragalus boeticus*, a3: *Astragalus hamosus*, a4: *Calicotome villosa*, a5: *Hedysarum coronarium*, a6: *Hedysarum capitatum*, a7: *Hippocrepis unisiliquosa*, a8 : *Lathyrus ochrus*).



Figure 1b. Illustration of the different Fabaceae collected different stations (b1: *Lotus edulis*, b2: *Lotus ornithopodioides*, b3: *Lotus creticus*, b4: *Lotus cytisoides*, b5: *Lotus parviflorus*, b6: *Lupinus luteus*, b7: *Melilotus sulcatus*, b8: *Medicago arborea*, b9: *Medicago murex*, b10: *Medicago orbicularis*, b11: *Medicago intertexta*, b12: *Medicago ciliaris*, b13: *Medicago scutellata*, b14 : *Medicago truncatula*, b15 : *Medicago polymorpha*, b16: *Ononis variegata*).



Figure 1c: Illustration of the different *Fabaceae* encountered in the different stations (c1 : *Ononis mitissima*, c2: *Retama raetam*, c3: *Scorpiurus muricatus*, c4: *Trifolium campestre*, c5: *Trifolium nigrescens*, c6: *Trifolium isthmocarpum*, c7: *Trifolium tomentosum*, c8: *Trifolium fragiferum*, c9: *Trifolium scabrum*, c10: *Trifolium stellatum*, c11: *Trifolium cherleri*, c12 : *Trifolium angustifolium*, c13: *Vicia sativa*, c14: *Vicia lutea*, c15 : *Vicia sicula*, c16: *Vicia disperma*).

Diversity indices: Species richness

Our sampling in studied localities allowed us to identify 40 species divided into 15 genera. The genera *Trifolium* and *Medicago* are the most diversified and are characterized by the presence of 9 to 8 species corresponding to 43% of the species encountered. The genera *Lotus* and *Vicia* are represented by 5 to 4 species. The genera *Astragalus*, *Hedysarum* and *Ononis* are bispecific, while monospecific genera are represented by about 36% of the genera listed. Figure 2 illustrates the diversity of the *Fabaceae* genus in relation to the different sites visited in northern Tunisia.

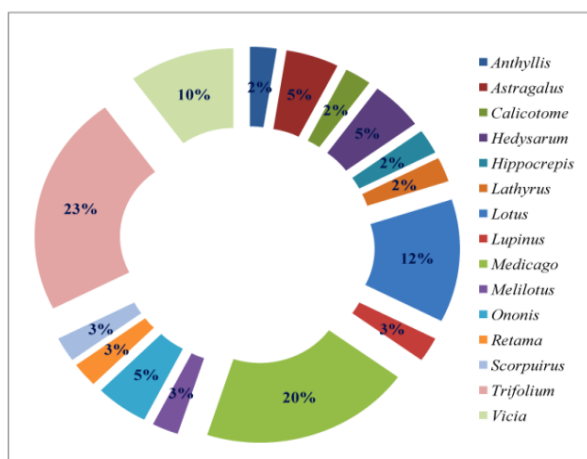


Figure 2. Specific diversity of *Fabaceae* encountered in the nine studied stations of northern Tunisia

Thus, the results showed the presence of the genus *Lotus* and the genus *Trifolium* in the 3 visited sites of the governorate of Bizerta, the genus *Anthyllis* and that of *Retama* characterize the region of Rimel and are considered as the predominant genera in this site. The genus *Astragalus* is present in the governorates of Bizerta and Beja but is absent in Jendouba. The genus *Calicotome* is present in one site in the governorate of Bizerta and in two sites in the governorate of Jendouba. It is also represented by a large population in the region of Ain Draham. In addition, the genus *Hedysarum* and the genus *Lotus* have been found in all governorates and appear to be markers of northern Tunisia. However, *Hippocrepis*, is present only in the area of Medjez El Bab in the governorate of Beja. The genus *Medicago* is present in all sites except Cap Blanc and the genus *Lupinus* was found only in Tabarka in the governorate of Jendouba. On the other hand, the genus *Scorpiurus* is present in one site in the governorates of Bizerta and Beja but is absent in Jendouba. This genus is abundant in the region of Menzel Abderrahmen but poorly described in Oued Zarga. Also, the genus *Trifolium* exists in all sites except that of Ain Draham. Finally, the genus *Vicia*, considered among the best known forage legume, has been described in the Rimel site of Bizerta governorate and in the Tabarka site of Jendouba governorate but is absent in Béja.

Table 2 summarizes the presence or the absence of forage legumes in the studied localities.

Simpson's index

In our study, we used the Simpson diversity index represented by 1-D. The maximum of diversity is being represented by the value 1, and the minimum of diversity is represented by the value 0 (Schlaepfer and Bütler, 2002). Thus, the values of 1-D varied between a maximum of 0.909 and a minimum of 0.499. The maximum value was recorded at Tabarka since it is the richest area in Forage legumes. In contrast, the minimum value was recorded at Ain Draham. This index also confirms the specific wealth in the governorate of Béja compared to other governorates, the average for this governorate is equal to 0.854, followed by the governorate of Bizerta by an average of 0.792 and finally the governorate of Jendouba by an average equal to 0.722 (Table 3).

Shannon-Weiner diversity index

The average values of the Shannon diversity index for the three sites are as follows: 1.883 bits in Bizerte, 2.052 bits in Beja and 1.601 bits in Jendouba. This shows that the diversity index is significantly higher in Beja than in Bizerta and Jendouba. In this study, the value of the highest Shannon index was found at 2.546 in Tabarka indicating the co-dominance of several species. The lowest value was found at Ain Draham 0.692 because the individuals encountered belong to only two species (Table 3).

Similarity: Jaccard Index

In order to compare *Fabaceae* population studied in the 3 governorates and in the 9 sites visited, we used the similarity coefficient of Jaccard. The latter takes into account the presence or absence of the species. The highest value of the recorded Jaccard index is 45.45% between Bou Salem and Chott Zouaraa, with the presence of 5 common species to both sites, followed by Menzel Abderrahmen and Medjez El bab with a similarity equal to 43.75%. The

R: Rimel, CB : Cap Blanc, MA: Menzel Abdrahman, MB: Mjez el Bab, Z: Zouaraa, OZ: Oued Zarga, T: Tabarka, A: Ain Draham, B: Bou Salem

Table 3. Shannon-Weiner, equitability and Simpson diversity indices of forage legumes species found in Northern Tunisia

	Bizerte			Béja			Jendouba		
	Rimel	Menzel Abrrahman	Cap Blanc	Mjez el Bab	Zouaraa	Oued Zarga	Tabarka	Ain Draham	Bou Salem
Species richness	13	13	7	10	10	9	16	2	6
Number of legumes	1684	1441	364	802	522	545	833	122	555
Shannon index	1.966	2.169	1.514	2.205	2.159	1.791	2.546	0.692	1.566
Equitability	0.766	0.845	0.778	0.957	0.937	0.815	0.918	0.999	0.873
Simpson index	0.823	0.858	0.696	0.881	0.872	0.810	0.909	0.499	0.759

Discussion

It is widely accepted that biodiversity depends on the hydrological regime, geological and climatic conditions (Vyas & Joshi, 2013). For legumes, the most important genera known are *Medicago*, *Trifolium*, *Hedysarum*, *Astragalus* and *Lotus*. Edaphic factors are more important than climatic factors in determining their distribution in an environment appropriate to their growth (Andrew & Hely, 1960). Thus, the genus *Medicago* is represented by a large number of species in the Mediterranean region. The genus *Trifolium* is represented in the Mediterranean region by a large number of taxa whose perennials are infrequent in the southern Mediterranean. This genus generally grows in North Africa in wet meadows and lowlands (Abdelguerfi & Laouar, 2004). The genus *Hedysarum* is quite common in the Mediterranean basin. *Hedysarum coronarium* appears to be the most promising taxon, with good summer dormancy, high forage quality, pasture tolerance, and vegetative propagation associated with natural reseeding (Sulas et al., 1999). The genus *Astragalus* is widely represented at the level of the Mediterranean basin, several species of different interests grow in different environments. Certain taxa, by their morphology (thorny), and others by their toxicity manage to remain in the courses subjected to overgrazing. In addition to the pastoral interest of some taxa, species of this genus may be a source of adaptation genes in some environments. *Lotus* genus is also widely represented around the Mediterranean basin. The species are quite numerous and some of them can play an important role in improving pastoral and / or forage production. *Lotus creticus* has a good salt resistance and can withstand brackish water (Foury, 1954). In the present work, we found 40 species of forage legumes, which shows that *Fabaceae* family is fairly well represented in northern Tunisia and this is concerning richness species. This result is in agreement with that obtained by (Averti Ifo et al., 2016).

Considering the Shannon diversity index, our study showed a low diversity of *Fabaceae* in the sites studied since the value of the highest Shannon index found is equal to 2.546. However, the

value of the Shannon value diversity Index could reach up to 5 in habitats rich in species. The lack of species within sites could be explained by the fact that they have disappeared from culture and arable land because of several factors, including overgrazing, intensification of culture, agricultural techniques used such as deep plowing, cultivation and over-use of herbicides and environmental changes (Gounot, 1958; Mathison, 1983).

However, some forage legume species are classified as species that prefer risky habitats such as crops (cereals, legumes) where threats of genetic erosion are important. Therefore, abiotic factors can affect the diversity of plant species. Indeed, it seems that the low winter temperatures that characterize the sites at altitude are constraints for the expansion of annual legumes in the Mediterranean region (Ewing, 1995). In Morocco, the distribution of annual legumes appears to be influenced mainly by soil pH and texture, and incidentally by altitude and precipitation (Beale et al. 1991). For this, Bounejemate et al. (1992) recommend that endangered species sites should be surveyed at regular intervals to ensure their presence or disappearance. In addition, the similarity dendrogram showed the following results:

Ain Draham site is isolated from other sites, probably because of the presence of the cork oak (*Quercus suber* L.), a dense layer of trees that inhibits the development of herbaceous plants.

- Tabarka is distinguished from other sites, which is explained by the fact that this site is the only one located near the fields of wheat, barley and pear.

- The sites of Bou Salem and Chott Zouraa and those of Medjez El Bab and Menzel Abderrahmen are the most similar. For the first group, this similarity would probably be due to their presence near a wetland (Oued Medjerda basin in Bou Salem and the Sidi Barrak dam in Chott Zouraa). For the second group, the observed similarity may be due to their presence in olive groves.

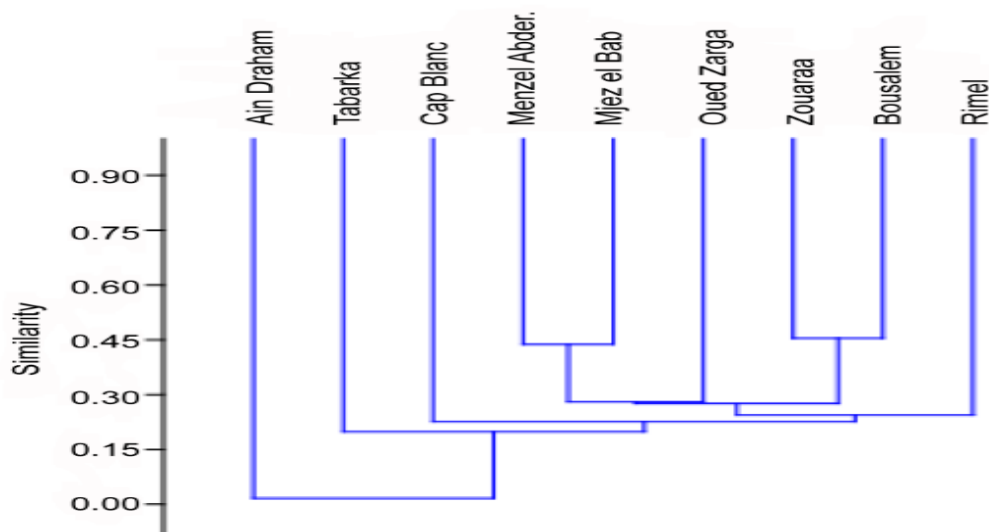


Figure 3. Dendrogram of similarity of *Fabaceae* in the various sites surveyed in northern Tunisia.

Conclusion

In the present work, we were interested in the evaluation and valorization of plant genetic resources of interest for forage in northern Tunisia. A study was carried out on the biodiversity of *Fabaceae* and their geographical distribution as well as on the ecological characterization of the natural habitat of spontaneous *Fabaceae* using different ecological indices. The floristic inventory carried out in the stations shows the presence of 40 different species of forage legumes divided into 15 genera, some of which exist simultaneously in the three governorates surveyed. Thus, we have identified widespread distribution species such as *Trifolium campestre* and *Trifolium tomentosum* as well as the genera *Hedysarum* and *Medicago* that appear to be present in the three governorates studied. We have also found species with a restricted geographical distribution reflecting a good heterogeneity of species dependent on habitats such as *Anthyllis vulneraria* in Rimel in the governorate of Bizerte and *Hippocrepis unisiliquosa* in the governorate of Beja in Medjez El Bab.

The analysis of the Simpson index confirms that the richest value belongs to the governorate of Beja compared to other governorates. The average diversity index of Simpson in the governorate of Beja is equal to 0.854, followed by the governorate of Bizerte equal to 0.792 and finally the governorate of Jendouba equal to 0.722. The values of this index range between a maximum of 0.909 and a minimum of 0.499. The maximum value was recorded at Tabarka because it is the richest area of *Fabaceae*. In contrast, the minimum value was recorded at Ain Draham.

The analysis of the Shannon diversity index revealed differences between governorates and within each governorate. In the governorate of Bizerte, the highest value of H' was registered at Menzel Abder rahman with 2.169 bits, followed by Rimel with 1.966 bits and Cap Blanc with a value of 1.514 bits. In the governorate of Beja, the highest value of H' was recorded in Medjez El bab, followed by Chott Zouara and then Oued Zarga with 1.791 bits corresponding to the minimum value. The governorate of Jendouba showed a high H' value recorded at Tabarka equal to 2.546 bits, followed by Bou Salem with 1.966 bits and finally Ain Draham with a minimum value corresponding to 0.692 bits.

Jaccard similarity index calculated for the population in the different studied stations showed that these sites are not very similar to each other. The closest sites are those of Bou Salem and Chott Zouaraa, where the value of the registered Jaccard index is 45.45%, followed by the sites of Menzel Abderrahmen and Medjez El Bab with a similarity of 43.75%.

Jaccard similarity index showed that Ain Draham is far from other sites and that the similarity values between the populations found on the different sites can be attributed to biotic and abiotic factors (climate, altitude, soil, surrounding vegetation, etc.).

Therefore, one of the major difficulties is that the majority of species have few recorded specimens to model their ranges. This difficulty can be overcome by coordinating the collaboration of local and international authorities. Now we call for a legume diversity assessment through collaboration among various scientists and additional financial support for a global project.

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