Journal of Animal & Plant Sciences (J.Anim.Plant Sci. ISSN 2071-7024)
Vol.52 (2) : 9495-9502 https://doi.org/10.35759/JAnmPlSci.v52-2.6

Diversity analysis of spontaneous populations of *Moraea sisyrunchium* (Iridacea) in different bioclimates of Tunisia

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Key words: *Moraea sisyrinchium*, spontaneous, Tunisia, morphological diversity.

Submission 05/01/2022, Publication date 31/05/2022, http://m.elewa.org/Journals/about-japs/

1 ABSTRACT

The survey of the north and the Sahel region of Tunisia enabled the collection of five spontaneous populations of the *Moraea sisyrinchium* (*Iris sisyrinchium* L.) plant. This North Africa endemic plant species is present in various plants associations from the humid to the arid bioclimatic and colonizes diverse habitats from high altitude to lagoons. This article intends to analyse the morphological diversity of these populations. For preserving their biodiversity, the study of the ornamental spontaneous species becomes essential. The results displayed a large diversity (p = 1 ‰) among the different *Moraea sisyrinchium* populations for the parameters: leaf length, stalk length, perianth tube length, ovary length, anther length and seed diameter. Important intra-population diversity was demonstrated. The Shannon-Weaver index is high (> 0.8) for the most of studied parameters.

2 INTRODUCTION:

Tunisia is located in the north of Africa and is largely open to the Mediterranean. This region counts up to 22,500 species of vascular plants (Myers and *al* .2000), 11,700 species were found in the hotspot region including Tunisia (Thompson et *al* 2005). Despite its relatively small size, Tunisia has a high level of biodiversity. This is due to its special geographic location. Different species of Iris are used as ornamental flowers, add to this many of them are the resources that produce perfumes in the cosmetics and beauty industry (Demir and Çelikel, 2018). A great diversity was detected on Iris sp. So many new cultivars can be produced by the technique of hybridization. These ornamental species are usually used in landscape and in cut flowers (Asgari *et al.,* 2021). There are many studies that interest edible plant diversity but there are a few studies with interest on spontaneous ornamental plants diversity in Tunisia. Tunisian Iridaceae resources are important. *Morea sisyrinchium* species, subject of this study, was considered the most ancient Iris species in the world (Lynch, 1903). It is the most interesting, in particular, if consider its ecological flexibility (different habitats: hills, mountains, sebkha). This study aims the analysis of the morphological diversity of different Spontaneous populations of *Moraea sisyrinchium* in different climate in Tunisia. This species is endemic to northern Africa; it is known, for a long time, for its medicinal benefits. However, this species is included in the list of taxa by rarity rating with respect to IUCN regression in France (red book of endangered flora of France) and in Tunisia (unpublished research).

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Photo 1: *Moraea sisyrinchium* (Marabouts: Environmental conservation vectors in North Africa « sacred sites of conservation »)

3 MATERIALS AND METHODS

A large germplasm searching expedition was carried out in different regions of Tunisia (North and the Sahel) in order to collect and characterize *Morea sisyrinchium* spontaneous populations. Five populations were identified. A soil analysis of the different collection sites was done. For the analysis of morphological diversity, a sample of thirty (30) individuals from each population was studied. The morphological characterization of iris populations was based mainly on quantitative descriptors studied over two successive years. It concerns the parameters of vegetative, flowering and reproductive organs (Table 1).

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Photo 2: Prospect sites: a: olive trees (Beja), b: lagoon of Maaoura (Nabul), C: hill in Hamamamet, d: Djebel Ressas (Morneg)

Table 1: Parameters of morphological characterization

Parameters	Unity	Code
Leaf length Length of the flower stalk Flower height Flower diameter Number of levels Ovary length Perianth tube length Anther length Filament length Bulb diameter Number of seeds / fruit Seed diameter	Cm cm cm cm 1 to 4 cm cm cm cm cm number mm	LL FS FH FD LN OL PL AL FL BD SN SD

Intra-population and inter-population variability was measured for each descriptor by analysis of variance (ANOVA). The multivariate analysis method carried during this study is the Principal Component Analysis (PCA). The frequencies of morphological traits are processed by the Shannon diversity index- Weaver (Efombagn *et al.,* 2009) according to the formula:

$$H' = -\sum_{i=1}^{k} Pi \ln Pi$$

Pi : frequencies counted for the i th class, k is the number of phenotypic classes determined from the values of the analysis of variance (ANOVA), according to the model of Jaradat *et al.*(2004).

4 RESULTS

4.1 Characterization of *Moraea sisyrinchium* populations: The distribution and characterization of *Moraea sisyrinchium* collection sites in Tunisia are presented in Table 2. It appears that this species has an important ecological plasticity, in fact it colonizes different soils, even a sebkha and thrives under different bioclimates. Analysis of the chemical characteristics of soils relating to the different collection sites (Table 3) proves a great ecological flexibility of the species. Indeed, *Moraea sisyrinchium* can survive in different types of soil, even the most saline: 434.48 mg / kg (lagoon of Maamoura) and the poorest in organic matter: 0.41% (Sousse).

Table 2: Characteristics of *Moraea sisyrinchium* distribution sites.

Population code	Location	Latitude/longitude	Bioclimatic stage (MEDD, 2006)	Reliefs	Vegetation ground occupation
B	Beja	36 ° 48´N, 9°2´E	Humid	East facade of a hill	Olives trees Pasturage
H	Hammamet	36°24N, 10°30`E	Semi -arid with mild winters	Planer zone with embankment	Olive grove Cereals Pasturage
M	Maamoura	36°29´N, 10°49´E	Semi -arid with mild winters	lagoon side	Spontaneous succulents' plants Barely field
R	Djebel Ressas	36°36´N, 10°20´E	Sub-humid	Mountain and basin	Rosmarinus
S	Sousse	35°56´N, 10°33´E	Semi -arid with mild winters	Small hill	Spontaneous vegetation

Table 3: Edaphic characteristics of collection sites for Iris species: electrical conductivity

Locality	CE (ms/cm)	pH	OM (%)	N (%)	K^+ (mg/kg)	Ca^{2+} (mg/kg)	Na^+ (mg/kg)	P_2O_5 (mg/kg)
Beja	1.35	7.01	0.97	2.43	11.8	6200	75.9	17.9
Dj Ressas	2.95	8.2	3.88	1.52	43.1	13400	96.6	18.3
Hammamet	28.2	7.38	0.67	0.31	31.4	14200	43.45	61.3
Maamoura	22.15	8.55	1.75	0.72	90.2	11600	434.48	34.7
Sousse	1.3	7.66	0.41	0.21	27.5	15600	62.07	67.7

(EC), pH, percentage of organic matter (OM), percentage of nitrogen (N), calcium (Ca^{2+}), sodium (Na^+) and phosphorus (P_2O_5).

4.2 Morphological diversity analysis: Analysis of variance with two classification criteria (populations and plants) (table 4) applied to all the parameters studied, reveals the existence of a significant inter-population variability for all the parameters, except for the height and the diameter of the flower. A low variability within-population is reported for the parameters: lengths of anther and filament, number of levels and bulb diameter.

4.3 Correlations between the studied parameters: Examination of the correlation matrix (table 5) shows that the majority of correlations exceed 0.30. Strong correlations (r = 0.50 to 0.87) have been reported. A strong significant correlation (r = 0.87) was demonstrated between the length of the ovary (OL) and the length of the perianth tube (PL). Another strong correlation (r = 0.71) was noted between the height and the diameter of the flower.

Table 4: Mean values and degrees of significance of the differences observed within and inter-populations for the various parameters studied.

Parameter	CM	Means	F calculated (intra)	Pr (intrapopulation)	F calculated (inter)	Pr (interpopulations
LL	185.34	29.95±0.565	1.72	0.09 NS	8.02	<0.0001**
FS	109.64	30.59 ±0.580	1.75	0.08 NS	8.78	<0.0001**
FD	0.347	13.33±0.232	0.66	0.78 NS	0.73	0.49 NS
FH	0.343	3.06 ±0.321	1.27	0.27 NS	0.5	0.69
LN	0.801	1.42 ±0.532	2.88	0.002 *	1.74	0.0037 *
OL	0.771	1.64 ±0.696	1.22	<0.29NS	19.83	<0.0001 **
PL	0.540	1.95 ±0.715	0.98	0.48 NS	23	<0.0001 **
AL	0.179	1.52 ±0.504	4.08	0.007 *	7.93	<0.0001 **
FL	0.183	0.99±0. 573	3.14	0.004 *	4.08	0.007 *
BD	1.791	1.64 ±0.579	**3.27**	0.0016*	2.34	0.07 *
SN	1225.16	60.52 ±0.398	1.11	0.37 NS	1.11	0.02 *
SD	0.093	1.26 ±0.603	2.10	0.03 NS	8.98	<0.0001 **

CM: squares, Pr: probability, *: significant differences, **: highly significant at 1%, NS: non-significant differences.

Table 5: Pearson correlation matrix of parameters measured on plants of different *Moraea sisyrinchium*. Populations

Parameter	LL	FS	FD	FH	PL	OL	AL	FL	SN	BD	SD	LN
LL	1											
FS	0.505**	1										
FD	0.410**	0.365*	1									
FH	0.404*	0.369*	0.713**	1								
PL	0.487**	0.634**	0.552**	0.514**	1							
OL	0.487**	0.643**	0.496**	0.440*	0.87**	1						
AL	0.443*	0.456*	0.415*	0.416*	0.65**	0.58**	1					
FL	0.161	0.290	0.128	0.339*	0.28	0.28	0.45*	1				
SN	0.298	0.274	0.152	0.038	0.38*	0.38*	0.25	0.15	1			
BD	0.231	0.028	0.166	0.367*	0.26	-0.18	0.12	-0.01	0.23	1		
SD	0.036	0.154	-0.101	-0.126	-0.10	-0.07	-0.11	0.07	-0.08	-0.06	1	
LN	0.323	0.189	0.041	0.010	0.05	0.07	-0.02	-0.01	0.21	0.08	0.2	1

*: significant correlation at the 5% level; **: highly significant correlation at the 0.01% level.

4.4 Principal component analysis (PCA):

The first three axes of the principal component analysis absorb 98.7% of the total variability, which testifies to a good structuring of the variability within the species. Axis 1 represents 74.4% of the total variability (Table 6). It is positively correlated to the leaf length, the flower stalk, the ovary length and the perianth tube. Axis 2 represents only 13.3% of the total variability. It is mainly defined by the parameters of filament length and seed diameter. Axis 3 represents 11% of total variability. It is negatively correlated with the length of the stalk and positively correlated with the seed diameter parameter. The graphic representation of the dispersion of the five populations on the plane designed by the first two principal components (87.7% of the total variability) reveal a divergence between the different populations materialized by the dispersion of points representative of the populations studied (Figure 1). The populations of Hammamet, Maamoura and Béja are correlated with axis 1 on the positive side. On the positive side of axis, 2 are the populations of Maamoura, Hammamet and Dj Ressas. The latter is far from the two others because it is on the negative side of axis 1. On the negative face of axis 2, thera are the populations of Béja and Sousse. The populations of Hammamet and Maamoura are probably native from the same geographic region.

Table 6. Definition of the three first axes of PCA

		Axe 1	Axe 2	Axe 3
Own value		8.18	1.46	1.22
Proportion		74.4	13.3	11
% cumulated		74.4	87.7	98.7
Definition of axes	Variable	Coefficient of correlation		
	LL	**0.318**	-0.243	0.247
	FS	**0.33**	-0.021	**0.266**
	FD	0.346	0.014	**-0.127**
	FS	0.235	0.22	-0.62
	PL	**0.344**	0.00054	0.153
	OL	**0.346**	-0.068	-0.102
	AL	**0.327**	0.213	-0.205
	FL	0.21	**0.65**	0.094
	SN	0.328	-0.116	0.047
	LN	0.275	-0.044	-0.046
	BD	0.33	-0.22	0.151
	SD	-0.074	**0.596**	**0.597**

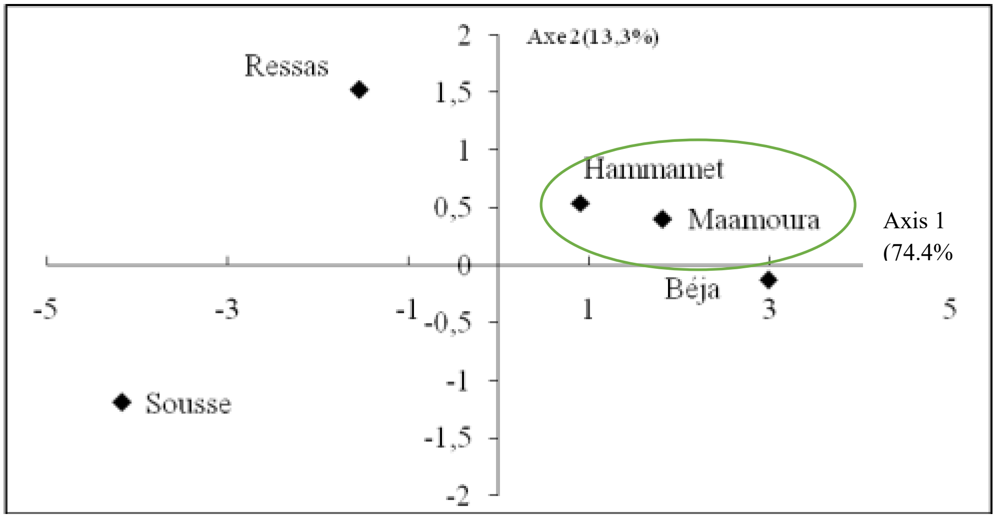

Figure 1. Projection of *Moraea sisyrinchium populations* in the factorial plane (1-2) of principal component analysis (PCA).

The group analysis revealed two branches that meet at a distance Euclidean of 1.25. The first group is formed by the populations of Béja, Maamoura and Hammamet. Cluster 2 includes the populations of Dj Ressas and Sousse, which show a lower value for most of the parameters studied.

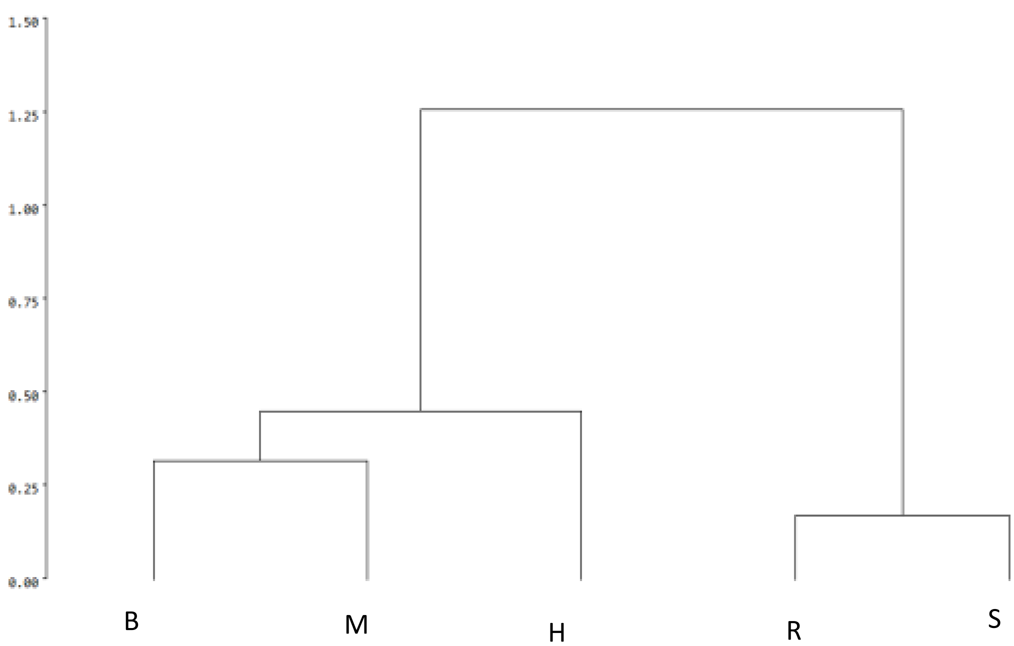

Figure 2: Dendrogram of morphological dissimilarity of *Moraea sisyrinchium* populations

4.5 Phenotypic diversity (H '): Shannon –Weaver index: The phenotypic diversity estimated by the Shannon-Weaver index reveals a high value for most of the morphological parameters studied. The diversity index. The highest phenotypic (H '= 0.936) is observed at the level of the length of the ovary (OL) parameters. The lower value (0.241) is calculated for the bulb diameter. All values remaining are greater than 0.8, except those estimated for flower height (0.567) and the seed number parameter (0.663) (Table 7).

Table 7: Shannon-Weaver diversity index (H ') calculated for the different parameters and for the five populations of *Moraea sisyrinchium.*

Character	Phenotypical diversity index (H')
LL	0.750
HL	0.807
FD	0.881
PL	0.886
OL	0.936
AL	0.858
FL	0.848
LN	0.567
SN	0.663
SD	0.809
BD	0.241

The estimated total phenotypic diversity (H'T) for all parameters and all populations combined is of the order of 0.773. Morphological diversity estimated by population (Table 8) shows that the diversity within each population is high and the highest value is recorded in the population of Béja (H '= 0.863). Population from Maamoura has the lowest phenotypic diversity index (0.611).

Table 8: Means values of Shannon Weaver diversity index measured for all populations.

Population	Diversity index (H')
Beja	0.863
Hammamet	0.839
Maamoura	0.611
Dj Ressas	0.642
Sousse	0.691

5 DISCUSSION

The morphological characterization made it possible to highlight a great population variability response of spontaneous *Moraea sisyrinchium* for the various parameters studied and which manifested mainly through the length of the leaves, the length of the flower stalk, the length of the perianth tube, the length of the filament and the ovary, as well as the number and seed diameter. Principal component analysis shows that populations of Beja, Hammamet and Maamoura always tend to regroup. These three populations, belonging to two different climatic stages, are characterized by the vigour of their seedlings. This phenotypic variability between populations could result from a strong heterogeneity genotypic but also from the influence of the environment. The study of enzymatic polymorphism in Tunisian populations of *Moraea sisyrinchium* (Ferjani *et al.,* 2012) has shown that morphological diversity is due to a great intrapopulation genetic diversity.

6 CONCLUSION

This study falls within the framework of conservation and development of local plant *genetics* resources of spontaneous *Moraea sisyrinchium* populations in Tunisia. it has been demonstrated that these spontaneous *flowers* are natural resources which deserve to be better studied for a possible use in landscape ornamentation given their great diversity and their ecological flexibility.

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