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Analysis of diversity among wild gladiolus (*Gladiolus* sp.) accessions using morphological traits

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Abstract

In Tunisia, the local germplasm of wild Gladiolus have been currently damaged by severe genetic erosion, pollution, urbanization and bad farming practices. The aim of the present study was to prospect, collect and assess morphologically 15 accessions of this species from several areas in Tunisia. The identification and characterization of 300 individuals (20 samples per each accession), was performed utilizing 20 morphological traits. In this study, morphological traits showed a large variability for wild Gladiolus species. Cluster analysis revealed two major clusters: the first includes the accessions with populations of *Gladiolus italicus*, the second consists of all the rest, the populations with seeds wing. The Principal Component Analysis of the measured morphological characters revealed that the 3 primary components included 70.5% of total variability. The diversity is currently threatened by genetic erosion. The sustainability of Gladiolus in Tunisia is necessary using a rapid program of conservation *in situ* and *ex situ*.

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Introduction

The genus *Gladiolus* belongs to the subclass of Lilidaes part of the class of monocots in the order of the Liliales and the family Iridaceae (Poittier-Alapetite, 1954). This is the principal genus of the family Iridaceae (Cohat, 1993). The taxonomy of the family is based mainly on the morphology, anatomy, embryology and chromosome numbers (Raycheva, 2011).

The Iridaceae is a large family with over 2000 species, many of which are endemic to South Africa and produce attractive flowers and foliage (Ascough, 2011). Approximately, 40 species and hybrids from 12 genera have been successfully micropropagated for the floricultural industry (Ascough *et al*., 2009).

Gladiolus is one of the largest genera in the Iridaceae family with possibly as many as 300 species. Almost all of these grow in sub-Saharan Africa (Goldblatt and takei, 1997); less than twenty species are endemic to Europe and neighbouring countries. Since the days of ancient Greece, the gladioli are said to be cultivated. History reveals that it is known since 1578, as evidenced by a record in Lyte's Nievve Herball, first were introduced into France and soon after that it spread to England, Germany, Holland and North America. These were the only wild species as garden gladioli three hundred years ago **(**Cantor *et al*., 2010). Tunisian flora has three wild species of gladioli and genetic heritage interest: *Gladiolus italicus* Mill. (*G. segetum* Ker-Gawl), *Gladiolus dubius* and *Gladiolus communis* subsp. *byzantinus* (Mill.) that covers some areas in the middle of fields and the meadows (LE FLOC'H *et al*., 2010).

Gladiolus italicus Mill. is distinguished by its brown tunics bulb, formed by thin fibers and welded its flower spike elongated, the upper part of the perianth being larger than its neighbors and it is spaced from the other three lower parts being substantially equal, anthers shorter than the net, the fruit is a triquetra capsule. The seeds are angular, pyriform, globose and wingless (Poittier-Alapetite, 1954).

Gladiolus dubius Guss. is characterized by a stem robust derived from 30 to 60 cm. The glabrous, tunic of the bulb consists of thin fibers, parallel and non-crosslinked at the top; sturdy stem, with leaves rather large cluster couplet, unilateral, 5-8 flowers spathes shorter lower that the flower perianth divisions obtuse and mute, the narrower side, oblong corner; anthers shorter than the net, and acute ear divergent stigmas dilated almost imperceptibly from the base; capsule obovate, obtusely angled keeled. The seeds are wing. (Bock, 2012).

Gladiolus communis subsp. *byzantinus* (Mill.) is not considered in Tunisian flora. Represent a complex unstable hybridogene between *G. dubius* and *G. italicus* (LE FLOC'H *et al*., 2010).

Iridaceae is a family of perennial, rhizomatous or bulbous plants with almost worldwide distribution. They inhabit diverse natural habitats, exhibit a high adaptability and wide variability of their physiological and morphological features, which makes investigations of their taxonomy, evolutionary history and the phylogenic relations a serious challenge (Ellis, 1997).

The present work focused on the study of different morphological parameters, related to leaves, flowers and fruits, of 15 accessions of wild Gladiolus species in Tunisia.

Material and methods

Plant material

During summer 2011, a collection of 15 accessions of wild gladiolus was performed on five different sites. Each site was subdivided into three blocks. The collection of each accession was made to sample the best variability (Figure 1).

Morphological Measurement

Gladiolus plants were collected in different regions on the territory of Tunisia. 15 wild accessions were measured in this study (table 1). These populations represent 3 species recorded in northern and central Tunisia; Measurements were taken during the peak of flowering season (mid-March to early May). The measured plants were chosen randomly within each accession.

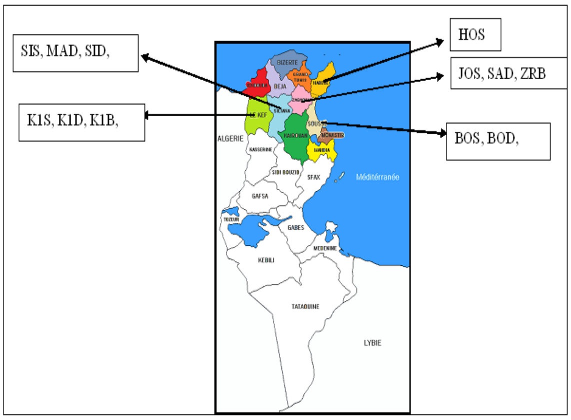

Fig. 1. Location of the 15 accessions of wild species of Gladiolus.

A morphological description was conducted to identify and characterize the species and to find distinctive characters. This characterization has focused on different parts of the plants. 20 morphological traits related to leaves, flowers and fruits were studied (table 2).

Statistical analysis

For all parameters, the analysis of variance (one-way ANOVA) was used to determine the differences between accessions. A comparison of the mean values was made using the Duncan's multiple range test ($P < 0.05$). Multivariate relationships among accessions were revealed through a principal component analysis (PCA) using a correlation matrix derived from the significant characters.

The squared Euclidean distance was used to perform cluster analysis. Statistical analyses were computed using SPSS 13.0 statistical software.

Results

Morphological study

To study the morphology of gladiolus plants in its natural environment, many parameters were measured (H, Nlf, LlF, Wlf and Alf). The analysis of variance showed highly significant differences between accessions. Plants of ZRB were the most vigorous with a height of 73.8 cm and a number of leaves of 3.7 (table 3). The leaf length varied from 28.5 for BOS to 46.6 cm for K1D. The accession SIS showed the highest leaf width (1.85 cm) and area (30.3 cm^2).

Means followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan test.

Table 1. Locations of wild *Gladiolus* species studied for morphological traits.

Code	Location	Region	Latitude	Longitude	Altitude (m)
BOS	Bouficha	Sousse	36.283	10.417	13
JOS	El Jouf	Zaghouan	36.317	10.217	197
HOS	Haouaria	Nabeul	36.967	10.917	45
SIS	Siliana	Siliana	36.050	9.300	633
K1S	Le Kef	Le Kef	36.183	8.867	498
BOD	Boufícha	Sousse	36.283	10.417	13
SAD	Saouaf	Zaghouan	36.217	10.150	158
MAD	Makthar	Siliana	35.867	9.150	1059
SID	Siliana	Siliana	36.050	9.300	633
K1D	Le Kef	Le Kef	36.183	8.867	498
AAB	Ain Rahma	Sousse	36.283	10.417	13
ZRB	Zriba	Zaghouan	36.317	10.217	197
SIB	Siliana	Siliana	36.050	9.300	633
K1B	Le Kef	Le Kef	36.183	8.867	498
K2B	Le Kef	Le Kef	36.183	8.867	498

Table 2. Morphological traits studied in wild Gladiolus species.

Character number	Code	Character descriptive value
1	H	Height of plant (cm)
2	Nlf	Number of leaves
3	Llf	Length of leaf (cm)
4	Wlf	Width of leaf (cm)
5	Alf	Area of leaf (cm^2)
6	DC	Diameter of corm (mm)
7	WeC	Weight of corm (g)
8	FC	Form of corm (1 globular, 2 flatten)
9	DS	Diameter of seed (cm)
10	LW	Length of Wing (mm)
11	LC	Length of capsule (cm)
12	NSC	Number of seeds per capsule
13	WS	Weight of 100 seeds (g)
14	FS	Form of seed (1:non winged, 2: winged)
15	DFS	Diameter of floral stem (mm)
16	DF	Number of flowers
17	BF	Beginning of flowering (1:early, 2: medium, 3: late)
18	CF	Main color of flowers (1: purple, 2 :pink)
19	WF	Width of flower (1: narrow, 2: medium, 3: broad)
20	FVF	Shape in front view of flower (1: rectangle, 2 diamond, 3 variable)

The characters related to fruit are shown in table 4. Analysis of variance showed highly significant differences between accessions for all parameters except weight of 100 seeds (WS). The highest corm diameter (26.12 mm) and weight (11.88 g) were observed for BOS. The accessions AAB, ZRB, SIB, K1B and K2B showed the most winged seeds.

Table 3. Mean values, standard deviations and significance degree of differences between 15 accessions of wild Gladiolus for leaf characteristics.

Accessions	H	Nlf	Llf	Wlf	Alf
BOS	54.3 ± 7.21^{ef}	3.6 ± 0.52^{b}	28.5 ± 7.74^{e}	1.43 ± 0.35^{abc}	22.70 ± 2.57^{b}
JOS	54.4 ± 7.23^{ef}	3.6 ± 0.52^{b}	32.9 ± 5.28^{cde}	1.71 ± 0.5^{ab}	27.64 ± 7.33^{ab}
HOS	49.5 ± 8.44^{f}	3.6 ± 0.52^{b}	39.2 ± 8.05^{bc}	1.71 ± 0.43^{ab}	26.1 ± 9.2^{ab}
SIS	57.5 ± 4.58^{cdef}	3.8 ± 0.42^{ab}	36.7 ± 6.48^{bcd}	1.85 ± 0.38^{a}	30.36 ± 6.82^{a}
K1S	$55.8\pm6.46d^{ef}$	3.5 ± 0.53^{b}	29.4 ± 4.06^{e}	1.51 ± 0.43^{abc}	27.09 ± 6.06^{ab}
BOD	63.1 ± 9.23^{bcde}	4 ± 0.67^{ab}	43.5 ± 5.06^{de}	1.42 ± 0.2^{abc}	22.81 ± 2.11^{b}
SAD	63.8 ± 11.14^{bcd}	4 ± 0.47^{ab}	39.5 ± 5.68^{ab}	1.61 ± 0.52^{abc}	25.86 ± 5.94^{ab}
MAD	54.8 ± 12^{a}	4.3 ± 0.67^{a}	31.2 ± 5.71^{bc}	1.69 ± 0.21^{ab}	26.80 ± 7.83^{ab}
SID	53.9 ± 5.9^{ef}	4.3 ± 0.67^{a}	39.8 ± 5.63^{de}	1.83 ± 0.49^{a}	29.86 ± 6.75^{a}
K1D	69.3 ± 10.61^{ab}	4 ± 0.67^{ab}	46.6 ± 11.39^{abc}	1.79 ± 0.52^{a}	25.69 ± 6.63^{ab}
AAB	67.3 ± 10.09^{ab}	4 ± 0.47^{ab}	38.5 ± 5.66^{a}	1.42 ± 0.5^{abc}	24.60 ± 5.16^{ab}
ZRB	73.8 ± 9.8^{a}	3.7 ± 0.48^{b}	41.6 ± 8.03^{bc}	1.6 ± 0.45^{abc}	26.49 ± 6.13^{ab}
SIB	65.9 ± 11.58^{abc}	3.9 ± 0.57^{ab}	40.2 ± 9.46^{bc}	1.32 ± 0.38^{bc}	26.10 ± 5.48^{ab}
K1B	68.5 ± 8.73^{ab}	3.8 ± 0.63^{ab}	37.7 ± 4.52^{bcd}	1.34 ± 0.41^{bc}	25.73 ± 5.12^{ab}
K2B	66.6 ± 9.52^{ab}	3.6 ± 0.52^{b}	41.6 ± 7.65^{bc}	1.2 ± 0.26^{c}	25.67 ± 5.65^{ab}

Means followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan test.

Analysis of variance for flower parameters showed a highly significant differences between accessions for all parameters. The most discriminating ones were the diameter of floral stem (6 mm for JOS) and the number of flowers (12 for BOD). The dominate color is the purple. The accessions BOS and BOD were the earliest (table 5).

Principal Component Analysis

The Principal Component Analysis (PCA) of the measured morphological characters revealed that the 3 primary components included 70.5% of total variability (43.4% for the first main component, PCA1, 15.26% for PCA2 and 11.8% for PCA3) (figure 2). The characters highly correlated to the first axis (r> 0.6) were: the height of plant (H), the length of leaf (Llf), the diameter of seed (DS), the Length of wing (LW), the form of seed (FS), the beginning of flowering (BF), the main color of flowers (CF), the width of flower (WF) and the shape in front view of flower (FVF).

Table 4. Mean values, standard deviations and significance degree of differences between 15 accessions of wild Gladiolus for fruit characteristics.

	DC	WeC	FC	DS	LW	LC	NSC	WS	FS
BOS	26.12 ± 1.98^{a}	11.88 ± 2.57^{a}	1	3.18 ± 0.11^{a}	1.57 ± 0.52^{e}	14.45 ± 1.51^{cd}	24.5 ± 6.17^{ab}	1.59 ± 0.01^{b}	1
JOS	17.26 ± 4.32^{cde}	7.92 ± 3.4^{cde}	1	2.99 ± 0.18^{ab}	1.91 ± 0.58^{e}	14.32 ± 2.46^{cd}	25.2 ± 5.98^{ab}	1.6 ± 0.01^{b}	1
HOS	16.12 ± 4.05^{e}	6.92 ± 3.1^{de}	1	3.07 ± 0.19^{ab}	1.58 ± 0.49^{e}	15.29 ± 2.13^{bcd}	30.2 ± 2.7^{a}	1.59 ± 0.04^{b}	1
SIS	18.52 ± 4.32^{cde}	8.33 ± 3.39^{cde}	1	3.071 ± 0.09^{ab}	1.91 ± 0.58^{e}	15.87 ± 0.99^{abcd}	24.5 ± 5.36^{ab}	1.58 ± 0.01^{b}	1
K1S	19.42 ± 6.57^{cde}	6.95 ± 2.64^{de}	1	3.1 ± 0.09^{ab}	1.61 ± 0.35^{e}	15.29 ± 1.6^{bcd}	23.1 ± 6.64^{b}	1.58 ± 0.01^{b}	1
BOD	24.91 ± 3.31^{a}	9.57 ± 2.79^{abcd}	2	2.84 ± 0.5^{bc}	3.74 ± 0.66^{d}	14.4 ± 3.12^{cd}	28.9 ± 4.95^{ab}	1.41 ± 0.09^{cd}	2
SAD	21.06 ± 3.64^{bc}	11.29 ± 2.79^{ab}	2	2.7 ± 0.24^{cd}	4.38 ± 0.7^{c}	17.29 ± 3.25^{ab}	26 ± 5.25^{ab}	1.42 ± 0.09^{c}	2
MAD	19.23 ± 2.09^{cde}	8.81 ± 2.08^{bcde}	2	2.86 ± 0.35^{ab}	3.90 ± 0.56^{cd}	17.78 ± 2.89^{a}	23.7 ± 6.8^{b}	1.51 ± 0.1^{bc}	2
SID	16.53 ± 2.39^{de}	10.53 ± 2.47^{abc}	2	2.46 ± 0.28^{def}	4.01 ± 0.52^{cd}	16.40 ± 1.49^{abc}	24.1 ± 5.04^{b}	1.49 ± 0.01^{bc}	2
K1D	20.79 ± 5.14^{bcd}	7.73 ± 3.22^{cde}	2	2.61 ± 0.19^{cde}	3.99 ± 0.49^{cd}	16.39 ± 1.46^{abc}	26.2 ± 5.2^{ab}	1.41 ± 0.1^{cd}	2
AAB	23.64 ± 4.59^{ab}	8.97 ± 2.18^{bcde}	1	2.31 ± 0.36^{f}	6.22 ± 0.95^{a}	15.53 ± 1.56^{abcd}	24.1 ± 7.06^{b}	1.31 ± 0.03^{de}	2
ZRB	17.34 ± 3.58^{cde}	9.05 ± 3.03^{abcde}	1	2.3 ± 0.24^{ef}	6.19 ± 0.67^{a}	15.76 ± 2.54^{abcd}	24.6 ± 6.13^{ab}	1.22 ± 0.09^{ef}	2
SIB	16.92 ± 4.66^{cde}	8.28 ± 3.69^{cde}	2	2.38 ± 0.16^{ef}	6.32 ± 0.56^{a}	16.05 ± 3.23^{abcd}	26.4 ± 6.02^{ab}	1.99 ± 0.02^{a}	2
K1B	17.85 ± 3.78^{cde}	7.8 ± 3.02^{cde}	2	2.61 ± 0.22^{def}	5.84 ± 0.5^{a}	13.90 ± 2.07^{d}	28.8 ± 4.37^{ab}	1.19 ± 0.001^{f}	2
K2B	17.50 ± 4.92^{cde}	6.3 ± 2.62^{e}	1	2.8 ± 0.31^{bc}	5.22 ± 0.9^{b}	17.56 ± 2.94^{ab}	24.6 ± 5.6^{ab}	1.20 ± 0.01^{f}	2

Means followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan test.

Table 5. Mean values, standard deviations and significance degree of differences between 15 accessions of wild Gladiolus for flower characteristics.

	DFS	DF	BF	CF	WF	FVF
BOS	5.35 ± 1.01^{abc}	8.6 ± 2.84^{bcde}	1	1	1	1
JOS	6.01 ± 0.71^{a}	11 ± 4.32^{ab}	2	1	1	1
HOS	5.92 ± 1.03^{ab}	7.7 ± 2.26^{cdef}	3	1	1	1
SIS	5.12 ± 1^{abc}	10.1 ± 1.97^{abc}	3	1	1	1
K1S	5.57 ± 1.1^{abc}	8.9 ± 3.57^{abc}	2	1	1	1
BOD	4.8 ± 1.55^{bc}	12 ± 2.75^{a}	1	2	3	2
SAD	5.7 ± 0.82^{abc}	8.8 ± 1.55^{bcd}	2	2	3	2
MAD	5.2 ± 1.14^{abc}	10.4 ± 2.99^{ab}	2	2	3	2
SID	5.5 ± 1.27^{abc}	9.9 ± 3.25^{abc}	2	2	3	2
K1D	6 ± 1.49^{ab}	12.2 ± 3.49^{a}	2	2	3	2
AAB	5.55 ± 1.01^{abc}	6.7 ± 1.49^{def}	2	1	2	3
ZRB	5 ± 1.15^{abc}	6 ± 2.26^{ef}	2	1	2	3
SIB	4.6 ± 1.26^{c}	7.1 ± 2.02^{def}	3	2	2	3
K1B	5.4 ± 1.07^{abc}	6.9 ± 1.2^{def}	2	2	2	3
K2B	5.78 ± 0.96^{abc}	5.2 ± 1.69^{f}	2	1	2	3

Means followed by the same letter(s) are not significantly different at P= 0.05 according to Duncan test.

The second axis was positively correlated with the number of leaves (Nlf), the width of leaf (Wlf), the form of corm (FC) and the number of flowers (DF). PC3 explained 11.8% of the variability. It was positively correlated with the diameter of corm (DC) and negatively correlated with the area of leaf (Alf) (table 6).

Cluster Analysis

The morphological analysis was based on different characters that showed a high polymorphism with 15 accessions of wild Gladiolus. The dendrogram was based on a squared Euclidian distance clustered accessions into two major groups (Figure 3). The first cluster was constituted with five accessions: BOS, JOS, HOS, SIS and K1S d=12.63. It was characterized by a large wingless seeds (diameter > 3mm), the front view of flower was in a narrow vertical rectangle, a large difference in width between the dorsal and the upper lateral tepal and the capsules was as long as wide.

Table 6. Definition of the first three components of PCA made on the basis of morphological traits of the wild Gladiolus species.

	PCA1	PCA2	PCA3
Eigen values	8.6	3.05	2.3
% of variance	43.4	15.2	11.8
Cumulative %	43.4	58.6	70.5
H	0.86	-0.11	0.09
Nlf	0.38	0.82	0.13
Llf	0.64	0.28	0.01
Wlf	-0.48	0.70	-0.27
Alf	-0.23	0.40	-0.81
DC	-0.08	-0.05	0.88
WeC	-0.03	0.32	0.57
FC	0.41	0.67	0.29
DS	-0.85	-0.23	0.09
LW	0.98	-0.02	-0.05
LC	0.29	0.43	-0.41
NSC	0.11	-0.09	0.33
WS	-0.45	0.15	-0.07
FS	0.92	0.33	0.11
DFS	0.01	0.42	0.07
DF	-0.48	0.70	0.34
BF	0.99	-0.11	-0.06
CF	0.99	-0.11	-0.06
WF	0.99	-0.11	-0.06
FVF	0.99	-0.11	-0.06

The rest of accessions formed the second cluster at d=16.57. This accession had the highest plants, capsules were more longer than wide. It was characterized by small seeds (diameter < 3mm) which were very winged, and that can reach up to 6 mm.
This cluster was divided into two subgroups. The first subgroup, detached at d=9.73, comprised the accessions AAB, ZRB, K1B, K2B and SIB. It was distinguished essentially from the remaining genotypes by a front view of flower in a wide vertical diamond and by an equal width between the dorsal tepal and the upper lateral tepals. The second subgroup contained the accessions MAD, SID, SAD, BOD, and K1D d=13.42. These accessions were characterized by very variable proportions clones of capsules (can go from one extreme to the other), fairly large seeds (diameter > 3mm without wing) to wing always greater than in *G. italicus* and always smaller than in *G. dubius*.

Discussion and Conclusions

The genus *Gladiolus* was located in the north and the center of Tunisia and it was absent in the south. These results confirm those of Matoussi (2007) who reported the presence of this genus in the mentioned regions. However, in some regions the genus *Gladiolus* is becoming increasingly rare. Among the factors involved in the extinction of the species in an area, we include agricultural practices such as weeding and plowing which are a threat to this plant considered messicole. Runoff and erosion, can also be the cause of the disappearance of the plant at each site and can even induce its reappearance in a new location (Matoussi, 2007).

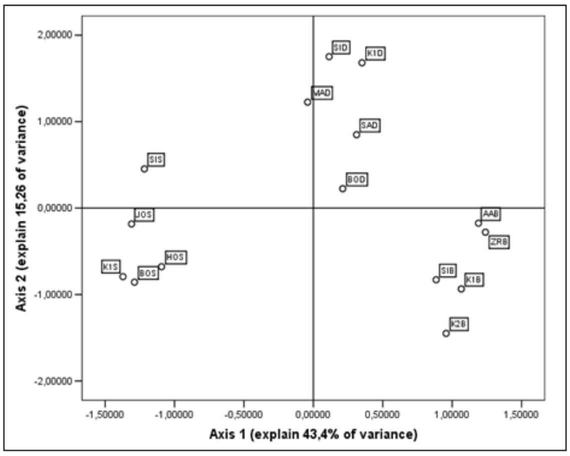

Fig. 2. Plot of the first and the second principal components resulting from a PCA of the wild Gladiolus species using morphological characters.

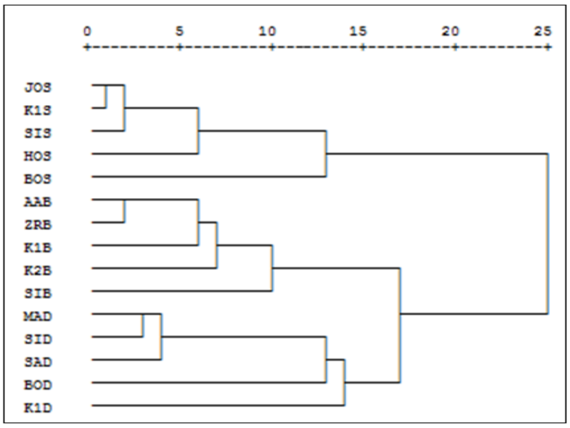

Fig. 3. Dendrogram (based on squared Euclidian distance) of wild Gladiolus species performed using morphological characters.

The study of the natural habitat of wild gladioli showed that it is a species messicole in Tunisia. This specie resists in low winter temperatures (Halvery, 1985). In this study, morphological traits showed a large variability for wild Gladiolus species. The plant height is shorter in HOS compared to other accessions, may be explained by the proximity of the sea and soil salinity. The number of leaves per plant (3 to 5), while this number (7 to 9) from *Gladiolus* hybrid (Cohat, 1988). The number of flower 12 for K1D and the earliest flowering (early march) is observed in BOS and BOD, this accessions in the region of Bouficha. The beginning of flowering is late in the region Siliana (SIS and SIB), this parameter is dependent on the light, the temperature, the shading exercised by the culture and the accompanying plants (Matoussi, 2007). The earlier flowering can be improved by the vernalization of bulbs. Lopez *et al.*, (2003) obtain an early production by placing the corms of *Gladiolus tristis* at a temperature of 5°C for 5 weeks. Among the 20 variables analyzed, those of high discriminating level were: height of plant (H), length of leaf (Llf), diameter of seed (DS) length of wing (LW), form of seed (FS), beginning of flowering (BF), main color of flowers (CF), width of flower (WF) and shape in front view of flower (FVF). Results of PCA based on morphological traits showed that the 3 primary components included 70.5% of the total variability.

The cluster analysis showed a high degree of diversity in the germplasm of Tunisia native gladioli. The first group formed accessions from *Gladiolus italicus*. This species characterized by a large seeds (diameter > 3 mm) with a reduced wing, an apical triangle and a front view of flower in a vertical narrow rectangle. The second group was devised in two subgroups, the first one included MAD, SID, SAD, BOD and K1D. The accessions is characterized by a small seeds (< 3 mm diameter) with a around wing, pole robust derived from 30 to 60 cm, sturdy stem with leaves large (SID 1,83 cm and K1D 1,79 cm) and the most number of flowers is obtained for BOD (12 flowers). In agreement with the morphological description of *Gladiolus dubius* of Bock (2012). The second one included the accessions AAB, ZRB, K1B, K2B and SIB were distincted by large seeds (diameter > 3mm).

The differentiation of geographical races into new species is promoted by two major processes: random genetic drift and natural selection (Grant, 1981). When natural selection is predominant, we may expect populations in close proximity to be morphologically more similar than those far apart because the macroenvironments (and therefore selective forces) are likely to be more similar over shorter than over long distances (Endler, 1977). When genetic drift is the main mechanism, such a relationship between phenotypic similarity and geographical distance is less likely. In plants, morphological characters have been investigated along various environmental gradients, such as latitude/longitude (Allen *et al.,* 1996), climate (Passioura and Ash, 1993), rainfall (Sandquist and Ehleringer, 1998), soil and nutrients (Wilson, 1991). Further studies are needed involving chemical, biochemical, and molecular markers (Raycheva *et al.*, 2011). They would clarify the genetic variation at the molecular level in these genus. The Iridaceae family is a taxonomically difficult to analyze and a phylogenetically poorly understood family. The generic boundaries, species affiliations, and phylogenetic relationships vary from one author to another (Rodriguez and Catedral, 2003). To ensure the exactness of evaluation, only variables with strong genetic control should be quantified in morphological traits analysis (Pragya *et al.,* 2010).

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