MORPHOLOGICAL AND KARYOLOGICAL STUDIES IN TWO WILD IRIS SPECIES (IRIDACEAE) OF TUNISIA

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Abstract

Morphological and cytological variation among two wild *iris* species (*iris juncea* (poir) and *iris sisyrinchium.* (l)) from tunisia was studied. Initially, morphological traits analyzed concern floral and vegetative characters. Analysis of variance showed significant differences. Higher variation coefficient belongs to flower height, flower diameter, filament length, seed diameter, and level number. Pearson coefficients correlations between different characters were positive and highly significant ($p< 0.0001$) for flower diameter and flower heigth. Factor analysis showed that only two axes define 100% of variance among characters. Secondly, in cytological variation each species had different karyotypic formula such as 2n = 32 = 30sm + 2st for *iris juncea* with a satellite in pair number 2; 2n= 24 = 18 st + 6 sm for *iris sisyrinchium* beja‘s population and 2n=26=18 st + 8sm for *iris sisyrinchium* hammamet population.

Keywords: Cytological, *iris juncea*, *iris sisyrinchium*, morphological, wild

Introduction

The monocot iridaceae family comprises approximately 2050 species distributed among 67 genera, with a major center of radiation in the southern african sahara, including madagascar. Over 1130 species occur there, of which almost 1000 are restricted to southern africa. In contrast some 335 species occur in eurasia including north africa and the canary islands, about 290 species occur in the new world and just 36 species occur in australasia (Goldblatt 2000; Goldblatt et al. 2008). Rich coloring and extreme diversity

are the distinctive characteristics of more than 300 iris species. Under diverse ecological conditions in their broad territory, irises grow as mesophytes, merophytes-cryophytes,psammophyte, calcifuges and as calcifiles (Rodionenko 1987). The iridaceae family is taxonomically difficult to analyze and phylogenetically poorly understood. The genetic boundaries, species affiliations, and phylogenetic relationships vary from one author to another (Rodriguez and Catedral 2003). The extreme diversity of morpho-ecological characteristics, the evidence of convergent evolution in many traits used for systematic reconstructions and a particularly chromosomal complex pattern of iris species are complicated. Cytogenetic researches based on chromosomes studies have been conducted as a useful tool in providing data with regard to the chromosomes numbers and composition (martinez et al. 2005). *Iris* has considerable polyploidy as well as disploidy with x=12 and 10 are the most common numbers. Disploidy and aneuploidy appear to have an important role in the chromosome evolution of iridaceae. Polyploids tend to reduce the number of duplicate sites due to diploidization mechanism and gene silencing (Leitch and Bennett, 1997). Systematic studies related to *iris sisyrinchium* haven't been found and this species is endemic to tunisia, algeria and libya (Cuénod *et al.* 1954). *Iris juncea*, especially found in the north of africa (maroc, algeria, libya) and sicilia (Cuénod et al. 1954).

In the present study, we tried to realize a morpho-cytological characterization of these iris species with regards to their ornamental importance.

Materials and methods

Plant material: the plants (number = 180) were collected from their biotope and planted in the research field of borj cedria ecopark (tunis–tunisia). Two *iris sisyrinchium* populations were collected from distinct geographical regions of Tunisia (separated distances equaling one hundred eighty kilometers) (table 1). One *iris juncea* population was identified in hammamet region with the *iris sisyrinchium* species.

Table 1. climate and soil characteristic of studied iris locality (ec: soil electric conductivity, om: soil organic matter percentage).

Locality	Climate region	Latitude/longitude	Ph	Ec	Om (%)
Beja (b)	Humid	36°73' n/9°83'n	7.01	1.35	2.43
Hammamet (h)	Sub-humid	36°24' n/10°32'e	7.38	2.82	0.67

Experimental design

Morphological traits

Morphological measurements were taken during the peak of flowering season (end-february to mid april) from 2007 to 2009. The populations were scored for 12 morphological characters (table 2).

Table 2. Morphological characters recorded in *iris* populations.

Character/code	Unit
Leaf length (ll)	Cm
Stem length (sl)	Cm
Flower heigth (fl)	Cm
Flower diameter (fd)	Cm
Levels number (ln)	1 to 4
Ovary length (ol)	Cm
Perianth tube length (pl)	Cm
Anther length (al)	Cm
Fillet length	Cm
Bulb diameter	Cm
Seed number/fruit	Number
Seed diameter	Mm

Pearson correlation was performed to determine the interrelationships between traits. Principal component analysis (pca) was utilized to show the patterns of variation of quantitative variables among populations. Statistical analyses were performed using the sas 9.1.

Karyological analysis

Chromosome analyses were performed using root tips pretreated with 0.10% colchicine at room temperature for 2-3h. The material was mixed then in carnoy 1 solution (3:1 v/v) for 24 h at 4°c. Root tips were hydrolyzed in 1 n hcl at 60°c for 15-25 min and then washed, stained with schiff reagent for 2h (Jahier, 1992 modified). Photomicrographs of well-spread metaphase images were captured with a cooled ccd camera using a microscope. Chromosomes were arranged from the longest to the shortest, and were designated with the arabic numerals (Stebbins, 1971).

The following traits for each chromosome pair: s (short arm), l (long arm), t (total chromosome length), arm ratio (r=l/s) and ci (centromeric index=l/t) were calculated and used to classify the chromosomes according to levan et al. (1964). In addition, mean chromosome length (mcl), mean total relative length (trl) and interchromosomal symmetry index (a) of watanaabe et al. (1999) were calculated. The chromosomes pairs were arranged according to their length. Karyotype asymmetry was estimated using the asymmetry index of huziwara (1962), called also, total form index (tf) was calculated according to the following formula: total sum of shorts arm lengths/ total sum of chromosomes lengths.

Data concerning the long arm chromosomes length (l), short arm lengths (s), total chromosomes length (t) and centromeric index (ci) were submitted to analysis of variance using the metaphasic plaques as replications, for each species. Mean separations of each character within populations was performed using the test of duncan at 5%. Idiograms were made using the mean values of chromosomes length (statistical). The sas 9.1 package was used for statistical analysis.

Results

Morphological analysis

Quantitative traits results were summarized in table 3. It shows an important variability in flower diameter, flower height, filament length and seed diameter ($p<0.0001$). The variability was lesser in bulb and leaf traits.

Table3. Mean, standard deviation (std), maximum (max) and minimum (min) for the quantitative evaluated traits.

Code	Mean	St d	Min	Max
Ll	32.68	8.12	19	50
Sl	32.78	6.92	25	48
Fd	4.18	1.36	2.27	7
Fh	3.85	1.39	1.8	7.8
Pl	2.25	0.28	1.6	2.7
Ol	2.00	0.44	1	2.95
Al	1.61	0.28	1	2
Fl	1.21	0.44	0.5	2
Ln	1.37	0.68	1	3
Sn	60.87	23.02	28	117
Bd	1.58	0.51	1	3.6
Sd	1.23	0.24	0.78	1.74

Correlations between traits were calculated (table 4). The strongest positive correlations were between flower diameter and flower height ($r^2=0.856$), perianth tube length and ovary length ($r^2=0.661$). These correlations were highly significant ($p<0.0001$).the negative correlation within some traits cannot be attributed to the peculiar properties of the individual plants such as genetic variations, but may be explained by the genetic and the developmental relationships between some combinations of traits (Wanli and Zhangcheng, 1998; Rahimi et al. 2011).

Table 4. Correlation coefficient between quantitative traits.

Code	LL	SL	FD	FH	PL	OL	AL	FL	LN	SN	BD	SD
LL		0.12	-0.198	-0.067	-0.338	-0.096*	-0.425	-0.197	0.363	0.183	0.196	0.085
SL			-0.187	-0.029	0.046	0.145	-0.388	-0.150	-0.157	0.069	-0.151	0.176
FD				0.856**	0.239	0.274	0.040	0.520*	-0.370	-0.270	-0.091	-0.192
FH					0.239	0.313	0.028	0.553**	-0.356	-0.214	0.069	-0.260
PL						0.661**	0.331	0.179	-0.454*	-0.222	0.128	-0.296
OL							0.256	0.214	-0.236	-0.087	-0.046	-0.232
AL								0.266	-0.187	0.030	0.079	-0.156
FL									-0.422*	-0.107	-0.191	-0.04
LN										0.167	0.111	0.359
SN											0.384	0.071
BD												0.042

* significant at $p\leq 0.05$ and** significant at $p \leq 0.01$.

The pca results (table 5) showed the correlation of each character with the principal components, the percentage of variation explained by these components, the variability explained and accumulated by the axes. In our counts, 100% of variation was accumulated by the two first axes. The first axes represented 63.81% of variation. It was positively correlated with flower diameter, flower height, perianth tube length, ovary length, anther length and filament length level. In this axis, the traits with the most important contribution were related to the flower length and flower height (r^2=0.361). The second axis (36.19% of variability) was highly positively correlated with anther length (r^2=0.427) and negatively correlated with flower seed number (r^2=-0.450). The total amount of variability accounted for the two principal components was 100%; this high percentage indicated that the traits showed a strong association (rahimi et al. 2011).

Table 5. Correlation of the analyzed traits with the two first pca principal axes

Descriptor	**Axe 1**	**Axe 2**
Ll	-0.21	0.39
Sl	-0.34	0.15
Fd	0.36	-0.01
Fh	0.36	-0.02
Pl	0.23	0.36
Ol	0.22	0.38
Al	0.16	0.42
Fl	0.35	-0.11
Ln	-0.34	0.17
Sn	-0.27	0.31
Bd	-0.12	-0.45
Sd	-0.35	-0.10

Karyological analysis

Iris juncea: the number of chromosomes was found to be 2n =32 with 15 pairs of submedian (sm) and 1 pair of subterminal (st). This number was reported by Martinez et al. (2010). The chromosome length varied from 3.86 to 6.89 µm.

The karyotype formula: 2n= 32= 30sm + 2st. One satellite was observed in these species (fig 1). Arms ratio of the chromosomes ranged from 1.87 to 3.12. Total chromosome length was about 82.92 µm and mean of total relative length (trl) consisted of 3.12. The mean value of centromeric index was 0.3 and the percentage of total form (tf) was about 29.54%. The interchromosomal asymmetry of Watanabe et al. (1999): a was 0.4; it indicated a symmetrical karyotype (table 6).

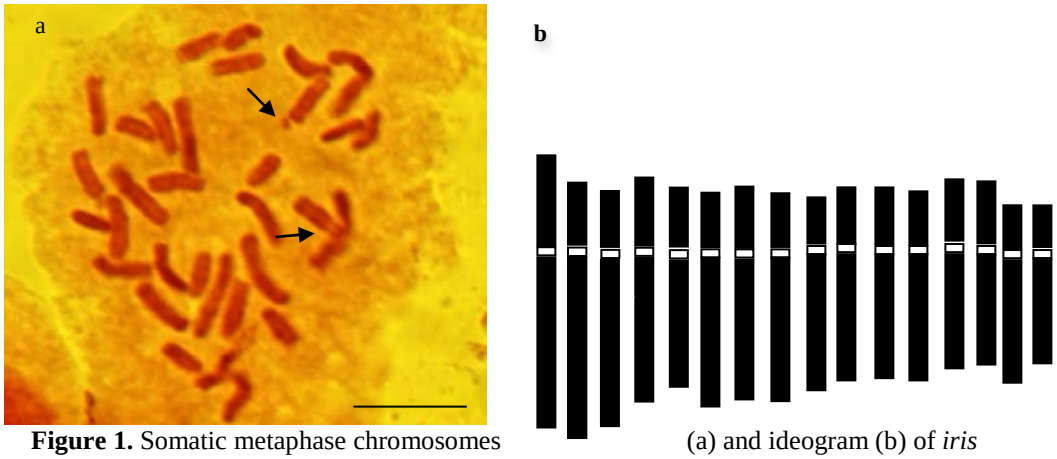

Figure 1. Somatic metaphase chromosomes (a) and ideogram (b) of *iris juncea (the bars represent 10 µm)*. The arrows represent the secondary construction.

Table 6. Chromosomes details of *iris juncea*.

Pairs	T	L	S	Ci	Arm ratio (r)	Chromosome type
1	6.89±2.0	4.49±1.07	2.4±0.98	0.35	1.87	Sm
2	6.41±1.9	4.77±1.71	1.64±0.46	0.26	2.91	Sm
3	5.87±1.26	4.39±0.71	1.48±0.56	0.25	2.97	Sm
4	5.68±1.14	3.8±0.58	1.88±0.64	0.33	2.02	Sm
5	5.38±1.35	3.34±0.53	1.55±0.48	0.29	2.15	Sm
6	5.37±1.19	3.94±1.06	1.43±0.67	0.27	2.76	Sm
7	5.32±1.37	3.70±0.93	1.62±0.45	0.30	2.28	Sm
8	5.22±1.37	3.8±1.21	1.42±0.38	0.27	2.68	Sm
9	4.98±0.31	3.77±0.5	1.21±0.20	0.24	3.12	St
10	4.89±0.66	3.34±0.53	1.49±0.41	0.30	2.24	Sm
11	4.76±2.21	3.27±1.85	1.49±0.41	0.31	2.19	Sm
12	4.75±0.96	3.34±0.83	1.41±0.25	0.30	2.37	Sm
13	4.63±1.0	3.02±0.21	1.62±0.87	0.35	1.86	Sm
14	4.54±1.25	2.91±0.93	1.63±0.45	0.36	1.79	Sm
15	4.37±0.67	3.23±0.63	1.14±0.19	0.26	2.83	Sm
16	3.86±0.26	2.77±0.151	1.09±0.31	0.28	2.54	Sm
Total	82.92	57.88	24.50			
Mean	5.18	3.62	1.53	0.3		
Tf%	29.54					
Trl	3.12					
A	0.40					

T: total chromosome length; l: long arm; s: short arm; r: arm ratio= l/s; ic: centromeric index; trl: total relative length, tf: total form ; a= mean (l-s/l+s).

Iris sisyrinchium: for hammamet population, the chromosome number was found to be 2n= 26: 9 pairs of the chromosomes are st-type and 3 pairs of sm-type. Their length varied from 6.43 to 11.59 µm. Arms ratio of the

chromosomes ranged from 1.72 to 4.88. This population had the biggest chromosomes. The second pair represented a satellite (fig 2c).

The total relative length (trl) mean was 3.85. The mean value of centromeric index was 0.21 and the percentage of total form tf is about 12.63%. The interchromsomal symmetry index of Wtanabe et al (1999) a was 0.57 (table 7). The mitotic metaphase cells of beja population consisted of 24 chromosomes: 9 pairs of sm-type and 3pairs of st-type. The chromosome length ranged from 5.74 to 8.13 µm. Such us in the first population arms ratio of the chromosomes ranged between 1.70 and 4.88. The total relative length means (trl) was 1.58. The centromeric index mean value was 0.28. The total form percentage (tf%) was 38.71% and a was 0.44 (table8).

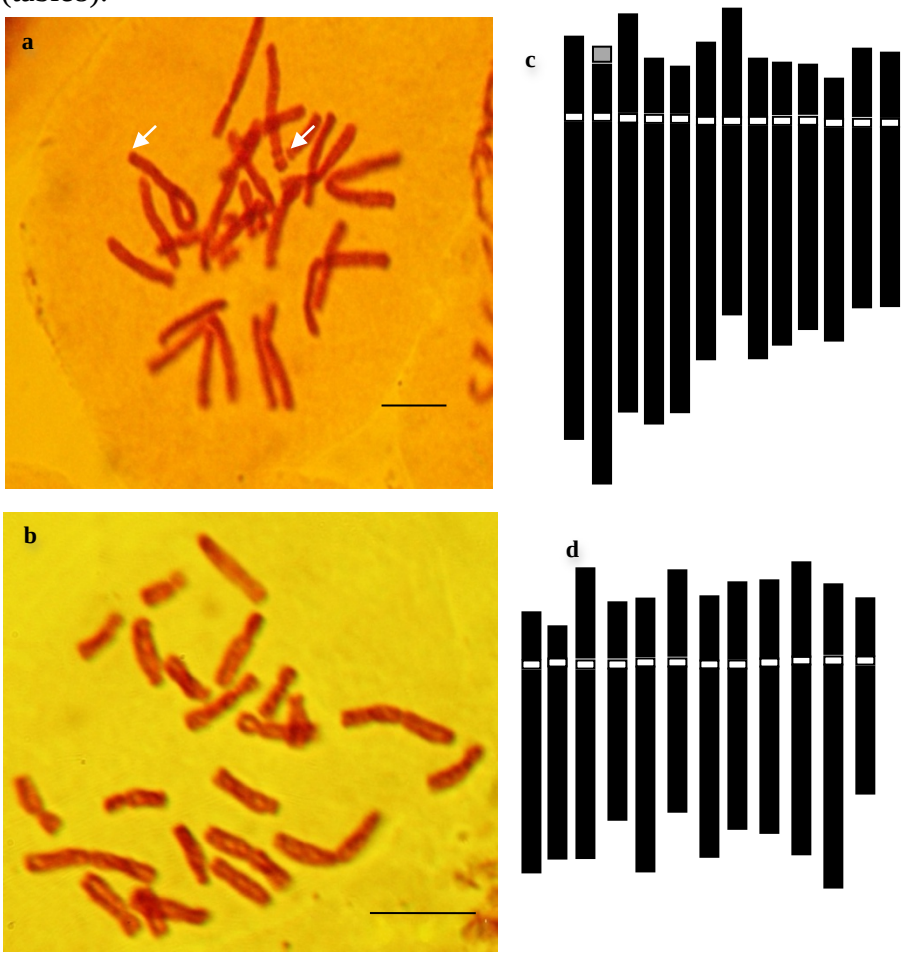

Figure 2. Somatic metaphase chromosomes (a,b), ideogram (c,d) of *iris sisyrinchium populations* of hammamet and beja respectively *(the bars represent 10µm)*. The arrows represent the secondary construction.

Table 7. Chromosomes details of *iris sisyrinchium* (population of hammamet).

Pairs	T	l	S	Ic	Arm ratio (r)	Chromosome type
1	11.59 ± 4.16	9.62±3.1	1.97±1.06	0.17	4.88	St
2	11.17± 4.65	9.53±4.48	1.64±0.17	0.15	5.81	St
3	9.35 ± 2.67	6.76±2.75	2.59±0.08	0.28	2.61	Sm
4	9.32 ± 4.24	7.89±4.25	1.42±0.002	0.15	5.56	St
5	8.81 ± 2.12	7.6±1.35	1.21±0.746	0.14	6.28	St
6	8.25±0.96	6.290±0.10	1.96±0.86	0.24	3.21	St
7	7.83±0.78	4.95±0.50	2.88±1.28	0.37	1.72	Sm
8	7.10±0.67	5.75±0.86	1.35±0.19	0.19	4.26	St
9	7.66±0.51	6.11±0.51	1.55±0.99	0.20	3.94	St
10	6.71±1.65	5.42±1.54	1.27±0.10	0.19	4.27	St
11	6.67±2.35	5.6±2.37	1.07±0.04	0.16	5.23	St
12	6.5±1.89	4.72±1.53	1.77±0.36	0.27	2.67	Sm
13	6.43±3.31	4.74±2.89	1.69±0.46	0.26	2.80	Sm
Total	107.39					
Mean	8.26	6.54	1.72	0.21		
Tf%	12.63					
Trl	3.84					
A	0.57					

Table 8. Chromosomes details of *iris sisyrinchium* (population of beja).

Pairs	T	L	S	Ic	Arm ratio (r)	Chromosome type
1	8.13± 4.13	5.61 ±2.08	2.52±2.36	0.31	2.23	Sm
2	7.73±1.44	5.89±1.53	1.84±0.56	0.24	3.20	St
3	7.53±1.69	5.03±0.93	2.5±0.79	0.33	2.01	Sm
4	7.32±2.94	4.97±1.54	2.35±0.93	0.32	2.11	Sm
5	7.28±1.14	5.42±1.54	1.86±0.50	0.26	2.91	Sm
6	6.68±1.13	4.997±0.69	1.686±0.72	0.25	2.98	Sm
7	6.62±0.58	4.55±0.41	2.07±0.37	0.31	2.20	Sm
8	6.57±2.45	5.34±2.39	1.23±0.64	0.19	4.34	St
9	6.25±1.84	4.24±1.17	2.01±1.31	0.32	2.11	Sm
10	6.12±1.58	5.08±1.42	1.04±0.27	0.17	4.88	St
11	6.10±1.94	3.84±0.47	2.26±1.63	0.37	1.70	Sm
12	5.47±4.13	4.0±2.08	1.47±2.36	0.27	2.72	Sm
Total	81.8					
Mean	6.82	4.91	1.90	0.280		
Tf%	38.71					
Trl	4.24					
A	0.44					

T: total chromosome length; l: long arm; s; short arm; r: arm ratio=l/s; ic: centromeric index=s/t; trl: total relative length tf: total form = $(\sum s/\sum t)*100$; a=mean (l-s/l+s).

Discussion

Morphological study

We have signaled at this report significant variation at five traits within the twelve traits analyzed, between *iris juncea* and *iris sisyrinchium* population. Furthermore, *iris juncea* had a big flower with an attractant yellow color and a good held in vase. These characteristics make this species available for cut flower culture. In contrast, *iris sisyrinchium* species set a bigger number of flowers but these one were fugaceous. An important advantage characterized these species is their ecological flexibility, in fact, iris plants vegetate even in poor soils and in different climates.

Karyological study

Considering the results obtained by other authors, our counts of 2n = 32 for *i. Juncea*, fit into the chromosome numbers found in the literature for these species (Martinez et al. 2010). The chromosome number of *iris sisyrinchium* species found in literature is about 2n = 24, it concords with the result obtained for beja population. The 24 chromosome number was reported by others authors such as simonet (1932). Chakhgari et al. (2013) reported 32 chromosomes for iranian *iris sisyrinchium*. Goldblatt and takei (1997) suggested that in the early evolution of the iridaceae, there was a burst of polyploidy followed by descending dysploidy in many genera. So, the chromosome number 2n= 26, obtained for the hammamet population, can be explicated with a polyploidy then dypsloidy, if we know that this species coexisted with *iris juncea* in this region. This species need to advanced cytotaxonomical methods for diagnosing basic set of chromosomes.

The iridaceae are variable in chromosome numbers, sizes and morphologies (Kenton and Heywood 1984). Such variability may be related to cycles of polyploidy and disploidy reduction in both neotropical (Goldblatt et al. 1998) and old world species (Goldblatt and Takei, 1993). *Iris juncea* species presented a karyotype with submetacentric and subterminal chromosomes. Three metaphasic cells, from the five cells analyzed, presented the same number of sm and st chromosomes. In the two other cells, the number of sm chromosomes decreased to view metacentric chromosomes. The increase of karyotypic asymmetry occurs due to the change of the centromere to terminal or subterminal position, and to differences in the relative size of the chromosomes of the complement, making the karyotype more heterogeneous (Stebbin, 1971). Many authors advanced that the basic number, in the genus *iris*, vary from 6 to 12 and Martinez et al. (2010) have affirmed that *iris juncea* is a tetraploïd, so the karotypic formula of this species is 2n= 4x= 32. In iris, changes in basic chromosome numbers are frequent, the ancestral base number, however, remains uncertain (Goldblatt and Takei, 1997).

Conclusions

The present study highlights an important variability, as soon as morphological and cytological character, between the two species*; iris juncea* and *iris sisyrinchium* populations. So, these species need more investigation even when we know their innumerable biochemical and ornamental virtues, add to their menaced being.

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